REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
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UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
DEPARTEMENT DE BIOCHIMIE
****
LABORATOIRE DE PHYTOBIOCHIMIE ET D'ETUDE DES PLANTES MEDICINALES

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
****
UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY
****
LABORATORY FOR PHYTOBIOCHEMISTRY AND MEDICINAL PLANTS STUDIES

PRELIMINARY SCREENING OF SIX MEDICINAL PLANTS FOR ANTICONVULSANT ACTIVITY AND FURTHER EVALUATION OF *Annona muricata* FOR ANTICONVULSANT AND RELATED PHARMACOLOGICAL EFFECTS

THESIS
Submitted and defended in Fulfilment of Requirements for the Award of the Doctorat/Ph.D Degree in Biochemistry

Par : **DONGMO NGUEPI Mireille Sylviane**
Master of Science (MSc) in Biochemistry

Sous la direction de
FEKAM B. Fabrice
Professor, University of Yaounde I

Année Académique : 2017

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DEPARTEMENT DE BIOCHIMIE
DEPARTMENT OF BIOCHIMIE

ATTESTATION OF CORRECTION OF DOCTORAT/Ph.D THESIS

We, the undersigned, members of the jury of the thesis defense of **Doctorat/Ph.D** of Madame **DONGMO NGUEPI Mireille Sylviane**, Matricule number **09R1341**, following authorization N°/017-099/UYI/VREPDTIC/DAAC/DEPE/SPD of the Rector of Yaoundé I University of the 26th January 2017, certify that the corrections required from the candidate during this assessment made on the 09th March 2017 have actually been done and that this document can be accepted in its current form.

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Yaoundé , 30 JUIL 2018 .....

President of Jury

Examiner

Head of Department

Mouridjo Fewou Paul D3C,D.Etat
Professor
Enzymology-Toxicology

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Yaoundé , ……

President of Jury **Examiner**

Head of Department

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ANNEE ACADEMIQUE **2017/2018**
(Par Département et par Grade)

DATE D'ACTUALISATION: 25 Avril 2018

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13	NGONDI Judith Laure	Maître de Conférences	En poste
14	NGUEFACK Julienne Epse KENFACK	Maître de Conférences	En poste
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20	DAKOLE DABOY Charles	Chargé de Cours	En poste
21	DEMMANO Gustave	Chargé de Cours	En poste

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35	LUNGA Paul KAILAH	Chargé de Cours	En poste
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26	GOUNOUE KAMKUMO Raceline Epse FOTSING	Chargé de Cours	En poste
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33	NOAH EWOTI Olive Vivien	Chargé de Cours	En poste
34	LEKEUFACK FOLEFACK Guy Benoît	Chargé de Cours	En poste
35	NGOUATEU KENFACK Omer BEBE	Chargé de Cours	En poste
36	NGUEGUIM TSOFACK Florence	Chargé de Cours	En poste
37	NGUEMBOCK	Chargé de Cours	En poste
38	NJUA Clarisse YAFI	Chargé de Cours	En poste
39	TADU Zéphirin	Chargé de Cours	En poste
40	YEDE	Chargé de Cours	En poste
41	ETEME ENAMA Serge	Assistant	En poste
42	KANDEDA KAVAYE Antoine	Assistant	En poste
43	KOGA MANG'Dobara	Assistant	En poste
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19	NGOUO Lucas Vincent	Chargé de Cours	En poste

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27	NOUKEU KOUAKAM Armelle	Assistant	En poste
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18	ACAYANKA Elie	Chargé de Cours	En poste
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24	KENNE DEDZO Gustave	Chargé de Cours	. En poste
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26	MAKON Thomas Beauregar	Chargé de Cours	En poste
27	MBEY Jean Aimé	Chargé de Cours	En poste
28	NDI Julius NSAMI	Chargé de Cours	En poste
29	NDOSIRI Bridget NDOYE	Chargé de Cours	En poste
30	NJOYA Dayirou	Chargé de Cours	En poste
31	NYAMEN Linda Dyorisse	Chargé de Cours	En poste
32	PABOUDAM GBAMBIE Awaou	Chargé de Cours	En poste
33	TCHAKOUTE KOUAMO Hervé	Chargé de Cours	En poste
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13	KEUMEDJIO Félix	Maître de Conférences	En poste
14	KOUAM Jacques	Maître de Conférences	En poste
15	MBAZOA née DJAMA Céline	Maître de Conférences	En poste
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17	NGO MBING Joséphine	Maître de Conférences	En poste
18	NOUNGOUE TCHAMO Diderot	Maître de Conférences	En poste
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24	EYONG Kenneth OBEN	Chargé de Cours	En poste
25	FOTSO WABO Ghislain	Chargé de Cours	En poste
26	KAMTO Eutrophe Ledoux	Chargé de Cours	En poste
27	NGONO BIKOBO Dominique Serge	Chargé de Cours	Chef Cell/ MINRESI
28	NOTE LOUGBOT Olivier	Chargé de Cours	CEA/MINESUP
29	OUAHOUO WACHE Blandine Marlyse	Chargé de Cours	En poste
30	TAGATSING FOTSING Maurice	Chargé de Cours	En poste
31	ZONDEGOUMBA Née NKWENGOUA T. Ernestine	Chargé de Cours	En poste
32	NGOMO Orléans Epse SIELECHI	Chargé de Cours	En poste
33	NGINTEDO Dominique	Assistant	En poste
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5	CHEDOM FOTSO Donatien	Chargé de Cours	En poste

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7	KOUOKAM KOUOKAM Etienne Appolin	Chargé de Cours	En poste
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9	MOTO MPONG Serge Alain	Chargé de Cours	En poste
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11	TINDO Gilbert	Chargé de Cours	En poste
12	TSOPZE Norbert	Chargé de Cours	En poste
13	WAKU KOUAMOU Jules	Chargé de Cours	En poste
14	ABESSOLO ALO'O Gislain	Assistant	En poste
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11	DOUANLA YONTA Hermann	Chargé de Cours	En poste
12	FOMEKONG Christophe	Chargé de Cours	En poste
13	KIANPI Maurice	Chargé de Cours	En poste
14	KIKI Maxime Armand	Chargé de Cours	En poste
15	MBAKOP Guy Merlin	Chargé de Cours	En poste
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17	MBEHOU Mohamed	Chargé de Cours	En poste
18	MBELE BEDIMA Martin	Chargé de Cours	En poste
19	MENGUE MENGUE David Joe	Chargé de Cours	En poste
20	NGUEFACK Bertrand	Chargé de Cours	En poste
21	POLA DOUNDOU Emmanuel	Chargé de Cours	En poste
22	TAKAM SOH Patrice	Chargé de Cours	En poste
23	TCHANGANG Roger Duclos	Chargé de Cours	En poste
24	TCHOUNDJA Edgar Landry	Chargé de Cours	En poste

25	TETSADJIO TCHILEPECK Mesmin Erick	Chargé de Cours	En poste
26	TIAYA TSAGUE N. Anne- Marie	Chargé de Cours	En poste
27	DJIADEU NGAHA Michel	Assistant	En poste
28	MBIAKOP Hilaire George	Assistant	En poste
29	NIMPA PEFOUKEU Romain	Assistant	En poste
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11	BOUGNOM Blaise Pascal	Chargé de Cours	En poste
12	NJIKI BIKOÏ Jacky	Chargé de Cours	En poste
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6	WOAFO Paul	Professeur	En poste
7	PEMHA Elkana	Professeur	En poste
8	TCHAWOUA Clément	Professeur	En poste
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11	DJUIDJE KENMOE Gemaine épse ALOYEM KAZE	Maître de Conférences	En poste
12	EKOBENA FOUDA Henri Paul	Maître de Conférences	*Chef Dépt UN*
13	EYEBE FOUDA Jean Sire Armand	Maître de Conférences	En poste
14	FEWO Serge Ibraïd	Maître de Conférences	En poste
15	HONA Jacques	Maître de Conférences	En poste
16	MBANE BIOUELE Cesar	Maître de Conférences	*En poste*
17	NANA NBENDJO Blaise	Maître de Conférences	En poste
18	NJANDJOCK NOUCK Philippe	Maître de Conférences	*Chef Serv. MINRESI*
19	NOUAYOU Robert	Maître de Conférences	En poste
20	OUMAROU BOUBA	Maître de Conférences	*En poste*
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22	SIEWE SIEWE Martin	Maître de Conférences	En poste
23	SIMO Elie	Maître de Conférences	En poste
24	ZEKENG Serge Sylvain	Maître de Conférences	En poste
25	ABDOURAHIMI	Chargé de Cours	En Poste
26	BODO Bernard	Chargé de Cours	En poste
27	ENYEGUE A NYAM Françoise épouse BELINGA	Chargé de Cours	En poste
28	EDONGUE HERVAIS	Chargé de Cours	En poste
29	FOUEDJIO David	Chargé de Cours	*Chef Cellule MINADER*
30	MBINACK Clément	Chargé de Cours	En Poste
31	MBONO SAMBA Yves Christian U.	Chargé de Cours	En poste
32	MVOGO Alain	Chargé de Cours	En Poste
33	NDOP Joseph	Chargé de Cours	En poste
34	OBOUNOU AKONG Marcel Brice	Chargé de Cours	*Dir.acad/Univ.Int.Etat Cam-Congo*
35	TABI Conrad Bertrand	Chargé de Cours	En poste
36	TCHOFFO Fidèle	Chargé de Cours	En poste
37	VONDOU DERBETINI Appolinaire	Chargé de Cours	En Poste
38	WAKATA née BEYA Annie Sylvie	Chargé de Cours	*Chef Serv. MINESUP*
39	WOULACHE Rosalie Laure	Chargé de Cours	En poste
40	CHAMANI Roméo	Assistant	En Poste
41	MELI'I Jorelle Larissa	Assistant	En Poste
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38	TCHAPTCHET TCHATO De Pesquidoux I	Chargé de Cours	En poste
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Répartition chiffrée des enseignants permanents par Département
(25 Avril 2018)

Département	**Nombre d'enseignants**				
	Pr	**MC**	**CC**	**ASS**	**Total**
BC	05 (1)	11 (4)	21 (11)	03 (2)	**40 (18)**
BPA	11 (1)	09 (3)	20 (8)	03 (0)	**43 (12)**
BPV	04 (0)	09 (2)	10 (3)	04 (4)	**27 (9)**
CI	09 (1)	8 (1)	16 (2)	1 (1)	**34 (5)**
CO	9 (0)	13 (3)	10 (3)	1 (0)	**33 (6)**
IN	2 (0)	1 (0)	10 (0)	13 (2)	**26 (2)**
MA	3 (0)	4 (0)	19 (1)	4 (0)	**30 (1)**
MB	2 (0)	5 (1)	6 (2)	0 (0)	**13 (3)**
PH	8 (0)	16 (1)	15 (3)	2 (1)	**41 (5)**
ST	4 (0)	14 (2)	22 (3)	2 (0)	**42 (5)**
Total	**57 (3)**	**90 (17)**	**149 (36)**	**33 (10)**	***329 (66)***

Soit un total de : 329 (66) dont
- Professeurs 57 (3)
- Maîtres de Conférences 90 (17)
- Chargés de Cours 149 (36)
- Assistants 33 (10)
- ( ) = Nombre de femmes.

Le Doyen de la Faculté des Sciences

AWONO ONANA

DEDICATION

To my parents: Dongmo François and Dongmo Kenfack Marie

My family, especially my brothers and sisters: Judith, Valerie, Eric, Gervais, Joel, Christelle

My spouse Christian Tchapga

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Behavioural scientists do things in groups, as indeed, no man is an island. Aware that I have had contacts with a great deal of people, benefiting from them in different ways, I wish to express my profound gratitude to all of them.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

AAE: Ascorbic Acid Equivalent

AA Amino acid

AED Anti epileptic drug

AGE: Advanced glycation end product

AlCl: *Alchornea cordifolia* leave,

$AlCl_3$ Aluminium chloride

AnMr: *Annona muricata* roots

AnMfl : *Annona muricata* flower

AnMs: *Annona muricata* seed

AnMl : *Annona muricata* leaves

AnMpf: *Annona muricata* pulp of fruit

AnMtw: *Annona muricata* twigs

AnMp: *Annona muricata* pericarp

AnMsb : *Annona muricata* stem bark

AnMs: *Annona muricata* seed,

AnMtw: *Annona muricata* twig,

AnSl: *Annona senegalensis* leave,

AnStw: *Annona senegalensis* twig,

BCG Bromocresol green

CAM Cameroon

CBZ Carmabazepine

Ca Calcium

Cu Copper

CNS Central nervous system

CH_2Cl_2 Methylene chloride

CH_3COONa Sodium acetate

DeA: *Desmodium adescendes*

DMSO Dimethyl sulfoxide

DNA Deoxy ribo nucleic acid

DPPH 1,1-Diphenyl-2- picrylhydrazyl

DZP Diazepam

EtOAC Ethyl acetate

FETAX Frog Embryo Teratogenesis Assay-Xenopus

FiTl: *Ficus.thoningii* leave,

FiTsb: *Ficus.thoningii* stembark,

FRAP: Ferric Reducing Antioxidant Power Assay

GAE Gallic Acid Equivalent

GABA Gamma-aminobutyric acid

GABA-A Gamma-aminobutyric acid-A

GABA-B Gamma-aminobutyric acid-B

HCG Human Chorionic Gonadotropin

HNC Herbier national du cameroun

H_2O Water

H_2O_2 Hydrogen peroxide

HCl Hydrochloric acid

GEPR Genetically epilepsy-prone rat

GSH Glutathione

GSSG Glutathione disulfide

K Potassium

LD50	Lethal dose 50
MDA	Malondialdehyde
MES	Maximum electroshock
MeOH	Methanol
Mg	magnesium
MnSOD	Manganese-superoxide dismutase
Na	Sodium
NaOH	Sodium hydroxide
$NaNO_3$	Sodium nitrate
Na_2CO_3	Sodium carbonate
NH_4OH	ammonia
NO	Nitric oxide
OD	Optical density
OECD	Organization for economic cooperation and development
PHB:	Phenobarbital,
PTZ:	Pentylenetetrazol
PTX:	Picrotoxin

QE:	Quercetin Equivalent
RAGE	Receptor for advance glycated end product
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SNP	Sodium nitroprusside
TEAC:	Trolox Equivalent Antioxidant Capacity
TC:	Tonicoclonic
TLC	Thin layer chromatography
USA	United states of America
ViDsb:	*Vitex doniana* stembark,
Vit C	Vitamin C
WHO	World health organization
Zn	Zinc

LIST OF TABLES

LIST OF FIGURES

LIST OF IMAGES

LIST OF APPENDICE

ABSTRACT

Annona muricata is a small tree or shrub which can reach 8 m of height found in Africa and other parts of the world. Different parts of the plant are used in traditional medicine for the treatment of diseases such as epilepsy. This study investigated the anticonvulsant, antioxidant, antiglycation, analgesic and safety properties of *A. muricata*.

Plant collection was done in the center region and the plants collected evaluated for their anticonvulsant activity using pentylenetetrazole (PTZ). *A. muricata* (AnM) was selected to further the work. After qualitative and quantitative phytochemical analysis, two widely used animal models of epilepsy PTZ and picrotoxin (PTX) were tested against the extracts and fractions of AnM. Acetic acid, formalin and the hot plate test were used to investigate its antinociceptive effects and naloxone for the involvement of opioid receptors. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO), were among the antioxidant tests used in this study. Ribose, glucose and glyoxal were used to evaluate the antiglycation activity and the adverse effect evaluated.

After anticonvulsant screening of plants with PTZ, *A. muricata* displayed the highest activity at 200 mg/kg b.w. Consequently, different organs from *A. muricata* were tested using Pentylenetetrazol (PTZ) and Picrotoxin (PTX) induced seizure in mice. Leaves, roots and twigs in both PTZ and PTX tests were able to delay seizure onset and reduce convulsion duration significantly ($p<0.001$), while they also significantly increased total protein and albumin values to normal values ($p<0.001$). The roots which displayed the highest activity were subjected to fractionation. Of all the fractions and the compounds tested, J18 at all the doses tested significantly delayed the appearance of seizure ($p<0.001$). Qualitative phytochemical screening showed that the different parts of the plant extract contained metabolites as tannins and flavonoids, while the fractions contained alkaloids, saponin, steroid and triterpernes. The quantitative phytochemical analysis showed that the highest content of polyphenols, flavonols and flavonoid, was obtained in leaves, with a value of 230.8 ± 2.386 GAE/g, 6.571 ± 0.048 mg/g ($p<0.001$) and 9.962 ±0.870 QE/gof extract ($p<0.01$) respectively.

In the DPPH test, the maximum percentage of inhibition was from root extract with a percentage of inhibition of 87.89%; while the scavenging activity of OH was greater in leaves and twigs (69.96%). In the FRAP assay, the root extract presented the highest activity. The

percentage of inhibition of NO was highest in leaves, and a slight difference in the activity of the extracts was observed in the ABTS test.

In the acetic acid pain model, *Annona muricata* roots (AnMr) and leaves (AnMl) at 200 mg/kg significantly ($p<0.001$) reduced mice writhings with respectively 64.65% and 53.84%. Further investigation of AnMr showed that in the 1st phase of the formalin test at 200 mg/kg it significantly ($p<0.5$) reduced the time for licking or biting the paw by mice (65.44%); but the effects were reduced by co-administration of naloxone. In the 2nd phase of the formalin test, the time of licking or biting the paw by the mice was significantly reduced at 200 mg/kg ofAnMr with a value of 56.80% ($p<0.05$). On the hot plate test, only the same dose of AnMr significantly ($p<0.5$) inhibited the time of staying on the plate with a percentage of inhibition of 119.01%.

The formation of advanced glycation end product (AGE) was significantly reduced by ribose after 5 days at concentrations ranging from 20 to 95 µg/ml ($p<0.001$) while the same pattern was observed with glucose at 5, 10, and 95 µg/ml ($p<0.001$). In the second step using glyoxal, except the concentration of 10 µg/ml, all the tested doses of AnMr extract were able to significantly reduce the formation of AGE ($p<0.001$).

In the acute toxicity test, 30 min after administration the behavior of animals such as locomotion was modified and after an hour some of the animals treated with AnMPf (pulp of fruit) and AnMs (seeds) died, giving LD_{50} values of 2.31 g/kg and 4.53 g/kg for AnMs and AnMPf respectively. After the 2nd day, the surviving mice were recovering. Using the *Macaca mulatta*, monkey, rhesus kidney cells (LLCMK2) for the cytotoxicity, the compounds J17 and P106 were safe products with IC_{50} values higher than the concentrations tested during the 3 days of observation. The developmental toxicity using the FETAX test shows that J17 and J19 at the lowest concentration tested did not empeds the development of embryos, while P106 was responsible for the delay in development observed.

The results indicate that the different plants tested, especially *A. muricata* has anticonvulsant effect in the different models used, exhibiting analgesic and antiglycating effects and antioxidant properties that support its use in traditional medicine. Meanwhile further investigations on characterization of active extracts/fractions and understandings of their mechanisms of action are required.

Key word: anticonvulsant, antioxidant, antiglycation, analgesic, *A. muricata*.

RESUME

La médicine traditionnelle en Afrique a longtemps été considérée comme partie intégrante du quotidien des populations et un héritage socio-culturel. Les plantes médicinales à l'exemple d'*Annona muricata* sont utilisées par les tradipraticiens camerounais pour le traitement de l'épilepsie, une maladie neurologique qui peut affecter les personnes de tous les âges, et dont la prevalence est élevée dans les pays en voie de developement. Malgré de bons pronostics en termes de contrôle des crises, beaucoup de patients n'ont pas accès aux antiepileptiques disponibles, et font par conséquent recours aux plantes medicinales et aux herbarlistes pour controler leur mal, particulièrement dans les pays pauvres d'Afrique. Cette étude a porté sur l'evaluation des propriétés anticonvulsivantes de six plantes de la pharmacopée camerounaise, puis sur l'évaluation de l'activité anticonvulsivante d'*Annona muricata* (AnM), et les effets pharmacologiques associés.

La collection des plantes a été effectuée dans differents villages de la région du Centre (Cameroun) et leurs extraits utilisés pour lévaluation de l'activité anticonvulsivante par le pentylenetetrazole (PTZ). Compte tenu des résultats préliminaires, *A. muricata* a été retenu pour les études ultérieures. Afin de confirmer l'activité anticonvulsivante, 2 modèles couramment employés pour induire des crises de type épilepsie, le PTZ et la Picrotoxin (PTX) ont été utilisés pour évaluer l'activité des diferents extraits et fractions d'AnM et le flumazenil a été utilisé pour determiner le mécanisme d'action. Les tests à l'acide acétique, la formaline et la plaque chauffante ont été utilisés pour déterminer les effets antinociceptifs. Le naloxone a été utilisé pour déterminer l'implication des recepteurs opioides. Les tests au DPPH, ABTS, ORAC, FRAP, OH et NO ont été utilisés dans le cadre de la détermination de l'activité antioxidante au cours de cette étude. Le ribose, le glucose et le glyoxal ont été utilisés pour déterminer l'activité antiglycante des extraits, tandis que le rotarod, la toxicité aigüe, la cytotoxicité et la toxicité developementale ont été évalués dans le cadre de l'évaluation des effets secondaires.

Après le screening anticonvulsivant avec le modèle PTZ en utilisant les extraits de *Ficus thoningii*, *Desmodium adescendes*, *Alchornea cordifolia*, *Annona senegalensis et Annona muricata*, AnM a présenté la meilleure activité à 200 mg/kg (écorce), en augmentant l'intervalle d'apparition des crises et en protégéant les animaux des convulsions. Cette plante a par conséquent été retenue pour le reste de l'étude. Dans la suite, différents organes de *A.*

muricata: feuilles, racines, écorces, graines, pulpe de fruit et péricarpe ont été testés en utilisant les crises induites par le PTZ et la PTX chez la souris. Les feuilles, les racines et les écorces ont présenté les meilleures activités dans les deux tests en augmentant le l'intervalle d'apparition des crises, réduisant la durée des crises de manière significative ($p<0.001$); de même les taux de protéines totales et de l'albumine ont été ramenées à des valeurs normales ($p<0.001$). Parmi les fractions issues des racines, J18 a significativement augmenté l'intervalle d'apparition des crises à toutes les doses testées ($p<0.001$), suivie de J16 à 75 mg/kg, J20 à 150 mg/kg et le produit P106 à 20 mg/kg.

Le criblage phytochimique qualitatif a révelé les tannins, flavonoides, stéroides, terpenoides, anthocyanins, lipides, saponins, phénols, huiles essentielles, anthraquinones, coumarines et alcaloïdes dans les différents organes de la plante. L'analyse phytochimique quantitative a montré que la teneur maximale en polyphénols, flavonols et flavonoides se retrouve dans les feuilles avec des valeurs respectives de 230.8 ± 2.386 GAE/g, 6.571 ± 0.048 mg/g ($p<0.001$) et 9.962 ±0.870 QE/g d'extrait ($p<0.01$). Des ions tels que Ca, Mg, K, et Na ont été retrouvés à des pourcentages différents dans les extraits. Le flumazenil, un antagoniste du récepteur GABA a réduit le temps d'apparition des crises chez la souris.

Dans le test au DPPH, le pourcentage maximum d'inhibition de 87.89% a été obtenu avec l'extrait des racines à la concentration maximale testée de 300µg/ml; tandis que l'activité antiradicalaire vis-à-vis des ions hydroxyls (OH^-) était plus élevée avec l'extrait les feuilles et des écorces (69.96%) en comparaison aux racines (59.74%). Pour ce qui est du test de FRAP, les racines avaient l'activité la plus élevée. Le pourcentage d'inhibition de NO^- a été élevé avec l'extrait les feuilles, tandis qu'une faible différence de l'activité des extraits a été observée dans le test de l'ABTS.

Dans le test de douleur à l'acide acétique, les racines (AnMr) ainsi que les feuilles (AnMl) d'*A. muricata* à 200 mg/kg ont réduit de façon significative ($p<0.001$) le temps de tortillement chez la souris, avec des valeurs de 64.65% et 53.84%. Des investigations poussées avec AnMr ont montré que lors de la 1ère phase du test à la formaline, la dose de 200 mg/kg a significativement réduit ($p<0.5$) le temps de léchage ou de morsure de sa patte par la souris de 65.44%; mais ces effets ont été réduits après la co-administration du naloxone, un antagoniste. Dans la 2nde phase du test à la formaline, le temps de léchage ou de morsure de la patte a été significativement réduite par l'extrait (AnMr) à la dose de 200 mg/kg avec une valeur de 56.80% chez la souris ($p<0.05$). Dans le test à la plaque chauffante, la même dose

de AnMr a significativement ($p<0.5$) inhibé le temps de retenue sur la plaque avec un pourcentage d'inhibition de 119.01%.

L'activité antiglycante de AnMr en utilisant le glucose et le ribose dans la 1ère phase, a montré une réduction significative de la formation des produits terminaux de la glycation par le ribose après 5 jours à des concentrations de 20 et 95 µg/ml ($p<0.001$), tandis que les mêmes résultats ont été observés avec le glucose à 5, 10, et 95 µg/ml ($p<0.001$). Dans la seconde phase en utilisant le glyoxal, à l'exception de la concentration de 10 µg/ml, toutes les doses de AnMr ont été capables de réduire la formation des produits terminaux de la glycation ($p<0.001$).

Concernant la neurotoxicité, 30 min après administration de l'extrait, la réduction de la locomotion a été observée, particulièrement à la dose 400 mg/kg de AnMr ($p<0.01$) ainsi que le produit P106, mais cet effet disparait avec le temps, les animaux étant capables de rester sur la barre rotative jusqu'à la fin de la période d'observation. Dans le test de toxicité aiguë, 30 min après l'administration, le comportement des animaux comme la locomotion a été modifié et une heure plus tard certains animaux du groupe traités avec AnMPf et AnMs sont morts. Deux jours plus tard, les survivants ne présentaient plus aucun signe de toxicité. Ceci a permis d'avoir des doses léthales à 50% (DL_{50}) de 2.31 g/kg et 4.53 g/kg respectivement pour AnMs et AnMPf, les autres extraits étant non toxiques car aucune mortalité n'a été observée jusqu'à 2 g/kg. Les tests de cytotoxicité ont montré que les fractions J17 et P106 étaient non cytotoxiques contre les cellules normales de rein de singe (LLCMK2) avec des IC_{50} plus élevées que les concentrations testées pendant les 3 jours d'observation. La toxicité developmentale par le test FETAX a montré que les fractions J17 et J19 à la plus petite dose testée ne présentent pas d'effets néfastes pour la croissance des embryons tandis que le produit P106 est responsable du retard de developement obvervé chez les embryons.

Les résultats obtenus indiquent que les plantes étudiées, particulièrement *A. muricata*, possède une activité anticonvulsivante, analgésique, antioxidante et antiglycante, justifiant ainsi son utilisation par la médecine traditionnelle. Néanmoins des investigations futures sont nécessaires pour une meilleure compréhension des mécanismes mises en jeu dans l'activité des extraits et fractions testés.

Mots clés: anticonvulsivant, antioxidant, antiglycation, analgésique, *A.muricata*.

INTRODUCTION

INTRODUCTION

Epilepsy is collectively designated to be a group of central nervous system disorder characterized by spontaneous occurrence of seizures generally associated with the loss of consciousness and body movements **(Moshi *et al.,* 2005)** Epilepsy is the most common neurological disorder worldwide and affects about 40 million people withabout 80% of people living in the developing world **(Njamnshi *et al.,* 2010)**. In Cameroon, the disease is present in the Central region of the country with a prevalence of 6% in Badissa **(Njamnshi *et al.,* 2009b).** Other foci were also detected the NorthWest region –particularly Batibo with a prevalence of 1.8% **(Nsame, 2003)** and the Western region in the Khoung-Khi division with a prevalence of 7.0% **(Dongmo, 2003)**. This high prevalence has led to the creation of a national epilepsy control programme.

Epilepsy is a sudden imbalance between the excitatory and the inhibitory system **(Pedersen *et al.,* 2009)**. Over excitation of excitatory amino acids is a commonly accepted mechanism for the genesis of epilepsy **(ilhan *et al.,* 2005a)**. The activation of excitatory amino acid receptors can trigger the formation of reactive oxygen species (ROS). These increased ROS not only causes long-lasting seizure formation, but if not arrested, can lead to neuronal death. Furthermore many experimental studies have demonstrated that oxidative stress occurs as a consequence of prolonged epileptic seizures, suggestingthat oxidative stress may play an important role in seizure-induced brain damage **(Ilhan *et al.,* 2005b)**. The negative effects of oxidative stress may be mitigated by the consumption of antioxidants to delay or inhibit the oxidation of lipids and other molecules **(Guttierez *et al.,* 2012)**. Some molecules, such as reducing sugars, proteins, nucleic acids or lipids through non enzymatic reactions, produce advanced glycated end products (AGE). Advanced glycated end product induce oxidative stress and molecular cross linkages that cause cellular and tissue damage by impairing proteinfunction and clearance **(Harris *et al.,* 2014)**.

Currently available anti epileptic drugs (AEDs) do not provide cure nor prevent relapse and are often accompanied by debilitating adverse effects including sedation, blood dyscrasias, teratogenesis **(Chindo *et al.,* 2009)**. Among the epileptics, only 20% of them are on treatment **(Njamnshi *et al.,* 2010)** and about a third of patients are refractory to pharmacotherapies **(Alagpulinsa *et al.,* 2010)**. Furthermore, there is currently no drug available which completely prevents the development of epilepsy (e.g. after head trauma) **(Temkin, 2001)** and all currently available AEDs drugs are synthetic molecules. Receptors

involved are mainly GABA and glutamate, but some studies have revealed the importance of opioid receptors in the regulation and modulation of brain excitability **(Becker *et al.*, 1994**). Epilepsy and pain have common mechanism and several anticonvulsants are clinically used to treat painful disorders **(Pace *et al.*, 2013),** such as neuropathic pain, a type of pain were neuronal hyperexcitability and corresponding molecular changes have many features in common with the cellular changes in certain forms of epilepsy **(Amabeoku, 2007)**. Despite substantial progress made in the treatment of neurological diseases, epilepsy remains a significant therapeutic challenge **(Chindo *et al.*, 2009)**.

According to World Health Organization (WHO), greater than 80% of the total world's population depends on traditional medicines in order to satisfy their primary health care needs **(Pieme *et al.*, 2014)**. The use of medicinal plants for the treatment of human diseases is an ancient practice and has greatly increased in recent years **(Aiyelero, 2012)**. In recent times, scientific study of their effects has flourished. Despite the availability of rich synthetic drugs, plants remains– even today a fundamental ingredient of health-care. In developing countries, the practice of medicine still relies heavily on plant extracts for the treatment of human ailments **(Adeyemi *et al.*, 2007)**.

Several reports also tend to show that two-thirds of plant derived natural products are effective antioxidants that can reduce the oxidative stress in cells and therefore be useful in the treatement of many human diseases **(Krishnaiah *et al.*, 2011)**. Many medicinal plants with a long history of use in folk medicine in different countries against a variety of diseases have turned out to be rich sources of antioxidants **(Ramkisoon *et al.*, 2012)** and antiglycating agents, due to the presence of many constituents that have been demonstrated to be able to suppress AGE formation **(Adisakwattana *et al.*, 2014)**.Membrane lipids are particularly susceptible to oxidation due to their high concentration of polyunsaturated fatty acids and their association in the cell membrane with enzymatic and non-enzymatic systems able to generate free radical species **(Yagi *et al.*, 2013)**. Excessive production of free radicals accelerates non-enzymatic protein glycation reactions **(Ramkisoon *et al.*, 2012)**. Hence, studies on natural antioxidants have gained increasingly greater importance

The development of new pharmacological agents has become a major goal in epilepsy research. Considering the great reliance on traditional medicinal plants for treatment of diseases and the potential for drug discovery, it becomes relevant to search for effective and relatively safe plant medicines through scientific validation of claims about their use in

traditional medicine. Therefore this study focuses on the study of medicinal plants with anticonvulsant, analgesic, antioxidant and antiglycating properties.

Hypothesis: Cameroonian flora contains some medicinal plants that are useful for the treatment of epilepsy.

In order to verify this claim, the following objectives were pursued.

Objectives

General objective: Contribute to the valorization of selected Cameroonian medicinal plants commonly used in the treatment of epilepsy

Specific objectives

- Collection and preliminary screening of plants used in traditional medicine for anticonvulsant activity.
- Exploration of the anticonvulsant activity of the selected medicinal plant *Annona muricata* extracts and its fractions.
- Evaluation of the antioxidant, antiglycation and analgesic properties of *Annona muricata* extract
- Study of the acute toxicity, effect on motor function, cytotoxicity and teratogenicity of the extracts and fractions

CHAPTER I: LITERATURE REVIEW

I.1-Epilepsy

I.1.1 Definition

The name epilepsy is derived from the Greek word « epilambanein » meaning to seize **(Gopal, 2010)**, to attack **(Chakir, 2007; WHO, 2001)**, to be overwhelmed by surprise **(WHO, 2005)**. Epilepsy is collectively designated to a group of central nervous disorders characterized by spontaneous occurrences of seizures generally associated with the loss of consciousness and body movements **(Muhizi, 2002)**. For **Sridharan (2002)**, epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. Epilepsy is a major neurological disorder characterized by recurrent, spontaneous brain seizures or convulsions **(Sander *et al.*, 1996)**.

I.1.2 Epidemiology

Epilepsy is a common neurologic condition that affects at least 40 million people worldwide **(Njamnshi *et al.*,** 2010**)**. Its prevalence in developing countries is generally higher than in developed countries **(Stafford *et al.*, 2008)**. Approximately 1% of the world's population has epilepsy, the second most common neurological disorder after stroke **(Porter and Meldrum, 2001)**. It is a universal disease since it has no social, racial, sex or geographical boundaries **(Hauser and Annegers, 1993; Chakir, 2007; WHO, 2001)**. In the world about 5% of the population will have seizures during its life **(Chakir, 2007)**. According to **Sridharan (2002)**, 3 to 5% of the population has a seizure sometimes in their life and half to one percent of the population have active epilepsy. According to many studies around the world, the prevalence is around 8.2‰ for the overall population. However this can be an underestimation, because some studies in underdeveloped countries, suggest a higher prevalence, higher than 10‰ **(Gourieet *et al.*, 1999)**.

In developed countries, an epidemiological study suggests a prevalence of 6.8‰ in the USA **(Amole *et al.*, 2009)**, while in France, the prevalence is 0.5% **(Arpeije, 2001)**; in UK, 300000 persons are affected **(Sridharan, 2002)**. It is probable that this prevalence is high in less developed countries **(Sander and Shorvon, 1996)**, because of higher incidence of antecedent factors such as brain infections **(Amole *et al.*, 2009)**.

In developing countries, higher prevalence rates ranging from 14-57 per thousand have been reported from some South American and African countries **(Sridharan, 2002).** In

Cameroon a survey conducted in the Mbam valley revealed a prevalence of 4.9‰ **(Pepoumi *et al.*, 2002).** There are 5,500,000 persons with epilepsy in India **(Sridharan, 2002)**, while in Morocco there are 300,000 epileptics, 21‰ in Senegal, 18.6‰ in Kenya **(Chakir, 2007)**. Studies in Latin America, revealed prevalence between 17 and 22 ‰ **(De Bittencourt *et al.*, 1996)**. A study conducted in Pakistan, revealed an overall prevalence of 9.99 ‰ **(Khatri et al., 2003).** These prevalences indicate 80% of the burden of epilepsy in the developing world **(WHO, 2005)**.

Annual incidence rate of epilepsy is 50-70 per 100,000 in industrialized countries and 100-190 per 100,000 in resource-poor countries **(Walczak *et al.*, 2001; Dhir *et al.*, 2006)**. The incidence varies greatly with age, with high rates in early childhood, low levels in early adult life and an escalating second peak in people higher than 65 years of age **(WHO, 2005**)

I.1.3 Impact

Epilepsy is one of the non-transmittable diseases widespread in the world, and this mainly in low income countries with regard to demographic growth **(Chakir, 2007)**. Recent studies suggest a six-fold increase risk of epilepsy-related deaths mainly in Africa .This is higher than the two-to-three fold increase reported in developed countries **(Christianson *et al.*, 2000; Diop et *al.*, 2005).** Though the reasons are not clear, in fact, data suggest that people from socio-economically deprived backgrounds in developed countries are more likely to develop epilepsy **(Heaney *et al.*, 2002; Sander, 2003).**

It is a disease traditionally considered as serious, due to urine and saliva emitted during seizure and which are considered contagious **(Arborio and Dozon, 1998)**. This neurological disorder is viewed as a shameful disorder and has severe social implications in African communities as it carries a stigma with a profound impact on quality of life in developed as well as developing regions **(Baskind and Birbeck, 2005).** Epileptics therefore suffer more from these stigmas than the disease itself **(WHO, 2000)**. Sufferers are often shunned and discriminated against with respect to education, employment and marriage **(Baskind and Gretchen, 2005; Stafford *et al.*, 2008)**. In countries with old supernatural beliefs, epileptics have to face discrimination and social rejection. Even in developed countries, epileptic seizures remain a taboo and those suffering avoid talking about it **(Baskind and Gretchen, 2005)**. In Haiti, for example, epilepsy had been attributed to voodoo possession and was treated by local mambos **(Chakir, 2007)**. These disabilities vary between rural and urban regions, with rural residents suffering greater **(Baskind and Gretchen, 2005)**.

Thus, out of 50 million epileptics in the world, 35 did not have access to an adequate treatment, due to nonexistent services, or epilepsy is not considered as a medical problem, or a cerebral affection that can be treated, especially in developed countries **(WHO, 1997)**. Therefore, it is a heavy sanitary and socio-economic burden that needs management as stressed by the WHO objective **(WHO, 1999)**.

I.1.4 Physiopathology

Strategies for the development of new AEDs are essentially based on the fact that epilepsies are due to disequilibrium between the excitatory and inhibitory transmission in the brain **(Oliveira *et al.*, 2008; Gandhimathi, 2008)**. Seizures are due to the shock of many nervous cells, at the same time and abnormally; this electric shock are sudden excessive and generally brief **(Biraben, 1998)**.

Neurons are interconnected in a complex network in which each individual neuron is linked through synapses with hundreds of others. A small electrical current is discharged by neurons to release neurotransmitters at synaptic clefts to permit communication with each other. Normally, these neurotransmitters will contribute to the ions (sodium, calcium, potassium…) exchange between the inner and the outer media **(WHO, 2001)**. More than a hundred neurotransmitters or neuromodulators have been shown to play a role in neuronal excitation. However, the major excitatory neurotransmitter in the brain is glutamate and the major inhibitory neurotransmitter in the brain is gamma-amino butyric acid (GABA). An abnormal function of either of these could result in a seizure **(Dhir *et al.*, 2006)**. The higher glutamatergic neurotransmission seems to be a signal of epileptogenesis and can be explain by mechanisms of excitotoxicity, the loss of cells during chronic epilepsy and the abnormal dendritic morphology observed in the temporal lobe of epileptics. An increase in glutamate and aspartate concentrations is associated with seizures **(Parent *et al.*, 1997)**. An excited neurone will activate the next neuron whereas an inhibitory neuron will not. A normal neurone discharges repetitively at a low baseline frequency, and it is the integrated electrical activity generated by the neurons of the superficial layers of the cortex that is recorded in a normal electroencephalogram. If neurones are damaged, injured or suffer electrical or metabolic insult, a change in the discharge pattern may develop. In the case of epilepsy, regular low-frequency discharges are replaced by bursts of high-frequency discharges usually followed by periods of inactivity. An epileptic seizure is triggered when a whole population of neurons discharges synchronously in an abnormal way **(BFE, 2003)**. This abnormal discharge

may remain localized or it may spread to adjacent areas, recruiting more neurons as it spreads **(Alagpulinsa, 2010)**.

In some cases seizures can be announced by "signal symptoms" and characterized for example by a strange taste, a bad feeling, and in some cases by an internal shiver **(Chakir, 2007)**. Further analysis shows that the blockade of post-synaptic gamma-amino butyric acid receptors or an inhibition of GABA synthesis is the principal origin of brain discharge. According to **Bienvenu *et al.*, (2002)**, an epileptic attack can be triggered by a sensory stimulus, which is specific for individuals; complex phenomenon of neurons recruitment triggers a reaction in chain and thereby the propagation of seizures **(BFE, 2003)**.

I.1.4.1 Neurotransmitters and epilepsy

A neurotransmitter is a substance released by one neuron and acting rapidly, briefly and at short range on the membrane of an adjacent neuron, producing a change in conductance which either increases or decreases the excitability of the post synaptic cell **(Rang and Dale, 1991)**. Major neurotransmitters implicated in epilepsy are GABA, glycine and glutamate (Bradford, 1995). It is therefore important to emphasize their role as neurotransmitters in epileptogenesis, since they are the major inhibitory and excitatory transmitters in the central nervous system, respectively **(Alagpulinsa, 2010).**

i- GABA

GABA is the major inhibitory neurotransmitter in the mammalian brain **(Rang and Dale, 1991; Saransaari *et al.*, 2005)**; an increase in its level in brain has a variety of CNS dependent effects including anticonvulsant effect **(Kumar and Gandimathi, 2003)**. It has been shown to contribute to over 40% of the synapses in the cortex alone and its inhibition is thought to be an underlying factor in epilepsy **(Gale, 1992; Amole *et al.*, 2009)**. It is formed in the GABAergic terminal axon and liberated in the synapse where it acts on one of the two types of receptors: GABA-A receptors which are ionic channels acting as ligands and controling the enter of Cl^- ions in the cell, and GABA-B receptors which are metabotropic protein G coupled receptors which increase the conductance of K^+ while reducing Ca^{2+} **(Treiman, 2001; Sperk *et al.*, 2004)**. The widespread distribution of GABA and the fact that virtually all neurons are sensitive to its inhibitory effect, suggest that its function is ubiquitous in the brain **(Rang and Dale, 1991).**

GABA plays an important role in regulation of neuronal excitability by interaction with specific membranes **(Teuber *et al.*, 1991)**; and impairment of GABA function produces seizures **(Olsen *et al.*, 1997)**. Studies show that GABA plays the role of neurotransmitter in about 30% of all CNS synapses **(Pal *et al.*, 1999);** the discovery of many GABAergic synapses in the CNS indicates that its absence could induce epilepsy **(Bradford, 1995)**. It exerts its major inhibitory effect via GABAA receptor (which is a ligand-gated ion channel), by increasing neuronal membrane conductance for chloride ions causing membrane hyperpolarization, resulting in reduced neuronal excitability and most rapid inhibition in brain **(Sieghart, 1992)**. GABAA receptor consists of five subunits that form a chloride ion channel **(Macdonald *et al.*, 1994)**. The subunits consist of various subtypes and pharmacological studies have shown that individual subunits and subtypes confer different sensitivities to agents acting on GABAA receptors **(Neelands *et al.*, 1998)**. It is postulated that exposure of GABA to postsynaptic receptors for a brief GABAA receptor is target for many important neuroactive drugs including antiepileptic drugs benzodiazepines and barbiturates **(Scholze *et al.*, 1996; Sieghart, 1992)**. It is therefore evident that GABA is a critical inhibitory transmitter and seizures can rapidly be elicited by pharmacological disruption of GABAergic mechanism **(Feldman *et al.*, 1991)**.

ii- Glycine

Glycine is an inhibitory neurotransmitter **(Lopez *et al.*, 2001)** mainly in the spinal cord, acting on its own receptor, which functionally resembles the GABAA receptor **(Rang and Dale, 1991; Salih and Mustafa, 2007)**. It is an amino acid found in higher concentrations in certain presynaptic elements of the spinal cord and functioning as inhibitory mediator in many synapses of these regions **(Guyton, 1989)**.

iii- Glutamate

Glutamate and GABA are quantitatively excitatory and inhibitory most important neurotransmitters in the mammalian brain **(Pedersen *et al.*, 2009)**. Glutamate is the most important excitatory neurotransmitter in all rapidly conducting relay pathways of the motor and sensory systems of the outer tube of the central nervous system **(Rang and Dale, 1991)**. It produces fast or prolonged synaptic excitation and triggers various calcium dependent processes in the target cells, including production of nitric oxide **(Bienvenu *et al.*, 2002)**. It is released from brain-tissue by electrical stimulation or by potassium-evoked depolarization **(Rang and Dale, 1991)**. Glutamate acts via two types of receptors, ionotropic glutamate receptors (iGluR) which are ligand-gated cation specific channels and metabotropic glutamate

receptors (mGluR) which are G-protein-coupled receptors **(Chapman, 2000; Mares *et al.*, 2004)**. Epilepsy may arise as a consequence of a dramatic release of glutamate from central nerve terminals **(Leonard, 2003)**, due to an abnormal absorption **(Meldrum, 1994; Fisher *et al.*, 2003)**. Glutamate is implicated in the mechanism of initiation and propagation of seizures **(Loscher, 1998).**

I.1.5 Etiology

There are many causes of epilepsy. Generally, an etiology is found in a quarter of epileptics **(Biraben, 1998)**. Meanwhile, two factors can be considered: genetic, predominant in primary epilepsy and lesions **(Cambier *et al.*, 2004)**.

I.1.5.1 Genetic

Genetic factors can cause recurrent abnormal synchronization and episodic hyperexcitability of neuronal networks through various mechanisms **(Steinlein, 2004)**. Epilepsy is hereditary in few cases; it is generally 4% of encountered cases **(LsCE, 2002; Biraben, 1998)**. Approximately 10% of children genetically predisposed become epileptics **(LsCE, 2002**), seizures occurring later in infancy **(Hoban, 1996)**.

In majority of cases, the heredity is multifactorial, but can be monogenic in certain cases, and the gene has been identified **(Cambier *et al.*, 2004; Jacobs *et al.*, 2009)**.

I.1.5.2 Lesional epilepsy

Epilepsy can result from a subjacent cerebral disease. Any cerebral disease can provokes epilepsy, but all patients suffering from the same cerebral disease will not have epilepsy **(OMS, 2001)**.

Epilepsy can also be caused by previous active pathology, such as birth trauma to the brain, during or following meningitis, trauma to the skull and brain later in life, cerebral abscesses, cerebral infarction, cerebral hemorrhage or subarachnoid hemorrhage **(Bienvenu *et al.*, 2002)**.

I.1.5.3 Infectious and parasitic

A brain infection can result in seizure at any age; it represents 3% of epilepsies **(Biraben, 1998)**. It is one of the higher incidences of the disease in developing countries **(OMS, 2001)**.Cysticercosis in an endemic area is characterized by multiple cerebral

calcifications and is a frequent cause of later epilepsy **(Fredy et Caille, 1995)**. After-effects of an infection (meningitis) can provoke epilepsy **(Epilepsie Canada, 2003)**. A febrile disease can in children trigger febrile convulsions; approximatively 3% of these children will later have epilepsy **(OMS, 2001)**.

Development of epilepsy in HIV patients is frequent. It can result from opportunistic complications (cerebral toxoplasmosis, cerebral lymphoma). In many cases (23-30%), the etiologic outcome is negative **(Fredy et Caille, 1995)**.

I.1.5.4 Traumatic epilepsy

A trauma can trigger seizures at any age **(OMS, 2001)**. A frequent complication of a serious cranial trauma gives rise to post-traumatic epilepsy which is about 5% of epilepsies **(Fredy et caille, 1995; Biraben, 1998)**. This can be due to brain operation, **(Epilepsy foundation, 2003)**, wound, general mother's illness which alters the fœtal development **(Epilepsie Canada, 2003)**. In this case, neuronal activity during foetal development is disturbed and seizures occur early in life and consequences can become serious few years later **(Chakir, 2007)**.

I.1.5.5 Tumoral epilepsy

Tumors are responsible for only 4% of epilepsies **(Biraben, 1998)**, in children 1 to 2% are concerned. Even if benign (80% of cases), they are the first symptom in about 40% of cerebral hemispheric tumours in children **(Cambier *et al.*, 2004; Fredy et Caille, 1995)**.

In adults, it is more frequent **(Cambier *et al.*, 2004)** and is about 16% of later epilepsies. Incidence here is function of the foci and the nature of the tumour **(Cambier *et al.*, 2004)**.

I.1.5.6 Vascular Epilepsy

Cerebrovascular diseases are one of the most frequent causes of epilepsies **(Hoban, 1996; De Bittencourt *et al.*, 1996)**. They generally occur after 50 years **(Cambier *et al.*, 2004)**, and can be indexed as involved in epilepsy in 5% of cases **(Biraben, 1998)**. The risk increases from 60 years. In these patients, the disease can be due to vascular injury, drug toxicity, metabolic disturbance and tumours **(Chakir, 2007)**. Apoplexy is a recurrent cause of epilepsy in elders. A brain bleeding which is another form of apoplexy can trigger later in life

seizures. A cardiac attack can deprive the brain temporarily from oxygen, with the same result **(Epilepsy foundation, 2003)**.

On the other hand, cerebral lesions from vascular origin can induce resistant epilepsy. Arteriovenal malformations are found in a third of partial epilepsies cases starting in infancy **(Cambier *et al.*, 2004)**.

I.1.5.7 Metabolic epilepsy

Some metabolic disturbances, modification of metabolism prevent nutrients passage to the brain and can provoke epilepsy **(Cambier *et al.*, 2004; Epilepsy foundation, 2003)**. Intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol are responsible for the greater number of epilepsies **(Bienvenu *et al.*, 2002)**, which can be isolated or status epilepticus **(Epilepsy Canada, 2003)**. Epilepsy due to biochemical imbalance of some elements that can leads to hypoglycaemia, hypocalcaemia anoxia, is one of the aspects of the cerebral impact; threshold varies according to individuals **(Cambier *et al.*, 2004)**.

Even if the etiology of epilepsy has been determined in some cases, the cause is still undetermined in 50 - 60 % of cases **(Epilepsy Canada, 2003)**.

- The cause of epilepsy cannot always be determined **(LFBE, 2003)**, normal subjects without decelable cerebral lesion can have epilepsy. The epileptogenic threshold abnormaly low in such patients could probably be due partly to genetic factors **(Biraben, 1998; Kwan and Brodie, 2007)**; in such cases, epilepsy is said to be idiopathic.

- Symptomatic epilepsy is linked to brain lesions which can develop or not, resulting from a vascular disturbance, difficult birth, abnormal metabolism **(Biraben, 1998; LFBE, 2003; Kwan and Brodie, 2007)**

- Cryptogenic epilepsies are presumably symptomatic but the etiology is unknown **(Biraben, 1998; Kwan and Brodie, 2007)**.

Epilepsy is often but not always, the result of an underlying brain disease. Any type of brain disease can cause epilepsy, but not all people with the same brain disease will have epilepsy. It is therefore suspected that such symptomatic seizures are more prone to having epilepsy due to biochemical or neurotransmitter factors **(Diop *et al.*, 2003)**.

I.1.6 Classification of seizure

The characteristic event in epilepsy is the seizure **(Rang and Dale, 1991; Duncan, 2002)**. Seizure types are organized firstly according to whether the source of the seizure within the brain is localized (partial or focal onset seizures) or distributed (generalized seizures) **(kabatende, 2005; Gopal, 2010)**.

I.1.6.1 Generalized seizure

Generalized seizures are categorised according to the effect on the body but all involves loss of consciousness. They arise from many independent foci or from epileptic circuits that involves the whole brain **(Gopal, 2010)**, both hemispheres are affected **(Bienvenu *et al.*, 2002)**. Clinical manifestations are immediately bilateral and symmetric. Seizures can be:

- Convulsive: it is the case of tonic, clonic, tonico-clonic
- Non convulsive, as absences, atonic or myoclonies **(Hoban, 1996; Biraben, 1998)**

A generalized tonic -clonic seizure, consists of an initial strong contraction of the whole musculature, causing a rigid extensor spasm. Respiration stops and defecation, micturition and salivation often occur. This tonic phase lasts for about 1 minute and is followed by a series of violent, synchronous jerks, which gradually dies out in 2-4 minutes **(Sudarsky, 1990, Rang and Dale, 1991)**. The patient stays unconscious for a few more minutes, and then gradually recovers, feeling ill and confused. Injury may occur during the convulsive episode **(Rang *et al.*, 2003)**.

I.1.6.2 Partial seizure

Partial seizures are categorized based on the extent to which consciousness is affected. If it is unaffected, then it is simple partial seizure; otherwise it is a complex partial seizure (psychomotor) **(Gopal, 2010)**. They usually arise from an epileptic focus, a small portion of the brain that serves as the irritant driving the epileptic response. The discharge begins locally and often remains localized **(Rang and Dale, 1991)**. These may produce relatively simple symptoms without loss of consciousnesses, such as involuntary muscle contractions, abnormal sensory experiences or autonomic discharge, or they may cause more complex effects on consciousness, mood and behavior, often termed psychomotor epilepsy **(Rang *et al.*, 2003)**. Psychomotor epilepsy is often associated with a focus in the temporal lobe; the

attack may consist of stereotyped purposeless movements such as rubbing or patting movements, or much more complex behavior such as dressing, walking or hair combing. The seizure usually lasts for a few minutes, after which the patient recovers with no recollection of the event **(Webster and Jordan, 1989, Rang and Dale, 1991)**.

Epilepsy should not be understood as a single disorder, but rather as syndromic with vastly divergent symptoms but all involving episodic abnormal electrical activity in the brain **(Gopal, 2010)**.

I.1.7 Diagnosis of epilepsy

Epilepsy is primarily clinical diagnosed based on a history taken from both the patient and any witnesses of seizures and a thorough physical examination by medical practitioners, as the physician will rarely have the occasion to witness seizures **(Biraben, 1998)**.

There are limited diagnostic testing options available. Generally, the most important component of a diagnosis for epilepsy is a comprehensive description of seizure events. Given that the person with epilepsy might be unconscious during a seizure and not remember the events, clinicians have to rely on eyewitness accounts. Unfortunately, the accuracy of seizure descriptions by witnesses is generally low and there are wide variations in descriptions **(WHO, 2005)**.

Two major categories of diagnostic testing are normally used.The first involves monitoring: The most common test is an EEG which is a recording, generally from the scalp, of the voltage arising in the cells of the brain, as it fluctuates over time. EEGs may assist in determining where in the brain the electrical activity that generated the seizure is occurring. Usually a person will not have a seizure during an EEG and not all forms of epilepsy have a distinct background EEG pattern so it is not unusual to receive a normal result. When diagnosis is difficult, people who are suspected of having epilepsy may have video telemetry (or monitoring) that involves an extended hospital stay for constant EEG and video monitoring. The expectation is that several seizures will occur during the period of monitoring which can then be reviewed in detail **(Biraben, 1998)**.

The second major category of diagnostic tests is imaging: These tests include computer tomography (CT) scans, magnetic resonance imaging (MRI) scans, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scans. These scans allow medical practitioners to determine if there are any lesions or abnormalities in the

patient's brain. It is recommended in some countries that, all people suspected of having a new diagnosis of epilepsy should have an EEG within four weeks and those suspected of having partial seizures or adult-onset epilepsy should have an MRI.

There should be a harmonious relationship between the patient and the neurologist in order to facilitate the recognition and the management of the epileptic **(Blume, 2003)**. The decision of the physician is based on the conviction that the patient really had epilepsy, the context of seizure onset and social preoccupations, because a misdiagnosis can have serious consequences. With all this information obtained, the physician will apreade the seizure type and choose an adequate treatment **(Biraben, 1998; Blume, 2003)** which could be pharmacotherapy, surgery or a special diet **(Epilepsy Canada, 2003)**.

I.1.8 Management of epilepsy

I.1.8.1 Pharmacotherapy

Once a person is diagnosed with epilepsy, the most common treatment is anticonvulsant medication. It is the major therapeutic intervention, the first usually tested **(Epilepsy Canada, 2003)**. Seizure mechanisms suggest many pathways in which these drugs can reduce or eliminate crisis. The goal of anticonvulsant medication treatment is to maximize seizure control and reduce seizure severity by maintaining the blood concentration at a high level **(Benbadis, 2003)**.

Avoidance of adverse reactions to the medication is also important, hence the focus on dose adjustment and new generation of antiepileptic drugs (AEDs). AEDs called olds controlled seizures in 50% of patients with partial seizures and 60-70% from those having generalized tonico-clonic seizures. Phenobarbital, Phenytoïn, Carbamazepine and Valproate are first line old AEDs **(Duncan, 2002)**. However, the same anticonvulsant medication is not equally effective for all individuals with the same diagnosis and often results in a variety of adverse reactions **(Brodie and Dichter, 1997)**.

Phenobarbital is the only barbituric used in the treatment of epilepsy. It is especially used against generalized tonico-clonic seizures. It increases inhibition of GABA while reducing the glutamatergic excitation **(Brodie and Dichter, 1997)**.

Phenytoin, which has a stabilizing effect on the neuronal membrane by blocking ionic channels have the same indications as Phenobarbital. It is useful but requires a control of blood flow, at least at the initial phase of the treatment, because the blood concentration

increases regularly and slowly until a certain dose up to which the increase of the plasmatic level is fast, yielding toxic manifestations **(Brodie and Dichter, 1997)**.

Carbamazepine and Valproate have a stabilizing effect on the neuronal membrane. They reinforce the GABA action and are effective against partial seizures **(Brodie and Dichter, 1997)**.

Benzodiazepine (BZD) such as diazepam and clonazepam act by interfering between BZD and GABA receptors and are most useful against status epilepticus **(Blume, 2003; Cambier *et al.*, 2004)**.

After these AEDs called old, it is ten years later that other AEDs were made available. These are Vigabatrin firstly in 1989; followed by Zonisamide, Lamotrigine, Gabapentine, Felbamate, Topiramate, Tiagabine, Oxcarbazepine and recently (2000), Levetiracetam **(Duncan, 2002)**. They are more effective on partial seizures.

As all medicine, AEDs present adverse effects. Their seriousness depends on the drug type, the dose and the patient's response **(Epilepsy Canada, 2003)**. The most frequent are: somnolence, irritability, ataxia, memory disturbance, hyperactivity in children **(Klitgaard *et al.*, 2002; Duncan, 2002)**. Some drugs can have a unique effect such as phenytoin which cause gum disease, CBZ which can reduce the white blood cell level. They are due to a depressive effect on the CNS; they are worse at the beginning of the treatment and during dose increment; but tend to reduce with the adaptation of the body to the AED **(Benbadis, 2002)**.

After five years of a successful treatment, drugs can be withdrawn in about 70% of children and 60% of adult without relapse. Therefore, one can with the agreement of the neurologist, progressively reduce the medication **(Brodie and Dichter, 1997)**.

About 30% of epileptics cannot favorably react to treatment **(WHO, 2001; LFBE, 2003)** in this case a surgical treatment can be applied.

I.1.8.2 Surgery

Surgical treatment can be considered in cases of pharmacoresistant epilepsies, particularly in young subjects **(cambier *et al.*, 2004)**. It consist for patients with seizures initiated partially, to delimit, define the functionality and remove the area of abnormal brain

tissue without provoking any unacceptable neither neurologic norneuropscychologic deficit **(Mousnier, 2000; Biraben, 2002)**.

About half of 30% of patients for which seizures cannot be controlled by medication can undergo a surgery. In USA, about 100 000 epileptics required a surgery, but until now only 3000 surgeries are done each year suggesting that this mode of treatment is still underused **(Benbadis, 2002)**.

Candidates for a surgery should be evaluated by a multidisciplinary team in order to be sure that language, memory or other neurological functions will not be affected **(Benbadis, 2002).**

Three types of surgery are currently used:

- The most frequent operation cures epilepsy by abolishing the region were seizures initiates; frequently, the anterior lobe of the brain.

- The second step, hemispherectomy, involves a large resection of one of the brain hemisphere.

- In the corpus callosotomy, connections between the two brain hemispheres are broken **(Benbadis, 2002)**.

An entirely new treatment involves the stimulation of the vague nerve, useful nerve linking the brain to the heart, lung, and stomach **(Benbadis, 2002)**. This is used when the surgical operation is not possible or the patient refuses. A surgery on the superior part of the chest is necessary to implement the stimulator which has the size of a cardiac pacemaker. The stimulator is programmed using a computer and settled according to each individual. Some patients can experience difficulty swallowing, throat ache. This method is more effective when used in combination with AEDs **(Benbadis, 2002; NSE, 2005)**.

Clinical research has found seizure reduction rates of between 50 and 80% after surgery, but it may require a large personal expense or distance of travel. Other therapies are therefore clearly needed **(Kossof and McGrogan, 2005)**.

I.1.8.3 Special diet

The ketogenic diet is a high-fat, low-carbohydrate and adequate protein diet that have been in use for childhood intractable epilepsy since 1924. This diet has the potential

advantage of theoretically being available everywhere and at lower costs than newer AEDs **(Kossof and McGrogan, 2005)**.

Patients on a ketogenic diet are advised to take a high-potency multivitamin to ensure adequate availability of nutrients. In addition, a high intake of fiber (more than 20 g daily) is recommended to reduce fluctuations in blood sugar levels. It is particularly effective in children with severe, symptomatic and generalized epilepsy, with multiple seizures and considerably harmful to brain **(Benbadis, 2002)**. This regimen is not easy to be followed up, is not applicable to adults and does not give vitamins or minerals necessary for a balance diet **(Benbadis, 2002; NSE, 2004)**.

With all these different treatments, epileptics are not always satisfied; thus the need for a better understanding of the disease and the need of new drugs.

I.1.9 Experimental epilepsy

The use of animal models has been an essential component in the discovery **(Hosseinzadeh, 2003; Cord *et al.*, 2008)** and development of new drugs for the treatment of epilepsy **(Hansen *et al.*, 2004; Pereira *et al.*, 2008)**. So, a number of experimental animal models have been developed to elucidate its pathophysiology **(Cord *et al.*, 2008)**.

I.1.9.1 Chemoconvulsants induced epilepsy

This method consists of the administration of a dose of convulsant solution to animals. Many convulsants are currently used: PTZ, Strychnine, etc.

i- Pentylenetetrazole

Prevention of PTZ-induced seizures in laboratory animals is the most commonly used initial screening test for recognizing anticonvulsant drugs or traditional herbs. It is a CNS convulsant that is thought to act at the picrotoxin site of the GABAA receptor **(Ahmadiani *et al.*, 2003; Hansen *et al.*, 2004; Moshi *et al.*, 2007)**.

PTZ test represents a validated model for human generalized myoclonic seizures **(Ahmadiani *et al.*, 2003)**, petit mal type of seizure **(Hema, 2009)** or absence **(Loscher *et al.*, 2002)**. Some studies revealed that PTZ diminishes the GABAergic tone **(Ahmadiani *et al.*, 2003)**. It has been reported to produce seizures by inhibiting GABA neurotransmission (Mahomed, 2006). According to **Mendes *et al.*, (2009),** it is a substance able to inhibit chloride conductance by binding to sites of GABAA receptor complex.

PTZ is a selective blocker of the chloride ionophore complex to the GABAA receptor and after repeated or single dose administration leads to a decrease in GABAergic function and to the stimulation and modification of the density or sensitivity of different glutamate receptor subtypes in many different brain regions **(Schoerder *et al.*, 1998; Rauca *et al.*, 1999)**. PTZ also triggers a variety of biochemical processes including the activation of membrane phospholipases, proteases and nucleases. Marked alterations in membrane phospholipid metabolism result in the liberation of free fatty acids, diacyl glycerol, eicosanoids, lipid peroxides and free radicals **(Ilhan *et al.*, 2006)**. The aim of such studies is to enhance our understanding of the processes leading to epilepsy and to identify drug targets for antiepileptogenesis **(Loscher *et al.*, 2000)**.

ii- Strychnine

Strychnine, a glycine receptor antagonist (**Ahmadiani *et al.*, 2003; Salih and Mustapha, 2008)**, has been demonstrated to have a well-defined mechanism of convulsant action by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine **(Biggio *et al.*, 1992; Ahmadiani *et al.*, 2003)** and thus increasing spinal reflexes **(Rang *et al.*, 1998; Adeyemi *et al.*, 2007)**.

iii- Picrotoxin

Picrotoxin, a selective non-competitive antagonist of GABAA at GABA receptor, has been widely implicated in epilepsy **(Rang *et al.*, 1998; Amole *et al.*, 2009)**. According to Nicoll, picrotoxin is a GABAA receptor antagonist which produces seizures by blocking the chloride ion channel linked to GABAA receptors, thus blocking the presynaptic inhibition mediated by GABA **(Ilodigwe *et al.*, 2010; Okakon and Nwafor, 2009)** and preventing the entry of these ions into the brain **(Quintans *et al.*, 2008)**, therefore, inducing convulsion through rapid summation of synaptic activity **(Dhir *et al.*, 2006)**. This process will in turn, inhibit GABA neurotransmission and activity in the brain **(Ojewole *et al.*, 2006; Heidari *et al.*, 2009)**.

iv- Other chemoconvulsants models

Other chemoconvulsants are:

- Bicuculline, a pure receptor antagonist of GABA receptor as assessed by ligand binding studies **(Dhir *et al.*, 2006)**

- Kainic acid is one of the most widely used animal models of temporal lobe epilepsy. It is an amino acid receptor agonist which induced limbic seizures **(Ahmadiani *et al.*, 2003)**.

Administration of kainic acid activates ionotropic glutamate receptors and selectively induces excitotoxic cell death **(Cord *et al.,* 2008)**. It is a model of partial focal seizures with complex symptomatology and secondary generalization from the limbic focus as well as a model of epileptogenesis after status epilepticus **(Pedersen *et al.,* 2010)**.

I.1.9.2 Electrical model: maximal electroshock seizures (MES)

MES is probably the best validated method for assessment of AEDs discovery in generalized tonic-clonic seizures **(Loscher *et al.,* 2002; Rollas and Kücükgüzel, 2007)**. This test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures **(Ahmadiani *et al.,* 2003)**.

PTZ or MES treatments results in rapid and transient expression of a large class of immediate early genes, many encoding transcription factors **(Cole *et al.,* 2002)**.

I.1.9.3 Genetic models

During the past decade, substantial progress has been made indelineating clinical features of the epilepsies and the basic mechanisms responsible for these disorders. Eleven human epilepsy genes have been identified and many more are now known fromanimal models **(Jacobs *et al.,* 2009)**.

Genetic animal models of epilepsy can be subdivided into animals with spontaneous mutations and animals, usually mice, with induced mutations **(Löscher, 1999)**. Models with spontaneous mutations can be further subdivided into mutant animals with reflex epilepsy, in which seizures are elicited by specific sensory stimulation, and animals with spontaneous recurrent seizures. Animals in which epilepsy results from spontaneous mutations such as audiogenic seizure susceptible mice and rats or rats with spontaneous spike-wave discharges (e.g. the Genetic Absence Epilepsy Rat from Strasbourg (GAERS)) are widely used to study the pharmacology of elicited or spontaneous seizures **(Löscher, 1999)**. Genetic models of absence epilepsy such as GAERS or lethargic mice with spontaneous spike-wave dischargesare clearly better suited to correctly predictdrug efficacy against non-convulsive seizures, it is a model of spontaneous **(Cole *et al.,* 2002; Nicolazo *et al.,* 2009)** and chronic seizures **(klitgaard *et al.,* 2002)**. Genetic absence seizure is known to originate from thalamocortical pathways that differ from auditory and visual pathways involved in the seizure generation in audiogenic and photosensitive animals **(Klitgaard *et al.,* 2002)**. It

represents an epileptic animal strain with a particular susceptibility for drug-induced adverse effects **(Loscher *et al.*, 2002)**.

In contrast, animals with induced mutations, such as transgenic or knockout mice, are rarely used for drug studies. In comparison to models of acquired partial epilepsy, most genetic animal models are models of generalized epilepsy that do not allow to search for drugs that prevent epilepsy, but these models have contributed significantly to our understanding of epilepsy mechanisms **(Noebels, 1999; Prasad *et al.*, 1999)**. Genetically epilepsy-prone rats (GEPRs) are models of generalized tonic/clonic epilepsy and have been used to study basic mechanisms of human epilepsy. GEPRs exhibit audiogenic seizures in response to acoustic stimulation **(Shin *et al.*, 2011)**.These diverse methods demonstrate that alteration of neuron's function can undergo many mechanisms: blockade of excitatory or inhibitory neurons, disturbance of metabolism **(Cambier *et al.*, 2004)**.

Many studies reveal that seizures are mediated by the oxidative stress. There is emerging evidence that focuses on the role of exidative stress in seizures. A growing body of evidence has suggested that reactive oxygen species and reactive nitrogen species generation may underlie the convulsant and neurotoxic effect of PTZ **(Aldarma *et al.*, 2010)**.

I.2 Oxidative stress

I.2.1 Definition

Oxidative stress describes the pathologic condition in which the balance of oxidant generation and detoxification is tipped toward a pro-oxidant state **(Lie fan *et al.*, 2000; Maibam *et al.*, 2010)**. In a normal cell there is an appropriate pro-oxidant/antioxidant balance. The shifting of this balance in favor of the former, either due to increased production of oxygen species or diminished levels of antioxidants, results in a state known as oxidative stress **(Devi *et al.*, 2008)**.In this state, antioxidant defenses are overwhelmed, reactive species accumulate, and damage to nucleic acids, proteins, and membrane lipids **(Golden and Patel, 2009)** and the supporting extracellular matrix ensues **(Jesberger and Richardson, 1991)**, leading to cells senescence and death **(Lie Fan *et al.*, 2000; Maibam *et al.*, 2010)**.

I.2.2 Free radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals **(Valko *et al.*, 2007)**. Under

pathological conditions or when certain drugs are present, larger amounts of free radicals than normal may be formed **(Jesberger and Richardson, 1991)**. They are highly reactive substances capable of giving rise to chain reactions, i.e. reactions that involve a number of steps, each of which forms a free radical that triggers the next step. There are three phases: initiation, propagation and termination, and there are different free radical species: oxygen-centred radicals (ROS), nitrogen-centred radicals (RNS), carbon-centred radicals and sulphur-centred radicals **(Buonocore *et al.*, 2006)**.

Free radicals are in reality, substances that have one or more unpaired electrons in their outer shell. They are produced during various biochemical reactions of aerobic metabolism in the body **(Ilhan *et al.*, 2006; Valko *et al.*, 2007)** or as a result of occasional leakage **(Adedapo *et al.*, 2008)**. Their endogenous sources are leakages from mitochondria, immune system, enzymatic and autooxidation reactions. They are extremely reactive and can bind various molecules that lead to changes in DNA bases, injury to various proteins, and lipid peroxidation of membrane lipids. As a result, they lead to tissue injury (**Jesberger and Richardson, 1991)**.

I.2.2.1 Types of free radicals

A free radical is an atom or molecule that has one or more unpaired electron. Its consequent tendency to acquire an electron from other substances makes it highly reactive **(Devi *et al.*, 2008)**. The term reactive oxygen species includes both oxygen radicals and certain non-radicals that are oxidizing agents and/ or easily converted into radicals (singlet oxygen) **(Devi *et al.*, 2008)**.

i- Reactive oxygen species

Radicals derived from oxygen represent the most important class of radical species generated in living systems **(Valko *et al.*, 2007)**. Reactive oxygen species (ROS) are generated in various cellular compartments as a consequence of normal metabolism **(Golden and Patel, 2009)**. ROS such as superoxide anion, hydrogen peroxide and hydroxyl, nitric oxide and peroxinitrite radicals plays an important role in oxidative stress related to the pathogenesis of various important diseases **(Finkel and Holbrook, 2000; Lie Fan *et al.*, 2005)**.

i-1- Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is generated by the dismutation of the superoxide radical or by special oxidases found in the cell or in organelles such as peroxisomes **(Jesberger and Richardson, 1991)**. The activation of excitatory amino acid receptors (NMDA: N-methyl-D-aspartate) leads to intracellular calcium accumulation and nitric oxide synthase activation, producing nitric oxide. An increasing amount of superoxide anion interacts with nitric oxide to yield the highly toxic peroxynitrite anion **(Devi *et al.,* 2008)**. A steady-state level of superoxide and H_2O_2 is always present in cells as a result of normal metabolism.

Hydrogen peroxide has been implicated recently as an intracellular messenger that affects cellular processes including protein phosphorylation, transcription and apoptosis **(Choi *et al.,* 1998)**. H_2O_2 itself is not a free radical, but undergoes autooxidation to become an extremely reactive hydroxyl radical **(Jesberger and Richardson, 1991)**.

i-2- Hydroxyl radical

Hydroxyl radical has a high reactivity, making it a very dangerous radical **(Buonocore *et al.,* 2010)**; when produced *in vivo* •OH reacts close to its site of formation **(Valko *et al.,* 2007)**. Hydroxyl radical readily reacts with DNA, membrane lipids, and protein **(Golden and Patel, 2009)**. It is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone **(Valko *et al.,* 2007)**. Superoxide and hydroxyl radicals are involved in a large number of degenerative changes, often associated with an increase in peroxidative processes and linked to low antioxidant concentration **(Mates, 2000)**.

i-3- Superoxide anion

Superoxide radical is one of the free radicals **(Jesberger and Richardson, 1991)**. It is formed by the addition of one electron to dioxygen **(Valko et al., 2007)**. It is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase enzyme **(Jesberger and Richardson, 1991)**. Superoxide anion radical (O_2^-), a major radical in cellular systems, producer enzyme may increase through the proteolytic conversion of xanthine dehydrogenase to XO and produce enormous amount of O_2^-.Other sources of reactive species are neutrophils and other phagocytic cells **(Devi *et al.,* 2008)**.

ii- Reactive nitrogen species

Reactive nitrogen species (RNS) also play a role in oxidative stress. Nitric oxide (NO•) is an abundant reactive radical that acts as an important oxidative biological signaling

molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation **(Bergendi *et al.,* 1999)**. Nitric oxide (NO) acts as a signal-transduction molecule in vasodilation **(Melov *et al.,* 2005)**, neuronal signaling **(Arancio *et al.,* 1996)** and nitrosylation which is proposed to be a redox-sensitive protein modification involved in signal transduction. NO• has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system **(Valko *et al.,* 2007)**. NO also reacts with superoxide in a reaction that is diffusion limited, forming peroxynitrite (ONOO-), a powerful oxidant. This reaction can serve as a mechanism to control levels of superoxide or NO or both, affecting cell signaling by NO. Additionally, peroxynitrite and its breakdown products can react with proteins, resulting in nitration of tyrosine residues. Both superoxide and nitric oxide are produced in the course of the inflammatory response, leading to the formation of nitrotyrosine, which can be used as a molecular footprint of nitrosative stress in inflammation **(Golden and Patel, 2009)**. Nitric oxide (NO) has contradictory roles in cellular systems such as an oxidant or sometimes a scavenger of $O2^{\circ-}$**(Fadillioglu *et al.,* 2003; Ilhan *et al.,* 2004)**.

Overproduction of reactive nitrogen species is called nitrosative **stress (Klatt and Lamas, 2000; Ridnour *et al.,* 2004)**. This may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function **(Valko *et al.,* 2007)**.

I.2.3 Oxidative stress markers

The most important effect of free radicals is lipid peroxidation due to the attack membrane lipids by ROS **(Fadillioglu *et al.,* 2003; Ilhan *et al.,* 2004**), which causes disruption of cell membrane leading to cell death **(Satisha *et al.,* 2007)**. Malondialdehyde is an indicator of lipid peroxidation by free radicals, and is the end-product, thus serves as an index of lipid peroxidation **(Akyol *et al.,* 2002; Sarsilmaz *et al.,* 2003)**. Therefore it is an oxidation product **(Ilhan *et al.,* 2006)**. The prevention of lipid peroxidation is an essential process in all the aerobic organisms, as lipid peroxidation products can cause DNA damage. Increased lipid peroxidation and decreased antioxidant protection frequently occurs **(Mates and Sanchez-Jimenez, 1999)**.

Exposure to free radicals from a variety of sources has led the body to develop a series of defense mechanisms **(Cadenas, 1997)**. Defense mechanisms against free radical-induced oxidative stress involve:

(i) Preventive mechanisms,

(ii) Repair mechanisms,

(iii) Physical defences, and

(iv) Antioxidant defences **(Valko *et al.*, 2007)**

I.2.4 Antioxidants

Under normal physiological conditions tissue injury caused by free radicals is controlled by endogenous antioxidant defenses **(Ilhan *et al.*, 2006; Maibam *et al.*, 2010)**. This include on one hand detoxifying enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase **(Halliwel, 1995)** and on the other hand non enzymatic antioxidants as ascorbic acid, Vitamin E, glutathione (GSH), carotenoids, flavonoids, and other antioxidants **(Valko *et al.*, 2007; Devi *et al.*, 2008)**. They are substances that either directly or indirectly protects cells against adverse effects of drugs and toxic radical reactions **(Halliwell, 1995)**.

The components of the defense systems that have evolved to reduce and contain the injury from free radical attack include the structural conformation of the membranes and the DNA strands, several enzymes and a few free radical scavenger antioxidants **(Jesberger and Richardson, 1991)**.

I.2.4.1 Enzymatic antioxidants

The FRs produced in the normal transfer of electron are prevented from doing damage to cellular components by being bound to enzyme system during generation. These organelles also contain the enzymes super oxide dismutase (SOD), glutathione peroxidase and catalase which are the most important antoxidant enzymes**(Jesberger and Richardson, 1991)** to help prevent toxic levels of FRs from developing **(Devi et al., 2008;Maibam et al., 2010)**.

i- Super Oxide Dismutase

It is an intracellular antioxidant enzyme **(Akyol *et al.*, 2002)**. There exist three forms of SOD: Cu Zn SOD found in the cytosol, whereas MnSOD containing SOD is localized in

the mitochondrial matrix. An extracellular form of Cu Zn SOD is expressed at low levels in plasma and extracellular fluids **(Devi *et al.*, 2008)**. They are a group of metalloenzymes that catalyze the transformation of the superoxide radical into hydrogen peroxide **(Jesberger and Richardson, 1991)**. It is a potent protective enzyme that can selectively scavenge the $O_2^{\circ-}$ into H_2O_2 **(Ilhan *et al.*, 2005a)**. It catalyzes the degradation of hydrogen peroxide to produce water and oxygen **(Sarsilmaz *et al.*, 2003; Devi *et al.*, 2008)**. All three forms catalyze the dismutation of superoxide anion to hydrogen peroxide, thereby reducing the risk of hydroxyl radical formation.

ii- Catalase

Catalase is an enzyme, present in most aerobic cells, that catalyzes the reduction of hydrogen peroxide to water and oxygen **(Jesberger and Richardson, 1991)**. It decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound **(Valko *et al.*, 2007)**.

Superoxide dismutase and catalase are responsible for degradation of superoxide and H_2O_2, respectively, and the balance between these antioxidant enzymes is relevant for cell and neuronal functions **(Freitas, 2009)**.

iii- Glutathione peroxidase

Glutathione peroxidase is a peroxidase that catalyzes the reduction of hydroperoxides by glutathione **(Jesberger and Richardson, 1991)**. It is a selenium containing enzyme which acts on reduced glutathione and hydrogen peroxide to produce oxidized glutathione (GSSG) and water **(Akyol *et al.*, 2002; Sarsilmaz *et al.*, 2003)**. In addition to eliminating hydrogen peroxide, it also participates in pathways responsible for the detoxification of lipid peroxy radicals **(Devi *et al.*, 2008)**.

Reduced glutathione: GSH is both a nucleophile and a reductant that can react with electrophilic or oxidizing species before the latter can interact with nucleic acids or proteins **(Jesberger and Richardson, 1991)**. This system not only works as peroxide scavengers, but also to regulate the redox state of the cells **(Mates *et al.*, 2000)**. During scavenging the ROS, GSH is oxidized and forms glutathione-protein mixed disulfides; hence, the cell's ability to reduce or synthesize GSH is the key to how effectively the cell can manage the oxidative stress **(Mates *et al.*, 2000)**.

I.2.4.2 Non enzymatic antioxidants

Among non-enzymatic antioxidants are glutathione, α-tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene (vitamin A), albumin, bilirubin, and uric acid **(Jesberger and Richardson, 1991)**.

i- Glutathione

It is present in most mammalian tissue and plays an important role in the cell's defenses against free radicals, peroxides. In the brain, GSH is localized almost exclusively in astroglial cells.It is thought to also exist in nerve terminals and some neurons **(Abe *et al.*, 2000)**. The fact that GSH is released from brain cells and present at significant levels in the cerebrospinal fluid implies that GSH also functions as a neurotransmitter or neuromodulator in the brain **(Abe *et al.*, 2000)**.

ii- Vitamins C and E

Ascorbate (vitamin C) will reduce free radicals with the concurrent formation of dehydroascorbate. Vitamin E, α-tocopherol, is the most common member of this group of tocopherols. Vitamin E is often referred to as a chain-breaking antioxidant because it blocks the chain reaction process that propagates the peroxidation cascade along a membrane. The complex tocopherol molecule is able to stop free radical propagation by accepting the odd electron from the radical. Vitamin E appears to be especially important in protecting membrane phospholipids from free radical attack. It may act most efficiently at high oxygen concentration **(Jesberger and Richardson, 1991; Devi *et al.*, 2008).**

iii- Beta carotene

Beta carotene is a metabolic precursor of vitamin A and is accumulated in high concentrations in the membranes of certain tissues such as the retina. Beta carotene will quench both excited species and react directly with free radicals **(Devi *et al.*, 2008)**. Especially important is the fact that it operates most effectively at low oxygen tensions and thus seems to operate with vitamin E in a synergistic way. It is able to inhibit the formation of malondialdehyde (MDA) and block lipid peroxidation **(Jesberger and Richardson, 1991)**.

I.2.4.3 Natural antioxidants

A number of known antioxidants as well as yet unknown antioxidants are supposedly present in plants. These antioxidants are going to do a lot of good to human health by sequestering the hazardous free radicals which are generated due to physiological errors in the

cells **(Maibam *et al.,* 2010)**. A number of other endogenous substances, some of which were previously thought to be metabolic waste products, can also function as antioxidants. These include metal ion chelators which are able to reduce the formation of free radicals by preventing metal catalyzed Fenton-type reactions. Particularly important are the iron- and copper-binding proteins that act as intracellular storage sites for these metals (**Jesberger and Richardson, 1991)**.

Several epidemiological studies have been undertaken, which have established a beneficial link between polyphenol intake and lower disease risk with many of the clinical benefits being attributed to both the antioxidant properties of polyphenols **(Rahman, 2008)**. The high content of polyphenolic compounds and flavonoids in different parts of various medicinal plants have the antioxidant properties **(Maibam *et al.,* 2010)**.

These antioxidants have the important role to protect the body from oxidative stress and therefore diseases. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica*,*Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins **(Khalaf *et al.,* 2008)**. Spices and herbs are therefore recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging **(Khalaf *et al.,* 2008)**.

I.2.5 Oxidative stress and epilepsy

The harmful effect of free radicals causing potential biological damage is termed oxidative stress **(Valko *et al.,* 2007)**. Several studies have proven that oxidative stress has been implicated in seizures induced by pilocarpine, kainic acid **(Freitas, 2009)** and PTZ induced kindling **(Ilhan *et al.,* 2005)**.

Free radicals and neuronal hyper excitability have been postulated as causal agents or mediators in an increasing number of pathological conditions including epilepsy **(Jesberger and Richardson, 1991; Devi *et al.,* 2008**). Neurons are especially vulnerable to free radicals attack and impaired defenses or exposure to FR can lead to neuronal death. Free radicals

contribute to neuronal loss in cerebral ischemia and hemorrhage and may be involved in the degeneration of neurons in epilepsy **(Jesberger and Richardson, 1991).**

The role played by free radicals in epileptic discharges of origins other than iron is currently unknown but it is possible those free radicals may be involved in some types of seizures and that the action of free radicals may help to explain phenomena such as kindling **(Jesberger and Richardson, 1991)**.

There is emerging evidence that focuses on the role of oxidative stress in epileptic seizures **(Aldarma *et al.*, 2010a)**. ROS have been implicated in the initial phases of seizure-induced pathology **(Bruce and Baudry, 1995)**. The capacity of the system to quench chemical species capable of lipid peroxidation during epileptogenic discharge may determine the extent of seizure activity **(Jesberger and Richardson, 1991)** and several studies have reported oxidative stress in different brain regions following experimental seizures **(Frantseva *et al.*, 2000)**. Induction of epileptic seizure activity can occur when the generation of free radicals is augmented, scavenging of free radicals or repair of oxidatively modified macromolecules decreases or both **(Singh *et al.*, 2003)**. Studies demonstrate that prolonged seizure acutely result in oxidative damage to lipids, DNA and susceptible proteins **(Aldarmaa *et al.*, 2010a)** and that membrane alterations due to lipid peroxidation can change membrane electrophysiological properties such as ion gating **(Jesberger and Richardson, 1991)**. The ability of antioxidant in protecting against seizures manifestation and the accompanying biochemical changes further highlights a role of free radicals in seizures **(Gupta and Chaudry, 2002; Tan *et al.*, 1998)**. This generation of seizure is associated with changes in the intracellular levels of antioxidant and oxidants **(Devi *et al.*, 2008)**.

The high rate of oxidative metabolism coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage **(Mates *et al.*, 2000; Devi *et al.*, 2008)** and therefore particularly susceptible to oxidative stress **(Mates, 2000).** The brain normally produces relatively large amounts of ROS, such as superoxide, hydroxyl radical, nitric oxide, nitrite, nitrate and H_2O_2 **(Hazra *et al.*, 2009)**. The brain is one of the organs that is highly susceptible to oxidative stress, since it consumes a hight amount of the body's oxygen, as it derives its energy almost exclusively from oxidative metabolism of the mitochondrial respiratory chain **(Coyle, 1993)**. These free radicals have been suggested to be the most likely candidate responsible for producing the neuronal changes mediating the behavioral deficits in neurodegenerative diseases **(Frantseva**

***et al.,* 2000)**. Epilepsy is not classified as a neurodegenerative disease per se, but seizure activity leads to cell loss and neurodegeneration, which contribute to disease progression. Epilepsy can be age-related, developing in response to age related diseases such as stroke. Evidence exists of oxidative stress accumulating in epilepsy. Status epilepticus causes a depletion of glutathione **(Liang and Patel, 2010)**. Prolonged seizure activity results in excitotoxicity, which leads to an influx of extracellular calcium, a dysregulation of intracellular calcium, and mitochondrial ROS production **(Reynolds and Hastings, 1995)**. The cell death that results is dependent on superoxide production, and that superoxide is produced by the mitochondria. The cell death may activate an inflammatory response, resulting in more oxidative damage **(Rong *et al.*, 1999; Golden and Patel, 2009)**.

The resulting hydroxyl radicals are highly toxic **(Hazra *et al.*, 2009)** and react with non-radical molecules, transforming them into secondary free radicals. This reaction takes place during lipid peroxidation of cell membranes and produces hydro peroxides **(Mc Cord, 1995; Hazra *et al.*, 2009)**. Peroxidation of membrane lipids caused by an increase in the generation of free radicals or a decrease in the activities of antioxidant defense system has been suggested to be critically involved in seizure control **(Singh and Pathak, 1990; Martens *et al.*, 1995)**. Apart from the hydroxyl radicals, H_2O_2 generated by the action of SOD is highly toxic by itself **(Hazra *et al.*, 2009).**

The brain is more vulnerable to injury by lipid peroxidation products than other tissues **(Naffah *et al.*, 2001)** because,

- It generates very high levels of ROS due to its very high aerobic metabolism and blood perfusion, and it has a relatively poor enzymatic antioxidant defense;
- It is enriched in lipids that are preferentially susceptible to oxidative damage;
- The damaged neuronal DNA in the adult brain cannot be effectively repaired since there is no DNA replication **(Ilhan *et al.*, 2005b)**.

Moreover, lipid peroxidation is an index of irreversible neuronal damage of cell membrane phospholipid and it has been suggested as a possible mechanism of epileptic activity **(Freitas, 2009)**. Free radical generation can induce seizure activity by inactivation of glutamine synthase or by inhibition of glutamate decarboxylase leading to decrease of GABA, an inhibitory neurotransmitter **(Satisha *et al.*, 2007)**.

Oxidative injury in the brain is increasingly recognized as a common pathway of cellular injury in many acute neurological insults including ischemia-reperfusion and epileptiform brain activity, and in more chronic disease states such as Parkinson's or Alzheimer's disease **(Dexter *et al.*, 1994; Sperk, 1994; Beni and Moretti, 1995)**.

I.3 Protein glycation

I.3.1 Definition

Glycation or the Maillard reaction is the non-enzymatic adduct formation between amino groups (predominantly the ε-amino group of lysine and the guanidine group of arginine) **(Baynes *et al.*, 1989)** and carbonyl groups of reducing sugars **(Odjakova *et al.*, 2012)**, leading to the formation of fluorescent advanced glycation end products (AGEs) that can be identified by increasing fluorescent intensity **(Chayaratanasin *et al.*, 2015)**.

I.3.2 Different stages in protein glycation

The Maillard reaction is subdivided into three main stages: early, intermediate, and late.

In the early stage, glucose (or other reducing sugars such as fructose, pentoses, galactose, mannose, xylulose) react with a free amino group of biological amines, to form an unstable aldimine compound, the Shiff base **(Odjakova *et al.*, 2012; Bartosz and Bartosz, 2015)**. Then through an acid-base catalysis, this labile compound undergoes a rearrangement to a more stable early glycation product known as Amadori product **(Guttierez *et al.*, 2010; Perera *et al.*, 2013)**. Because the Maillard reaction is non-enzymatic, the variables which regulate its velocity *in vivo* are the glucose and protein concentrations, the half-life of the protein, its reactivity in terms of free amino groups, and the cellular permeability to glucose.

In the intermediate stage, viadehydratation, oxidation and other chemical reactions, the Amadori product degrades to a variety of reactive dicarbonyl compounds such as glyoxal, methylglyoxal, and deoxyglucosones which, being much more reactive than the initial sugars, act as propagators of the reaction, again reacting with free amino groups of biomolecules.

In the late stage of the glycation process through oxidation, dehydratation and cyclization reactions, irreversible compounds, called Advanced glycation end products (AGEs) are formed. The AGEs are yellow-brown, often fluorescent and insoluble adducts that accumulate on long-lived proteins thus compromising their physiological functions

(Odjakova *et al.,* 2014).The association of AGEs with disease is thought toreflect AGE-dependent changes in protein structure and function.These are (i) changes in protein structure arising from loss oflysyl, arginyl or N-terminal ionization, introduction of ionizedAGE structures and changes in local hydrophobicity **(Reddy and Beyaz, 2006)**, (ii)resistance to proteolysis by AGE-mediated cross-linking and(iii) binding to cell-surface receptors (AGE receptors) and otherproteins (lysozyme, lactoferrin) specifcally with associated cellactivation or impairment of protein function **(Ahmed andThornalley, 2002)**. Glycation of proteins can interfere with their normal functions by disrupting molecular conformation, altering enzymatic activity, reducing degradation capacity, and interfering with receptor recognition **(Odjakova *et al.,* 2014)**.

Three routes have been proposed for AGEs formation: 1) autoxidative pathway in which sugars give rise to reactive products by autoxidation, 2) Amadori rearrangement, **(Odjakova *et al.,* 2014)**and 3) formation of a Schiff base **(Adisakwattana*et al.,* 2014)**. Reactive oxygen species (ROS) in the presence of trace levels of catalytic redox-active transition metal ions also contribute to AGEs formation. The process includes oxidative steps and is therefore called glycoxidation **(Guttierez *et al.,* 2011; Odjakova *et al.,* 2014)**.

I.3.3 Glycation,oxidative stress and epilepsy

Abundant evidence exists that an excessive productionof reactive oxygen species (ROS) and reactive nitrogen species are generated during glycation and glycoxidation. The production of ROS causes the oxidation of amino acid residues of protein to form a carbonyl derivative, which diminishes the oxidative defence of protein by eliminating the thiol groups **(Chayaratanasin *et al.,* 2015)**. ROS is increased from protein glycation reaction, by the reaction of methylglyoxal (a degrade compound from Amadori product) withlysine in the presence of metal ions (Fe3+ and Cu2+) which gives hydroxyl radicals, this in the early stages of glycation **(Chayaratanasin *et al.,* 2015)**. The interaction of AGEs with receptors for AGEs (RAGE) directly activates multipleintracellular signaling, gene expression, and the secretorypro-inflammatory molecules accompanied by increasingfree radicals that contribute towards pathologic complicationsrelated to neurological diseaes **(Adisakwattana et *al.,* 2014)**.AGEs are known to beProinflammatory **(Bartosz and Bartosz, 2015)**, and increasing evidence indicates that activation of inflammatory processesin the brain is a common feature of various epileptic disorders **(Zurolo *et al.,* 2011; Iori *et al.,* 2013)**. Recent reports have also suggested thatmetal-catalyzed oxidation reactions play a major role inaccelerating the rate of

AGE formation **(Price *et al.*, 2001; Chetyrkin *et al.*, 2008)**. Therefore, agents with antiglycation and antioxidantproperties may retard the process of AGE formation bypreventing further oxidation of Amadori products andmetal-catalyzed glucose oxidation **(Chen *et al.*, 2011)**.

I.3.4 Antiglycating agents

Both synthetic compounds and natural products have been evaluated as inhibitors against the formation of advanced glycation end products (AGEs). The synthetic AGEs inhibitors so far discovered are divided into three classes: (a) carbonyl trapping agents which attenuate carbonyl stress; (b) metal ion chelators, which suppress glycoxidations; and (c) cross-link breakers that reverse AGE cross-links **(Odjakova *et al.*, 2014)**.

Despite of their inhibitory capacities against the formation of AGEs, many synthetic inhibitors of AGEs formation such as Aminoguanidine **(Adisakwattana*et al.*, 2014)**, carnosine, pyridoxamine **(Panaskar *et al.*, 2013)**,were withdrawn from clinical trials due to relatively low efficacies, poor pharmacokinetics, and toxicity**(Adisakwattana*et al.*, 2014; Odjakova *et al.*, 2014)**.On the other hand natural products have been proven relatively safe for human consumption and many plant extracts have been tested for their ability to prevent AGEsformation **(Chayaratanasin *et al.*, 2015)**.Some important compounds such as phenolics, oligo- and polysaccharides, carotenoids, unsaturated fatty acids and many others have been reported to possess anti-glycating activity **(Odjakova *et al.*, 2014; Bartosz and Bartosz, 2015)**.

I.3 Pain and epilepsy

I.3.1Generalities

Pain is a complex event that is uniquely experienced by each individual. It is considered to be a sensation resulting from any tissue-damaging stimulus and is essential for survival **(Kabatende, 2005)**. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” **(Merskey, 1994)**.Pain is also an unpleasant subjective incident that is the net effect of a complex communication of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes **(Boakye, 2009)**. Pain can affect all areas of a person’s life including sleep, thought, emotion, and activities of daily living. Since there are

no reliable objective markers for pain, the patients are the only ones to describe the intensity and quality of their pain **(Boakye, 2009)**. Pain in many cases represents the only symptom for the diagnosis of several diseases. It is associated with various diseases, inflammatory conditions, tissue trauma and surgical interventions **(Alagpulinsa, 2010)**. It often has a protective function and is essential for survival.

Pain is often called "chronic" when it lasts longer than 6 months. Pathophysiological nociceptive pain occurs when tissue is inflamed or injured; it may appear as spontaneous pain, as hyperalgesia or allodynia, and be effectively treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and opiates (Schaible *et al.*, 2004). Pain caused by neurone injury or neuropathic pain in the peripheral or central nervous system is less effectively managed by these drugs and often, anticonvulsants or tricyclic antidepressants are used (Alagpulinsa, 2010).

Pain has constantly been described as a symptom. However, current advances in the understanding of neural mechanisms have confirmed that unrelieved pain may lead to changes in the nervous system and as such pain, particularly chronic pain, may be considered a disease in itself **(Smith *et al.*, 2001)**. Pain is categorized according to its duration, location and etiology. Pain receptors, unlike the more specialized receptors of the other senses, can be activated by a variety of stimuli, including heat, cold, electrical impulses, pressure of stretching, cuts or tears and chemical irritants **(Kabatende, 2005)**.

Several distinct types of pain have been described based on their pathophysiology: nociceptive, inflammatory, neuropathic, and functional.

-Nociceptive pain is a transient pain in response to a noxious stimulus at nociceptors that are located in cutaneous tissue, bone, muscle, connective tissue, vessels, and viscera. Nociceptors may be thermal, chemical, or mechanical. The nociceptive system extends from the receptors in the periphery to the spinal cord, brain stem, and to the cerebral cortex where pain sensation is perceived **(Baron and Treede, 2007; Messeguer *et al.*, 2006; Suardiaz *et al.*, 2007)**.

-When tissue damage occurs despite the nociceptive defense system, inflammatory pain ensues. The body now changes focus from protecting against painful stimuli to protecting the injured tissue. The inflammatory response contributes to pain hypersensitivity

that serves to prevent contact or movement of the injured part until healing is complete, thus reducing further damage **(Anseloni and Gold, 2008; Harvey and Dickenson, 2008)**.

-Neuropathic pain is defined as spontaneous pain and hypersensitivity to pain associated with damage to or pathologic changes in the peripheral nervous system; or pain originating in the central nervous system (CNS), that which occurs with spinal cord injury, multiple sclerosis, and stroke **(Baron and Treede, 2007; Garcia-Larrea and Magnin, 2008)**. Chronic neuropathic pain is a form of pain that is caused by neural injury and persisted at least 3 to 6 months or longer. It is a severe clinical problem with limited treatment options. Pathological pain or pain of neuropathic origin is typically resistant to conventional treatments. Anticonvulsants have been used in these situations due to the belief that they are effective in alleviating certain forms of neuropathic pain and even in acute pain. It is reported that different therapeutic agents such as tricyclic antidepressants, anticonvulsants, membrane stabilizers can alleviate neuropathic pain **(Alagpulinsa, 2010; Karimi *et al.*, 2011)**.

-Functional pain, a relatively newer concept, is pain sensitivity due to an abnormal processing or function of the central nervous system in response to normal stimuli **(Nielsen and Henriksson, 2007)**.

In order to better understand pain and its mechanism of action, there are many models available for investigating the antinociceptive activity of drugs including anticonvulsants with potential antinociceptive activity **(Alagpulinsa *et al.*, 2010)**.

I.3.2Effects of anticonvulsants on animal models of pain

Tissue injury and inflammation generate bradykinin, which not only activates nociceptors, but increases their sensitivity by triggering prostaglandin production. The process of pain transduction, gating and modulation involves neurotransmitters (L-glutamate, N-methyl-D-aspartic acid, γ-amino butyric acid) ionic channels (calcium and sodium), and neuropeptides (calcitonin gene-related peptide, substance P) **(Amoateng, 2011)**. These mechanisms make anticonvulsants useful in the treatment of many neuropathic and even acute pain conditions **(Alagpulinsa, 2010)**. Acute pain model was used to investigate the antinociceptive effect of topiramate and it shows that they were effective in these models. It has also been shown in animal models that gabapentin prevents nociceptive responses from hyperalgia in animal modelsand also has analgesic actions in clinical reports **(Amoateng, 2011)**. There are many models of pain available for investigating the antinociceptive activity

of drugs including anticonvulsants with potential antinociceptive activity. These include chemical and thermal methods in rodents **(Alagpulinsa, 2010)**. The formalin test which is the most commonly used is the most predictive of acute pain **(Le Bars *et al.*, 2001)** and a valid model for clinical pain **(Vissers *et al.*, 2003; Costa-Lotufo *et al.*, 2004)**, involves intradermal injection of formalin solution **(Le Bars *et al.*, 2001)**. The formalin model is the most used. Injection of formalin solution into the paw of a rat or a mouse causes spontaneous behaviours, persistent pain caused by peripheral tissue injuries and inflammation of the cells **(Sunday, 2008)**. It is a model for antinociceptive with potential anticonvulsant activity.

I.4 Previous study on plants extracts against epilepsy, oxidative stress and protein glycation

In many cultures, including African and Asian cultures, epilepsy is commonly treated with herbal preparations from a wide variety of plants.

Traditional medicine remains an important part of the health system in many societies despite the availability of well-established alternatives **(Balamuragan *et al.*, 2009)**. Recently focus on ethnopharmacology research has been increased all over the world and a growing body of evidence has indicated immense potential of medicinal plants as alternative and complementary therapies for many human ailments **(Visweswari *et al.*, 2010)**. Besides a number of allopathic medications available, there is considerable evidence of an increase in demand for medicinal plants **(Madhu *et al.*, 2009)**. Medicinal plants and herbs contains substances known to modern ancient civilization for their healing properties and they were the sole sources of active principles capable of curing human ailments **(Devadas, 2001)**. Medicinal and aromatic plants have claimed unique place for themselves fromimmemorial times due to their therapeutic values of prevention and cure of diseases, maintenance of health, longevity and happiness to the suffering humanity **(Muralidhar *et al.*,1996)**.

The vegetal world is an important target in the process of discovery of new compounds **(Chindo *et al.*, 2009; Pedersen *et al.*, 2009)**. Today a renewed interest for the use of plants for the treatment and relief of many diseases such as epilepsy and the possible involved mechanism as oxidative stress and protein glycation is observed **(Heidari *et al.*, 2009, Perera *et al.*, 2013)**. Sure enough, recently the properties of medicinal plants are investigated all over the world due to their potential pharmacological activities, their low toxicity and their economic viability **(Ilhan *et al.*, 2005)**. Plants extracts can be source for

natural and safe products. These extracts fractions and pure compounds are used in the treatment of epilepsy and have proved anticonvulsant properties that need further investigations **(Raza *et al.*, 2001, Quintans *et al.*, 2008)**. In the other hand, many plants have also being used due to their antioxidant and antiglycating properties **(Mahomoodally *et al.*, 2012)**. Ethnobotanical studies in Mali by **Pedersen *et al.*, (2009)**; **Ediriweera *et al.*, (2007**) in Sri lanka; by **Jiofack *et al.*, (2009 and 2010), Tsabang *et al.*, (2016)** in Cameroon; **Muazu and Kaita (2008)** in Nigeria; **Moshi *et al.*, (2000)** in Tanzania; **Birkumar *et al.*, (2002)** in India; **Quintans *et al.*, (2008)** in Brasil reveals the uses of many plants in the traditional pharmacopea for the treatment of diverse ailments, among them those of the central nervous system in general and particularly epilepsy.

In Africa particularly, according to a report by WHO, more than 80% of the population recourse to traditional medicine for primary health care **(Muazu and Kaita, 2008)**. Over 7,500 species of plants are estimated to be used by 4635 communities for human and veterinary health care. About 1000 plants have been used in the Indian system of medicines **(Beulah *et al.*, 2004)**. Plants having active constituents have a direct pharmacological action on our body including various organs. One such major organ is the brain, so complex that still only few drugs are approved by drug authorities for epilepsy (**Shubini *et al.*, 2008)**. Despite this, many plants are currently used by local populations particularly in Africa, Asia, and SouthAmerica. Some of these plants have been tested in order to confirm their folkloric use against epilepsy.This is the case of the aqueous extract of *Nauclea latifolia* leaves **(Bum *et al.*, 2009)**, the ethanolic extract of *Astragalus mongholicus* roots **(Aldarmaa *et al.*, 2010)**. Plants are not only used for their anticonvulsant activity but also for their antiglycating and antioxidant activities. This is the case of the aqueous extract of *Mesona Chinensis*, **(Sirichai *et al.*, 2014)**, *Clitora ternatea* flower petal aqueous extract **(Chayaratanasin *et al.*, 2015)**.

I.5 Plants used in this study

I.5.1 *Ficus thonningii* (Moraceae)

Also called strangler fig, *Ficus thonningii* Blume (Moraceae) is used as a medicinal plant in Northern and Southern Nigeria. The species is widely distributed in upland forest, open grassland, riverine and rocky areas and sometimes in savannah. It occurs naturally from the Democratic Republic of Congo and Tanzania in the north to the Eastern Cape in South Africa **(Orwa *et al.*, 2009)**. Trees are relatively drought resistant. It is also found in Nigeria,

Sierra Leone, Togo, Liberia, Ivory Coast and Cameroon. It is an evergreen tree 6-21 m, with a rounded to spreading and dense crown **(Orwa *et al.*, 2009)**. In the natural state it commences as an epiphyte and is generally propagated by stake which grows rapidly. Sometimes epiphytic, often a strangler; trunk fluted or multi stemmed. Bark on young branches hairy, with a stipular cap covering the growth tip, but smooth and grey on older branches and stems, lenticellate, often with aerial roots hanging down from branches; the whole plant exudes copious, milky latex often turning pinkish. Leaves are simple, glossy, dark green, thin and papery or slightly leathery, margin smooth, elliptic or obovate, sometimes rather elongated or slightly oblanceolate, grouped at ends of twigs; leaves axils can have figs which can be red or yellow **(Orwa *et al.*, 2009)**. *F. thonningii* is a sacred or emblemic tree among several tribes in northern Nigeria. The Chawai (a tribe in Zaria, Nigeria), before a hunt set the bush on fire by the ritual method of drilling two pieces of *F. thonningii* sticks **(Dalziel, 1987; Sunday, 2008)**. The bark is important in local medicine, and it is used in treating colds, sore throat, dysentery, wounds, constipation, and nose bleed and to stimulate lactation. Latex is used for wound fever, while an infusion of the root and fiber is taken orally to help prevent abortion. Powdered root is taken in porridge to stop nosebleed; the milky latex is dropped into the eye to treat cataracts **(Orwa *et al.*, 2009)**. In northern Nigeria, it is used to cure pain associated with fever and for treating wounds **(Sunday, 2008)**. In Cameroon, leaves are used for the treatment of malaria **(Titanji *et al.*, 2008)**.

Studies by **Musabayane *et al.*, (2007)**, revealed that the ethanolic extract of stem bark of *F. thonning*ii was effective on blood glucose, cardiovascular and kidney function of rats and on kidney cell line of the proximal and distal tubes at 60, 120 and 240 mg/kg. The antimicrobial activity of the methanolic extract of stem bark against *Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa*, *Salmonella typhi* (Gram-negative), *Staphylococcus aureus* and *Streptococcus spp.* (Gram-positive), sensibly inhibited the growth of these micro organisms at 10 mg /ml against pseudomonas and 1.25 mg/ ml against remaining organisms tested for the minimal inhibitory concentration and was 2.5 mg/ ml for *Staphylococcus aureus* and that of *Streptococcus spp*. was found to be 0.625mg/ ml concerning the minimal bactericidal concentration **(Usman *et al.*, 2009)**. The phytochemical tests revealed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins and tannins **(Usman *et al.*, 2009)**.

Image 1: *Ficus thonningii* (Dongmo, 2010)

I.5.2 *Desmodium adescendes* (Fabaceae)

Desmodium adscendens (Sw.) DC. (Fabaceae (alt. Leguminosae) subfamily: Faboideae) is one of the important species belonging to the genus Desmodium which have been used extensively as traditional medicines in India and Africa, respectively over a long period of time and their uses have been well documented. The genus Desmodium is represented by nearly 450 species distributed in the tropical and subtropical countries throughout the world, except in Europe and New Zealand, of which about 20–25 species are found in India **(Rastogi *et al.*, 2011)**.

Desmodium adscendens is herbaceous, stems slender angular, leaflets ovate-oblong acute subrepand bracts minute setaceous, joints small as long as broad. Stem erect, clothed when young with fine short spreading grey hairs. Stipules large, lanceolate, acuminate, not amplexical; leaflet membraneous or subcoriacous, green and smooth above, grey clothed with short adpressed hairs beneath; long; narrowed gradually to a point. Racemes copious, lax, lateral and terminal, the latter reaching a foot long, usually simple; pedicle, ascending or spreading.Teeth lanceolate, longer than the tube **(Rastogi *et al.*, 2011)**.

It is called owondo bekon and is used in the center part of Cameroon as a general antidote against snake bite **(Noumi, 2004)**. In Cameroon, it is called tombolombo and leaves are used against catching cold **(Sandberg *et al.*, 2005)**. Decoction or infusion of the leaves, whole plant and roots is given orally and relieves abdominal and back pain; stops postpartum abdominal pain; alleviates digestive ailments in eastern Nicaragua **(Coe, 2008)**. In Southern Uganda, decoction is drunk to remove spells **(Ssegawa and Kasenene, 2007)**. In Ghana, the plant decoction is used to treat asthma and other diseases associated with smooth muscle

contraction. In the Congo, the healers use it in the management of several diseases, including fever, pain and epilepsy. In Mato Grosso, the plant is used only in the treatment of ovary inflammation **(N'gouemo *et al.*, 1996)**. *Desmodium adscendens* is used in the treatment of asthma in Ghana. In Mato Grosso, the plant is known as "amores do campo" or "carrapichinho" and in São Paulo and Rio Grande do Sul as "pega-pega". In Brazil it is easily found in the Northeast, Center West and Southeast regions **(Rastogi *et al.*, 2011)**.

The quality and the quantity of polyphenols, flavonoids, anthocyanins, and tannins in *D. adscendens* were evaluated by **Muanda *et al.*, (2011)**. In addition, the antioxidant capacity of these phenolic compounds is evaluated by ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic)), DPPH (2,2-diphenyl-1 picrylhydrazyl), and Cellular tests. The ethanolic extract of the leaves of *D. Adescendes* induced hypothermia and analgesic effect in mice. *D. Adescendes*also suppressed the tonic phase of convulsion and mortality induced by pentylenetetrazole (PTZ) in mice. In addition, the plant extract delayed the onset of PTZ forelimb clonus, and generalized limbic seizures induced by kainic acid, but did not affect either tonic convulsion induced by maximal electroshock in mice or the progression of limbic seizures towards the status epilepticus in rats **(N'gouemo *et al.*, 1996)**.

Image 2: *Desmodium adescendens* (Dongmo, 2010)

I.5.3 *Alchornea cordifolia* (Euphorbiaceae)

Alchornea cordifolia Muell. Arg. belongs to the family Euphorbiaceae and is distributed in secondary forests usually near water, moist or marshy places. It grows to a considerable height but is always of a shrubby or scrambling habit. It has long stalked cordate leaves and flowers in hanging racemes about one foot long. It is a small tree of many

stemmed, almost climbing shrub up to 5m high. Stems are armed with blunt spines; leaves are long- petiolate; broadly ovate, cordate at base, the apex is short acuminate, entire or slightly dentate margin, finely stellate-puberulous or slightly glabrescent beneath with glands in axils of basal nerves. Flowers are greenish while in lax pendulous spikes or raceme, styles long and permanent on mature fruits. Fruits are 2-celled, small, stellate pubscent **(Adeshina *et al.,* 2010)**. *Alchornea cordifolia* is an erect and bushy perennial shrub or small tree up to 4 meters high reproducing from seeds **(lamikanra *et al.,* 1990)**. It has long stalked cordate leaves and flowers in hanging racemes about one foot long. The plant grows to a considerable height but is always of a shrubby or scrambling habit. It is called Agyama in Ghana, Susu bolonta in Sierra Leone, Casamance bugong in Senegal, Tschiya in Togo, Bondji in Cameroon, particularly Aboue in Ewondo (Central Region of Cameroon), Dibobonji in Douala, Ewe ipa, Ubobo and Bambami in Nigeria **(Adeshina *et al.,* 2011)**. In Bamabara (Mali), it is called kounaninkala or dunféké **(Traore, 2005)**. A. cordifolia has been subjected to intensive phytochemical screening to determine its medicinal usage. It is used in Cameroon for the treatment of malaria **(Titanji *et al.,* 2008)**. An ethnopharmacological study conducted in the upper Nyong valley forest revealed that it is used against anaemia, dermatitis, panacea of withcraft, malaria, dysentery and toothache, ear ache **(Jiofack *et al.,* 2009; Jiofack *et al.,* 2010)**. *Alchornea cordifolia* is an important crude drug in indigenous system of medicine in the coastal regions of West Africa. A decoction of the leaves is taken as a remedy for veneral diseases and urethral discharges in the Cameroon Mountain and in Senegal **(Adeshina *et al.,* 2011)**. The infusion of the leaf of *A. cordifolia* is taken orally for urinary tract infection in Zaire **(Muanza *et al.,* 1994)**. For ringworm, the juice of the leaves and fruit is rubbed on the skin **(Okeke *et al.,* 1999)**. The plant is used for treating infected wound, while the infusion of the dried leaf of *A. cordifolia* is used for diarrhoea and cough in Zaire **(Muanza *et al,* 1994)**. In Ivory Coast, it is used to cure malaria, anemia; hypertension and cough **(Tra bi *et al.,* 2008)**

The plant has been shown to possess antibacterial, antifungal and spasmolytic properties **(Ayodele *et al.,* 2007)**. **Osadebe and Okoye (2003)** have shown that methanolic extract of *Alchornea cordifolia* leaves possesses anti-inflammatory activity, when given by intraperitoneal injection in the egg albumin induced rat paw oedema test (inhibition of 68.25% for 50mg/kg) and the topical inflammatory activity was inhibited by the plant extract **(Mangar *et al.,* 2001)**. The hepatoprotective activity of its ethanol extract against paracetamol induced toxicity has also been reported **(Ayodele *et al.,* 2007)**.

The ethanolic extract of leaves possess antioxidant properties; inhibit glutathione S-transferase activity in Acetaminophen-induced liver injury in rats **(Ayodele *et al.*, 2007)**. The methanolic and ethyl acetate extracts of the plant leaves exhibit antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* **(Adeshina *et al.*, 2010; Adeshina *et al.*, 2011)**, while the anti-inflammatory activity of fractions of *A. cordifolia* leaves were observed on mice, the methanolic extract being the most potent compared to hexane, ethyl acetate and water **(Manga *et al.*, 2004)**.

Compounds such as alkaloids, tanins, flavonoids, inulin and alchornin have been reported to be present in *A. cordifolia* **(Adeshina *et al.*, 2010)**.

Image 3: *Alchornea cordifolia* (Dongmo, 2010)

I.5.4 *Vitex doniana* (Verbenaceae)

Vitex doniana is a plant of the verbenaceae family. Also called black plum, it is a perennial shrub widely distributed in tropical West Africa, extending eastward to Uganda, Kenya and Tanzania in savanna and high rainfall areas. It is commonly known as Mfuru, Mgwobe (Tanzania), Munyamazi, Muhomozi (Uganda), Dinya, Tinya, Tunci (Fulani) **(Atawodi *et al.*, 2003; Iwueke *et al.*, 2006)**. *Vitex doniana* is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown and a clear bole up to 5 m. Bark rough, pale brown or greyish-white, rather smooth with narrow vertical fissures. The bases of old trees have oblong scales. Leaves are opposite, glabrous; their tips are rounded or emarginated. Flower petals white except on largest lobe, which is purple, in dense opposite and axillary cymes; flowers are small, blue or violet. Fruits are oblong, green when young, turning purplish-black on ripening and with a starchy black pulp **(Orwa *et al.*, 2006)**.

Among their medicinal uses, the fruit is used to improve fertility and to treat anaemia, jaundice, leprosy and dysentery. The root is used for gonorrhoea, and women drink a decoction of it for backaches. When boiled, these roots can treat tooth ache **(Matig *et al.*, 2006)**. The young tender leaves are pounded and the juice squeezed into the eyes to treat eye troubles **(Orwa *et al.*, 2006)**. Its bark is used to treat syphilis **(Jiofack *et al.*, 2009)**. The anti-hypertensive effect of extract of the stem bark of *V. doniana* has been reported. The extract exhibited a marked dose-related hypotensive effect in both normotensive and hypertensive rats **(Iwueke *et al.*, 2006)**. Extracts of stem bark of *V. doniana* have also demonstrated some level of in vitro trypanocidal activity against *Trypanosoma brucei brucei* **(Atawodi, 2005)**. The aqueous methanol extract has also exhibited anti-diarrhea activity **(Agunu *et al.*, 2005)**. The aqueous extracts of leave and stem antagonize the carbon tetrachloride induced liver injury in rats **(James *et al.*, 2010)**.

The aqueous extract of the root-bark induced sleep at the dose of 400 mg/kg and potentiated sodium thiopental action. This extract also presents an anticonvulsant and analgesic activities in rats **(Abdulrahman *et al.*, 2007)**. The methanol fraction of leaves presented an anti-inflammatory and analgesic activity, starting at 50 mg/kg and in a dose dependent manner **(Iwueke *et al.*, 2006)**. The ethanolic and aqueous extracts of leaves presented an antioxidant activity in dose dependent manner **(Agbafor and Nwachukwu, 2011)**.

Phytochemical investigation of water and ethanolic extracts of leaves reveals the presence of alkaloids, saponins, anthraquinones, tannins, flavonoids and terpenoids **(Agbafor and Nwachukwu, 2011).**

Image 4: *Vitex doniana* (www.pinterest.com)

I.5.5 *Annona muricata* (Annonaceae)

Annona muricata is a plant of the annonaceae family. It is commonly called "corosollier" and in some common languages in Cameroon, it is saba saba or elom, respectively in Bassa, Boulou or Ewondo **(Matig *et al.*, 2006)**.

Originally found in Antilles, *Annona muricata* has now naturalized and become established in many tropical countries of Africa were it is found near houses **(Matig *et al.*, 2006; Adewole and Ojewole, 2009)**. *A. muricata* is a small tree or shrub which can reach 8 m of height **(Matig *et al.*, 2006)**, with an open roundish canopy **(Adewole and Ojewole, 2009)**.

Its fruits are dark green and covered by weak spines and contain many dark seeds **(Matig *et al.*, 2006)**. These fruits are consumed and can be transformed into juice or cream **(Matig *et al.*, 2006)**.

The pulp of the fruit is used to calm anxiety or distress. In Cameroon, seeds added to leaves are effective against amibiases. Leaves are used against diarrhea and sleeplessness. This plant can also be used against high blood pressure and fever **(Matig *et al.*, 2006)**.

It is a medicinal plant that has been used as a natural remedy for a variety of illnesses. It is used to cure diabetes and high blood pressure by the Ivory Coast population **(Tra bi *et al.*, 2008)**. It is used in Gabon against dysentery and fever **(Lolke and Bodinga, 1990)**. The flowers used by the Indian community present an anticonvulsant property **(Quintans *et al.*, 2008)**.

The methanolic extract of these leaves were also investigated for their antihyperglycemic effect. This extract was effective against streptozotocin induced diabetes in rats at the dose of 100 mg/kg **(Olawale *et al.*, 2009)**. At the dose of 100 mg/kg and 300 mg/kg in mice, the ethanolic extract of *A. muricata* leaves were anticonvulsant **(N'gouemo *et al.*, 1997)**.

Some of the reported secondary metabolite isolated and characterized from various parts of the plant include: annonaceous acetogenins, lactones and isoquinoline alkaloids; tannins, coumarins, procyanidins, flavonoids, pentacyclic terpenoid saponins; p-coumaric acid, stearic acid, myristic acid, ellagic acid and phytosterols **(Adewole and Ojewole, 2009)**.

Image 5: *Annona muricata* (Dongmo, 2010)

I.5.6 *Annona senegalensis* (Annonaceae)

Annona senegalensis is also a plant of the annonaceae family. It is also known as*Annona arenaria* or *Annona chrysophylla* **(Matig *et al.*, 2006)**.

Usually called annone of savanna or Senegal, the local varies according to the region; it is falo in Bamenda, souli in Baya, doukwi in Bororo, ngwono in mandara. In the country, it is found in the periforestery savanna until the Adamaoua plateau. *A senegalensis* is found in the tropical regions of Africa. Shrub or small tree, it can have 10 m of height and 30 cm of diameter, the top is irregular, stembark grey. Leaves are simple, alternate, scented when crumpled. The fruits are bulging berry or ovoids, composed of many carpels linked. They are yellow or orange when matured. This plant possesses many seeds, flattened, smooth and brown clear in color **(Matig *et al.*, 2006)**.

The parts mostly used are roots, stem bark, leaves. Fruits are especially consumed by children and shepherds. Leaves are consumed by animals **(Matig *et al.*, 2006)**.

A study conducted by **Jiofack *et al.*, (2010)** reveals that the roots of this plant prepared in decoction are used against snake bite, gastritis and impotence, and treat madness **(Stafford *et al.*, 2008)**. *Annona senegalensis* is among the medicinal plants that have been documented to possess antibacterial effects. Also the ethnomedicinal uses of the plant in the treatment of wounds and infectious diseases such as diarrhea, periodontal and other oral infections had been reported. Furthermore, the anticonvulsant, sedative, and muscle relaxant as well as anti-inflammatory effects of the root bark extract and fractions of *A. senegalensis* have been reported **(Okoye *et al.*, 2012)**.

Metabolites of edible mushrooms from Nigeria (*Pleurotus pulmonarius* and *P. ostreatus*), cultured on a novel medium of yeast extract supplemented with an ethanolic extract of *A. senegalensis*, present an antileukemic activity at P<0.05 **(Olufemi *et al.*, 2012)**. The lipophilic fraction and the kaurenoic acid isolated from the root bark extract of A. senegalensis, was effective against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Staphylococcus aureus, Aspergillus niger* and *Candida albicans*,between 8.75 and 0.04 mg/ml for the fraction and the isolated compound respectively **(Okoye *et al.*, 2012)**. The total essential oil and its fractions from *A. senegalensis* have showed mild to moderate cytotoxicity in brine shrimp lethality bioassay with LC50 = 27.3 µg/ml, and against some human tumor cell lines as A549, HT29, MCF 7 and U251 **(Ahmed *et al.*, 2010)**. The acute and sub acute toxicity of the root bark extract reveals that extracts are safe at the lower doses tested, and calls for caution in use at higher doses in treatment **(Okoye *et al.*, 2012)**.

Phytochemical investigations revealed the presence of carbohydrate, alkaloids, reducing sugar, glycoside, saponins, flavonoids, resin, fat and oils, steroid, terpenoid and acidic compounds **(Okoye *et al.*, 2012)**.

Image 6: *Annona senegalensis* (Dongmo, 2010)

I.6 Toxicity

Living organisms are subjected to toxic effects from chemical compounds present in the environment, drugs and in food **(Schorderet, 1992)**. The toxicity of a substance can be defined as a property that it possesses to hurt or kill a living organism **(Serrano, 1990)**.

Toxicity can also be considered as induce disturbance in the organism by a substance taken at a relatively higher dose, in singly or many closer doses or by many small doses repeated for a long time **(Fabre and Truhaut, 1954)**. These foreign compounds in the bodyare capable of provoking cells, sometime mortality is dose-dependent, administration duration and the concentration of the compound responsible which arrive the action site **(craig and Stitzel, 1984; Reed and Fariss, 1984)**.

The acute toxicity of a substance relates to the adverse effects arising in a short time after administration of a unique dose or multiple doses shared out on a 24h period **(Dubick *et al.*, 1993)**. This study permits to know the smallest dose, which is administered in one intake; results in the death of 50% of animals in 24 to 48 h following the treatment. The maximal duration of the treatment is 14 days **(Serrano, 1990)**. Once ingested a large number of constituents present in plant extracts is transformed during their metabolism into reactive metabolites. The interaction of these metabolites with macromolecules of the organism can induce a variety of toxic effects.

Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic **(Fennell *et al.*, 2004)**. This is the case of Adracunculus essential oil which shown to be toxic at 1.3 ml/kg **(Sayyah *et al.*, 2004)**; *Anacardium occidentale* hexane extracts which present toxicity at 16 g/kg **(Tedong *et al.*, 2007)**.

CHAPTER II: MATERIALS AND METHODS

II.1 Plant collection and extract preparation

II.1.1 Plant collection

Six plants (*Annona senegalensis*, *Annona muricata*, *Ficus thonningii*, *Alchornea cordifolia*, *Desmodium adescendes*, *Vitex doniana*) were collected in August 2010, based mainly on semi-structured interviews with selected knowledgeable elders. Most of the interviews and discussions were conducted in the local language. Interviews were held in a place where the informants were most comfortable (in their homes). Information regarding the gathering, preparation and uses of medicinal plants for the management of epilepsy were obtained. Additional discussions were conducted with the informants in order to ascertain the other uses of the plants. At the end of each interview, specimens of plants mentioned for the management of epilepsy were collected and authenticated by a taxonomist at the national herbarium in Yaoundé, Cameroon. Voucher specimens for six medicinal plants with anticonvulsant activities were deposited at the national herbarium. All the participants in these interviews were located in Yaoundé and its surroundings, Kon-Yambetta, Mbalmayo (Centre region) and Ngoyang (South region). The following table (table 1) shows the identification numbers and parts of the plants used in the study.

Table I: List of plants used in this study

Name of the plant	Voucher specimen number	Extraction solvent	Part used
Alchornea cordifolia **(Euphorbiaceae)**	4682/SRFK	MeOH/CH_2Cl_2	leaves
Annona muricata **(Annonaceae)**	3289/HNC	EtOH	Seeds, twigs
Annona senegalensis **(Annonaceae)**	40060/HNC	MeOH/CH_2Cl_2	Twigs, leaves
Desmodium adescendes **(Fabaceae)**	10258/SRF/CAM	MeOH/CH_2Cl_2	whole plant
Ficus thoningii **(Moraceae)**	44042/HCN	H_2O	Leaves, stembark

Vitex **(Verbenaceae)**	***doniana***	21147/SRF/CAM	MeOH/CH_2Cl_2	stembark

II.1.2 Plant extraction

Leaves, seeds, twigs, and/or stem barks of various plants or whole plants were separated, shade dried and ground using a blender. 100g of *A. cordifolia* leaves powder, *A. senegalensis* twigs and leaves powder, *D. adescendes* (whole plant), and *V. doniana* stembark powder were each macerated in 1L of MeOH/CH_2Cl_2 (1:1). 100g of *A. muricata* seeds and twigs powder and *F. thoningii* leaves and stembark powder were as well macerated in 1L of EtOH and water respectively. 72 h after maceration, all were filtered using Whatman paper n°1. The aqueous filtrate was dried in an oven (Memmert) at 45ºC for 48 hours. The organic solvent filtrates were concentrated under reduced pressure using a rotary evaporator (BÜCHI Rotavapor) at 40°C then 65° C for the MeOH/CH_2Cl_2 (1:1) extract and at 80ºC for the ethanolic extract.

A. muricata was separated in different organs comprising the seeds, leaves, fruit pulp, stem bark, flowers, pericarp, twigs and roots and the ethanolic extracts were obtained as previously stated.

II.1.3 Fractionation of the ethanolic extract from *A.muricata*

The roots extract was submitted to 95% ethanol extraction for 48 h to have the ethanolic extract. The ethanolic extract was partitioned between H_2O and CH_2Cl_2, leading to H_2O layer and CH_2Cl_2 layer. The CH_2Cl_2 layer was submitted to vacuum evaporation.The CH_2Cl_2 fraction of root of *A. muricata* (31.67g) was subjected to column chromatography over silica gel (Merck, 230-400 mesh) and eluted with n-hexane, n-hexane/EtOAc, EtOAc and EtOAc/MeOH, in increasing order of polarity. One hundred and forty four fractions of 250 ml were collected and subsequently combined according to their TLC profiles on pre-coated silica gel 60 F plates, developed with n- hexane/EtOAc and CH_2Cl_2 /MeOH mixtures, to give five subfractions (J16-J20) and a product (P106).

II.2 Phytochemical analysis

II.2.1 Qualitative phytochemical analysis

A small quantity of the different extracts and fractions was used to determine the presence of alkaloids, phenols, flavonoids, tannins, saponins, anthocyanins, anthraquinones, steroids, essential oils, triterpenes, glycosides, coumarin and lipids using standard methods **(Harbone, 1976; Sofowora, 1993; Trease and Evans, 1996, Mukherjee, 2006).**

II.2.1.1 Alkaloids

The detection of alkaloids was done by boiling 2 g of the plant extract in 10% NaOH. The observation of a white turbidity or precipitate indicates the presence of alkaloids **(Harbone, 1976).**

II.2.1.2 Phenols

To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols **(Harbone, 1976)**

II.2.1.3 Flavonoids

Ten milliliters of extract was heated with 5 ml of ethyl acetate over a steam bath for 3 min. The mixture is filtered and 4 ml of the filtrate is shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids **(Sofowora, 1993; Igwe, 2004).**

II.2.1.4 Tannins

Two grams of the plant extract was added to 15 ml of distilled water. The mixture is heated in steam bath for 5 minutes and filtered. Few drops of 3% ferric chloride were added to the filtrate, we will observe a black- blue (blue dark) for gallic tannins or black- green (green black) if catechics tannins are present **(Harbone, 1976)**

II.2.1.5 Saponins

One gram of the plant extract was heated in 15 ml of distilled water and then filtered. The filtrate (10 ml) is vigorously shaken in a test tube for 15 seconds. The formation of a foam column of at least 1 cm height which persists indicates the presence of saponines **(Harbone, 1976).**

II.2.1.6 Anthocyanins

One gram of the extract was mixed with 15 ml of HCl 1% and boiled. The variation of color from orange-red to orange-blue characterized the presence of anthocyanins **(Odebeyi and Sofowora, 1978; Harbone, 1976).**

II.2.1.7 Anthraquinones

One gram of the extract was diluted in 10 ml of benzene and filtered. To the filtrate is added 5ml of NH4OH and the mixture homogenized. The development of a red coloration is characteristic of anthraquinones **(Odebeyi and Sofowora, 1978).**

II.2.1.8 Sterols

Sterols were detected using the Liebermann-Buchard test for sterols. To 0.2 g of the powder extract are added 10 ml of chloroform and the mixture shaken to ensure dissolution. 2 ml of acetic acid is added to the solution and cooled well in ice followed by addition of concentrated sulfuric acid carefully. Blue green ring indicate the presence of sterols **(Sofowora, 1993).**

II.2.1.9 Terpenoids

They are detected using the Salkowski test: 2 ml of chloroform are added to 10 ml of a 10 % w/v solution of the extract and shaken. 3 ml of concentrated sulfuric acid are carefully added to form a layer. A reddish brown coloration is formed at the chloroform water interface **(Trease and Evans, 1996).**

II.2.1.10 Essential oils

The presence of volatile oil is detected by boiling 5g of the air- dried powder of the plant with 500 ml of distilled water for 3 h. Appearance of yellow colour indicate the presence of volatile oil **(Mukherjee, 2006).**

II.2.1.11 Coumarins

Place with a pastor pipette a drop of extract dissolved with methanol on silica flow on a glass plate, then cover the stain with NaOH 10%, then, heat the plate. Fluorescence on an UV lamp whith the wave height between 254 and 336 nm, indicates the presence of coumarins **(Harbone, 1976).**

II.2.1.12 Lipids

A drop of the aqueous extract is put on a filter paper. A translucent stain reveals the presence of lipids

II.2.2 Quantitative phytochemical analysis

II.2.2.1 Total phenol determination

Principle

The colour of Folin-Ciocalteau reagent changes from yellow to blue upon the detection of phenolics in extracts which is normally due to the chemical reduction of tungsten and molybdenum oxide mixtures in the reagent **(Blainski *et al.,* 2013; Hue *et al.,* 2012)**.

Procedure

The total phenol content was determined by the Folin–Ciocalteu method as described by **Wolfe *et al.,* (2003).** The reaction mixture contains: 200 µl of diluted plant extract, 800 µl of freshly prepared diluted Folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture will be diluted to 7 ml with deionized water. Mixtures will be kept in the dark at ambient conditions for 2hr to complete the reaction. The absorbance at 765nm will be measured. Galic acid was used as standard and the results were expressed as mg galic acid (GAE)/g of extract

II.2.2.2 Determination of total flavonoid content

Principle

Aluminium chloride ($AlCl_3$) reacts with OH groups of phenols to form a yellow stable complex which absorbs at 510 nm **(Bouras and Houchi, 2013)**.

Procedure

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to a known method using quercetin as a standard as described by **Ordon *et al.,* (2006)**. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510nm. The results were expressed as mg quercetin (QE)/g of extract

II.2.2.3 Determination of total Flavonols

Principle

In ethanolic solution, flavonols are chelated by $AlCl_3$, and the absorbance is read between a wavelenght of 380 and 460 nm (**Kumaran and Karunakaran, 2007)**.

Procedure

To 2.0 mL of sample (standard), 2.0 mL of 2% $AlCl_3$ ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 hr at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/ ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve, where x was the absorbance and was the quercetin equivalent (mg/g).

II.2.3 Determination of mineral content of the plant

-Basic cations Ca, Mg, K, and Na were extracted by dry ashing in a muffle furnace at 500^{o}C, diluted using a dilute acid mixture of HCl/HNO_3 and analyzed using the atomic absorption spectrophotometer **(Benton and Vernon, 1990)**. Results were reported as a %.

II.3 Experimental animals

Healthy, male and female Balb/c mice (Mus musculus) of 6 to 8 weeks, weighing 20 to 24g were used. They were reared in the animal house of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 (Cameroon). The animals were kept and maintained under laboratory conditions of temperature, humidity and light (day and night) and were allowed free access to food (standard pellet diet) and water ad libitum. All the animals were fasted for 16h, but still allowed free access to water, before the commencement of experiments. Animals were separated in groups of 6 mice for experimentation. Experimentation was conducted following the guidelines for the care and use of laboratory animals (NIH, 1996)

II.4 Assessment of anticonvulsant activity

II.4.1 Pentylenetetrazole-induced seizure test

The convulsion inducer, Pentylenetetrazole (PTZ) and the positive treatment controls, Phenobarbital (PHB) and Diazepam (DZP) were dissolved in normal saline. All compounds

were prepared freshly each time and administered intraperitoneally (*ip*) in a volume of 0.1ml/10 g body weight. Control animals received the same volume of vehicle.

The obtained extracts were kept at 4°C and diluted as needed for each day of our experiment. *F. thoningii* and *V. doniana* extracts were dissolved in water, while *A. cordifolia* and *D. Adescendes* extracts were dissolved in DMSO/tween 20 and the remaining extracts were dissolved in olive oil.

Thirty one experimental groups of 6 mice each were used for anticonvulsant evaluation using the method of **Hosseinzadeh and Parvadeh (2004)** with minor modifications. The first, second and third groups of mice received PTZ at a dose of 80 mg/kg, 1hour after administration of vehicle (DMSO/Tween 20, olive oil) and normal saline as negative controls. Phenobarbital, in increasing doses of 10, 20, 40 mg/kg, *ip*, was used as reference anticonvulsant drug for comparison and was administered 30 minutes before PTZ challenge (80 mg/kg). The rest of animals were treated with three different doses of each extracts ranging from 200 to 1000mg/kg, 60 minutes before injection of PTZ. The animals were individually placed in plastic boxes and observed immediately after PTZ injection for a period of 30 min. The latency period (the average length of time between drug administration and seizure onset) and duration of myoclonic jerks, as well as the percentage of protection against incidence of seizures and mortality were recorded.

Among the plants harvested and screened, *Annona muricata* because of its highest activity was after preliminary investigation using pentylenetetrazole induced seizures for study of anticonvulsant properties selected for further study. The differents extracts were diluted in olive oil and the animals were receiving doses ranging from 200 to 400 mg/kg. The positive control consists of DZP (1mg/kg) and PHB (40 mg/kg).

II.4.2 Picrotoxin-induced seizure test

Animals were divided into 4 groups of 6 animals and treated as in the case of PTZ-induced seizure test, except that mice were administered picrotoxin, 8mg/kg *ip*.

The observation was made as described by **Ilhan *et al.*, (2005)** wih minor modifications. Immediately after injection of the convulsant, mice were placed into clear plastic observation chambers and observed for a period of 30 minutes.

Generally, following injection of PTZ and PIC, mice displayed walking and sniffing types of behavior and periods of immobility. At the same instance the animal displayed myoclonic jerks followed by a general seizure. Since some animals died due to effects of the seizure, after this period they were monitored for another 30 minutes only to assess mortality. The seizure duration was calculated as the sum of these multiple seizures for each animal to be assessed as one combined fit. Subsequently, latency to first seizure onset, total seizure duration, the number of seizure episodes and mortality were recorded for each subject. The anticonvulsant property of the plant was assessed by its ability to delay the onset of seizure.

After observation, animals were killed by decapitation and the blood collected for total protein and albumin determination.

II.4.3 Determination of total protein and albumin content

The blood collected in dry eppendorf tubes was submitted to centrifugation at 4000 rpm for 15 min. The serum obtained was preserved at – 20° C until the test day.

II.4.3.1 Determination of total protein content

The Biuret method described by **Gornall *et al.*, (1949)** was used for the determination of total proteins in the serum.

Principle

Active hydrogen of the peptide bonds is substituted by a metallic ion (Na+, Cu2+) in alkaline medium. This reaction is characterized by the formation of blue- violet coloration with the intensity being proportional to the content in proteins.

Procedure (presented in table II below)

Table II: Procedure for the determination of total proteins

Tube No	**1**	**2**	**3**
Tube name	Blank	Standard	Test
Distilled water	20 µl	-	-
BSA (1 mg/ml)	-	20 µl	-
Test (serum)	-	-	20 µl
Biuret reagent	980 µl	980 µl	980 µl
Incubation at room temperature for 30 minutes and read OD at 540 nm			

$$\text{Total protein concentration } \left(\frac{g}{l}\right) = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration}$$

II.4.3.2 Determination of albumin content

The method described by **Doumas *et al.*, (1971)** was used for measuring the serum albumin level using the albumin kit (Fortress diagnostics).

Principle

Serum albumin binds with bromocresol green indicator in an acid medium to form a green BCG (bromocresol green) complex, the amount of which is directly proportional to the albumin concentration present in the sample, with a maximum absorption between 620 and 640 nm.

Procedure (showed in table III)

Table II: Procedure for the determination of albumin content

Tube No	**1**	**2**	**3**
Tube name	Blank	Standard	Test
Distilled water	5 µl	-	-
Standard (45 mg/ml)	-	5 µl	-
Test (serum)	-	-	5 µl
Albumin reagent	1000 µl	1000 µl	1000 µl
Incubation at room temperature for 5 minutes and read OD at 630 nm			

$$\text{Albumin } \left(\frac{g}{l}\right) = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration}$$

II.4.4 Investigation of the involvement of benzodiazepine receptors in anticonvulsant activity

We studied the effects of a selective benzodiazepine receptor antagonist, flumazenil, on the anticonvulsant activity of *A.muricata* in order to investigate their probable involvement. Mice were separated into six groups of 6 animals each. In the first group, mice were given flumazenil (2 mg/kg) 5 min before the administration of *A. muricata* (200 mg/kg) and 65 min before the injection of PTZ. In the second group, the animals received flumazenil (2 mg/kg) 5 min before the administration of diazepam (1 mg/kg). Also, three groups were respectively injected with diazepam (1 mg/kg *i.p*), flumazenil (2 mg/kg) and normal saline 30 min and 1hour (for the saline) before the administration of PTZ (80 mg/kg *i.p*) respectively.

Following the experimental setup, observation and documentation of convulsion-like behavior, seizure onset for each group was recorded during an hour **(Hosseinzadeh and Parvardeh, 2004)**.

II.5 Analgesic activity of plant extract

The animals were distributed in differents groups of 6 mice each. Except for the hot plate test where each mouse serves as its own control, in the remaining tests the control group received saline, while the test groups received differents doses of the extracts ranging from 200-400 mg/kg. The positive control groups received Aspirin, Morphine or Indomethacin. Naloxone was used as an antagonist in the last group.

II.5.1 Acetic acid-induced abdominal writhing

This test was carried out according to the method described previously by **Ojewole and Amabeoku (2007)** with minor modifications. The mice used were separated in control and test groups. The ethanolic extract (100, 200 and 400 mg/kg) or vehicle (olive oil) were administered to the mice before intraperitoneal (*i.p.*) injection of acetic acid (1% in saline solution, 10 ml/kg) 1hour after treatment with different compound. Aspirin (100 mg/kg, *i.p.*) employed as reference drug, was dissolved in distilled water. Morphine (5mg/kg, *i.p.*) another reference drug, was also used and administered 30 min before acetic acid (AA). Naloxone (1 mg/kg) was administered in the group of animals treated with morphine and the group of mice treated with extract which exhibit the best activity, this 15 min prior the administration of extract or morphine. The number of writhes (abdominal contractions) was counted during the 30 min first minutes following acetic acid injection. This test mean reaction time value was subsequently used to determine percentage of inhibition, based on the following formula:

$$\text{Percentage of inhibition of (\%)writhing} = \frac{(\text{Test mean} - \text{control mean})}{\text{Control mean}} \times 100$$

II.5.2 Formalin test

In the formalin test **(Santos and Calixto, 1997; Cha *et al.*, 2011)**, groups of mice were treated orally with vehicle or AnMr (100, 200,400mg/kg).After 1hour, each mouse was treated with 20µl of 2.5% formalin (subplantar) into the right hind-paw. The duration of paw licking (s) was used as an index to measure the pain response during the 0–5min period (first phase, neurogenic) and the 15–30min period (second phase, inflammatory) after formalin

injection. Morphine and indomethacin were used as positive control drugs and were administred 30 min (*i.p.*) and 1h (*p.o.*) respectively before the test. Naloxone was used as an antagonist to elucidate the possible mechanism of action of the plant extract as previously stated in the AA test.

II.5.3 Hot plate assay

The hot-plate test measured response latencies according to the method described by **Ojewole and Amabeoku (2007)** with minor modifications. Each mouse was placed separately on a DS-37 model socrel. Insight hot-plate maintained at 55±2°C and the time between placement of the animal on the hot-plate and the occurrence of either the licking of the hind paws, or jump off from the surface was recorded as the response latency and as an indication of the animal's response to heat-induced pain. The reaction time taken for each mouse was recorded in seconds. Each mouse served as its own control. Thus before treatment its reaction time was determined. The mean reaction time of all the mice used was pooled to obtain the control mean reaction time. Only mice with an initial nociceptive response between 7 and 10 s were used for additional experiments. The cut-off time for the hot plate latencies was set at 50 s. Animals were treated with either oil, AnMr (100, 200, and 400 mg/kg, *p.o*) 1 hour or morphine (5 mg/kg, *i.p.*), reference drug, 30 min before the experiments.Naloxone (1mg/kg) an antagonist was used and administered 15 min before experimentation. After different treatments the reaction time was taken at 30, 60, 120, 180 and 240 minutes. These reaction times were pooled for mice used in each treatment group and the final test mean reaction time for each treatment group was calculated. This test mean reaction time was subsequently used to determine percentage thermal pain relief or protection, based on the following formula:

$$\text{Protection against thermal pain (\%)} = \frac{(\text{Test mean} - \text{Control mean})}{\text{Control mean}} \times 100$$

II.6 Determination of antioxidant potential of plant extracts

II.6.1 Scanvenging activity of DPPH radical

Principle

This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and

the absorbance change at λ= 517nm is measured. The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity **(Pathiranan and Shahidi, 2005)**.

Procedure

This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and the absorbance change at λ= 517nm is measured. The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity. Briefly, in 3 mL of each diluted extract, 1mL of methanol solution of DPPH 0.1 mM is added. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank **(Pathiranan and Shahidi 2005)**. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

$$\text{Radical scavenging activity (\%)} = \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \times 100$$

II.6.2Nitric oxide scavenging activity

Principle

Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates NO, which reacts with oxygen to produce nitrite ions.Thechromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylenediamine dihydrochloride is measured **(Gaikwad *et al.*, 2011)**.

Procedure

Nitric oxide scavenging activity was determined according to Griess Illosvoy reaction. The reaction mixture contained: 10 mM SNP (Sodium nitro prusside) in 0.5 M phosphate buffer, pH 7.4, and various doses (50-250 µg/ml) of the test solution in a final volume of 3 ml). After incubation for 60 min at 37°C, Griess reagent (a-napthyl-ethylenediamine 0.1% in water and sulphanilic acid 1% in H3PO 45%) was added. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with α-napthyl-ethylenediamine was measured spectrophotometrically at 540nm. Ascorbic acid was used as a positive control **(Garratt 1964)**. Nitric oxide scavenging ability (%) was calculated by using the formula:

$$\text{Percentage of radical scavenging activity} = \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \times 100$$

II.6.3 Hydroxyl radical scavenging activity

Principle

Hydroxyl radicals generated by the phenanthroline system - Fe^{2+}- H_2O_2 (Fenton's reaction) are trapped by antioxydants, producing a variation of absorbance at 560 nm **(Yu *et al.*, 2004)**.

Procedure

In 1.5 mL of each diluted extract, 60 µL of $FeCl_3$ (1 mM), 90 µL of 1,10-Phenanthroline (1 mM), 2.4 mL of 0.2 M phosphate buffer, pH 7.8 and 150 µL of H_2O_2 (0.17 M) was added respectively. The mixture was then homogenized and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the radical scavenging activity of each extract was calculated from the equation below:

Percentage of radical scavenging activity = [(OD control - OD sample)/OD control] x 100

II.6.4 Antioxidant capacity determined by radical cation ($ABTS^+$)

Principle

This test is based on the ability of an antioxidant to stabilise the blue–green chromophore ABTS (2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) by decolorizing it to colourless ABTS due to the trapping of a proton by the antioxidant **(Alam *et al.*, 2013)**.

Procedure

ABTS assay was based on a previously described method by **Re *et al.*, (1999)** with slight modifications. ABTS radical cation ($ABTS^+$) was produced by the reaction of a 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was stored in the dark at room temperature for 12H before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. After addition of 25 mL of extract in ethanol (5, 10, 20, 40 and 80 mg/mL) or Trolox standard to 2 mL of diluted $ABTS^+$ solution, absorbance at 734 nm was measured at exactly 6 min. The decrease in absorption was used for calculating TEAC values. A standard curve was prepared by measuring the reduction in absorbance of $ABTS^+$ solution at different concentrations of Trolox. Appropriate blank measurements were

carried out and the values recorded. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC)

II.6.5 Total antioxidant activity by Ferric Reducing Antioxidant Power assay (FRAP)

Principle

This is a colorimetric assay that measures the ability of the compound to reduce the intense blue ferric tripyridyltriazine complex to its ferrous form, thereby changing its absorbance **(Baradinath *et al.*, 2010)**.

Procedure

The FRAP was determined using the method of **Benzie and Strain (1996)** which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant compounds. The fresh FRAP reagent consist of 500 mL of acetate buffer (300 mM pH 3, 6), 50 mL of 2, 4, 6- Tri (2-pyridyl)-s-triazin (TPTZ) (10 mM), and 50 mL of $FeCl_3 \bullet 6H_2O$ (50 mM). The colorimetric measurement was performed at 593 nm and the reaction was monitored up to 12 min on 75 µL of each extract and 2 mL of FRAP reagent.

II.7 Glycation of proteins

Albumin-derived advanced glycation endproducts (AGEs) were measured using fluorometry as described previously by **Perera *et al.*, (2000)** and **Khwanta *et al.*, (2014),** with minor modifications.

Principle

Any sample giving fluorescence equal to the fluorescence of BSA/sugar implied that there was no inhibition of glycation; whereas, any sample giving fluorescence lower than that of BSA/sugar indicated that there was inhibition of glycation by the extract present. Aminoguanidine was included as a positive control **(Mahomoodally *et al.*, 2012)**.

Procedure

100 µl of BSA (100 mg/ml) was incubated with 100 µl of sugar and 10 µl of different concentrations of extract (5-95 µg/ml) diluted in methanol. 100 µl of PBS in the presence of 0.02% NaN_3 was used as the sample control and 10µl of amminoguanidine (6.76 mg/ml) as positive control. A negative control was carried out at the same time with BSA (100 µl), PBS (100 µl) and methanol (10 µl) under the same conditions.

The sugars used were glucose (288 mg/ml), ribose (30 mg/ml) and glyoxal (10 µg/ml). The mixtures were prepared in a microplate and the plate incubated at 37°C, during 5 days for the test with ribose and 7 days for the tests with glucose and glyoxal. After the incubation the plate are read at wavelenght of 370nm and 440nm.

Results were expressed as µg/ml according to the percentage of inhibition

Percentage of inhibition = [(OD control - OD sample)/OD control] x 100

II.8 Evaluation of the adverse effect of plant extract

II.8.1 Acute toxicity test

After evaluation of the anticonvulsant properties, the acute toxicity of the plant extract was determined following the **OECD** guideline for testing of chemicals **(2001).**

After a fasting period of 12 hours, mice received orally, using an esophageal borer the extracts dissolved in olive oil.

Before determination of the effective lethal dose (LD50), an approximate lethal dose of each extract was initially determined during a preliminary study realized by the scale method using a small number of mice (2 per dose) and increasing doses of extracts. Briefly, 2 mice were force-fed with a given dose and observed for 24hours for any mortality. This helped us in determining the gap between the maximal non-lethal dose and the minimum lethal dose. Anindepth study was there after realized using three levels of doses in the established range, with a bigger number of animals in each group **(Ghosh, 1984)**. For this, animals were distributed in three groups of 6 mice each. Increasing doses of extracts between 1 and 5 g/kg were chosen for the determination of the LD50. The control group was treated only with olive oil.

After treatment animals were observed during 7 hours for any immediate sign of toxicity, then on a daily basis during three days for any change such as excitation, tiredness, appetite, mobility, fur status, tail and stool aspects, sensitivity to noise, suffocation and mortality. During this period, the numbers of deaths were counted in each group.

Starting from the day of force-feeding the animals, they were observed for 13 days.

The percentage of mortality at each dose level was transformed in probit values (Ghosh, 1984), then the LD50 was determined as described by **Randhawa (2009).**

II.8.2 Effect on motor function (neurotoxicity)

The effect of the various treatments on motor function was assessed using the rotarod test. The experiment was performed as previously reported by **Pieretti *et al.*, (1999)** with a Rota-rod apparatus (Ugo Basile 7560; Milano, Italy) consisting of a bar with a diameter of 3 cm, subdivided into five compartments by disks of 240 mm in diameter. The bar rotated at a constant speed of 16 revolutions per min. Latency to fall from the bar is automatically recorded in seconds. Mice that stayed on the bar for more than 60 seconds were given the maximum score, 60 seconds. A preliminary selection of mice was made on the day of the experiment excluding those that did not remain on the Rota-rod bar for two consecutive periods of 60 seconds each. The integrity of motor coordination was assessed on the basis of the number of falls from the Rota-rod in 180 s. Selected animals were tested immediately at 30, 60, 90 and 120 min after administration of *A. muricata* extract, DZP (2mg/kg) or vehicle (10ml/kg.) **(Boakye, 2009; Taiwe *et al.*, 2012)**.

II.8.3 Cytotoxicity assay

II.8.3.1 Preparation of cells and test samples

Cytotoxicity was conducted according to the method designed by **Samje *et al.*, (2014)**. Monkey kidney epithelial cells (LLCMK2) were purchased from the American Type Culture Collection (ATCC, Virginia, USA) and proliferated in complete culture media at 37°C and 5% CO_2. Once the cells became fully confluent, the old media was swiftly decanted and the cells were dislodged using 0.125% trypsin and 0.5 mM EDTA in serum-free media. The dislodged cells were re-suspended in 10 ml of complete culture medium (RPMI 1640 with sodium bicarbonate, supplemented with 25mM HERPES, 0.3g γ-irradiated L-glutamine powder, 5% heat inactivated new born calf serum, 200 units/ml penicillin and 200 µg/ml streptomycin and 0.25 µg/ml amphoteracin B; pH 7.4) and centrifuged at 560 × g for 10 minutes to get rid of the trypsin. The last procedure was repeated once. The cell suspension (100 µl/well) was transferred into 96-well microtitre culture plates and kept in the CO_2 incubator for cells to grow and become fully confluent. This usually takes 3–5 days depending on the initial concentration of cells. The cells were mixed with different concentrations of plant fractions (375 – 1 µg/ml) and pure compound (20 - 0.367 µg/ml). Fractions and compound were dissolved in DMSO and stock concentrations were diluted in culture media giving a final DMSO concentration of 0.1%. This concentration of DMSO did not affect cell

viability. Control wells contained only the diluent and culture media. Experiments for each concentration tested were conducted in four wells.

II.8.3.2 MTT reduction assay

Principle

This biochemical evaluation was done using the MTT/formazan colorimetric assay. This assay is based on the ability of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically at 540 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel *et al.,* 2009).

Procedure

Fully confluent LLCMK2 cells were seeded in 96-well plates, and incubated at 37°C in a humid 5% CO_2 atmosphere. After 24 hours, the medium was replaced with new complete culture medium containing serially diluted fractions and compound to be tested. Following 72 hours of incubation, the cells were washed in PBS, and 500 µl/well of 0.5 mg/ml MTT (Sigma, USA) in serum free medium, were added and then incubated for 30 minutes under the culture conditions. Thereafter, the color development of the test compared with the control was observed **(Rodriguez *et al.*, 2014; Samje *et al.*, 2014)**.

II.8.4 Developmental toxicity

II.8.4.1 Chemicals and FETAX solution

All analytic-grade reagents, human chorionic gonadotropin (HCG), and tricaine were purchased from Sigma–Aldrich. The composition of the FETAX (Frog Embryo Toxicity Assay-Xenopus) solution was (mM): NaCl 10.69, $NaHCO_3$ 1.14, $CaCl_2$ 0.1, $CaSO_4.2H_2O$ 0.35, KCl 0.4 and $MgSO_4$ 0.62 in autoclaved water. The modified Barth's solution (MBS) was (mM): NaCl 88, $NaHCO_3$ 2.4, KCl 1.0, HEPES 10, $MgSO_4$ 0.82, $CaCl_2$ 0.41, and $CaNO_3$ 0.33 and PH adjusted to 7.4. The high stock solutions of fractions and isolated compound were prepared in DMSO first due to their low water solubility; then the DMSO stocks were diluted with FETAX solution to obtain the appropriate stock concentration. The final highest concentration of DMSO in test solutions was 0.5%. DMSO had been shown not to produce detrimental effects to developing Xenopus laevis embryos up to concentrations of 1% v/v **(Gardner *et al.*, 2016).**

II.8.4.2 Animals

Adult Xenopus were purchased and housed in an aquarium with filtered tap water at 18 ± 2 °C and an alternating 12-h light/dark cycle. The animals were fed a semi-synthetic diet

II.8.4.3 In vitro fertilization

Male xenopus was anaesthetized using tricaine. It was sacrificed and the obtained testes used for fertilization of eggs.

Ovulation was induced by injecting the female Xenopus with 650 IU HCG just under the skin in the evening and kept at 16°C. The next day, those females were made to lay eggs in 60-mm plastic dishes. The eggs were immediately fertilized with minced testes in 1X modified Barth solution after washing three times with 1XMBS. Following successful fertilization, the jelly coat was removed by swirling the embryos in a 2% cysteine solution. The embryos were then transferred to 0.1X MBS. Unfertilized eggs and dead embryos were removed and the viable embryos were maintained at 13°C until blastulae (stage 8) were formed.

II.8.4.4 FETAX assay

A FETAX assay was conducted to assess the developmental toxicity and teratogenic effects of *Annona muricata* (AnM) fractions based on the American Society of Testing Material (ASTM) guide (ASTM E1439-98) with minor modifications. Finely cleaved embryos in the blastula stage were selected and used to exclude the effects of spontaneous embryonic developmental problems. Embryos were exposed to different concentrations of AnM ranging from 300 µg/ml to 3 µg/ml for fractions and 20 µg/ml to 0.2 µg/ml for the isolated compound.

For each concentration, replicates of a maximum total of 47 embryos were tested. Embryos treated with DMSO and FETAX medium alone were used as controls. The embryos were incubated at ambient temperature until the end of the assay. The media were changed every day and dead embryos were removed. The embryos were counted after 24 h and 96 h. Each experiment was performed with separate clutches of embryos. At the end of the experiments, surviving embryos were fixed in 4% formaldehyde and observed under a light microscope to check for malformations using the Atlas of abnormalities **(Bantle *et al.*, 1991)**. Results of the FETAX assay were expressed according to teratogenic index (TI) values [TI = LC50 (concentration that is lethal to 50% of the embryos)/EC50 (concentration at which 50%

of the embryos are malformed)] to determine whether a compound was toxic or not **(Chae *et al.*, 2015)**. If the tested substance is toxic, then TI will be greater than or equal to 1.2.

II.9 Statistical analysis

The results of the preliminary study were subjected to analysis of variance (ANOVA), and post hoc significance testing with Dunnett's multiple comparison test using SPSS 16.0 software. P values less than 0.05 were considered as significant.

Except for the first anticonvulsant results analysed with SPSS, the data obtained were analyzed using GraphPad software Version 5.0 and expressed as mean±S.D. Statisticaly significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Tukey's post-hoc test. P-values less than 0.05 ($p < 0.05$) were considered as significant.

For the developmental toxicity, the teratogenic index (TI) value was calculated as the ratio of the 50% embryo-lethal concentration (LC50) versus the effective concentration that resulted in 50% of malformed larvae (EC50) among the surviving ones. Each LC50 or EC50 value was obtained with Graph-Pad Prism 5 software. When the TI value was greater than or equal to 1.2, the tested compound was regarded as toxic to embryos.

CHAPTER III: RESULTS AND DISCUSSION

III.1 RESULTS

III.1.1 Phytochemical analysis

III.1.1.1 Qualitative phytochemical analysis of extracts and fractions

Table IV below presents results of the qualitative phytochemical analysis of the differents parts of the plant.

Table IV: Qualitative phytochemical analysis of extracts

	Lipids	**Saponin**	**Alkaloid**	**Phenol**	**Flavonoid**	**Essential.Oil**	**Anthocyanin**	**Gallic tanin**	**Catechic tanin**	**Terpenoid**	**Anthraquinon**	**Sterol**	**Coumarin**
AnMsb	+	-	-	+	-	+	+	-	+	+	-	-	+
AnMr	+	-	+	+	+	+	+	-	+	+	+	+	+
AnMpf	+	+	+	+	-	+	+	-	-	+	+	-	+
AnMfl	+	-	-	+	+	+	+	-	-	-	-	+	+
AnMtw	+	+	+	+	-	+	+	-	-	-	-	-	+
AnMl	+	+	+	+	-	+	+	-	-	-	-	+	+
AnMs	+	+	+	+	-	+	+	-	-	-	-	-	+

AnMr : *Annona muricata* roots ; AnMfl : *Annona muricata* flower ; AnMs : *Annona muricata* seed ; AnMl : *Annona muricata* leaves; AnM pf: *Annona muricata* pulp of fruit; AnMtw: *Annona muricata* twigs; AnMp: *Annona muricata* pericarp ; AnMsb : *Annona muricata* stem bark ; + : present, - : absent

This table IV presents the existence of different metabolites in the plant extracts. Lipids, phenols, essential oils, anthocyanin and coumarin were observed in all plant parts, while gallic tanins were absent. Alkaloids were present in all plant parts except the pericarp and the flowers, while flavonoids were present only in roots and flowers and anthraquinonesonly in roots and fruit pulp. Catechic tannins on their part were absent in the similar organs as the terpenoids except the fruit pulp. Leaves, flowers and roots possessed sterols. Roots had the majority of secondary metabolites tested

Table III: Qualitative phytochemical analysis of fractions

	Fractions					
	J16	J17	J18	J19	J20	P106
Alkaloids	+++	+++	+	+	+	-
Saponins	+	+	+	++	++	-
Tannins	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Steroids	+++	+++	-	-	-	-
Triterpenes	+	++	+++	+++	+++	+++

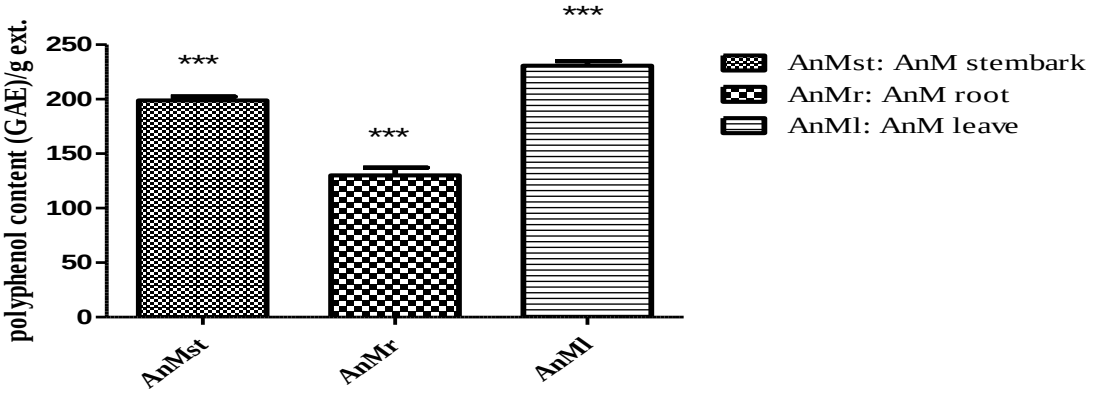
+++: abundant, ++: less abundant, +: slightly present, - : absent

Table V gives an overview of the presence of some secondary metabolites in fractions. According to this table, alkaloids, saponins, triterpernes were present in all the fractions, though in different amounts. On the contrary there were neither tannins, flavonoids nor glycosides in all the fractions.

The compound P106 obtained from the roots of the plant was quite rich in triterpenes.

III.1.1.2 Quantitative phytochemical of extracts

III.1.1.2.1Total phenol determination



(GAE)/g ext.: mg cafeic acid/ g dried extract

Figure 1: Polyphenol content of plant extract

As shown in the figure 1 above, the polyphenol content varied with the extracts. The highest content being in AnMl (230.8 ± 2.386 GAE/g) while the smallest polyphenol content (130.2 ± 4.017GAE/g) was in the roots. These values were all significantly different at p< 0.001.

III.1.1.2.2 Determination of total flavonoid content

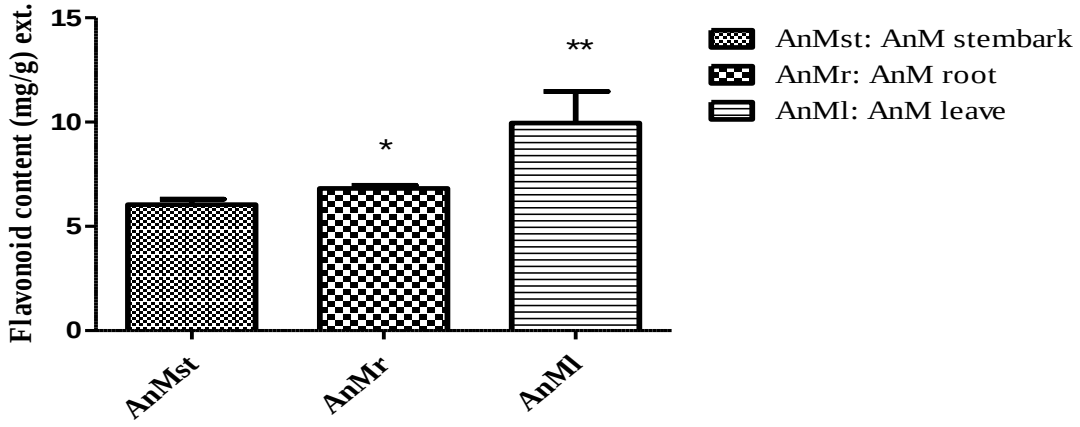
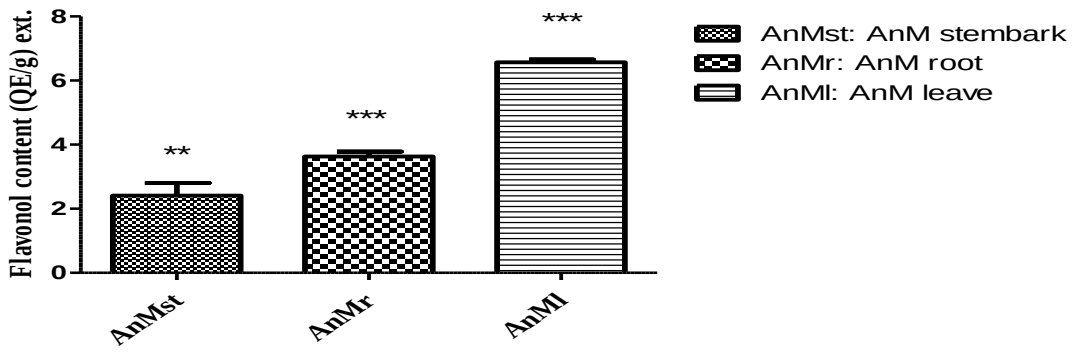


Figure 2: Total flavonoid content

Figure 2 presents the flavonoid content of the plant extracts. The values of the flavonoid content were 6.039 ± 0.156mg/g of dried extract, 6.824 ± 0.084 mg/g of dried extractand 9.962 ±0.870 mg/g of dried extract respectively for AnMst, AnMr and AnMl. The plant part containing the highest amount of flavonoids was the leaves, followed by roots. A significant difference was observed between AnMl and AnMst ($p<0.01$) and AnMl extract and AnMr extract ($p<0.05$). The content of AnMst and AnMr were not significantly different ($p<0.05$).

III.1.1.2.3 Determination of total Flavonol



(QE)/g dried ext: mg quercetin equivalent/g of dried extract

Figure 3: Flavonol content of plant extracts

Figure 3 presents the flavonol content of AnM. The flavonol contents were 2.409 ± 0.227 mg of quercetin equivalent/g of dried extract, 3.633 ± 0.087 mg of quercetin equivalent/g of dried extract and 6.571 ± 0.048 mg of quercetin equivalent/g of dried extract for AnMst, AnMr and AnMl respectively. A significant difference in flavonol content was observed among all the parts tested. A significant difference was observed between AnMr and AnMl ($p<0.001$), and between AnMst and AnMr ($p<0.01$). AnMl presented the highest content of flavonols.

III.1.1.3 Ions content in plant extracts

Table IVI: Ion content in plant extract (%)

Plant extract	Ca	Mg	K	Na
AnMr	1.03	0.08	0.47	0.011
AnMsb	0.22	0.09	0.30	0.005
AnMs	0.16	0.12	0.43	0.001
AnMpf	0.22	0.17	2.82	0.004
AnMp	0.19	0.09	1.26	0.002
AnMl	2.40	0.21	1.69	0.003

AnMr: *Annona muricata* roots ; AnMs : *Annona muricata* seed ; AnMl : *Annona muricata* leaves; AnM pf: *Annona muricata* pulp of fruit; AnMp: *Annona muricata* pericarp ; AnMsb : *Annona muricata* stem bark; Ca: Calcium; Mg: Magnesium; K: potassium; Na: Sodium

Table VI above presents the content of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} in the different organs of the plant. According to the results obtained, the leaves possess the highest content of Ca^{2+} and Mg^{2+}. Its K^{+} content was the second and the Na percentage was similar to that of the other organs. Roots on their part were the second organ rich in Ca^{2+}, but they were poor in Mg^{2+} and K^{+}, while being the organ with the highest Na content. The stem bark and the fruit pulphad the same amount of Ca^{2+} content; the Mg^{2+} content of sb was similar to the pericarp one. Stem bark has the smallest content of K^{+} and an average amount of Na^{+} in comparison with the other organs. Seeds were the organs with the smallest amount of Ca^{2+}. Its Mg^{2+} content was 0.12 while the K^{+} content 0.43 wascomparable to that of the roots. The Na content of this organ was the smallest among the organs tested. The Mg^{2+} content of pf was 0.17, the second content among the organs, but it has the highest amount of K^{+} and the Na^{+} content was 0.004. Pericarp content of Ca^{2+} was 0.19, while the Mg^{2+} content was the same as observed in sb. The k^{+} content was 1.26 and 0.002 for the Na^{+}.

III.1.2 Anticonvulsant activity

III.1.2.1 Pre-screening of selected plants for the anticonvulsant screening

Table V: Anticonvulsant activity of studied plants

Product	Dose	Seizure onset(s)	Clonic seizure onset(s)	Convulsion duration(s)	Death time(s)	Protection percentage
AlCl	500	59.67 ± 7.84^{b}	99.50 ± 6.95^{b}	50.83 ± 7.65^{a}	261,50±44.49	33.33
	700	57.67 ± 4.13^{b}	143.20 ± 59.05^{b}	98.00 ± 8.78^{ab}	682.67 ± 6.4^{a}	50
	1000	67.17 ± 11.83^{b}	155.20 ± 21.99^{b}	73.83 ± 9.94^{ab}	/	100
AnMs	200	70.50 ± 6.95^{b}	124.00 ± 11.46^{b}	56.83 ± 5.23^{a}	/	100
	400	84.33 ± 42.22^{b}	94.00 ± 1.41^{b}	93.00 ± 20.73^{ab}	186.67±12.22a	50
	800	71.50 ± 10.50^{b}	360.50 ± 0.70^{ab}	72.00 ± 10.13^{ab}	107.33 ± 24.82^{a}	50
AnMtw	200	132.67 ± 10.05^{a}	280.00 ± 14.14^{ab}	52.33 ± 6.65^{a}	/	100
	400	114.83 ± 5.70^{a}	229.33±31.00b	50.00 ± 7.04^{a}	/	100
	800	71.50 ± 8.52^{b}	146.00 ± 1.41^{b}	60.67 ± 6.56^{a}	917 ± 4.24^{a}	66.66
AnStw	200	69.83 ± 10.06^{b}	155.67 ± 7.37^{b}	46.00 ± 7.7^{a}	/	100

	400	69.17±9.49[b]	111.00±11.40[b]	49.50±9.64[a]	/	100
	800	89.00±19.75[ab]	186±81.34[b]	48.17±5.26[a]	183	83.33
AnSl	200	62.50±3.01[b]	98.50±7.32[b]	80.20±16.75[ab]	/	100
	400	61.50±3.01[b]	126.00±1.82[b]	55.00±8.57[a]	/	100
	800	102.50±12.19[ab]	209.00±4.96[b]	106.20±4.43[ab]	/	100
DeA	500	63.33±3.67[b]	133.40±5.85[b]	65.40±5.03[a]	/	100
	700	67.00±8.73[b]	64.00±18.60[ab]	59.40±26.86[a]	/	100
	1000	72.57±9.48[b]	83.60±18.87[ab]	72.60±9.15[ab]	503	83.3
FiTsb	200	74.25±4.34[b]	81.00±9.89[b]	146.00±13.92[ab]	/	100
	400	83.25±15.88[b]	132.25±63.20[b]	48.75±8.53[a]	/	100
	800	64.29±20.62[b]	165.33±15.34[b]	97.00±19.59[ab]	207.50±57.27[a]	66.66
FiTl	200	104.00±40.75[ab]	257.00±74.57[ab]	120.00±7.87[ab]	302.50±57.27	66.66
	400	98.00±25.80[ab]	286.00±8.18[ab]	47.25±9.25[a]	/	100
	800	84.83±19.40[b]	298.05±4.14[ab]	84.75±18.99[ab]	/	100
ViDsb	200	76.50±11.84[b]	121.75±39.30[b]	64.45±0.50[a]	328.00±15.68	33.33
	400	92.17±33.30[ab]	73.00±8.60[ab]	96.20±20.31[a]	343.25±56.64	33.33
	800	129.17±10.76[ab]	77.50±10.40[ab]	89.50±9.53[a]	395.75±85.45	33.33
PHB	10	58.29±1.11[b]	1468.00±10.08[b]	64.43±0.78[a]	560.29±3.81[a]	0
	20	67.00±3.57[b]	/	57.83±0.753[a]	1474.00±1.41[a]	66.66
	40	194.86±2.67[a]	/	43.00±2.16[a]	/	100
PTZ	80	49.00±2.29[b]	176.25±28.22[b]	127.5±2.12	378.44±27.68	33.33

Experimental data obtained are presented as means±SEM. /: animals were protected from the observed phenomenom; AlCl: Al.cordifolia leave, AnMs: A. muricata seed, AnMtw: A. muricata twig, AnSl: A. senegalensis leave, AnStw: A. senegalensis twig, DeA: D. Adescendes, FiTsb: F.thoningii stembark, FiTl: F.thoningii leave, ViDsb: V. doniana stembark, PHB: phenobarbital, PTZ: pentylenetetrazol; [a]: significant compared to PTZ; b: significant compared to PHB

Table VII presents the parameters observed after administration of plant extracts to convulsant, PTZ to mice that received 80 mg/kg of the chemical.

A. cordifolia leaves at doses tested did not significantly modify the time to seizure onset, nor the onset of tonicoclonic (TC) convulsions; but however the duration of convulsions was significantly reduced at all doses. All animals were protected from death at highest tested dose (1000 mg/kg), while 50% of mice were protected from death at 700 mg/kg.

Concerning *A. muricata*, seeds and twigs were tested at the same doses. Among these extracts, only twigs at the smallest doses tested (200 and 400 mg/kg) were able to delay seizures induced by PTZ in a significant manner, while the onset of TC seizures was significantly delayed at the highest dose tested for seeds (800 mg/kg) and the smallest for twigs (200 mg/kg). All doses of extracts these organs significantly reduced seizure duration and an entire protection was observed for the twigs and seeds at 200 mg/kg.

Twigs and leaves of *A. senegalensis* were also evaluated at doses of 200, 400 and 800 mg/kg. For these organs only highest doses significantly delayed seizure onset while the seizure duration was significantly reduced at all doses. None of these extracts significantly delayed the time of onset of TC convulsions compared to the negative control; but they were effective against the time of death. These extracts significantly delayed the onset of seizure as PHB at 10 and 20 mg/kg.

For *D. adescendens*, the entire plant was evaluated. The doses tested (500, 700 and 1000 mg/kg), significantly protected mice from death, but were ineffective in reducing the time to seizure onset nor the onset of the TC convulsions.

Leaves and stembarks of *F. thonningii* extracts were also evaluated and only leaves at the smallest dose tested (200 mg/kg), significantly delayed the seizure onset and the onset of TC convulsions.

Barks of *V. doniana* significantly delayed the time to seizure onset at all doses tested. Convulsion duration was significantly reduced compared to the negative control. Besides, the activity of this extract at 200 mg/kg was similar to that of PHB at 10 mg/kg. All tested doses exhibited a similar percentage of protection (33.33%) smaller than that of the positive control; PHB with values of 66 and100% at 20 and 40mg/kg respectively.

All the extracts tested significantly reduced the convulsion duration compared to the negative control. FiTl and FiTsb (800 mg/kg, 200 mg/kg), DeA at 1000 mg/kg, AnSl (800 mg/kg, 200 mg/kg) and AlCl (700 mg/kg, 1000 mg/kg), presented values far greater than

those of positive control PHB. Among the plants tested AnM presented the best results in delaying seizures and was therefore selected for further studies.

III.1.2.2 Study on *Annona Muricata*

III.1.2.2.1 Yield of the extracts

Table VII: Yield of different plant part extracts

Plant part	**AnMr**	**AnMfl**	**AnMs**	**AnMl**	**AnMpf**	**AnMtw**	**AnMp**	**AnMsb**
Yield (%)	9.76	2.8	2.25	14.5	2.6	6.85	8.04	7.6

AnMr: *Annona muricata* roots; AnMfl: *Annona muricata* flower; AnMs: *Annona muricata* seed; AnMl: *Annona muricata* leaves; AnM pf: *Annona muricata* pulp of fruit; AnMtw: *Annona muricata* twigs; AnMp: *Annona muricata* pericarp; AnMsb: *Annona muricata* stem bark

The analysis of table VIII revealed variations in yield ranging between 14.5 and 2.25%. The highest yield was obtained from leaves and the lowest from the seeds.

III.1.2.2.2 Anticonvulsant activity of extracts of *A.muricata* after PTZ induced seizures

Table IX: Effect of AnMl against PTZ induced seizures

Treatment	**Dose (mg/Kg)**	**Onset seizure(s)**	**Convulsion duration(s)**	**Total protein(g/l)**	**Albumin(g/l)**
PTZ	80	49.00±2.29	127.5±2.12	55.70±3.27	28.47±2.39
PHB	40	423.6±1.32***	22.75±2.12***	66.93±2.97**	35.05±1.93***
DZP	1	382.5±0.92***	17.50±0.83***	69.25±1.02***	37.13±4.39***
AnMl	400	201.1±2.29***	31.86±1.20***	64.79±2.87***	37.77±1.45***
AnMl	200	109.4±4.68***	35.86±1.20***	57.40±2.87*	34.51±1.01
AnMl	100	73.00±1.60***	104.4±1.12***	57.29±2.37*	35.14±2.52

PTZ: pentylenetetrazole, PHB: phenobarbital, DZP:diazepam, AnMl: AnM leave, ***: $p<0.001$, **: $p<0.01$, *: $p<0.5$

Table IX presents the effects of *A.muricata* leave extract (AnMl) after seizures induced by PTZ. The extract at all doses tested significantly delayed the seizure onset with the best activity observed at the dose of 400 mg/kg ($p<0.001$). In this model the reference drugs

used also delayed the onset of seizure, with Phenobarbital (PHB) being the compound with the better activity compared to Diazepam (DZP). Convulsion duration was significantly reduced in all groups compared to the negative control group treated with PTZ ($p<0.001$). The amount of protein in the negative control group was below the normal value, but after treatment with PHB it significantly increased to normal value ($p<0.01$). DZP (1mg/kg) and AnMl (400 mg/ kg), were also able to significantly increase the amount of protein ($p<0.001$). The level of albumin was significantly increased by PHB, DZP and leaves at 400 mg/kg $p<0.001$).

Table VII: Effect of AnMr against PTZ induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTZ	80	49.00±2.29	127.5±2.12	55.70±3.27	28.47±2.39
PHB	40	423.6±1.32***	22.75±2.12***	66.93±2.97**	35.05±1.93***
DZP	1	382.5±0.92***	17.50±0.83***	69.25±1.02***	37.13±4.39***
AnMr	400	187.67±1.96***	57.33±2.65***	64.32±2.67*	35.84±3.23***
AnMr	200	97.50±2.54***	55.33±5.78***	67.69±2.49*	38.61±0.82
AnMr	100	83.17±1.35***	64.17±3.43***	50.60±2.25	32.10±2.55**

PTZ: pentylenetetrazole, PHB: phenobarbital, DZP:diazepam, AnMr: AnM root, ***: $p<0.001$, **: $p<0.01$

Table X presents the effects of AnMr after PTZ induced convulsion in mice. At all doses tested the extract was able to significantly increase the time to seizure onset ($p<0.001$). This increase was dose dependent with the highest protective activity observed at the highest dose tested. Convulsion duration was significantly reduced at all doses tested when compared to the negative control group ($p<0.001$). There was no significant difference between the extracts at 400 mg/kg and 200 mg/kg ($p>0.05$). However, this difference was significant at $P<0.05$ for 400 mg/kg and 100 mg/kg while between 200 mg/kg and 100 mg/kg, the difference observed was also significant at $p<0.01$. No significant difference was observed between the groups treated with DZP and AnMr at 200 and 100 mg/kg but at 400 mg/ kg the difference was significative ($p<0.01$). PTZ treatment reduced the normal value of proteins, but after treatment with PHB or DZP it significantly rose to normal values ($p<0.01$ and $p<0.001$ respectively). Extract at 400 and 200mg/kg was also able to significantly increase the

protein levels ($p<0.05$). Albumin values were significantly increased by the reference drugs ($p<0.001$) as well as extracts. The extracts were able to increase the albumin level, with the best activity obtained at 200 mg /kg ($p<0.001$).

Table VIIII: Effect of AnMst against PTZ induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTZ	80	49.00±2.29	127.5±2.12	55.70±3.27	28.47±2.39
PHB	40	423.6±1.32***	22.75±2.12***	66.93±2.97***	35.05±1.93***
DZP	1	382.5±0.92***	17.50±0.83***	69.25±1.02***	37.13±4.39***
AnMst	400	65.83±1.24***	51.00±1.26***	64.17±3.02***	36.25±4.19***
AnMst	200	77.83±0.98***	33.17±1.94***	66.45±2.62***	35.33±5.22***
AnMst	100	64.33±1.20***	36.00±2.28***	57.05±1.81*	35.99±4.83***

PTZ: pentylenetetrazole, PHB: phenobarbital, DZP :diazepam, AnMst: AnM stembark, ***: $p<0.001$, *: $p<0.5$

Table XI, shows different parameters observed after treatment of mice with AnMst. The extract significantly delayed the time to seizure onset and this at all the doses tested ($p<0.001$). This activity was not dose dependent with the highest activity observed at 200 mg/kg. Convulsion duration was significantly reduced at all doses tested when compared to the negative control group ($p<0.001$). There were no differences between AnMst at 200 mg/kg and 100 mg/ kg ($p>0.05$). No significant difference in activity was observed between PHB and the extract at 100 mg/kg ($p>0.05$), but a significant difference in activity was observed at 200 mg/kg ($p<0.01$). Concerning the level of proteins, AnMst was able to significantly increase the level, $p<0.001$ for doses 400 and 200 mg/kg and $p<0.01$ for AnMst at 100 mg/kg. At 200 mg/kg the effect shown by the extract was similar that of PHB. As concerns the albumin levels, they were significantly increased ($p<0.001$) at all doses of extracts. There were no significant differences between the extract at 400 mg/kg and PHB or DZP ($p<0.05$).

Table IX: Effect of AnMpf against PTZ induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTZ	80	49.00±2.29	127.5±2.12	55.70±3.27	28.47±2.39
PHB	40	423.6±1.32***	22.75±2.12***	66.93±2.97***	35.05±1.93***
DZP	1	382.5±0.92***	17.50±0.83***	69.25±1.02***	37.13±4.39***
AnMpf	400	72.00±3.60***	76.67±3.33***	64.55±3.35***	28.59±2.81
AnMpf	200	59.17±1.19*	62.00±1.78***	62.01±1.60***	24.85±1.75
AnMpf	100	56.50±2.06	77.0±2.06***	55.60±3.70	22.28±2.29

PTZ: pentylenetetrazole, PHB: phenobarbital, DZP:diazepam, AnMpf: AnM pulp of fruit, ***: $p<0.001$, *: $p<0.5$

Table XII shows the effect of AnMpf after PTZ induced seizures in mice. The time to seizure onset was significantly increased by the extract at 400 mg/kg ($p<0.001$) and 200 mg/kg ($p<0.05$). At 100 mg/kg, the delay observed was not significant when compared to the negative control group. The seizure onset was not significantly different at 200 mg/kg and 100 mg/kg ($p>0.05$), while the difference in time to seizure onset observed between the extract at 400 mg/kg and 200 mg/kg was significant at $p<0.01$. The convulsion duration was significantly reduced at the doses tested ($p<0.001$) when comparing with PTZ group, but there were no significant difference between the groups treated with AnMpf at 400 and 100 mg/kg ($p>0.05$). The amount of protein was significantly increased by the extract except at 100 mg/kg ($p<0.001$); while at 400 mg/kg the difference observed was not significantly different ($p>0.05$) from PHB. As concerns albumin levels, only the reference drugs were able to increase the levels of albumin significantly ($p<0.001$) when compared to the negative control group.

III.1.2.2.3 Anticonvulsant activity of extracts of *A.muricata* after PTX induced seizures

Table X: Effect of AnMl against PTX induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP	1	627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMl	400	564.7±1.20***	50.17±0.44***	65.62±0.43***	32.40±0.13
AnMl	200	426.3±1.40***	54.67±2.33***	66.67±0.20***	31.66±0.55
AnMl	100	412.2±0.87***	39.0±0.89***	64.89±0.61***	31.54±0.50

PTX: picrotoxin, PHB: phenobarbital, DZP:diazepam, AnMl: AnM leave, ***: $p<0.001$, *: $p<0.5$

Table XIII shows the effect of AnMl after PTX induced seizures in mice. The time to seizure onset was significantly increased by the extract at all doses tested ($p<0.001$). The convulsion duration was significantly reduced by all extracts and products tested ($p<0.001$) when compared to the negative control group. As concerns the protein levels, all the compounds tested were able to rise up the amount of protein in comparison to the negative group ($p<0.01$). Albumin level was significantly increased by PHB, ($p<0.05$); there were no difference among the extracts nor between the extracts and DZP ($p<0.05$), but on comparison between extracts and PHB at significativity was observed at $p<0.01$.

Table XIV: Effect of AnMr against PTX induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP	1	627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMr	400	526.5±3.25***	50.33±0.84***	68.13±0.30***	33.27±0.20
AnMr	200	517.5±0.76***	59.83±1.01***	68.84±0.27***	33.10±0.55
AnMr	100	439.5±0.92***	51.00±1.39***	65.56±0.14***	32.48±0.21

PTX: picrotoxin, PHB: phenobarbital, DZP :diazepam, AnMr: AnM root, ***: p<0.001, *: p<0.5

Table XIV shows the effects of AnMr after PTX induced seizures in mice. The time to seizure onset was significantly increased by the extract at the doses tested (p<0.001). The seizure onset by AnMr at 400 mg /kg and 200 mg /kg was not significantly different from PHB (p>0.5). Convulsion duration was significantly reduced by the extracts (p<0.001), while the protein level was significantly increased (p<0.001). The level of albumin was significantly increased by PHB (p<0.05).

Table XI: Effect of AnMst against PTX induced seizures

Treatment	**Dose (mg/Kg)**	**Onset seizure(s)**	**Convulsion duration(s)**	**Total protein(g/l)**	**Albumin(g/l)**
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP	1	627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMst	400	523.3±1.60***	38.50±1.89***	66.50±0.17***	35.37±0.28
AnMst	200	440.7±3.28***	42.50±1.52***	58.21±0.18***	32.37±0.45
AnMst	100	403.0±1.36***	52.83±1.35***	57.92±0.72***	31.52±0.87

PTX: picrotoxin, PHB: phenobarbital, DZP: diazepam, AnMst: AnM stembark, ***: p<0.001, *: p<0.5

The study of the effect of AnMst after PTX induced seizure in mice showed that the extract significantly increase the time to seizure onset (p<0.001) (table XV). Convulsion durationwas significantly reduced by the extracts tested (p<0.001), but on comparison among the extracts, there was no significant difference between AnMst at 400 mg /kg and 200 mg/kg (p>0.05). The amount of protein was significantly increased by the extract tested (p<0.001). The albumin level was not significantly increased by the extract (p<0.05).

Table XIII: Effect of AnMpf against PTX induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP		627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMpf	400	480.0±1.34***	74.00±0.51	64.91±1.39***	35.85±0.82
AnMpf	200	474.3±1.08***	69.33±1.54*	64.07±0.63***	33.03±0.87
AnMpf	100	432.2±0.79***	63.50±1.54***	57.93±0.66***	23.70±0.80

PTX: picrotoxin, PHB: phenobarbital, DZP: diazepam, AnMpf: AnM pulp of fruit,***: p<0.001, *: p<0.5

The different doses of the extract tested were able to significantly increase the time to seizure onset (p<0.001) (table XVI). Convulsion duration was significantly reduced by the extract at 200 mg/kg and 100 mg/kg (p<0.01 and p<0.001 respectively). The protein level was significantly increased by theextract at the doses tested (p<0.001), whereas the level of albumin was not significantly increased by the extract (p<0.05).

Table XIII: Effect of AnMs against PTX induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP		627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMs	400	476.3±1.40***	51.33±0.61***	63.39±0.63***	36.62±1.13*
AnMs	200	451.7±1.17***	78.67±0.61	68.49±1.30***	36.48±0.72*
AnMs	100	424.3±0.80***	76.33±0.74	45.18±1.22***	27.05±0.93

PTX: picrotoxin, PHB: phenobarbital, DZP:diazepam, AnMs: AnM seed, ***: p<0.001, *: p<0.5

The extract at all thedoses tested was able to significantly increase the time to seizure onset at p<0.001 (table XVII). Convulsion duration was significantly reduced by PHB,

DZPdrugs and AnMs at 400 mg/kg ($p<0.001$). As concerns protein level, it was significantly increase by the extract ($p<0.001$). The level of albumin was significantly increased by theextract at 400 mg/kg and 200 mg/kg ($p<0.05$).

Table XIV: Effect of AnMtw against PTX induced seizures

Treatment	Dose (mg/Kg)	Onset seizure(s)	Convulsion duration(s)	Total protein(g/l)	Albumin(g/l)
PTX	8	373.7±3.72	73.50±1.05	45.80±0.35	28.86±0.32
PHB	40	523.4±2.76***	15.57±0.42***	64.0±1.59***	36.04±0.99*
DZP		627.2±1.77***	4.83±0.30***	62.92±1.99***	32.14±0.57
AnMtw	400	479.2±0.60***	54.67±1.40***	64.67±1.24***	36.51±0.68*
AnMtw	200	473.3±1.24***	56.83±1.18***	57.98±0.73***	36.12±0.88*
AnMtw	100	424.3±0.80***	72.00±0.77	59.22±1.45	25.91±0.70

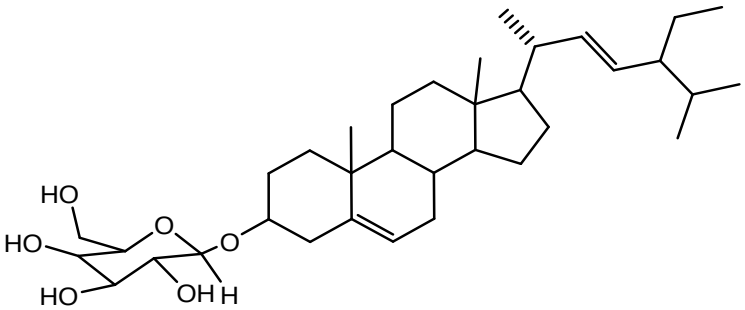
PTX: picrotoxin, PHB: phenobarbital, DZP:diazepam, AnMtw: AnM twig, ***: $p<0.001$, *: $p<0.5$

Table XVIII shows the time to seizure onset was significantly increased by the different doses of theextract tested ($p<0.001$).The convulsion duration was also significantly reduced by the extract ($p<0.001$), except the extract at 100 mg/kg.Protein level, was significantly increased by the extract except AnMtw at the lowest dose tested ($p<0.001$). The level of albumin was significantly increased by AnMtw at 400 mg/kg and 200 mg/kg ($p<0.05$).

At the end of this study, it was observed that among the different organs of the plant, AnMr was able to increase the time to seizure onset, reduce convulsion duration at a smaller dose than the other organs and was therefore submitted to fractionation.

Five fractions, namely J16, J17, J18, J19, J20 and a product P106 were obtained

The compound P106 presented as a form of whitish powder, was identified as 2-[17-(4-éthyl-1,5-diméthyl-hex-2-enyl)-10,13-diméthyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[α]phenanthren-3-yloxyl]-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol, mostly known as 3-O-β-D-glucopyranoside de stigmastérol which possesses the following structure as displayed in figure 4



2-[17-(4-Ethyl-1,5-dimethyl-hex-2-enyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy]-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol

Figure 4: Structure of AnMrP106: 3-O-β-D-glucopyranoside of stigmasterol

III.1.2.2.4 Anticonvulsant activity of fractions and product of *A.muricata* after PTZ induced seizures

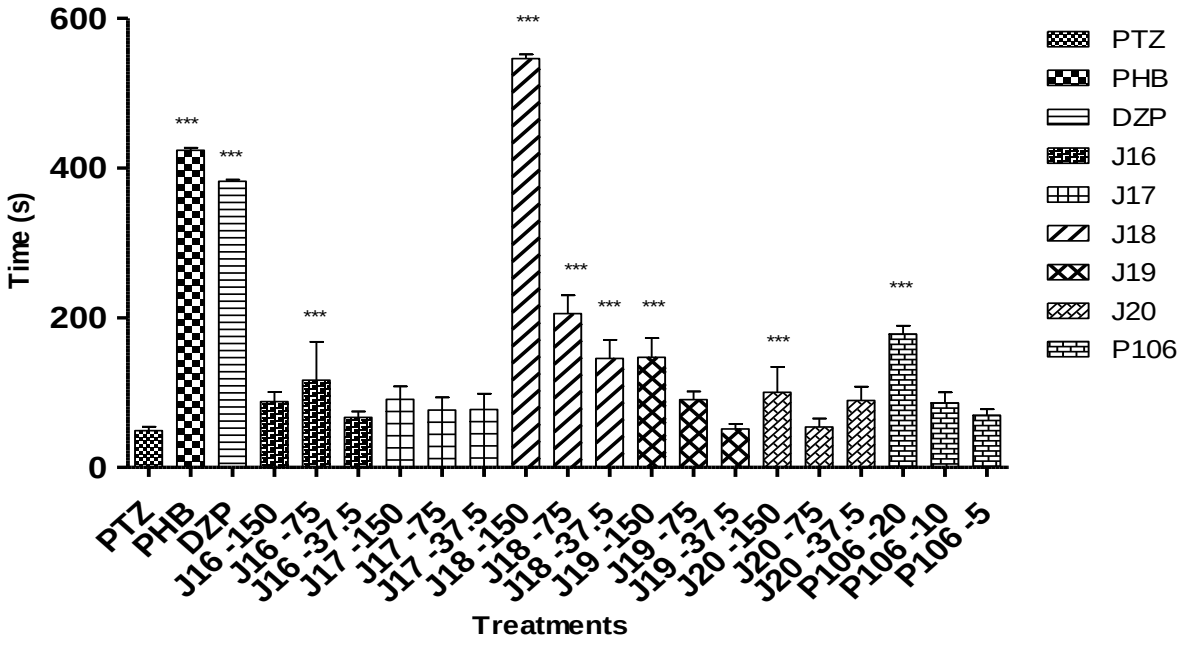


Figure 5: Effect of fractions and compound on time to onset after PTZ –induced seizures

Figure 5 presents the effects of fractions on the time to seizure after PTZ-induced seizures. Among the fractions and compound tested, J16 (75 mg/kg), J18 at all doses tested, J19 (150 mg/kg) and P106 (20 mg/kg) were able to significantly delay the onset of seizures comparedto negative control group ($p<0.001$). J20fraction also significantly delayed the onset

of seizures ($p<0.01$). J18 at 150 mg/kg presented the best protective activity, and the time to seizure onset was even longer than for the reference drug. Except for J18 fraction, none of the compounds protected more than the reference compound, and the difference between the compounds and the reference drugs was significant ($p<0.01$).

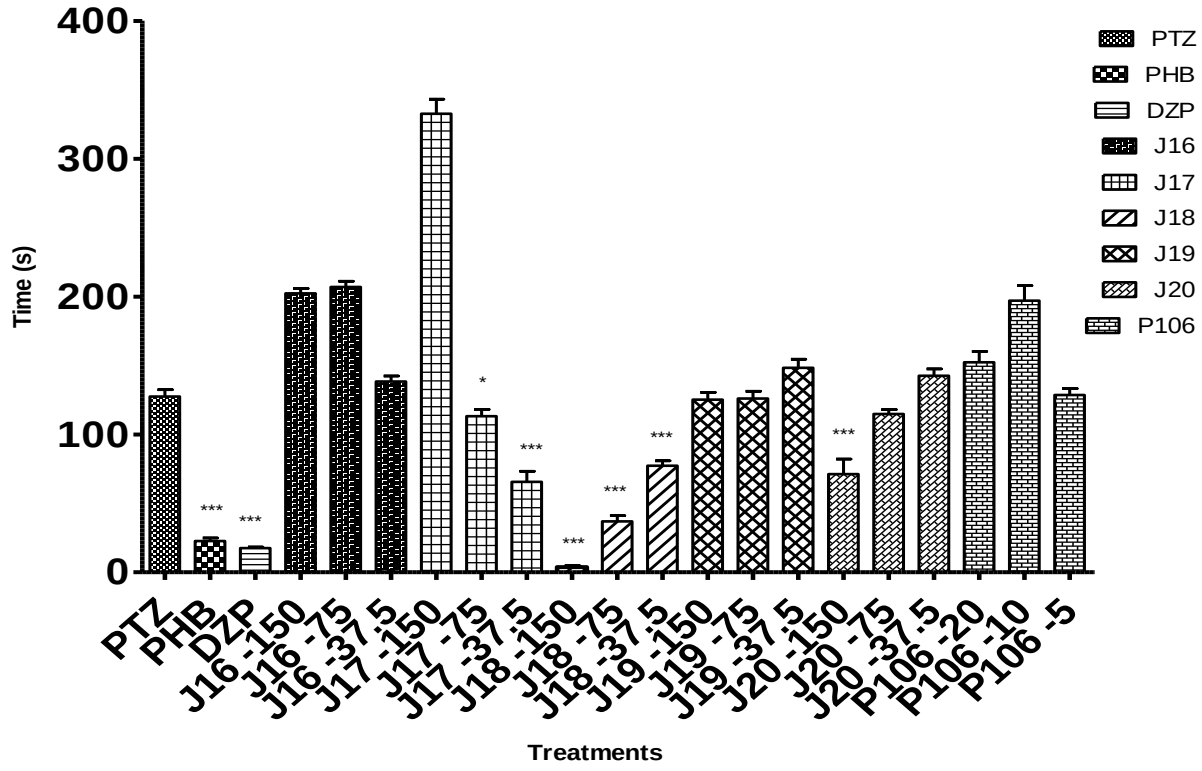


Figure 6: Effect of fractions and compound on convulsion duration after PTZ-induced seizures

Figure 6 shows the effects of fractions and compounds on convulsion duration after PTZ-induced seizures. Among the compounds tested, J17 (150 mg/kg and 37.5 mg/kg), J18 at all doses tested, J20 at 150 mg/kg were able to significantly reducethe duration of convulsion ($p<0.001$), while J17 at 75 mg/kg significantly increased time to seizure onset ($p<0.05$). The fraction J18 at the highest dose was able to reduce convulsion duration even more effectively than the reference compounds ($p<0.001$).

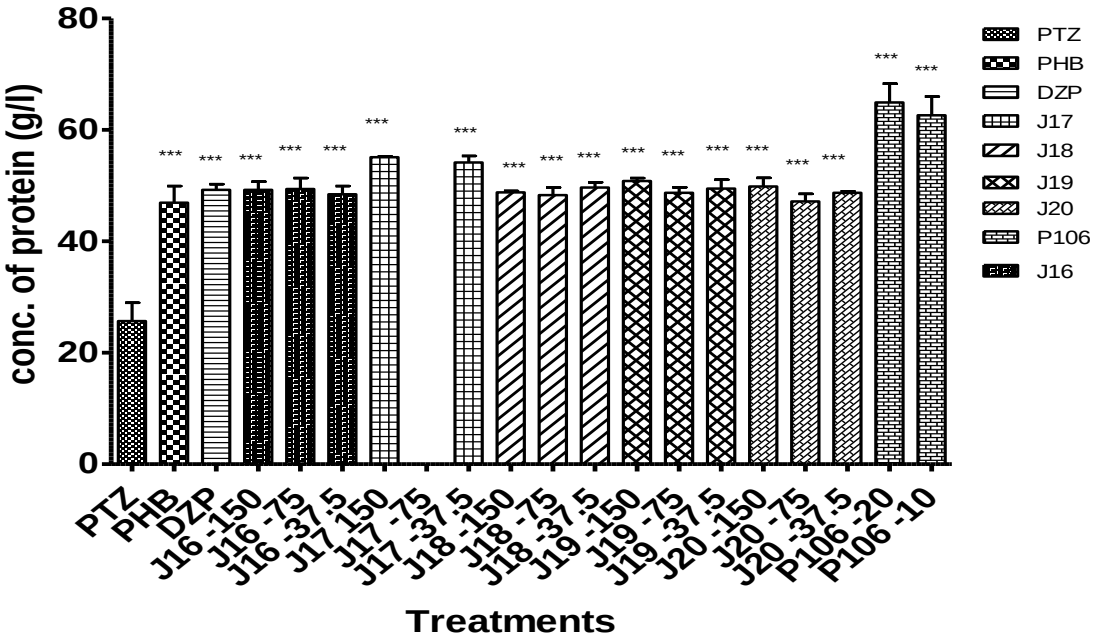


Figure 7: Effect of fraction and compound on protein levels after PTZ induced seizures

Figure 7 presents the effect of treatments on protein levelsafter seizure. Here, it can be seen that at all doses tested, the different compounds were able to increase the amount of proteins significantly ($p<0.001$). Except for the product P106 at the highest doses tested (20 and 10 mg/kg), the concentrations of proteins were higher than for the references drugs. The protein concentration of the fractions were not significantly different from the reference drugs ($p>0.05$).

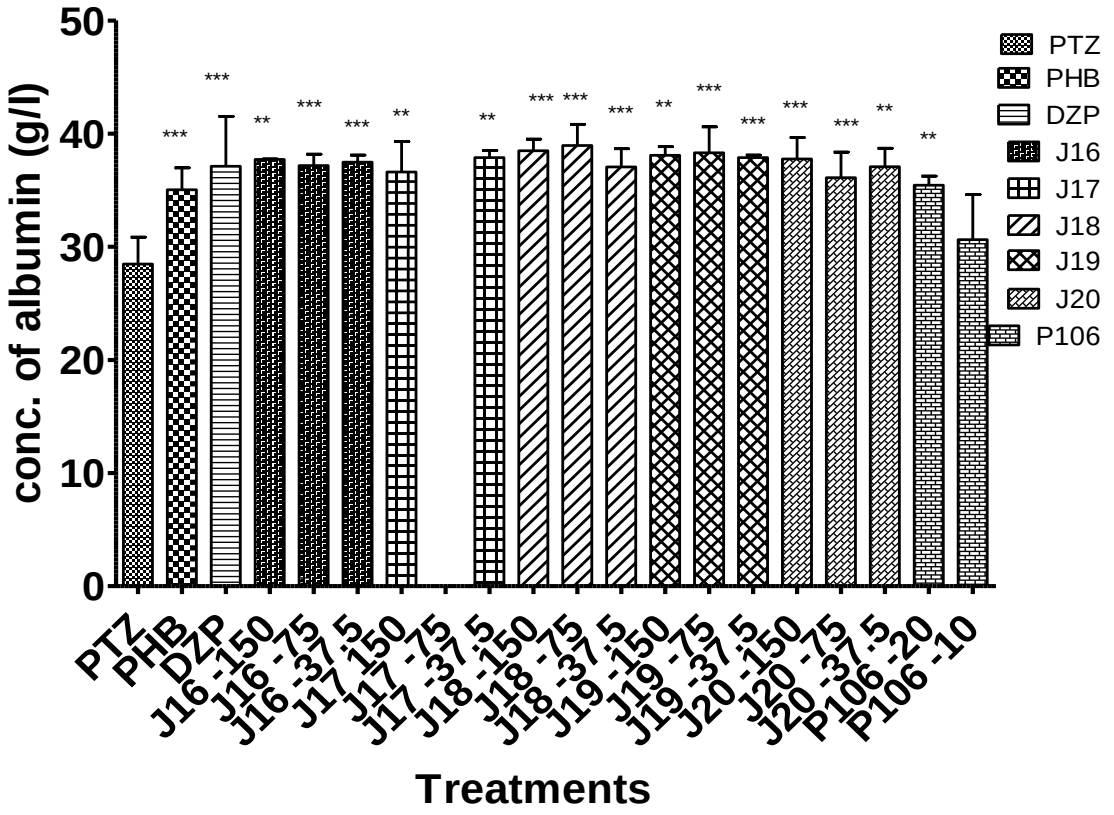


Figure 8: Effect of fractions and compound on albumin after PTZ-induced seizures

Figure 8 presents the effect of treatment on albumin levels. Practically all fraction and compound tested were able to significantly increase the level of albumin ($p<0.01$ and $p<0.001$), but there were no significant differences for P106 at 10 mg/kg.

III.1.2.2.5 Anticonvulsant activity of fractions and product of *A.muricata* after PTX induced seizures

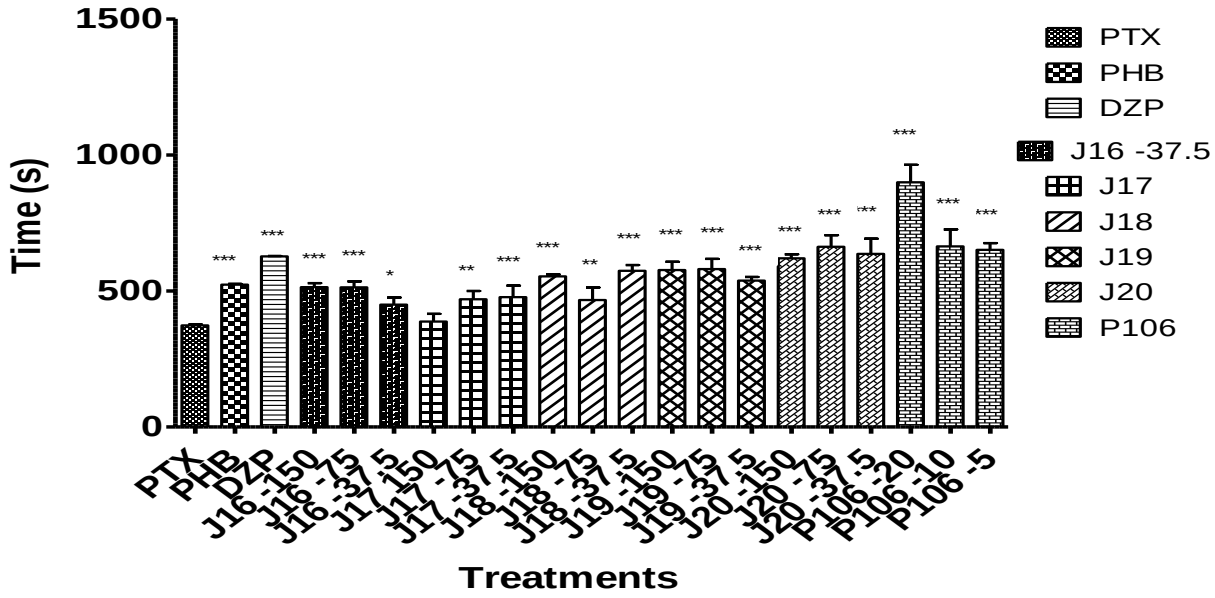


Figure 9: Effect of fractions and compound on time to onset after PTX-induced seizures

Figure 9 shows the effect of fractions and compound tested on time to seizure onset. Exception made by J17 at 150 mg/kg were no difference was observed ($p>0.05$), all the fractions and compound at all doses tested were able to significantly increase the time to seizure onset ($p<0.001$). The highest activity was obtained by P106 at 20 mg /kg ($p<0.001$) and the lowest by J16 at 37.5mg/kg ($p<0.05$).

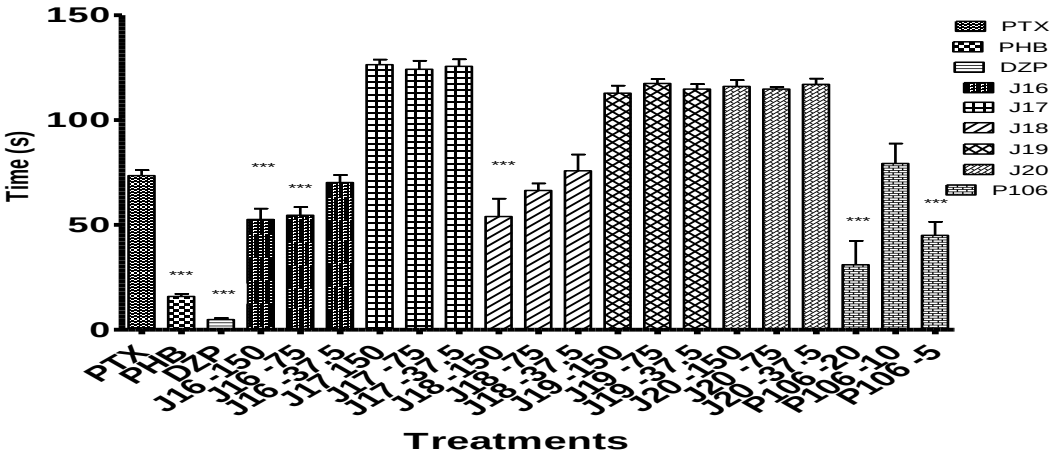


Figure 10: Effect of fractions and compound on seizure duration after PTX-induced seizures

Figure 10 shows the effect of fractions and compound tested on convulsion duration. Among the fractions and compounds tested only J16 at 150 and 75 ($p<0.001$) mg/kg, J18 at the highest concentration tested ($p<0.001$), and P106 at 20 and 5 mg/kg ($p<0.001$) significantly reduced the convulsion duration.

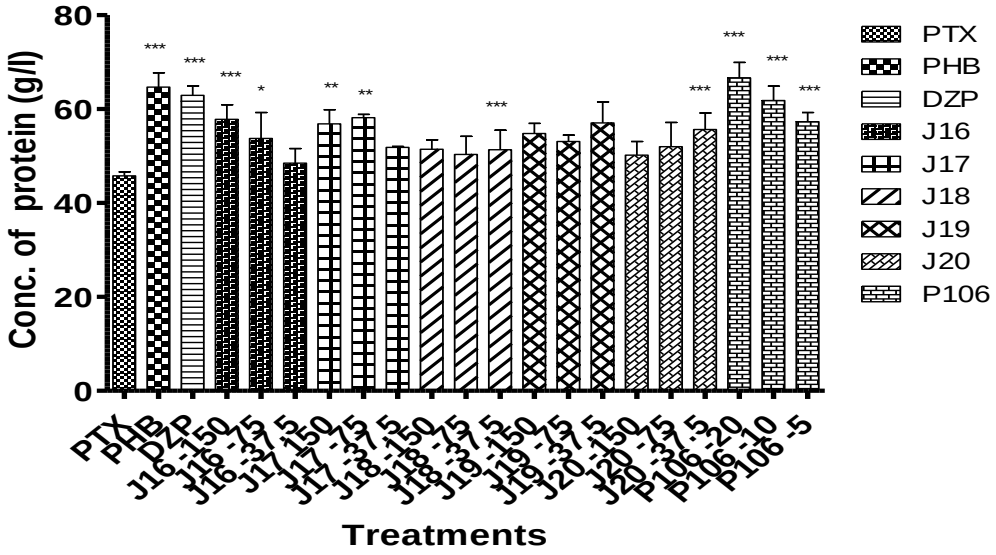


Figure 11: Effect of fractions and compound on protein levels after PTX-induced seizure

This figure 11 presents the effect of treatment on protein. Some fractions and pure compound were able to increase the concentration of protein after treatment. Particularly, J16 at 150 mg kg, J18 and J20 at 37.5 mg/kg, P106 at all doses tested ($p<0.001$), J17 at 150 and 75 mg/kg ($p<0.01$) and J16 at 75 mg/kg ($p<0.05$).

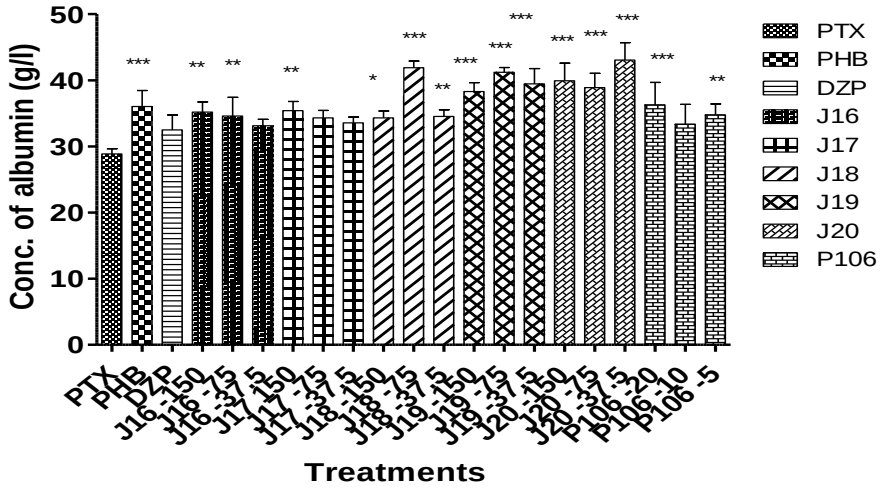


Figure 12: Effect of fractions and compound on albumin after PTX-induced seizure

Figure 12 shows the effect of treatment on albumin after seizures. J16 at 75 mg/kg, J17 at 150 mg/kg, J18 at 37.5 mg/kg and P106 at 5 mg/kg significantly increase the level of albumin ($p<0.01$). J18 at 75 mg/kg, P106 at 20 mg/kg, J19 and J20 at all doses tested, also significantly increase the level of albumin ($p<0.001$). The lowest significant activity was observed for J18 at 150 mg/kg ($p<0.05$).

III.1.2.2.6 Possible mechanism of action of the extract

Table XIX: Effect of flumazenil on anticonvulsant activity of *A.muricata* extracts

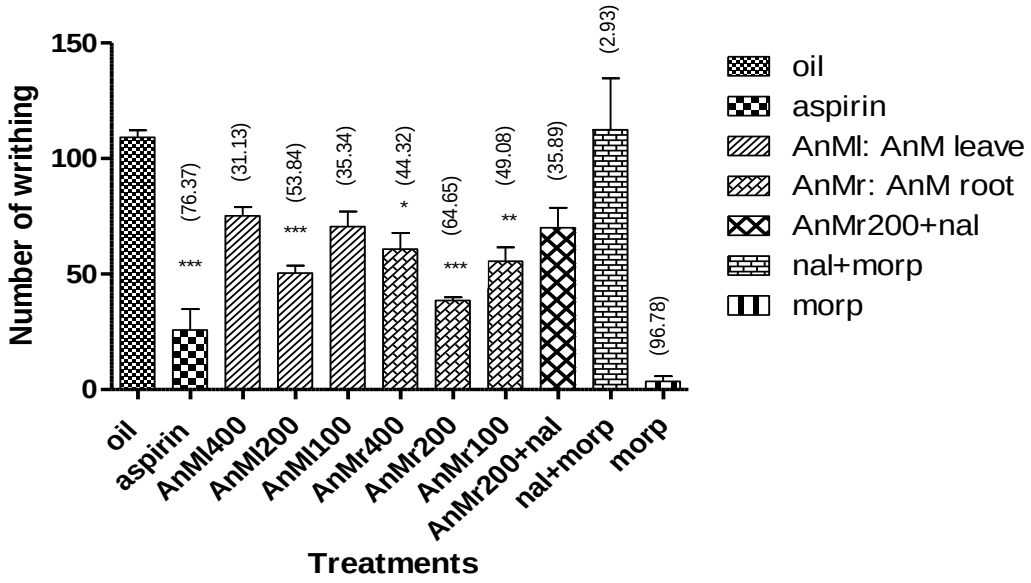
Treatment (dose)	Onset (sec)	Convulsion duration (sec)
Oil (10mg/kg)	49.00 ± 2.29	127.5 ± 2.12
Flu (2mg/kg)	43.33 ± 1.64	112.2 ± 1.28
DZP (1mg/kg)	382.5 ± 0.92***	17.50 ± 0.83***
DZP + Flu	51.67 ±0.49	104.16 ± 0.24
AnMl (400 mg/kg)	201.1 ± 2.29***	31.86 ± 1.20***
AnMr (400 mg/kg)	187.67 ± 1.96***	57.37 ± 2.65***
AnMl + Flu	50.50 ± 0.71	100.67 ± 1.85
AnMr + Flu	53.67 ± 1.06	102.79 ± 2.63

DZP: diazepam, Flu: flumazenil, ***:P<0.001, comparaison to flumazenil,

Table XIX shows the effect of flumazenil on anticonvulsant activity of *A. muricata* leave and root after PTZ-induced convulsion. The time to seizure onset was significantly reduced in the group of mice treated by flumazenil ($p<0.001$) in comparison to those who were not treated with flumazenil. As concerns the convulsion duration, it was significantly increased in the group of mice treated with flumazenil compared to the other groups ($p<0.001$). Flumazenil was also able to reverse the activity of Diazepam.

III.1.3 Antinociceptive activity of *A. muricata* extracts

III.1.3.1 Antinociceptive effect of *A. muricata* in Acetic acid induced pain



(): Percentage of inhibition

Figure 13: Antinociceptive effect of AnM extracts in Acetic acid induced pain

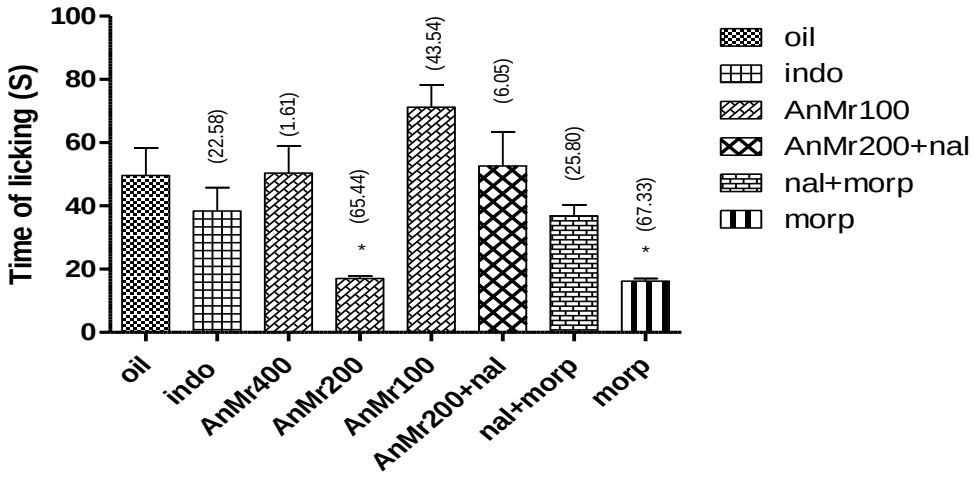
Figure 13 presents the effect of AnM extracts after acetic acid induced writhings in mice. Aspirin the analgesic compound, at 100 mg/kg significantly reduced the writhings ($p<0.001$). Morphine, another analgesic compound, profoundly reduced animal contractions ($p<0.001$). AnM leaves (200 mg/kg), significantly reduced writhings ($p<0.001$); followed by roots at all the doses tested, with the highest activity at 200 mg/kg ($p<0.001$). According to this preliminary test, roots which display the highest activity and morphine, were antagonized using naloxone. When comparing those groups to their respective ones without naloxone, an increase of writhings was observed in the group of mice treated with naloxone.

According to the percentage of inhibition of the compound after A.acid induced pain in mice, the best inhibition is observed in the group treated with morphine, followed by aspirin ($p<0.001$). Among the different extracts, the best inhibition was observed in the group treated with roots and leaves at 200 mg/kg ($p<0.001$).

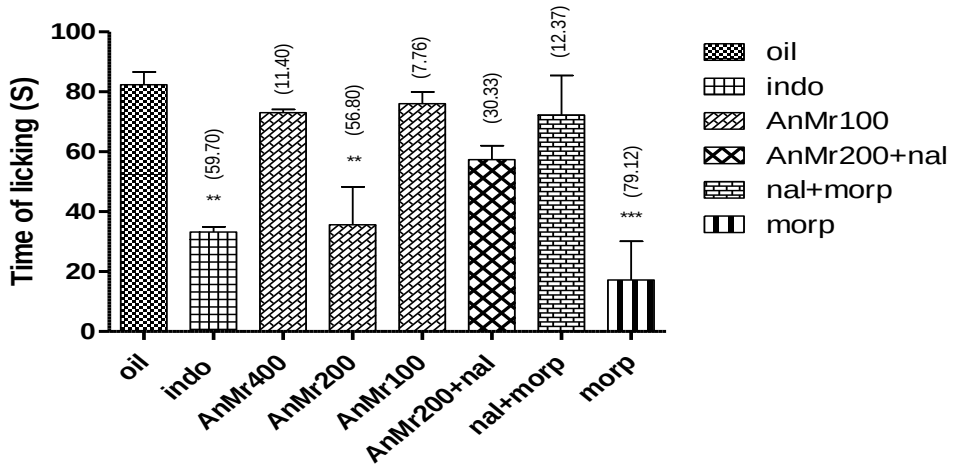
After this test AnMr which exhibited the best activity was used for subsequent other tests.

III.1.3.2 Antinociceptive effect of AnM root extract in formalin induced pain

A)



B)



(): Percentage of inhibition

Figure 14: Effect of AnMr extract in the first (A) and Second (B) phase of formalin induced pain

Figure 14 represents the effect of the root extract of AnM in the first phase (A) and the second phase (B) of formalin induced pain in mice. In the first phase, a significant difference was observed only for AnMr at dose of 200mg/kg ($p<0.5$) and morphine one of the reference drugs ($p<0.5$). At this stage, indomethacin another reference analgesic drug was not able to delay the time of licking or biting of the paw by the animals, even if the time of licking was

reduced compare to the negative control. Trying to elucidate the mechanism by which the extract exerts its action, naloxone an antagonist was used. Naloxone profoundly prevented the antinociceptive effect.

The percentage of inhibition at this stage was significant only for the groups treated with AnMr at 200 mg/kg and morphine ($p<0.05$).

In the second phase, the reference drugs, indomethacine and morphine significantly ($p<0.01$ and $p<0.001$ respectively) reduced the time of licking or biting of the paw by the animals. Among the different doses of extract tested, only the dose of 200 mg/kg significantly reduced the time of licking by the mice ($p<0.05$). The other doses tested, even if the time was reduced, were not significant. Trying to elucidate the mechanism of action of the extract, animals were pre-treated with naloxone and the results obtained showed that naloxone antagonized the effect of the administered drugs since the effect observed for compound when tested alone; disappear after the administration of naloxone.

The percentage of inhibitionis significanty different for morphine ($p<0.001$).This was the case for indomethacine the other reference drug and the root extract at 200 mg/kg ($p<0.05$)

III.1.3.3 Antinociceptive effect of *A.muricata* in hot plate induced pain

Table XX: Antinociceptive effect of AnMr in hot plate test induced pain

Treatment	**Dose**	**Response**	**Time**				
	(mg/kg)	pretreatment	0	30	60	120	180	240
AnMr	400	16.50±1.91 (0)	11.16±3.00 (29.69)	8.12±3.83 (50.78)	10.14±4.64 (38.54)	17.52±3.91 (6.18)	21.04±5.71 (27.51)	14.88±1.88 (9.81)
AnMr	200	8.94±0.96(0)	13.44±1.68 (50.33)	16.08±2.67 (79.86)	9.16±1.69 (2.26)	8.96±1.93 (2.23)	13.88±0.58 (55.25)	19.58±7.67* (119.01)
AnMr	100	13.02±1.61 (0)	17.59±3.24 (35.09)	18.52±8.52 (42.24)	13.70±4.10 (5.22)	23.88±7.2 (83.41)	12.38±2.38 (4.91)	20.42±1.62 (56.83)

Morp	5	11.59±1.92 (0)	5.18±1.07 (55.3)	40.08±5.00** (245.81)	17.32±3.46 (49.43)	8.66±3.28 (25.28)	15.50±8.99 (33.73)	7.48±1.75 (35.46)
Morp+nal	5+1	18.56±2.35 (0)	11.78±4.59 (36.53)	31.68±11.22 (70.68)	49.40±0.60** (166.16)	39.68±3.55 (113.79)	15.30±4.31 (17.56)	13.04±5.36 (29.74)
AnMr200+ nal	200	13.74±2.01 (0)	15.74±4.59 (14.55)	15.48±3.50 (12.66)	17.70±5.31 (28.82)	18.80±1.77 (36.82)	16.68±1.56 (21.39)	19.26±3.58 (40.17)

Table XX shows the effect of AnMr in the hot plate induced pain. Only the extract at 200 mg/kg, was able to counteract the heat significantly ($p<0.05$). Morphine, 30 min after its administration profoundly delay the time of remaining on the plate ($p<0.001$). In the group treated with naloxone, the activity of the extract disappears while the activity of morphine was still present after 1h of administration ($p<0.01$).

The percentage of inhibition varied according to time with the highest percentage of inhibition observed after the first hour after administration of the extract ($p<0.01$).

III.1.4 Antioxidant potential of plant extract

III.1.4.1 DPPH radical scanvenging potential

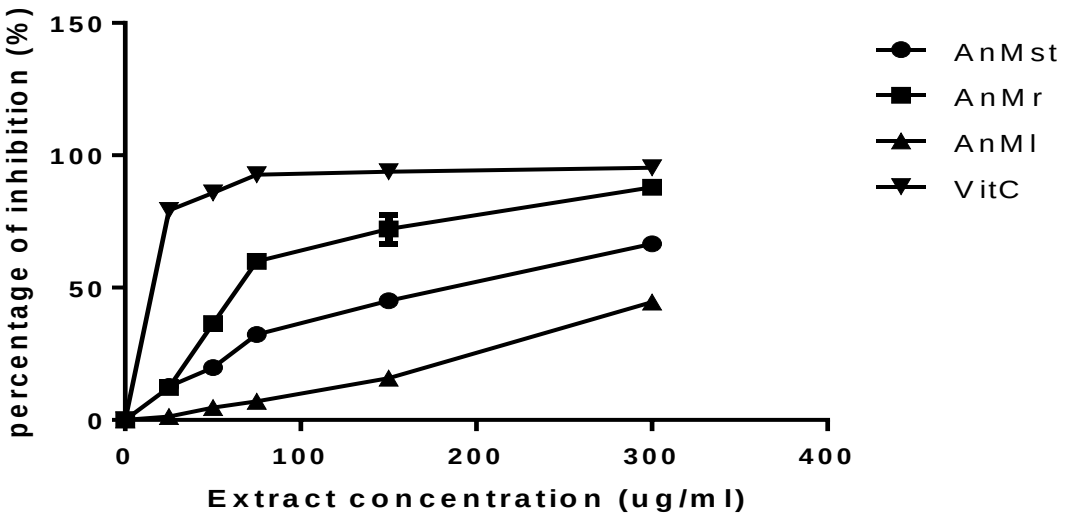


Figure 15: DPPH radical scavenging potential of plant extracts

Figure 15 presents the DPPH radical scavenging potential of the leaves, roots and stembark extracts of AnM. At the lowest concentration (25 µg/ml), the activity of the extracts

was not different, but as the concentration increases, an increase in percentage of inhibition was observed, with the highest inhibition being for AnMr (87.89%) at 300 µg/ml, followed by AnMst (66.55%). AnMl presented an activity which increased as concentration increased but this activity was lower compared to that of AnMst and AnMr extracts. Vitamin C, the positive control compound used presented the best activity with a percentage of inhibition of 95.26%.

III.1.4.2 Nitric oxide radical scavenging potential

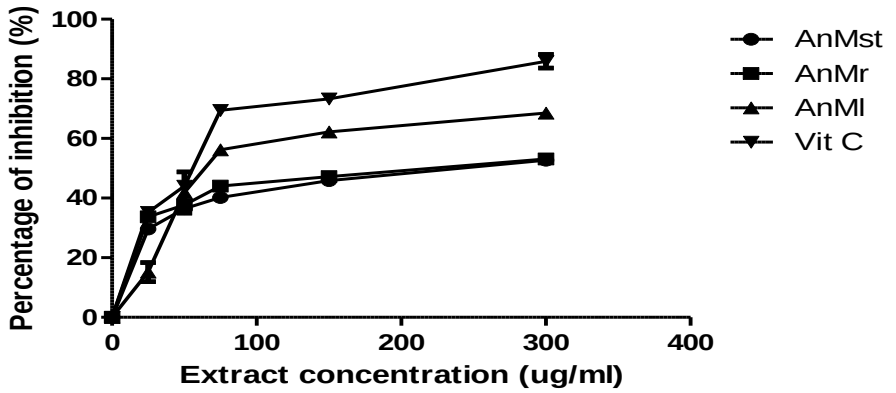


Figure 16: NO radical scavenging potential of plant extract

The NO radical scavenging activity is presented in figure 16. Here, we observed that the activity of each extract was different and this activity increased with the concentration. At 150 µg/ml, there were not significant differences between AnMr and AnMst extracts. The activities of these extracts at 300 µg/ml, were similar (53.10%). The scavenging activity of vitamin C was not too different from that of the extracts at the beginning, but rapidly increased and at the highest concentration tested (300 µg/ml), it reached 85.90%.

III.1.4.3 Hydroxyl radical scavenging activity

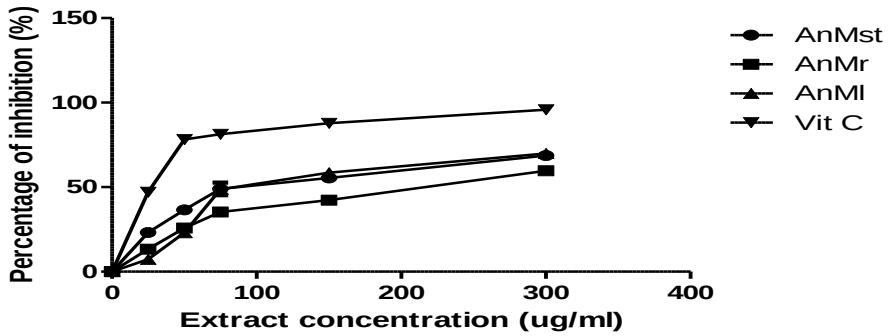


Figure 17: OH activity of plant extract

Figure 17 presents the hydroxyl radical scavenging activity of the plant extracts. Here, at 25 µg/ml the lowest concentration tested, activities of AnMl and AnMr were not too different, but as the concentration increased a difference in activity of plant extracts was observed with the highest activity being from AnMl and AnMst extracts with a scavenging value of 69.96% at 300 µg/ ml. The highest scavenging activity was with vitamin C, the reference compound with 97.78% at a concentration of 300 µg/ml.

III.1.4.4 Antioxidant capacity determined by radical cation (ABTS+)

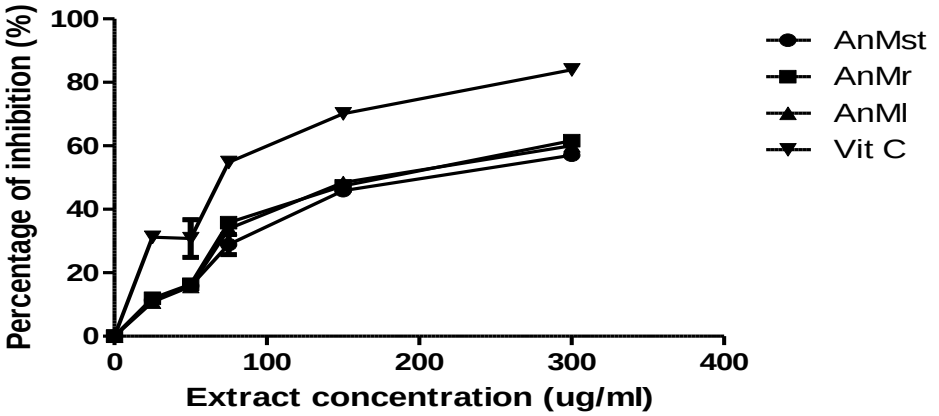


Figure 18: ABTS antioxidant activity of plant extracts

The ABTS scavenging antioxidant activity is presented in figure 18 above. In this figure, at the lowest concentration tested, the difference was not too visible, particularly for the extracts being tested, with the percentage of inhibition being around 10.82% at 25 µg/ml. As the concentrations increased particularly starting at 75 µg/ml, the activity was not clearly different. However at 150 µg/ml we obtained a percentage inhibition of 45.90% for AnMst, 47.38% for AnMr and 48.43% for AnMl. These percentages did not really change till the highest concentration tested (300 µg/ml).

III.1.4.5 Total antioxidant activity by Ferric Reducing Antioxidant Power assay (FRAP)

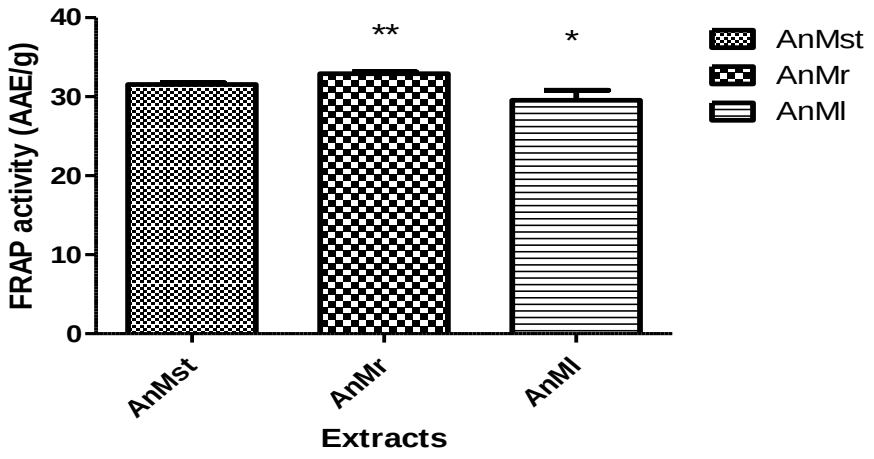


Figure 19: FRAP activity of plant extracts

Figure 19 presents the FRAP activity of extracts. The values obtained vary according to the extract with 31.55 ± 0.132, 32.93 ± 0.119 and 29.54 ±0.73 AAE/g of extract respectively for AnMst, AnMr and AnMl. The highest value was obtained with AnMr, the value being significantly different from that of AnMl ($p<0.01$), but not different from that of AnMst ($p<0.05$). AnMst the second extract according to this activity, was significantly different from AnMl ($p<0.05$).

III.1.4.6 Determination of IC50 of extracts for antiradical activities

Table XXI: Values of IC50 of the extracts of *A.muricata*

	DPPH	**OH**	**NO**	**ABTS**
AnMst	4.105±0.021**	3.325±0.272***	4.623±0.070***	4.500±0.139***
AnMr	2.800±0.084***	4.356±0.126***	4.415±0.166***	4.178±0.018***
AnMl	6.635±0.348	3.339±0.276***	3.065±0.247***	4.238±0.015***
Vit C	6.858±1.001	0.376±0.079	2.112±0.013	2.703±0.126

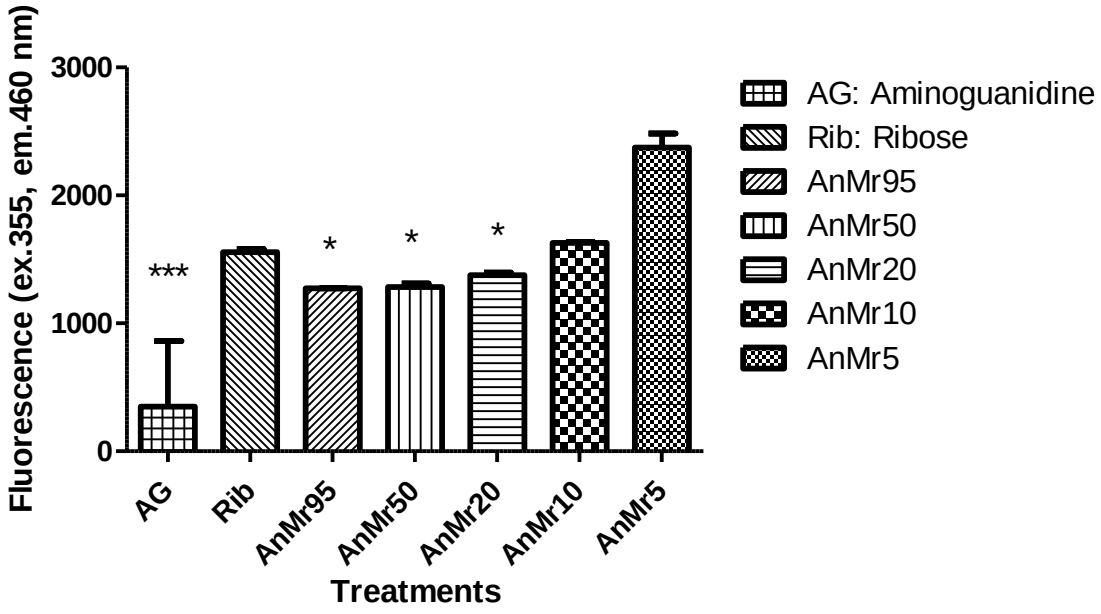
**: $p<0.01$; $p<0.001$

Table XXI presents the IC50 of the different extracts tested. According to this table, in the DPPH test, AnMr is the extract which presents the most effective IC50, followed by AnMst ($p<0.001$). In this test, no significance was observed between the IC50 values of

AnMst and AnMr ($p<0.01$), while a significant difference for AnMst and AnMl was observed ($p<0.01$). In the OH scavenging test the best activity was obtained with vitamin C the reference compound ($p<0.001$). Among the extracts tested, AnMst was the one with the best IC50 followedby AnMl; the values obtained were so close that there was no significant difference ($p>0.05$), while the differences observed between AnMr and AnMl, AnMr and AnMst were significant ($p<0.01$).In the NO scavenging test, AnMl was the extract with good activity, followed by AnMr; the values obtained showed no significant difference, while comparaison among the other extract was significant at ($p<0.001$). In the ABTS test, among extracts tested, AnMr presented a good IC50 value, followed by AnMl, but the difference observed was not significant; while the differences observed between AnMst and AnMl, AnMst and AnMr were significant. In all the tests done, except for the DPPH test where the IC50 of vitamin C was not different from that of the extract tested (AnMl), in the remaining test, it was always the compound with the best IC50 value.

III.1.5 Antiglycation activity of the plant extracts

III.1.5.1 Antiglycation activity of *A. muricata* in BSA incubated with ribose



***: $p<0.001$, *: $p<0.05$

Figure 20: Effect of AnMr extract on the formation of AGE in BSA incubated with ribose

Ribose is one of the sugars used in glycation studies. The formation of AGEs was monitored after 5 days by measuring the fluorescence intensity of the different solutions as

presented on figure 20. This figure presents the effect of AnMr extract on the formation of AGE in BSA incubated with ribose. According to this figure, the different concentrations of the extract except at 10 and 5 µg/ml were able to inhibit the formation of AGE significantly ($p<0.05$). In comparison among the different concentrations of the extract, there was no significant difference between the concentrations of 95, 50 and 20 µg/ml ($p>0.05$) which are the effective concentrations. The highest activity was observed with aminoguanidine, the reference compound ($p<0.001$).

III.1.5.2 Antiglycation activity of *A.muricata* in BSA incubated with glucose

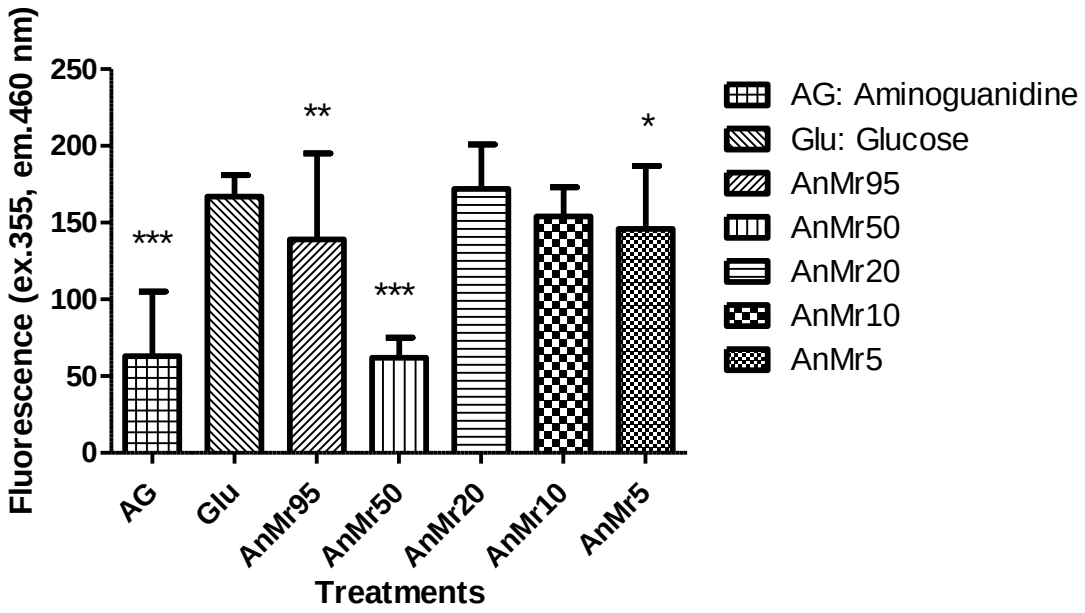
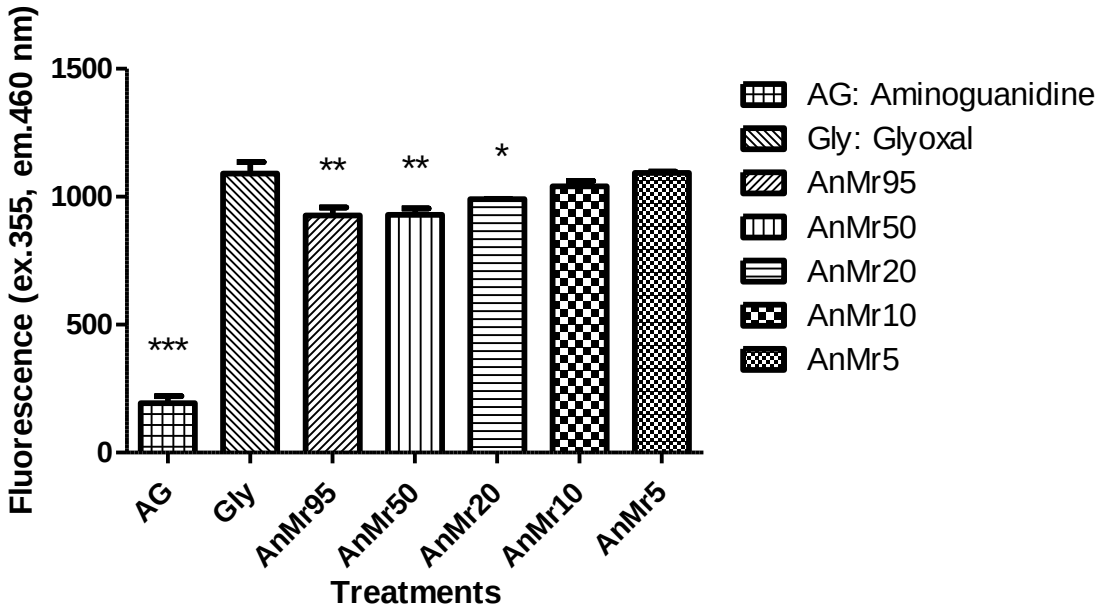


Figure 21: Effect of AnMr extract on the formation of AGE in BSA incubated with ribose

The BSA-glucose system employed is commonly used in non-enzymatic glycation studies. The figure 21 presents the antiglycation activity of the different doses of the extract on the BSA-glucose system. The formation of AGEs was monitored after 7days by measuring the fluorescence intensity of the BSA-glucose solutions. When BSA was incubated with glucose, a significant increase in fluorescence intensity was observed after 7 days of the experiment. After AnMr was added to the reaction media containing BSA/glucose, the fluorescence intensity significantly decreased in a concentration-dependent manner throughout the study period as presented on figure 21. On this figure, except the extract at concentrations of 50 and 20 µg/ml where there is no significant difference ($p>0.05$) when compared to the extract and the glucose, the other concentrations significantly ($p<0.001$) inhibit the activity of the glucose. In comparison among the different concentrations of the

extract the difference was significant ($p<0.001$) among the concentrations being tested, except between the concentration of 50 and 20 µg/ml.

III.1.5.3 Antiglycation activity of *A.muricata* in BSA incubated with glyoxal



***: $p<0.001$; **: $p<0.01$, *: $p<0.05$

Figure 22: Effect of AnMr extract on the formation of AGE in BSA incubated with glyoxal

Glyoxal is the end product of the second stage of glycation. The figure 22 above presents the effect of AnMr extract on the formation of AGE in BSA incubated with glyoxal after 7 days of incubation. The highest concentrations of the extract were able to inhibit activity of glyoxal in a significant manner ($p<0.01$ and $p<0.05$). The highest activity was observed by aminoguanidine the reference compound ($p<0.001$).

III.1.6 Adverse effect

III.1.6.1 Acute toxicity

III.1.6.1.1 Behavior of treated animals

Thirty minutes after treatment with the extracts, there were no visible changes. However there after, the displacement of animals was reduced, they gather together and showed tiredness. After approximately 2 hours of treatment, the animal fed with seed extract started to suffocate and die before the third hour post feeding. One hour later one of the animals fed withAnMpf died. The other animals were gathering together and their fur was

raised. They were tired and they were not able to eat. On the second day following treatment they were recovering and by the end of the two weeks, no sign of toxicity was anymore detectable.

Following observation of mortality for animals treated with seeds and the fruit pulp, other animals were therefore submitted to 1 and 2 g/kg of the same extracts. After receiving 2 g/kg of these extracts, there were five deaths in the group fed with seed extract and one death for the group treated with pf withing the 5 first hours of force-feeding. After administration of the dose of 1 g/kg, animals were gathered, tired, were not able to eat but no mortality was observed. The observable effects were not severe as with higher doses. On the second day the animals were recovering and at the end of the treatment period there were no more signs of toxicity. During the treatment and observation period their stool and tail aspects were not affected and they were reactive to noise, but their reaction to pinch was reduced as the dose increased.

III.1.6.1.2 Determination of the LD50

Results obtained at the end of the study are presented in the following tables. The percentage of mortality and the probit values are expressed according to administered doses.

Table XV: Percentage of mortality of mice following the administration of AnMs (A) and AnMpf (B)

Number of mice per group	Administered dose (g/kg)	Number of death	% of mortality	Probit value
		A (AnMs)		
6	5	6	100	8.71
6	2	5	83.3	5.98
6	1	0	0	1.90
		B (AnMpf)		
6	5	3	50	5.00
6	2	2	33.33	4.56
6	1	0	0	1.90

Using the different doses and the probit values, the LD50 of each extract was determined graphically as described by **Randhawa (2009)**.

From the analysis of obtained results, we can say that except the extract from leaves, roots, twigs and stem bark which were classified as non toxic as described by OECD (table XXIII), the seed and pulp of fruit extract present the LD50 of 2.31 g/kg and 4.53 g/kg respectively.

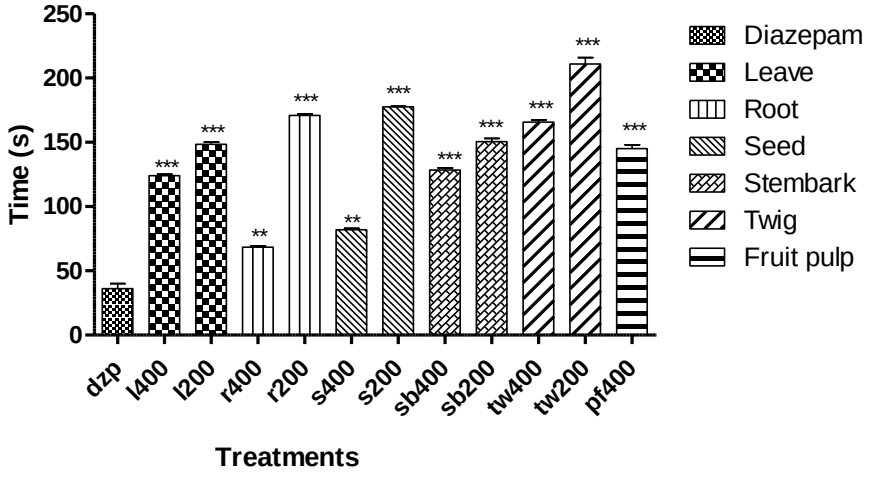
According to the obtained LD50 of seed and pf, they were classified as slightly and non toxic respectively.

Table XVI: Universal harmonized classification system of products according to their toxicity (OCDE, 2001).

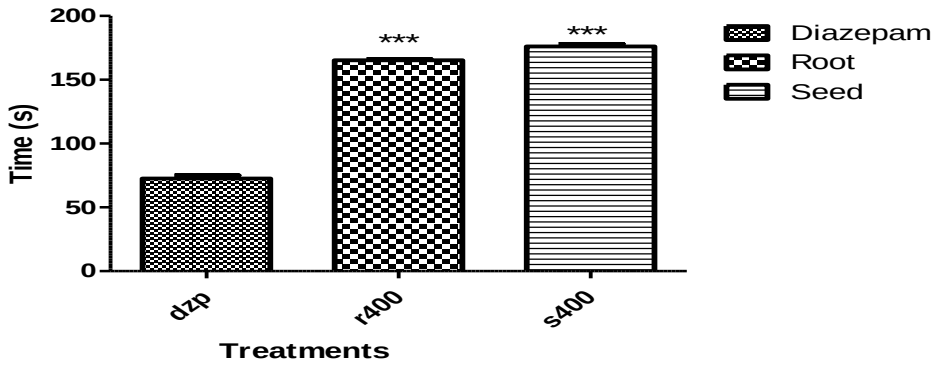
Range of dose (g/kg)	**Category**
≤ 0.005	Ultra toxic
0.005 – 0.05	Very toxic
0.05 – 0.3	Fairly toxic
0.3 - 2	Sligthly toxic
> 2	Non toxic

III.1.6.2 Effect on motor function

A)



B)



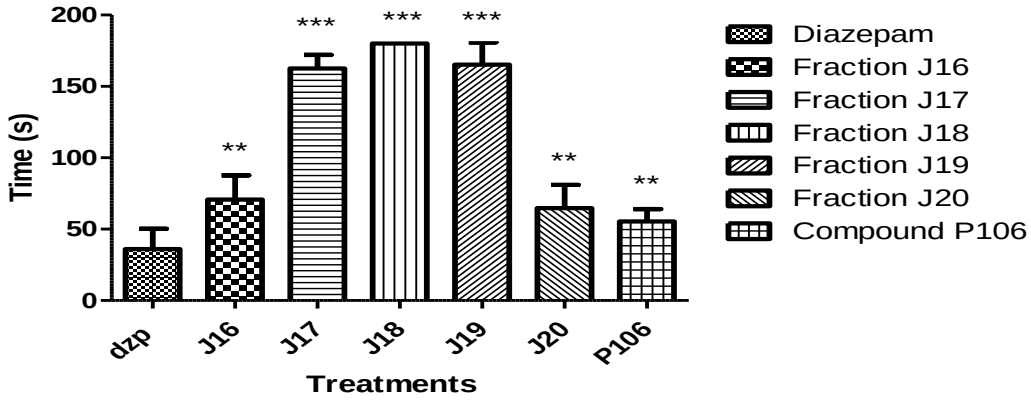
***, **Comparison between DZP and extracts ($p<0.001$, $p<0.01$)

Figure 23: Neurotoxicity of extracts 30 minutes (A) and one hour (B) after administration of extracts

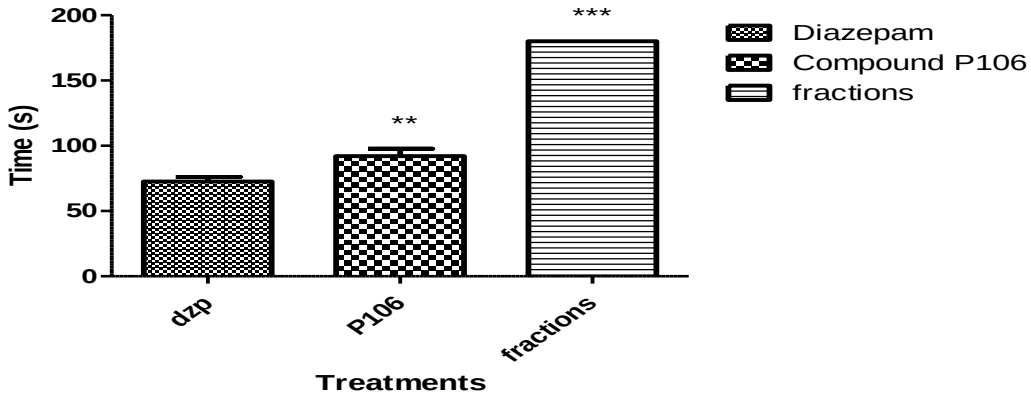
Figure 23 gives the effect of the different organs of AnM extract on motor coordination. This result shows that 30 min after administration, the highest doses of AnM did not alter the ability of the animals to remain on the bar. The time spent on the bar by treated animals was significantly different ($p<0.001$) from the time spent by the animals who received DZP. This effect decreases with time and after 60 min of treatment (fig 23B), the neurotoxicity was observed only for the highest doses of roots and seeds. At 100 mg/ kg, no neurotoxicity was observed for all the organs tested this in comparison with DZP (2mg/kg).

In comparison between extracts, animals treated with roots and seeds at the highest doses, have their time on the rod significantly reduced compared to the other extracts ($p<0.05$). One hour after administration, all the animals were able to remain on the bar for more than 30 min, except those treated with roots and seeds and at the highest dose. The time spent on the bar was significantly longer compared to DZP ($p<0.001$). Between extracts, those treated with seed were significantly ($p<0.05$) able to remain on the rod than those treated with roots.

A)



B)



***, **Comparison between DZP and extracts ($p<0.001$, $p<0.01$)

Figure 24: Neurotoxicity of fractions and product 30 minutes (A) and one hour (B) after administration

Figure 24A presents the ability of mice to remain on the bar 30 minutes after administration of fractions and pure product. According to this figure, animals treated with all the compounds were able to remain on the bar compared to DZP. The levels of significance were $p<0.001$ for fractionsJ17, J18 and J19; $p<0.01$ for J16, J20 and the pure product. Therewas no significant difference between the fractions J16, J20 and the compound P106 ($p>0.05$). The animals treated with J17, J18 and J19, were able to remain on the bar far longer than those treated with J16 ($p<0.001$).

Figure 24B presents the effects of the compound and fractions on the locomotor activity. One hour post administration the animals treated with the fractions were able to

remain on the bar during the period of observation compared to DZP ($p<0.001$), while the mice treated with P106 stayed more on the bar ($p<0.001$) than those treated with DZP and longer than the first 30 minutes of observation.

III.1.6.3 Cytotoxicity assay

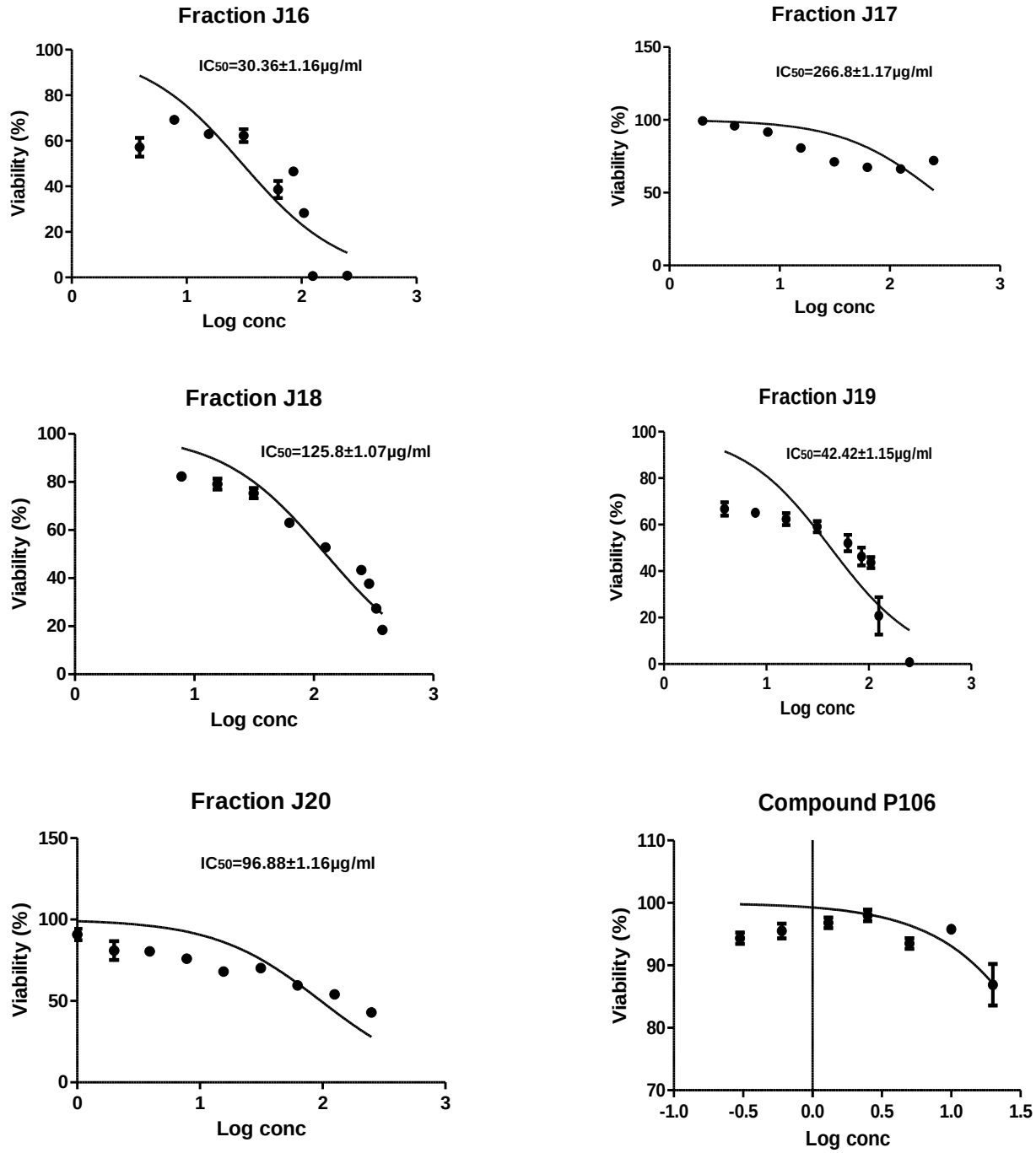


Figure 25: Cytotoxicity activity of fractions and compound from AnMr against LLCMK2 by MTT test

Figure 25 presents the cytotoxicity of fractions and compound from AnMr against the LLCMK2 by MTT test. The IC50 obtained were 30.36±1.16, 125.8±1.07, 42.42±1.15, 96.88±1.16 µg/ml respectively for J16, J18, J19 and J20. J16 was the most cytotoxic fraction, followed by J19. J17 in the range of concentrations tested did not give an IC50, thus it can be consider as slightly cytotoxic because the IC50 obtained was 266.8±1.17µg/ml which is out of the range of the concentrations tested. P106 on its own, till the end of the experimental period and at all the concentrations tested, did not present any sign of toxicity and was therefore not cytotoxic. It was observed that the decrease of viability was directly proportional to increase in concentration of compounds.

III.1.6.4 Developmental toxicity

Figure 26 below presents the mortality of embryo after treatment with the different fractions.

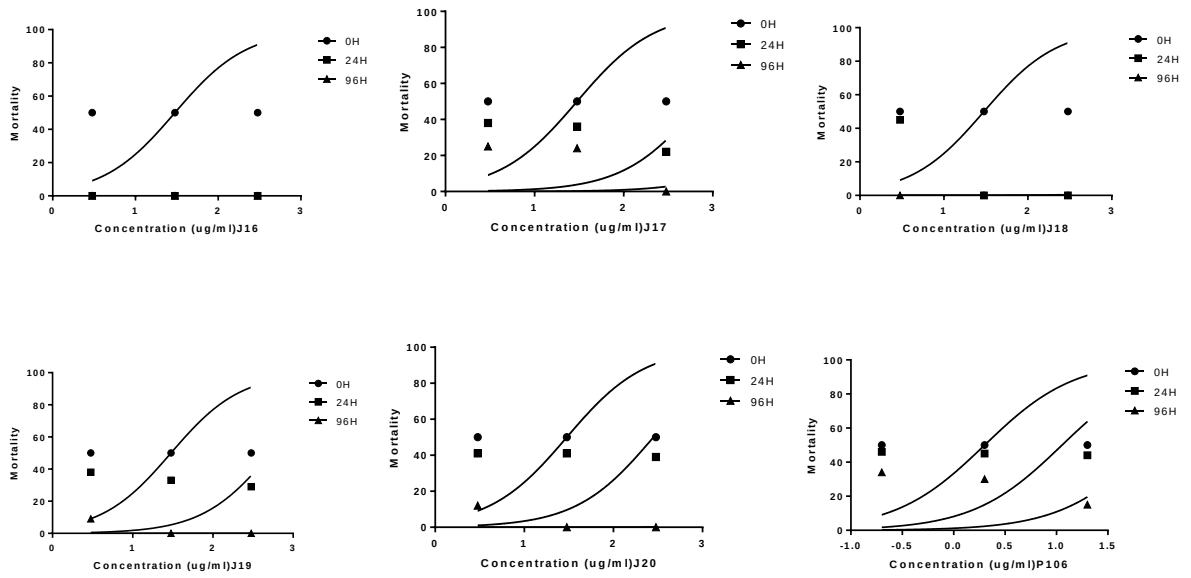
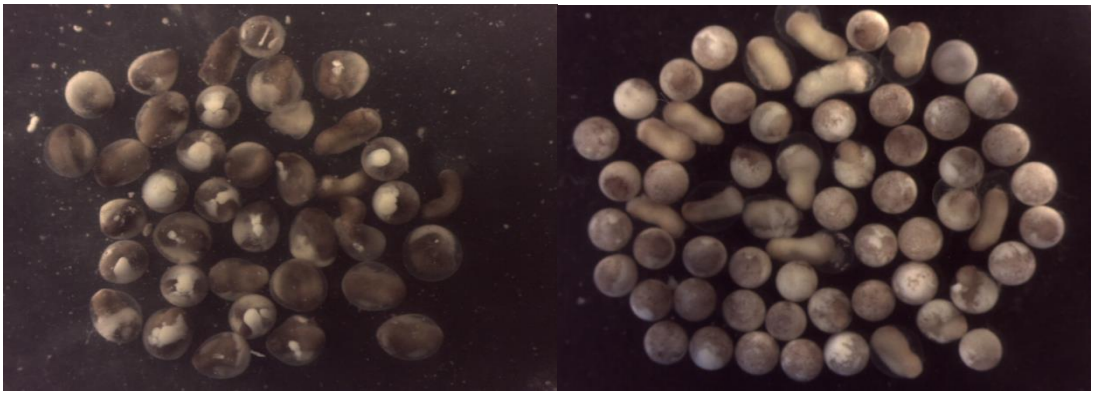
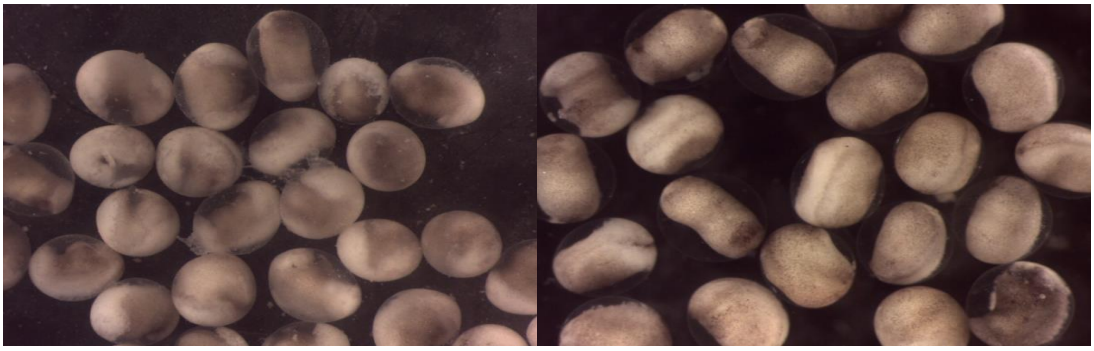


Figure 26: Concentration-effect curves for AnMr fractions and compound



A: J16 fraction at 300µg/ml B: J17 fraction at 300µg/ml

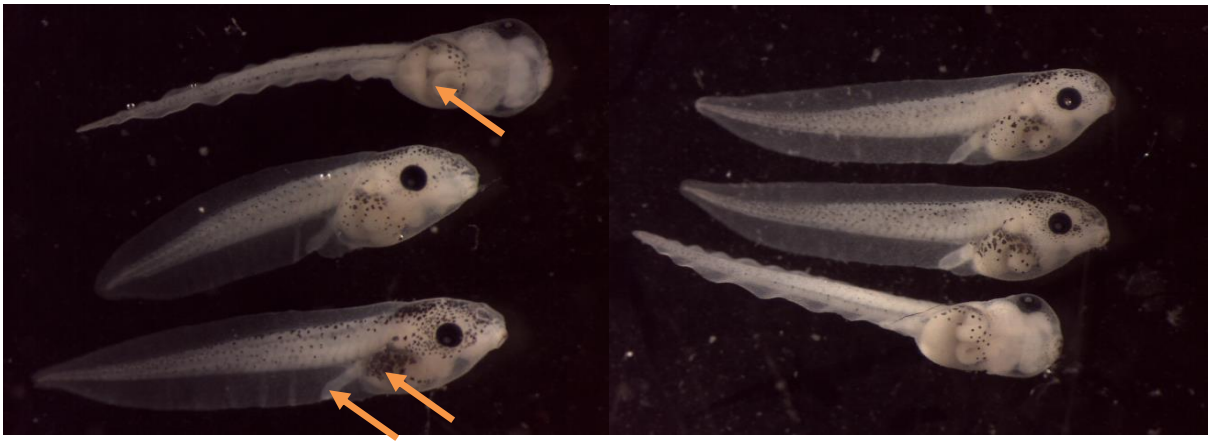


C: J18 fraction at 300µg/ml D: J19 fraction at 300µg/ml

E: J20 fraction at 300µg/ml F: Compound P106 at 20µg/ml

G: Control

Image 7: Embryos after 24 hours of treatment with *A. muricata* fractions at highest concentrations



A control B test

Image 8: Embryos at the end of the observation period (96h)

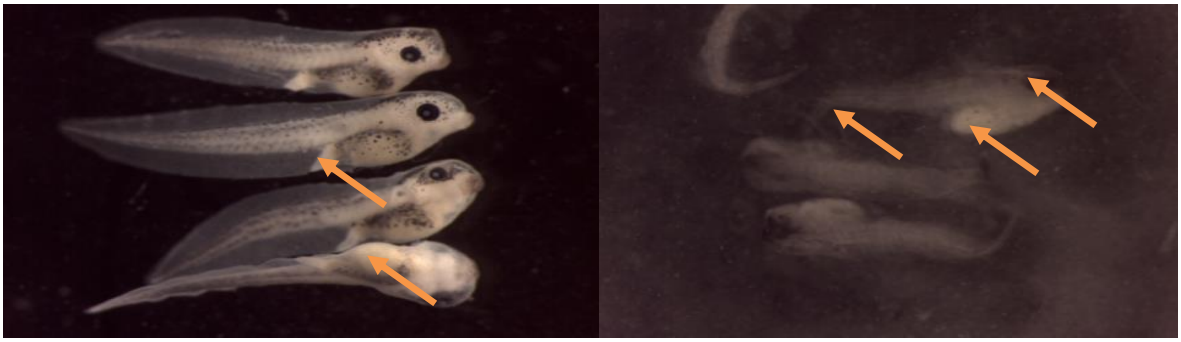


Image 9: Some malformations observed at the end of the observation period (96h)

Figure 26 shows the concentration-effect curves of the fractions and compound from AnMr. For the majority of fractions the development was stopped at the neurula stage. This was the case for J16 fraction where after 24h the embryos development was at the stage 13; for J17, J18 and J19 the development was stopped at stage 14, 20 and 21 respectively. For J20 and P106 the embryos developed till stage 25 and 28 respectively which correspond to the early tailbud stage (image 7).

The LC50 and EC50 were identified using the probit analysis were possible. The LC50 values obtained were 1.151, 277.522, 38.40, 2196.423, 384.232 and 65.28 µg/ml for J16, J17, J18, J19, J20 and P106 for the first 24h of the experimental period. The TI values obtained were between 0.006 and 5.76, the highest value being displayed by P106. At the end of the observation period, the values were 65.443 and 44.90 µg/ml for J17 and P106 (table XXIV). Due to the mortality of the embryos it was not possible to determine the LC50 of the remaining fractions and therefore the TI.

At the end of the observation period a developmental delay was observed in the group of embryos exposed to P106. The oedema were more present and the axial length seriously

reduced in the embryos exposed to the highest dose of P106; abnormal pattern of intestine coiling was observed after organogenesis at the end of the 96h of exposure (image 9). On the contrary, no adverse effect was observed in the other embryos which survive till the end of the experimental period (image 8).

Table XXIV: Endpoints at each exposure time to fractions of AnMr

Compound	LC50	EC50	TI
J16(24h)	1.151	ND	ND
J16(96h)	ND	ND	ND
J17(24h)	277.522	750.6	0.36
J17(96h)	65.443	10578	0.006
J18(24h)	38.40	ND	ND
J18(96h)	ND	ND	ND
J19(24h)	2196.423	534.4	4.11
J19(96h)	ND	3358	ND
J20(24h)	384.232	281.3	1.36
J20(96h)	ND	2516	ND
P106(24h)	65.28	11.33	5.76
P106(96h)	44.90	12.23	3.67

LC50: lethal concentration 50%; EC50: effective concentration 50%; TI: teratogenic index

III.2. DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body **(Omale and Friday, 2010)**. Yield of extracts from *A. muricata* parts varies between 2.25 and 14.5%. This demonstrates the variability of biosynthetic pathways of the plant. Leaves present the highest yield; this can be due to the fact that between all organs of *A. muricata*, they are the most concentrated in different secondary metabolites **(Pinto *et al.,* 2005)**. Sure enough, the majority of plant metabolites are transformed during photosynthesis at this level. This diversity of the different plant parts is manifested by the variation of the presence of secondary metabolites.

Epilepsy is a common neurological condition associated with an alteration in psychological, emotional and educational parameters **(Aldarmaa *et al.,* 2010)**. Prevention of PTZ induced seizures in laboratory animals is the most commonly used preliminary screening test for discovery of anticonvulsant drugs. It is mostly believed that inhibitors exert their action by acting as antagonists at the picrotoxinin-sensitive site at the γ-aminobutyric acid (GABAA) receptor complex **(Hansen *et al.,* 2004)**. Accordingly, **Huang *et al.,* (2001)** indicated that PTZ interacts with overlapping but distinct domains of the GABAA receptor. Within 2-4 seconds of PTZ administration, there is an increase in blood oxygen levels. This increase was observed at the thalamus, especially the anterior thalamic nuclei. The peak of this activity which is twofold greater than in all other thalamic areas is reached few seconds before the first seizure **(Brevard *et al.,* 2006)**. Extracts from *F. thonningii*, a plant of the Moraceae family presented an anticonvulsant effect against PTZ-induced convulsion. No previous scientific study revealed its anticonvulsant activity but however, **Noumi and Fozi (2003)** reported the use of a related plant (*Ficus sycomorus*) in combination with other plants such as *Asystasia gangetica*, *Ficus mucoso*, and *Aframamum melegueta* in Fongo-Tongo community in Cameroon to cure epileptsy. Beside this report, Tanzanian healers have been reported to boil the bark of *Ficus sycomorus* in the treatment of epilepsy **(Moshi *et al.,* 2005)**.

Another study conducted by **Sandabe *et al.,* (2003)** revealed that aqueous extract of *F. sycomorus* stem bark at the doses of 200 and 400 mg/kg protected laboratory animals from death while **Ibrahim *et al.,* (2008)**, using the flavonoid fraction of *F.sycomorus,* obtained an anticonvulsant activity against the PTZ and maximal electroshock seizure (MES) models at

10 mg/kg. **Singh and Goel (2009)**, working on another species of the same genus, *Ficus religiosa*, observed that the methanolic extract of figs at the maximum dose tested (100 mg/kg) was not effective against the PTZ model but was able to protect the animal from MES seizure starting at 25 mg/kg, the smallest dose tested. Similarly, the saponin fraction of the same plant at 1 mg/kg was able to decrease PTZ-induced seizures **(Singh *et al.*, 2013)**. Another species, *Ficus platyphylla*, studied by **Chindo *et al.*, (2009)** revealed that saponins extracted from the stem bark, tested at the minimal dose (25 mg/kg) were effective against *in vivo* and *in vitro* models of epilepsy.

In our study, leaves and stem bark of *F. thonningii* at 200 mg/kg, the smallest dose tested, significantly delayed the onset of seizures in general and tonicoclonic seizures in particular. At this dose, the stem bark extract entirely protected the animals, while 66.66% of mice were protected by the leaf extract. An ethnobotanical survey of epilepsy treatment in Fongo-Tongo village, Western region of Cameroon by **Noumi and Fozi (2003)**, presented the use of *D. Adescendes* leaves, *V. doniana* and *A. senegalensis* stem bark in the treatment of epilepsy by healers. They were effective when given to some epileptics who claimed to be cured after an average period of 18 months. The ethanolic extract of *D. Adescendes* tested at 300 mg/kg, was effective against clonic convulsions induced by PTZ as reported by **N'gouemo *et al.*, (1997)**. In our study, the ethanolic extract of *D. Adescendes* was able to protect mice from death at all doses tested, but was neither effective against seizure onset nor tonicoclonic seizures.

Concerning *A. senegalensis*, **Konate *et al.*, (2012)** reported the anticonvulsant property of the methanolic extract and aqueous fraction of the stem bark of *A. senegalensis* at 400 mg/kg. This result is different from what we obtained at the same dose and could be explained by the part of the plant used in our study or extraction solvent used. However, the activity observed at 800 mg/kg, particularly the onset seizure in our study confirmed the anticonvulsant property of this plant which did reduce the convulsion duration at all doses tested. *A. muricata* stem extract at 200 and 400 mg/kg significantly delayed seizures, while the onset of TC seizures was significantly delayed at 200 and 800 mg/kg for the stem and seeds respectively. Our findings are similar to those obtained by **N'gouemo *et al.*, (1997)** with the ethanolic extract of leaves of this plant at the dose of 300 mg/kg. Twigs and seeds significantly reduced seizure duration and conveyed entire protection at 200 mg/kg body weight in mice.

A. cordifolia leaves did not significantly modify the time to seizure onset, nor the onset of tonicoclonic (TC) convulsions, but significantly reduced the duration of convulsions.

In the contrary, **Pedersen *et al.*, (2009)** in a previuous study showed inhibition of spontaneous epileptic form discharges by the ethanolic extract of *A. cordifolia* leaves from Mali. This difference could be due to the difference in solvents used, or in the *in vitro* model compared to the *in vivo* approach used in our study. In fact, the *in vitro* active compounds could have been metabolized to non-active derivatives in the animal's system or did not cross the blood brain barrier. Otherwise, the active compounds present in the extract do not show effect on the PTZ model **(Pedersen *et al.*, 2009)**.

A comparison between the different extracts, shows that the extract *A. muricata* stem bark at 200 and 400 mg/kg, *A. senegalensis* stem bark and leaves at 800 mg/kg, *F. thonningii* leaves at 200 and 400 mg/kg and *V. doniana* at 400 and 800 mg/kg were able to delay seizure onset with the best activity observed for the *A. muricata* stem bark extract at 200 mg/kg. The protection offered by plant extracts might be dependent upon synergistic effects of constituents. Indeed, phytochemical analysis of components of these plants revealed the presence of alkaloids, saponins, tanins, anthraquinones, terpenoids, and flavonoids in the ethanolic and aqueous leaf extracts of *V. doniana* **(Agbafor and Nwachukwu, 2011)**. **Ayodele *et al.*, (2007)** working on the ethanolic extract of the leaves of *A. cordifolia*, found alkaloids, tannins, saponins, flavonoids and steroids. The authors also found a relationship between *A. cordifolia* intake and the prevention of neurodegenerative disease. A literature review conducted by **Rastogi *et al.*, (2011)**, revealed that *D. adscendens* contains triterpenoids, saponins, tetrahydroisoquinolones, phenyle thylamines and indole-3-alkyl amines isolated from the leaves. Three active components were identified as triterpenoid glycosides dehydrosoya saponin I (DHS-I), soya saponin I and soya saponin III. Dehydrosoya saponin, the major saponin compound of the plant was the most potent potassium channel opener. As in many plants from Annonaceae family, tannins, steroids and cardiac glycosides were the major compounds found in *A. muricata* **(Gajalakshmi *et al.*, 2012)**. **Arthur *et al.*, (2011)** working with the leaf aqueous extract of *A. muricata* also found that saponins and flavonoids are present while **Boyom *et al.*, (1996)** revealed the presence of sesquiterpernes, particularly β- caryophyllene. Concerning *A. senegalensis*, alkaloids, terpenoids, particularly monoterpenes were reported by **Boyom *et al.*, (1996)**. Resins, alkaloids, proteins, anthraquinones, saponins, sterols, glycosides and terpenes were also present in *A. senegalensis* **(Adzu *et al.*, 2005; Konate *et al.*, 2012)**.

DeA, FiTsb and AlCl showed no effects in our study despite traditional claims, particularly concerning the onset of seizures, but they were all able to reduce the convulsion duration. Differences in metabolism between rodents and humans might explain such

differences and justify the use in traditional medicine despite the lack of effect in the animal model **(Pedersen *et al.*, 2009)**.

As far as the mechanism of action is concerned, the extract either increases the threshold of PTZ- induced convulsion or protects mice against convulsions induced by PTZ. Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents and drugs that enhance inhibitory neuro-transmission by GABAA receptors (benzodiazepine, phenobarbital and valproate) **(Chindo *et al.*, 2009)**. The fact that the extracts protected animal against PTZ-induced seizures may suggest that they contain compounds that facilitate GABAergic transmission **(Ayanniyi and Wannang, 2008)**. Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, PTZ-induced acute seizures remains 'Gold standard' in the early stages of testing. The PTZ model is assumed to identify anticonvulsant drugs effective against "petit mal" seizures. The effects of extracts against PTZ-induced seizures thus suggest anticonvulsant efficacy against petit mal epilepsy in man **(Chindo *et al.*, 2009)**.

Further analyses revealed the effectiveness shown by the ethanolic extracts of *A. muricata* against acute seizure induced by chemical convulsants, pentylenetetrazole and picrotoxin suggests anticonvulsant effects **(Amole *et al.*, 2009)**. PTZ has been demonstrated to have a well defined mechanism of convulsant action reported to be the inhibition of gabaergic mechanism **(Hedge *et al.*, 2009)**.

Picrotoxin, on the other hand, is a selective non-competitive antagonist of gamma amino butyric acid (GABA) at GABAA receptor, which has been widely implicated in epilepsy **(Amole *et al.*, 2009)**. It appears to interfere indirectly with àttt&the tonic presynaptic inhibition action of GABA, thus induced convulsion through rapid summation of synaptic activity **(Dhir *et al.*, 2006)**. GABA is the major inhibitory neurotransmitter in the brain and its inhibition is thought to be an underlying factor in epilepsy **(Amole *et al.*, 2009)**. According to **Rang *et al.*, (2003)**, picrotoxin exerts its convulsant effect by blocking the GABAA receptor-linked chloride ion channel which normally opens to allow increased chloride ion conductance into brain cells following the activation of GABAA receptor by GABA **(Okokon and Nwafor, 2009)**.

The reference anticonvulsant drugs used in the present study, phenobarbitone (PBT) and diazepam (DZP), antagonized picrotoxin (PTX)-induced seizures. Postsynaptic GABAA-receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and

chloride-ion channels to form GABA-chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission.

The fact that the extract increased the threshold of seizures and protected animal against PTZ and PTX-induced seizures may suggest that the plant extract contains compound(s) that facilitate GABAergic transmission. It has been found empirically that drugs which inhibit PTZ-induced convulsions and raise the threshold for production of seizures are generally effective against absence seizures, whereas those that reduce the duration and spread of PTX-induced convulsions are effective in tonic-clonic seizures **(Ayanniyi and Wannang, 2008)**. Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents (ethosuximide) and drugs that enhance inhibitory neurotransmission by GABAA receptors (benzodiazepine, phenobarbital and valproate) **(Ayanniyi and Wannang, 2008)**. PTZ and picrotoxin interact with overlapping but distinct domains of the GABAA receptor **(Huang *et al.*, 2001)**.

Epilepsy has traditionally also been considered mainly a neuronal disease, with less attention to non neuronal cells until recently, when growing evidence suggests that astrocytes, microglia, blood-derived leukocytes, and blood brain barrier breakdown are involved in the pathogenesis of epilepsy **(Friedman *et al.*, 2009)**. Recent evidence indicates that leak of serum proteins (specifically albumin) through a dysfunctional blood brain barrier may be a key event in initiating specific signaling cascades within different elements of the neurovascular unit. Specifically, astroglia Upon blood brain barrier opening, the protein TGF-β (transforming growth factor) signaling pathway is activated, probably mediated by the binding of serum albumin to brain TGF-β receptor 2 and is involved in different types of brain injury by inflammatory pathway **(Friedman and Dingledine, 2011)**. Epileptics, unlike normal persons, between attacks show substantial variability in values for electrolytes, protein and nonprotein nitrogenous components, and other blood values **(Greene *et al.*, 2001)**. Animal models have demonstrated that an initial attack is associated to an important inflammatory response in neurons network. The relationship of cause to effect between inflammatory response and epilepsy are now better known. A key event is the rupture of the haemato encephalic barrier following an initial attack. The rupture of hemato encephalic barrier permits the entry in the neuronal tissue of plasma constituents of which albumin. Only the rupture of hemato encephalic barrier with deoxycholic acid or a direct injection of albumin in the brain is enough to create an epileptic center in the cortex. Albumin is transported in astrocytes, this will alter their functioning, particularly their capacity to pump

glutamate and extracellular potassium. Accumulation of glutamate and potassium in extracellular medium will depolarise neurons, and make them discharge action potentials and favours onset of seizures **(Bernard, 2011)**. A study by **Asano *et al.*, (1998)** and our study found that the level of albumin was decreased in mice subjected to seizures. Studies by **Vliet *et al.*, (2007)** showed that epileptiform activity can be induced by direct cortical application of albumin-containing solution in rats, suggesting that serum proteins play a role in the pathogenesis of focal epilepsies. Our results are in concordance with a study of **Janigro (1999)** who showed that in acute seizures, the level of albumin is decreased.

AEDs enhance GABA-mediated inhibitory activity, classically benzodiazepines and barbiturates **(Brodie *et al.*, 2011)**; benzodiazepines will increases the frequency of opening the receptor while barbiturates will increase the duration **(Giovanelli, 2011)**. In the PTZ test, clonic seizures are blocked by AEDs acting on the GABAA neurotransmitter system, such as barbiturates, benzodiazepines, valproic acid, and tiagabine, and by ethosuximide through its action on T-type calcium channels. Drugs effective in the PTZ test are considered to be likely to suppress myoclonic and/or absence seizures. Prediction based on these tests correlates with clinical results for most AEDs, but there are striking exceptions **(Brodie *et al.*, 2011)**. The anticonvulsant activities of extracts of AnM are similar with the work of **Heidari *et al.*, (2009)**, where the methanolic extract of *H. niger* was tested at differents doses and at 400 mg/kg the observed activity decreased.

The results of this study show that the ethanolic extract of *A. muricata* possesses anticonvulsant properties which are possibly mediated partly via facilitation of GABA transmission. These results suggest that the extracts of *A. muricata* may be beneficial in the management of absence and tonic-clonic seizures.

Emerging evidence focuses on the role of oxidative stress both as a consequence and cause of epileptic seizures **(Aldarmaa *et al.*, 2010)**. Antioxidants are substances that delay or inhibit oxidative damage to a target molecule. Antioxidants prevent cell and tissue damage as they act as scavenger. Phytochemicals are known to have a complex nature hence the antioxidant activities of plant extracts cannot be evaluated by a single method.

Quantitative phytochemical analyses were used to investigate the antioxidant potential of different extracts flavonoid, polyphenols and flavonol contents. Most antioxidants and medicinal properties of foods are credited to phenolics with flavonoids being the highest contributors **(Afolabi *et al.*, 2010)**. These total phenolics were determined using the principle

of the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium. The intensity of the absorption is equal to the sum of the individual contribution by the different classification of phenols in the samples. Phenolic compounds in plant extracts contribute significantly to their structure. Phenolics are composed of aromatic ring(s) bearing single or multiple hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance-stabilized phenoxyl radical **(Agyemang, 2013)**. Phenolics are also very important plant constituents because of their scavenging capability due to their hydroxyl groups. Flavonoids particularly, have the ideal structure for radical scavenging. They are more efficient antioxidants than vitamins C and E. Flavonoid antioxidant activity depends on its structure and may be determined by five factors: reactivity as a donor agent of H+ and electrons, stability of formed flavanol radical, reactivity compared with other antioxidants, capacity to chelate transition metals, and solubility and interaction with membranes. Sequestering activity is directly linked to the flavonoid oxidation potential and to the species to be scavenged. The smaller the flavonoid oxidation potential, the greater its activity as a free radical scavenger **(Aguiar *et al.*, 2012)**. It has been shown that various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies **(Lavanya *et al.*, 2010)**.

The antioxidant activity of AnM also evaluated using the DPPH, OH, NO, ABTS and FRAP radical scavengers methods.

DPPH (1,1-Diphenyl-2-picrylhydrazyl radical)is a simple, rapid and reliable method commonly used in the assessment of radical scavenging activities of plant extracts in vitro **(Munasinghe *et al.*, 2001; Ibrahim and Waheeb, 2012)**. The reduction capability of DPPH radicals was determined by the decrease in its absorbance which is induced by antioxidants **(Gaikwad *et al.*, 2011)**. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of *A. muricata*. This test showed that AnMr, AnMst and AnMl are good scavengers of DPPH, indicating that their compounds may contribute to neutralize the oxidant agents produced during inflammatory states **(Reynoso *et al.*, 2013)**.

Nitric Oxide (NO) is a diffusible free radical that plays many effective roles in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities **(Gaikwad *et al.*, 2011)**. Nitric oxide is generated from the decomposition of SNP (sodium nitroprusside) and measured by Greiss Reagent. SNP in aqueous solution at

physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be measured by the use of Greiss reagent **(Gaikwad *et al.,* 2011)**. A significant decrease in the NO radical is due to the scavenging activity of the extracts.

Hydroxyl radical (OH) is an extremely reactive oxidising radical, probably the most reactive radical **(Buonocore *et al.,* 2010)** that will react with most biomolecules at diffusion controlled rates. It has extremely short half-life but is capable of causing damage within a small radius of its site of production. A single hydroxyl radical can result in formation of many molecules of lipid hydro peroxides in the cell membrane, which may severely disrupt its function, and lead to cell death **(Sreedhar *et al.,* 2010)**.

The DPPH, OH, NO and ABTS (2,2′-azino-bis3-ethylbenzo-thiazoline-6-sulfonic acid diammonium salt) assays have been widely used to determine the free radical-scavenging activity of various plants and pure compounds **(Li *et al.,* 2009)**.

The FRAP method is based on the reduction of complexes of 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3 \bullet 6H_2O$), which are almost colourless, and eventually slightly brownish. This chemical forms blue ferrous complexes after its reduction **(Sochor *et al.,* 2010)**. Study by **Rauca *et al.,* (1999)** reveals that a linkage exits between the generation of tonic-clonic seizures and the increased formation of OH in the brain.

This study shows that the extract of AnM possesses antioxidant activity. This can be due to the presence of at least tannins, flavonoids and alkaloids content in these plants which are known to possess potent antioxidant activity. Hence, the observed antioxidant activity might be due to the presence of any of these constituents or due to a synergistic effect **(Mahomoodally *et al.,* 2012; Chen *et al.,* 2011)**.

Studies show an existence of a link between the antioxidant and the antiglycation activities. Albumin is a target protein for the glycation reaction due to its abundance in serum **(Chayaratanasin *et al.,* 2015)**. AGEs (advanced glycation end product) are heterogeneous group of molecules formed from the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids **(Golegaonkar *et al.,* 2015)**. In this study, three screening methods were used to measure the inhibitory effects of AnMr and aminoguanidine (AG) on formation of fluorescent AGEs in vitro. Aminoguanidine is one of

the most well-known synthetic prodrugs, but of limited practical application due to their toxicity and severe side effects **(Kwanta *et al.*, 2014)**.

In the present work, the different concentrations of AnM were found to inhibit the formation of AGE *in vitro*. It is suggested that the abilities to inhibit the formation of glycated end products is closely related to the abilities of the antioxidant properties of the plant extracts to scavenge radicals formed during the Maillard reaction which forms the basis of glycation. Interestingly, in the current work, AnMr extract that was found to possess the highest antioxidant properties was also found to possess antiglycation potential. Furthermore, the extracts do not possess antiglycation activities similar to the standard drug aminoguanidine, also known as pimagedine which is a nucleophilic hydrazine compound. Initially, it was thought that aminoguanidine prevented AGE formation by blocking carbonyl groups on Amadori products although it is now known to react with carbonyl groups from reducing sugars **(Mahomoodally *et al.*, 2012)**. Nevertheless the data obtained for the control in the test with glyoxal is similar to the value obtained by **Guttierez *et al.*, (2012)**. The glyoxal model represented the middle stage of protein glycation in which sugar is oxidized to α-dicarbonyl compounds such as methylglyoxal, glyoxal and 3- deoxyglucosome, which are more reactive in reacting with amino group of protein leading to AGE formation **(Kwhanta *et al.*, 2014)**. The study of **Poramin *et al.*, (2015)**, presents similar results to those we obtained in this work using glucose, despite the difference in the time(21 days and 7days). The study of **Shin *et al.*, (2015)** using glucose as a glycating agent for 3 weeks of work obtained an antiglycation activity lower than the activity of our extract. AG activity was comparable to the activity of AnMr; the isolated compound silibinin, on its own display results similar to our extracts at highest concentrations (10-100 µg/ml).

This indicates that AnMr may be able to inhibit the formation of advanced glycated end product and may also block the convertion of dicarbonyl intermediates to advanced glycation endproducts. In the test with glucose the data were not close; this can be due to the incubation duration, as Guttierez conducted his experiment in 14 days instead of 7 as in our study. These results suggest that AnM possesses an antioxidant and antiglycation potential. Therefore, various pharmacological effects ofAnM curative maybe closely correlated with its antioxidant and antiglycation activities.The difference in the antioxidant and antiglycation activities of the extracts may be due to the different phytochemical constituents present at different percentage **(Borah *et al.*, 2011)**.

It is now accepted that many anti-epileptic drugs can have an analgesic effect particularly in human neuropathic pain **(Jayaraman *et al.*, 2010)**. **Bonin and Konick (2013)** reveals that the enhancement of GABAA or glycine receptor activity or inhibitory drive can reverse pathological pain. In the same way, **Yehuda *et al.*, (1991)** in their work discovered that pain provides some elevation in the threshold of seizures; and that pain is connected to analgesia with different pathological conditions. The plants extracts were therefore tested for their antinociceptive activity.The effect of AnM extracts on peripheral nociception was determined using the acetic acid –induced writhing model which is a general non-selective model of antinociceptive studies **(Couto *et al.*, 2011; Ibrahim *et al.*, 2012)**, frequently use to estimate both the central and the peripheral analgesic effect of drugs **(Cha *et al.*, 2011)**. The acetic acid-induced writhing test has been associated with an increased level of prostaglandins (PGs), especially PGE2, in peritoneal fluids. PGs induce abdominal constrictions by activating and sensitizing peripheral chemosensitive nociceptors **(Cha *et al.*, 2011)** which are largely associated with the development of inflammatory pain **(Bley *et al.*, 1998; Cha *et al.*, 2011; Couto *et al.*, 2011)**. Therefore, one of the possible analgesic mechanisms related to this test is the inhibition of the COX enzyme. Non-steroidal anti-inflammatory drugs exert their peripheral analgesic potential through inhibition of PG synthesis, and in the present study, aspirin produced a significant decrease in the writhing response. AnM also showed potent inhibition of acetic acid-induced abdominal constrictions in a non-dose-dependent manner. The antinociceptive potential of AnM could be explained by its inhibitory action on COX-2 expression as described above. Another mechanism that might be involved include lipooxygenase (LOX) products in peritoneal fluids as well as the release of many inflammatory mediators such as bradykinin, substance P, TNF-a, IL-1b and IL-8. These inflammatory mediators excite the primary afferent nociceptors entering dorsal horn of the central nervous system **(Ferreira *et al.*, 2013)**. Therefore, it is likely that the opioid system is involved in the peripheral antinociceptive actions of the plant extract AnM.

The hot plate test, a thermal model of pain, is used to determine central antinociceptive activity **(Cha *et al.*, 2011)**. It is predominantly a spinal reflex or behavioral reaction **(Couto *et al.*, 2011)**. AnM extract show analgesic effect in this model, suggesting possible supra-spinal analgesic pathways. Morphine, an opioid agonist exhibits a rapid effect with a maximum peak in a short amount of time. In contrast, AnM extract reached the maximum level at different time according to the dose with the maximum at 120 minutes. This difference in the maximum analgesic effect could be explained by the method of administration or the

metabolic rate of each drug. After administration of naloxone the morphine's peak was shifted to 60 minutes, and disappear for the rest of the observation's time this indicates that morphine is a partial opioid agonist. For the extract, the highest activity was perceived at the end of the period of observation. The activity of extract was suppressed after administration of naloxone and this analgesic activity was not recovered till the end of the observation time, indicating that the activity may be mediated via activation of opioids receptors.

The results obtained in our experiments suggest that, AnM extracts possess centrally and peripherally-mediated analgesic activities. In order to obtain more specific evidence on the antinociceptive activity of AnM, other assays were performed on nociception induced by chemical stimuli. In this way, the formalin assay is a traditional test of nociception, which is sensitive to different classes of antinociceptive substances. The assay is considered a model of persistent pain, produced by intraplantar administration of the substance at 1% **(Rabelo *et al.*, 2013)**, which causes stimulation of nociceptors, and may be considered a model of biphasic behaviors indicative of pain (neurogenic and neuropathic pain). In addition to drugs that act at the central level, the anti-inflammatories interfere with this step **(Couto *et al.*, 2011)**.

The first phase (neurogenic pain) is caused by the direct chemical stimulation of nociceptive afferent fibers, predominantly C fibers **(Amaral *et al.*, 2007; Imam and Sumi, 2014)**. On the other hand, the second phase (inflammatory pain) results from the action of inflammatory mediators such as prostaglandins, serotonin, histamine and bradykinin in the peripheral tissues **(Cha *et al.*, 2011; Reynoso *et al.*, 2013)** and from functional changes in the spinal dorsal horn **(Dalal *et al.*, 1999; Imam and Sumi, 2014)**. The present study showed that morphine, a central analgesic drug, is effective in preventing both the early and late phases of formalin-induced nociception, whereas indomethacin, a NSAID (nonsteroidal anti-inflammatory drug), suppressed nociceptive activity in the later phase **(Ferreira *et al.*, 2013; Imam and Sumi, 2014)**. Drugs that act primarily as central analgesics inhibit both phases while peripherally acting drugs such as steroids and NSAIDs like indomethacin, aspirin and diclofenac inhibit only the second phase **(Yamamoto *et al.*, 2002; Couto *et al.*, 2011)**. In our experiments, AnM slightly inhibited the early phase of the formalin test. It was also observed that AnM reduced the duration of the paw licking time in the first phase and the second phase, demonstrating that AnM may suppress neurogenic and inflammatory nociception. These data provided further confirmation that AnM extract has a central mechanism of action, which was shown in the hot plate test. Furthermore, in agreement with the results from the acetic acid test, AnM also displayed peripheral analgesic effect as aspirin. Based on these findings, it may

be concluded that AnM has antinociceptive properties involving central and peripheral mechanisms **(Imam and Sumi, 2014)**. Besides that, the extract showed significant effect at the acetic acid-induced writhing method and at the second phase of the formalin method, indicating that antinociceptive effect occurred to the peripheral level. Thus, it can be concluded that AnM extract antinociceptive activities may be related to the modulation of release of inflammatory mediators involved in nociception **(Tjølsen *et al.*, 1992; Ferreira *et al.*, 2013)**.

One of the main strategies in nociception studies has been the search for opioid analgesics acting at opioid receptors outside the central nervous system (CNS), with the prospect of avoiding centrally mediated side effects as tolerance and dependence **(Vanegas and Tortorici, 2002; Couto *et al.*, 2011)**. For the assessment of opioid system involvement in the analgesic activity the mice were pre-treated with an opioid antagonist, naloxone. In this study, naloxone prevented the antinociceptive effect on both phases of the formalin test, as well in writhing and hot plate tests. Those results suggest that, at least part of the anti-hyperalgesic effect observed for the extracts is due to involvement of this system (μ-opioid) since naloxone reverted the antinociceptive activity **(Couto *et al.*, 2011; Habib and Waheeb, 2013)**. The percentage of inhibition varied according to the test and the compounds administered, with some percentages higher than 100%. This was observed by **Habib and Waheeb (2013)**, who obtained a percentage of inhibition of 145% in the hot plate test-induced pain. It is established that naloxone acts by antagonizing the action of endogenous opioids involve in pain or stress **(Dar *et al.*, 2005).** This activity can be due to the presence in the plant of alkaloids, terpene, and phenolic compounds. Since a review by **Carlini (2003)** emphasized on the presence of a large number of alkaloids, terpenoids, capsaicinoids, steroids, flavonoids, xanthines, tannins, xanthones, lignans, saponins, lactones, glycosides in *P. somniferum*, *C. sativa* and *Salix alba* L, plants with analgesic activity.

In summary, in several thermal and chemical nociception tests, AnM roots exhibited potent antinociceptive activities on both central and peripheral mechanism **(Nisar *et al.*, 2008)**. In addition, a combination test with naloxone revealed that AnM acts as a great opioid receptor agonist, particularly μ receptors **(Couto *et al.*, 2011)**. These endogenous opioids can regulate and modify processes of central excitability **(Becker *et al.*, 1994)**. **Yehuda (1991)**, discover that pain conditions contributed to some seizure protection. Based on these various properties, AnM may hold great promise for treating pain and seizures.

The present results show the presence of ions in all organs. With progress in neuropharmacological studies it is possible to identify the involvement of neurotransmitters (GABA and glutamate), as well as other alterations in membrane functions, receptors, ionic changes and alteration of neural networks that are involved in epileptogenesis **(Aguiar *et al.*, 2012)**. **Dekker (2002)** reveals that an electrolyte imbalance is a cause of seizure. Calcium plays a central role in neuronal excitotoxic reactions. It may flow excessively into cells; release from cell stores may be increased or the mechanism clearing calcium from cells may fail. Generally, any calcium flow into cells is likely to generate an excitotoxic condition. As far as neurons are concerned, any disturbance in calcium balance within and outside of the cells modifies membrane polarity and may induce epileptogenic activity.Sodium has a buffering effect on calcium. Modulating sodium flux may also exert a neuroprotective effect **(Arzimanoglou *et al.*, 2002)**.

To counteract seizures, many drugs are used. Traditional treatment is a good alternative, but the mechanism of action is usualy not known. Pretreatment with flumazenil inhibited the anticonvulsant effect of the extracts. Flumazenil reversed the effects of extracts on the increment of latency to seizures, and convulsion duration. It might therefore be assumed that extracts of AnM may exert their anticonvulsant effects through a GABAA–benzodiazepine receptor complex **(Abbasi *et al.*, 2012)**. These results are in agreement with those of other studies, such as the intra cerebrovascular administration of vitexin **(Abbasi *et al.*, 2012)** a flavonoid, as well as the intraperitoneal administration of Pasipay **(Nassiry *et al.*, 2007**), were the anticonvulsant activities were reversed by flumazenil. However, the anticonvulsant effects of Pasipay and vitexin were more potent than those of our extracts, and other possibilities for these anticonvulsant effects were discussed such as the families of plants. Not all compounds activities are mediated by GABA/A– benzodiazepine receptor complex as phytol a compound with anticonvulsant activity has the activity not altered after administration of flu **(Costa *et al.*, 2012).** Flu altered all the effects induced by administration of the extracts of AnM, suggesting that the extract contains a compound or compounds that interact with the benzodiazepine site in the GABA/A– benzodiazepine receptor **complex (Can and Özkay, 2012)**.

Almost all antiepileptic drugs show the signs of sedation, hypo or (less often) hyper-locomotion, ataxia, abnormal gait, reduced or inhibited righting reflexes and muscle relaxation in laboratory animals. These effects are commonly termed as neurotoxicity **(Loscher and Schmidt, 1988)**. In the lab neurotoxicity can be determined using rotarod,

chimney and inverted screen test. In our study we have used rotarod test to determine neurotoxic effects of AnM extract **(Singh and Goel, 2009)**.

The extract at 200 and 400 mg/kg show neurotoxicity 30 min after administration, their abilities to rapidly correct for normal position of the limb and body posture was modified. This inability to remain on the bar, decrease with time and 90 min post administration, no sign of neurotoxicity was observed and at 100 mg/kg all the animals remains on the bar for more than 3 min for all organs of the plant tested. This test is generally used to assess the side effect of drugs, including AED on motor functions **(Ilodigwe *et al.*, 2010)**. These results show that the extract caused impairment and neurological deficit at first administration.

Toxicological studies are helpful in evaluating in predictive manner, the risk for humans and animals, a voluntary or accidental exposition to a given chemical substance **(Adolphe, 1988)**.The different organs's extracts of *Annona muricata* modified the mice behaviour which culminated in death for group of animals treated with pf and seeds. Specifically their mobility was reduced, they were unable to feed, they were gathered together and their fur was dressed. These behaviours were dose-dependent. The slight modification observed may be due to a rapid assimilation of the extract by the animal. This reduced activity can be due to the sedative or tranquillizer effect of the extract **(Gatsing *et al.*, 2005)**, secondary effect usually encoutered in using AEDs, indicating their neurotoxicity **(Biraben, 1998)**. The mice were sensitive to noise during the test period; this can let us think that the extract administered can lacked depressive properties. At higher doses, animals were slightly sensitive to pinching; justifying the analgesic property of the extract.

This study revealed that all parts of the plant are not toxic; this is confirmed by **Larbie *et al.*, (2011)**, who after evaluating the acute toxicity of the aqueous extract of leaves of *A. muricata* found that they were not toxic at a dose of 5000 mg/kg. This difference can be justified by the place of harvesting the plant, the type of extract and the route of administration since **Adewole and Ojewole (2009)** working on the aqueous extract of leaves found it moderately toxic when using the intraperitoneal route. A review by **Carlini (2003)** on plants and the central nervous system, shows that *Ginkgo biloba* a plant which is generally used for amelioration of symptoms of anxiety, depression and memory deficits, can increase seizures, as observed with AnMs. In fact, there is evidence to suggest that some part of the plant may be epileptogenic (the seeds) while other parts (the leaves and the roots) may protect

against seizure activity. These effects can be due to the presence of some secondary metabolites in the extract. Among these substances are acetogenins, compounds found in the annonaceae family plant. Their presence in some parts of *A. muricata* can have an impact on dopaminergic neurones **(Lannuzel *et al.*, 2003; Yuan *et al.*, 2003)**. Meanwhile, according to OECD specification, we can say that none of the ethanolic extracts *of Annona muricata* were toxic, except a slight toxicity from seeds. Other authors studying the plant have come to the same conclusion particularly when working on leaves **(Larbie *et al.*, 2011)**.

The extracts with highest activity did not show any severe effect on laboratory mice. The few signs observed disappeared few minutes later and this may be attributed to the injection. On the contrary the fractions were cytotoxic to LLCMK2 cells with the highest IC50 being 125.8±1.07µg/ml, this for J18 among the fractions tested, while the Product P106 was not cytotoxic. This result, when compared to the acute toxicity suggests that the *in vitro* toxicity must not necessarily imply an *in vivo* toxicity. This was also observed on the study conducted by **Samje *et al.*, (2014)** on 2 plants of the Rubiaceae family: *M. lucida* and *C. laurinum*. This can be explained by the fact that the gastro intestinal pathway can play a detoxification role.

Until now no study has been done to evaluate the developmental toxicity (teratogenicity) of *A.muricata*. Toxicological tests using cells or mice are valuable for evaluating the toxicity and teratogenicity of drugs or chemicals. However, these assays have limitations. Cell-based systems are limited because they produce only *in vitro* results. Although mice are good animal models for various experiments, it is very difficult to directly observe teratogenic and toxic changes in utero during embryogenesis. Therefore, development of an alternative animal model system for toxicity and teratogenicity tests is required to overcome problems with current *in vitro* and animal models **(Chae *et al.*, 2015)**, the Frog Embryo Teratogenesis Assay: Xenopus (FETAX). The FETAX test provides a rapid, simple, and cost-effective method for performing preliminary evaluations of a compound's developmental toxicity **(Pekmezekmek *et al.*, 2014)**. Over other test it also has the advantage to evaluate a large number of paramaters in one study: mortality, malformation, and growth inhibition **(Martini *et al.*, 2012; Chae *et al.*, 2015)**. It is a test on organ generation and formation. Mechanisms associated with organ formation display a high degree of evolutionary conservation among animals. During the development of Xenopus laevis embryos, the initial 96 hours bears numerous resemblances to the developmental processes observed in the embryos of other animals, including humans; these 96 hours correspond to the first trimester

of a human embryo in terms of development. For this reason, assessment of drug toxicity and teratogenicity in Xenopus laevis embryos with the aid of the FETAX test is highly indicative of potential drug toxicity and teratogenicity for other animals **(Pekmezekmek *et al.*, 2014)**.
Anticonvulsants have been used to manage psychiatric conditions for over 50 years. A survey by **Cantilino *et al.*, 2014**, on many psychotic drugs revealed that anticonvulsants figures among the products hamful to pregnant women (82.7%). It is recognised that some, particularly valproate, carbamazepine and lamotrigine, are human teratogens; studies, mostly in women with epilepsy indicate that valproate and carbamazepine medications independently increase the risk of major congenital abnormalities when administered in the first trimester **(Langan *et al.*, 2013)**.

In our study, the growth of embryos exposed to higher concentrations of the products tested was observed to be significantly inhibited. On average, embryos did not proceed with development past the neurula for the majority of the fractions at the highest concentrations. Some abnormalities like ventral oedema were also observed. This work corroborates with various studies, where the congenital abnormalities were found to increase with dose **(Tomson *et al.*, 2001; Chae *et al.*, 2015)**. The growth delay observed was followed by the death of the embryos in most of the fractions tested. Those surviving after 24 hours were able to reach the stage 45 with apparently no malformations being observed in the majority of the tests. Nevertheless, embryos exposed to the highest concentration of P106 display some malformations; this malformation was severe to the embryos treated with P106 at 20 µg/ml. Except these malformations, in the other surviving embryos no adverse effect was observed; this can suggest that the products were acting by similar mechanism of action **(Fort *et al.*, 1997)**.

It was not possible to determine the teratogenic index (TI) values of all the compounds being tested; this is not an isolated case, since **Pekmezekmek *et al.*, (2015)** when studying the effect of nifedipine and ritodrine were not able to give the TI value of ritodrine. It was not possible to detemine these TI value for all the products due to the lethality of the embryos that empeded the observation of malformations. The observed effects were more pronounced with fractions than pure compound and with the concentration. The different LC50 and TI Values obtained indicate that fractions from AnMr have potential developmental toxic and teratogenic effects during Xenopus embryogenesis.

CONCLUSION AND PERSPECTIVES

CONCLUSION

The present work gives a glimpse in the anticonvulsant activity of *A. muricata*, *A. senegalensis*, *D. adescendes*, *V. doniana*, *F. thoningii* and *A. cordifolia*; followed by the anticonvulsant, analgesic, antioxidant, antiglycating and adverse effects of extracts and fractions of *A. muricata*, which are Cameroonian medicinal plants.

The preliminary study led to the selection of *A. muricata*, of which the twigs'extract displayed an anticonvulsant activity at 200 mg/kg against Pentylenetetrazol induced seizure. Futher studies revealed that leaves, twigs and roots extracts were active against convulsion induced by Pentylenetetrazol and Picrotoxin with the roots' extract being the most active anticonvulsant. The antagonism by flumazenil shows the involvement of BZD receptors in the disease. The fractions on their own particularly J18, J16, and P106 were able to impede convulsions.

Leaves, twigs and roots extracts were able to prevent pain induced by acetic acid, formalin and hot plate tests, with the highest activity obtained from roots at 200 mg/kg. The antagonist naloxone when used showed the implication of opioids receptors in pain. The antioxidant potential was displayed by the extracts of leaves, twigs and roots of *A. muricata* when using different assays such as NO, FRAP and OH. These extracts were also able to prevent the formation of advanced glycation end products in the first step of protein glycation using ribose and glucose, and the second step using glyoxal. The extracts, fractions and product from *A. muricata* therefore exhibited anticonvulsant, analgesic, antioxidant and advanced glycated end products scavenging.

The acute toxicity assay of *A. muricata* proved that many organs of this plant are safe by oral route in relation to their folkloric therapeutic dose; whereas some fractions such as J18 and J16 were cytotoxic and teratogenic.

PERSPECTIVES

At the end of this study, further work can be carried out:

-To determine the anticonvulsant activity using other *in vivo* and *in vitro* models and the implication of receptors in the disease to confirm its mechanism of action

-To determine the analgesic activity of extracts and fractions using other models of pain, in order to confirm the mechanism of action of the plant

-To evaluate the oxidative stress in epilepsy *in vivo*

-To evaluate the antioxidant activities *in vivo* and *in vitro* and antiglycation activities of fractions

-To evaluate the developmental toxicity of the fractions *in vivo* and to determine the different pathways involved

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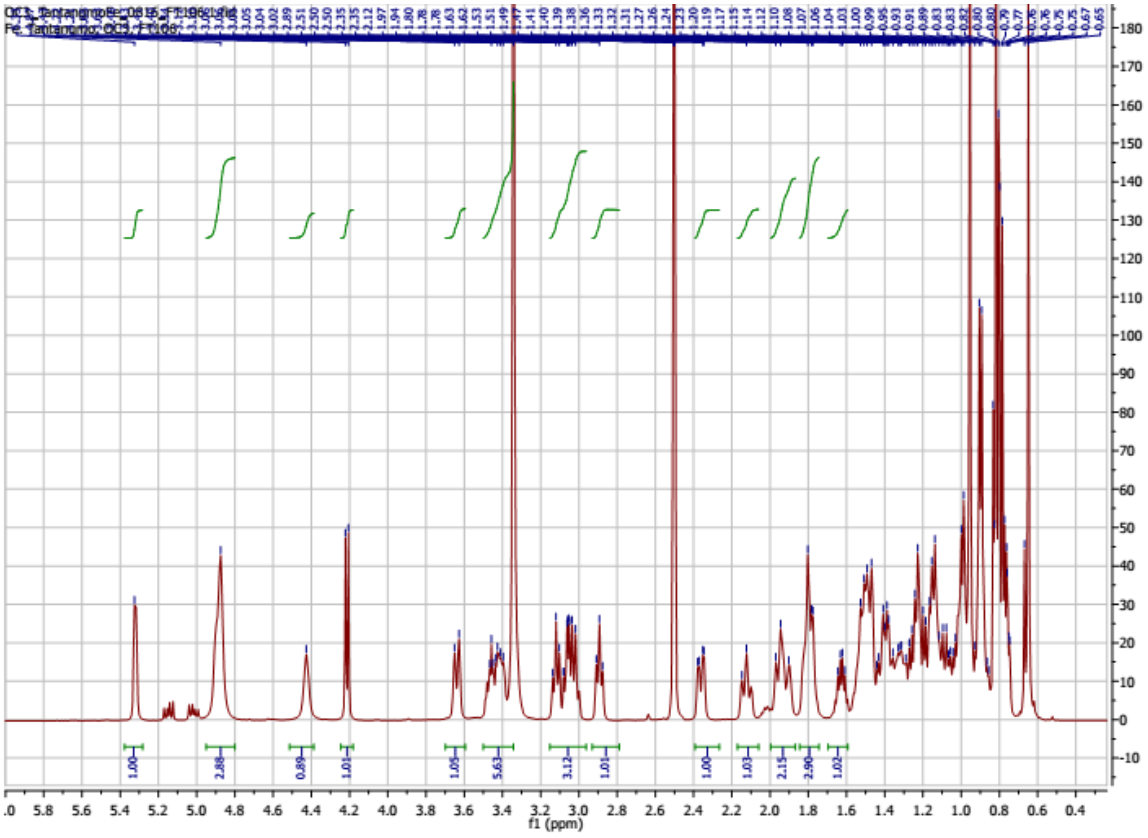
APPENDICES

APPENDICES

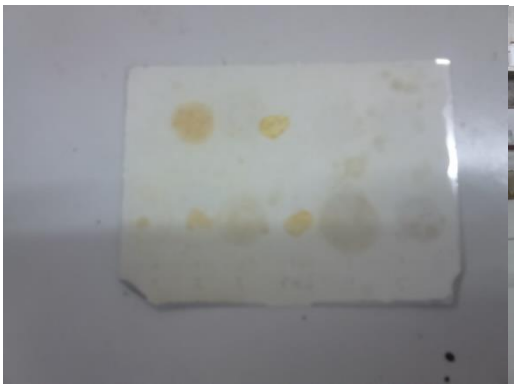
Appendice 1: Table of transformation of percentage to probit values

	0	1	2	3	4	5	6	7	8	9
0		2.67	2.95	3.12	3.25	3.35	3.44	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.62	4.64	4.67	4.70	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.98
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.38	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.56	6.65	6.75	6.88	7.05	7.33

Appendice 2: Spectrum of AnMr P106



Appendice 3: Phytochemical analyis of the fractions



Alkaloids

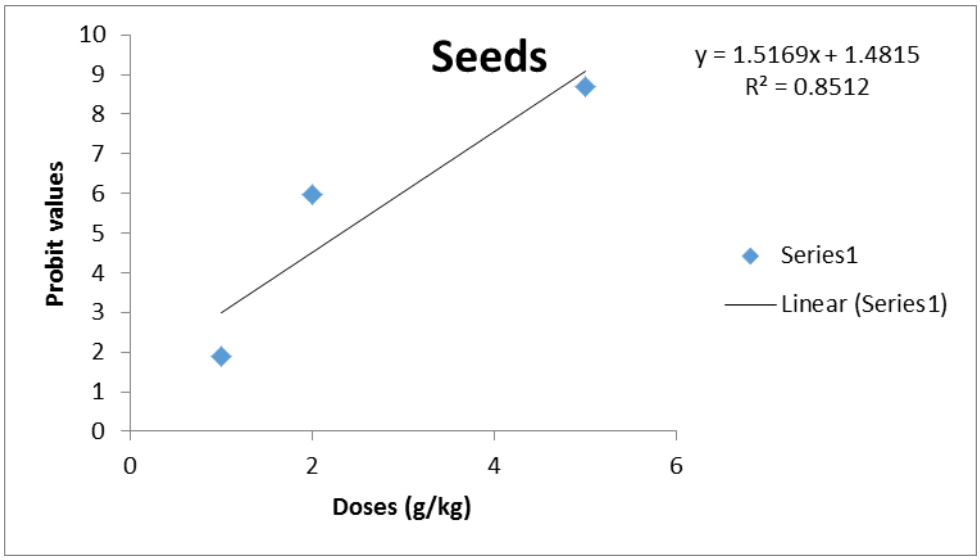
Triterpenes and steroids

Saponins

Tannins

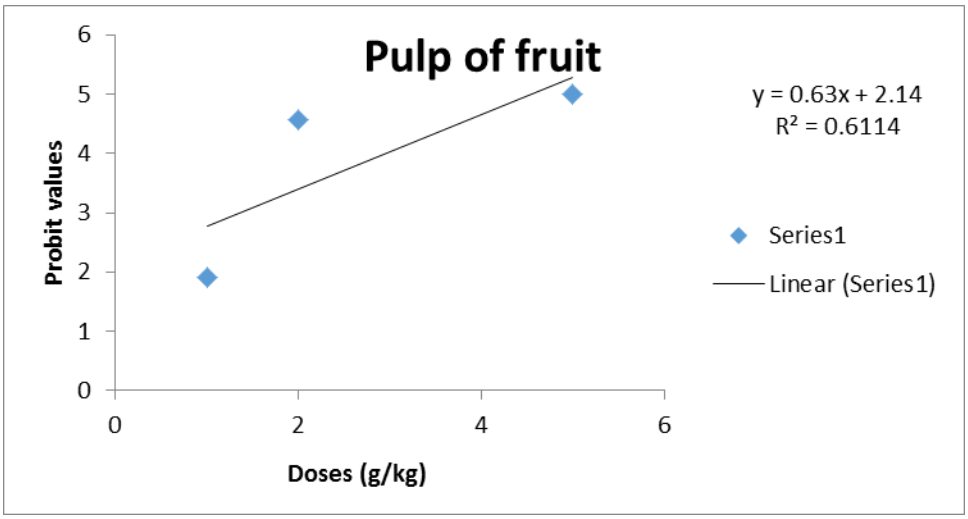
Glycosides

Appendice 4: Graphical determination of the LD50 of the seed extract



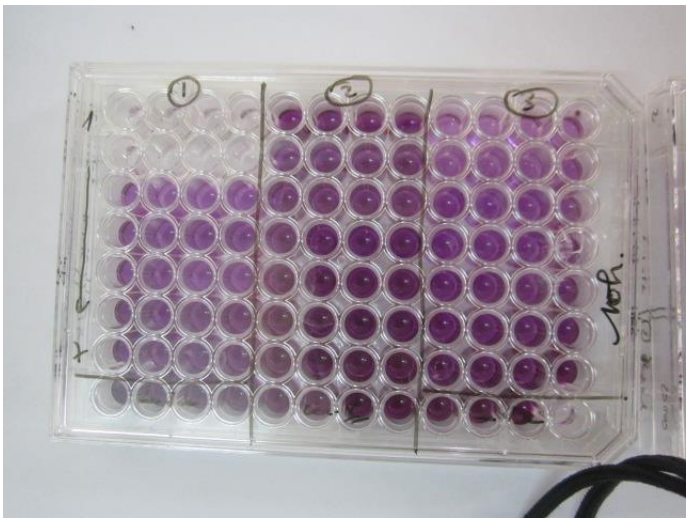
LD50= 2.31 g/kg

Appendice 5: Graphical determination of the LD50 of the pf extract



LD50=4.53 g/kg

Appendice 6: Plate showing variation in colour intensity as a result of different degrees of inhibition



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Anticonvulsant activity of extracts from six Cameroonian plants traditionally used to treat epilepsy

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ABSTRACT

Epilepsy remains one of the leading public health problems that affects about 50 million people worldwide, thus stressing the need for new anticonvulsant drug. This study was designed to evaluate the anticonvulsant activity against Penty lenetetrazole induced–convulsion in mice. Plants were extracted by maceration with water or organic solvents. The extracts were tested against PTZ-induced convulsion by measuring onset seizure, clonic seizure onset, convulsion duration, death time and percentage of protection. *A. cordifolia* leaf extract protected all animals from death at 1000 mg/kg. *A. muricata* stem extract delayed seizures at 200 and 400 mg/kg, while the onset of tonicoclonic (TC) seizures was significantly delayed at the highest doses tested for the seed extract (800 mg/kg). Stem and leaf extracts of *A. senegalensis* significantly delayed seizure onset at all doses. *D. adescendes* extract significantly protected mice from death. *F. thonningii* leaf extract at the smallest dose tested (200 mg/kg), significantly delayed the seizure onset and the occurrence of TC convulsions. Bark extract of *V. doniana* significantly delayed the seizure onset at all doses tested. The results obtained corroborate with the traditional claims that these plants can be a valuable source of new anticonvulsant compounds.

Keywords: Plant extracts, PTZ-induced seizures, anticonvulsant effect, mice.

INTRODUCTION

Epilepsy is one of the most common neurological disorders with no age, social, sexual or geographical boundaries (WHO, 2001). It affects about 50 million people worldwide (Moshi et al., 2005). This epilepsy prevalence far exceeds those in developing countries (Baskind and Birbeck, 2005). In Cameroon, epilepsy prevalence reaches 6% in some endemic areas (Njamnshi et al., 2009a) such as Badissa in the Center region (Njamnshi et al., 2009b). In most societies, epilepsy is regarded as possession by evil spirits, and is seen as a highly contagious and shameful disease (Moshi et al., 2005). This stigma has long been recognized as a major burden to people with epilepsy and their families especially in sub-Saharan Africa

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where the combination of poverty, social role expectations, limited medical care, and traditional beliefs coalesce to severely limit their live span (Baskind and Birbeck, 2005).

In the developed countries, where drugs are easily available, epilepsy responds to treatment in up to 70% of the patients (Ilodigwe et al., 2010). However, in developing countries, 75% of people with epilepsy do not receive the appropriate treatment (WHO, 2001) and their epilepsy remains uncontrolled, rendering the patients unproductive in all spheres of life. In the scarcity of such drugs, they mainly rely on traditional medicines as the only mains of control in both rural and urban areas (Moshi et al., 2005) of these countries. In fact, 80% of the population relies on traditional medicines or folk remedies for their primary health care need (Hedge et al., 2009). In Cameroon, traditional indigenous medicine still plays an important role in epilepsy management with up to 25% of epileptic patients relying on traditional treatment (Njamnshi et al., 2009c). Medicinal plants are believed to be an important source of new drugs and lead compounds with potential therapeutic effects. The inclusion of those with proven safety and efficacy in the healthcare programs of developing countries is encouraged by the WHO because of the great potential they possess in combating various diseases (Chindo et al., 2009). Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity (Hedge et al., 2009). In this line, the present work was designed to investigate the anticonvulsant activity of extracts from six medicinal plants traditionally used to treat epilepsy in Cameroon.

MATERIALS AND METHODS

Plant collection

The plant samples were collected in Yaoundé and its surroundings, Kon Yambettain the center region and Bafou in the western region of Cameroon during the month of August 2010. The plant species were identified at the Cameroon National Herbarium in Yaoundé where voucher specimens were deposited under the following identification numbers:

Alchornea cordifolia (Euphorbiaceae): 4682/ SRFK

Annona muricata (Annonaceae): 3289/HNC

Annona senegalensis (Annonaceae): 40060/ HNC

Desmodium adescendes (Fabaceae): 10258/ SRF/CAM

Ficus thonningii (Moraceae): 44042/HCN

Vitexdoniana (Verbenaceae): 21147/SRF/ CAM

Plants extraction and dosage preparation

Plants extraction

Leaves, seeds, stems, stem barks, twigs, or whole plants were harvested, shade dried and ground using a laboratory blender.100 g of powdered materials of *A. cordifolia* leaves, *A. senegalensis* twigs and leaves, *D. adescendes* (whole plant), and *V. doniana* stem barks were individually macerated in 1L of MeOH/CH_2Cl_2 (1:1) at room temperature for 72 hours.100 g of *A. muricata* seed and stem and *F. thoningii* leaf and stem bark powders were similarly macerated in 1L of EtOH and water respectively. Upon maceration, filtrates were prepared using Whatman paper n°1. The aqueous filtrate was dried in an oven (Memmert) at 45 ºC for 48hours. The organic solvents were evaporated under reduced pressure using a rotary evaporator (BÜCHI) at 40 °C then 65 °C for the MeOH/CH2Cl2 (1:1) extract and at 80 ºC for the ethanolic extract.

Extracts dissolution and dosage preparation

The extracts were diluted as needed for experiments each day. *F. thonningii* and *V. doniana* extracts were dissolved in water, while *A. cordifolia* and *D. adescendes* extracts were dissolved in DMSO/tween 20 and *A.*

muricata and *A. senegalensis* were dissolved in olive oil. Extracts were tested at doses of 1000, 700 and 500 mg/kg for *D. adescendes* and at 800, 400 and 200 mg/kg for *A. muricata, A, senegalensis, F. thonningii* and *V. doniana*. The convulsion inducer, Pentylenetetrazole (PTZ) and the positive control, Phenobarbital (PHB) were purchased from Sigma Chemical. They were dissolved in normal saline. PTZ was prepared freshly for each experiment and administered intraperiton eally (*ip*) in a volume of 10 ml/kg body weight. Control animals received the same volume of vehicle.

Animals

Healthy male and female Balb/c mice (*Mus musculus*) of 6 to 8 weeks, weighing 20 to 24 g were used. They were housed at the animal house of the Faculty of Medicine and Biomedical Science of the University of Yaoundé 1 (Cameroon). Animals were separated into groups of 6 mice for experimentation and were maintained under standard conditions with free access to food and water *ad libitum*. All animals were fasted for 16 h, but still allowed free access to water prior to experiments. Laboratory animal care and experimentation were performed under protocols approved by the animal care and use committee of the National Institute of Neurological Disorders and Stroke (NINDS) in strict compliance with the Guide for the care and use of laboratory animals of the National Research Council (National Research Council, 1996, National Academy Press, Washington, DC).

Evaluation of the anticonvulsant activity

A total of 31 experimental groups of 6 mice each were used for anticonvulsant evaluation using the method of Hosseinzadeh and Parvadeh (2004) with minor modifications.

Statistical analysis

All the results obtained were expressed as mean ± standard deviation and were subjected to analysis of variance (ANOVA), and post hoc significance testing with Tukey's multiple comparison test using Graph pad 5.0 software. Significance was tested at 0.05.

RESULTS

The results obtained from this study are presented and discussed below. The parameters recorded after administration of plant extracts and PTZ to mice at the dose of 90 mg/kg are summarized in Table 1.

From Table 1, it appears that *A. cordifolia* leaf extract at up to 1000 mg/kg did not significantly modify ($P > 0.05$) the time to seizure onset, nor the appearance of tonicoclonic (TC) convulsions; but the duration of convulsions was significantly reduced ($P<0.05$) at all doses compared to PTZ. All animals were protected from death at the dose of 1000 mg/kg, 50% of mice were protected from death at 700 mg/kg.

A. muricata stem extract at 200 and 400 mg/kg was able to delay seizures induced by PTZ in a significant manner ($P<0.05$), while the onset of TC seizures was significantly delayed ($P<0.05$) at the highest dose tested for seeds (800 mg/kg) and the smallest for stem (200 mg/kg). All doses of these extracts significantly reduced ($P<0.05$) seizure duration and an entire protection was observed for the stem and seed extracts at 200 mg/kg.

Twig and leaf extracts of *A. senegalensis* significantly delayed ($P<0.05$) seizure onset at 800 mg/kg while the seizure duration was significantly reduced ($P<0.05$) at all doses. None of these extracts significantly delayed ($P>0.05$) the time of onset of TC convulsions compared to the nontreated negative control, but they delayed the time of death. These extracts significantly delayed ($P<0.05$) the onset of seizure in a comparable manner to PHB at 10 and 20 mg/kg.

Table 1: Anticonvulsant activity of studied plants extracts.

	Dose (mg/kg)	Seizure onset (S)	Clonic seizure onset (S)	Convulsion duration (S)	Death time (S)	% Protection
			Extracts			
	500	59.67±7.84^{b}	99.50±6.95^{b}	50.83±7.65^{a}	261,50±44.49	33.33
AlCl	700	57.67±4.13^{b}	143.20±59.05^{b}	98.00±8.78^{ab}	682.67±6.4^{a}	50
	1000	67.17±11.83^{b}	155.20±21.99^{b}	73.83±9.94^{ab}	/	100
	200	70.50±6.95^{b}	124.00±11.46^{b}	56.83±5.23^{a}	/	100
AnMs	400	84.33±42.22^{b}	94.00±1.41^{b}	93.00±20.73^{ab}	186.67±12.22a	50
	800	71.50±10.50^{b}	360.50±0.70^{ab}	72.00±10.13^{ab}	107.33±24.82^{a}	50
	200	132.67±10.05^{a}	280.00±14.14^{ab}	52.33±6.65^{a}	/	100
AnMst	400	114.83±5.70^{a}	229.33±31.00^{b}	50.00±7.04^{a}	/	100
	800	71.50±8.52^{b}	146.00±1.41^{b}	60.67±6.56^{a}	917±4.24^{a}	66.66
	200	69.83±10.06^{b}	155.67±7.37^{b}	46.00±7.7^{a}	/	100
AnStw	400	69.17±9.49^{b}	111.00±11.40^{b}	49.50±9.64^{a}	/	100
	800	89.00±19.75^{ab}	186±81.34^{b}	48.17±5.26^{a}	183	83.33
	200	62.50±3.01^{b}	98.50±7.32^{b}	80.20±16.75^{ab}	/	100
AnSl	400	61.50±3.01^{b}	126.00±1.82^{b}	55.00±8.57^{a}	/	100
	800	102.50±12.19^{ab}	209.00±4.96^{b}	106.20±4.43^{ab}	/	100
	500	63.33±3.67^{b}	133.40±5.85^{b}	65.40±5.03^{a}	/	100
DeA	700	67.00±8.73^{b}	64.00±18.60^{ab}	59.40±26.86^{a}	/	100
	1000	72.57±9.48^{b}	83.60±18.87^{ab}	72.60±9.15^{ab}	503	83.3
	200	74.25±4.34^{b}	81.00±9.89^{b}	146.00±13.92^{ab}	/	100
FiTsb	400	83.25±15.88^{b}	132.25±63.20^{b}	48.75±8.53^{a}	/	100
	800	64.29±20.62^{b}	165.33±15.34^{b}	97.00±19.59^{ab}	207.50±57.27^{a}	66.66
	200	104.00±40.75^{ab}	257.00±74.57^{ab}	120.00±7.87^{ab}	302.50±57.27	66.66
FiTl	400	98.00±25.80^{ab}	286.00±8.18^{ab}	47.25±9.25^{a}	/	100
	800	84.83±19.40^{b}	298.05±4.14^{ab}	84.75±18.99^{ab}	/	100
	200	76.50±11.84^{b}	121.75±39.30^{b}	64.45±0.50^{a}	328.00±15.68	33.33
ViDsb	400	92.17±33.30^{ab}	73.00±8.60^{ab}	96.20±20.31^{a}	343.25±56.64	33.33
	800	129.17±10.76^{ab}	77.50±10.40^{ab}	89.50±9.53^{a}	395.75±85.45	33.33
			Controls			
PHB	10	58.29±1.11^{b}	1468.0±10.08^{b}	64.43±0.78^{a}	560.29±3.81^{a}	0
(ref	20	67.00±3.57^{b}	/	57.83±0.753^{a}	1474.00±1.41^{a}	66.66
drug)	40	194.86±2.67^{a}	/	43.00±2.16^{a}	/	100
PTZ	90	46.13±6.19^{b}	172.25±49.22^{b}	232.00±33.96	374.63±39.46	0

Experimental data obtained are presented as means±SEM. /: animals were protected from the observed phenomenom; *AlCl: Al. cordifolia leave, AnMs: A. muricata seed, AnMst: A. muricatastem, AnSl: A. senegalensis leave, AnStw: A. senegalensis twig, DeA: D. Adescendes, FiTsb: F. thoningii* stem bark, *FiTl: F. thoningii leave, ViDsb: V. doniana* stem bark, PHB: phenobarbital, PTZ: pentylenetetrazol, the chemical convulsant; (S): seconds; ^{a}: significant compared to PTZ; ^{b}: significant compared to PHB. The significativity was observed at P<0.05.

D. adescendens, at doses of 500, 700 and 1000 mg/kg, significantly protected (P<0.05) mice from death, but was ineffective in delaying either the seizure onset or that of the TC convulsions.

Leaf and stem bark extracts of *F. thonningii* significantly delayed (P<0.05) the seizure onset and the appearance of TC convulsions at 200 mg/kg.

The bark extract of *V. doniana* significantly delayed (P<0.05) the seizure

onset at all doses tested. Convulsion duration was significantly reduced compared to the nontreated negative control. Besides, the activity of this extract at 200 mg/kg was similar to that of PHB at 10 mg/kg. All tested doses exhibited comparable levels of protection (33.33%), but lower than that of the treated positive control; PHB with 66 and100% at 20 and 40mg/kg respectively.

All the tested extracts significantly reduced ($P<0.05$) the convulsion duration compared to negative control. *F. thonningii* leaves and stem bark (800 mg/kg, 200 mg/kg), *D. adescendes* at 1000 mg/kg, *A. senegalensis* leaves (800 mg/kg, 200 mg/kg) and *A. cordifolia* (700 mg/kg, 1000 mg/kg) presented values far greater than that of the positive control group treated with the reference drug, phenobarbital (PHB).

DISCUSSION

Epilepsy is a common neurological condition associated with an alteration in psychological, emotional and educational parameters (Aldarmaa et al., 2010).

Prevention of PTZ induced seizures in laboratory animals is the most commonly used initial screening test for discovery of anticonvulsant drugs. It is mostly believed that inhibitors exert the iraction by acting as antagonists at the picrotoxinin-sensitive site at the γ-aminobutyric acid ($GABA_A$) receptor complex (Hansen et al., 2004). Accordingly, Huang et al. (2001) indicated that PTZ interacts with overlapping but distinct domains of the $GABA_A$ receptor. Within 2-4 seconds of PTZ administration, there is an increase in blood oxygen levels. This increase was observed at the thalamus, especially the anterior thalamic nuclei. The peak of this activity which is twofold greater than in all other thalamic areas is reached few seconds before the first seizure (Brevard et al., 2006).

Extracts from *F. thonningii*, a plant of the Moraceae family presented a significant anticonvulsant effect against PTZ-induced convulsion. No previous scientific study had ever revealed its anticonvulsant activity but however, Noumi and Fozi (2003) reported the use of a related plant (*Ficus sycomorus*) in combination with other plants such as *Asystasia gangetica*, *Ficus mucoso*, and *Aframamum melegueta* in Fongo-Tongo community in Cameroon to cure epileptsy. Beside this report, Tanzanian healers have been reported to boil the bark of *Ficus sycomorus* in the treatment of epilepsy (Moshi et al., 2005). Another study conducted by Sandabe et al. (2003) revealed that aqueous extract of *F. sycomorus* stem bark at the doses of 200 and 400 mg/kg protected laboratory animals from death while Ibrahim et al., (2008), using the flavonoid fraction of F. *sycomorus,* obtained an anticonvulsant activity against the PTZ and maximal electroshock seizure (MES) models at 10 mg/kg. Singh and Goel (2009), working on another species of the same genus, *Ficus religiosa*, observed that the methanolic extract of figs at the maximum dose tested (100 mg/kg) was not effective against the PTZ model but was able to protect animal from MES seizure starting at 25 mg/kg, the smallest dose tested. Similarly, the saponin fraction of the same plant at 1 mg/kg was able to decrease PTZ-induced seizures (Singh et al., 2013). Another species, *Ficus platyphylla*, studied by Chindo et al. (2009) revealed that saponins extracted from the stem bark, tested at the minimal dose (25 mg/kg) were effective against *in vivo* and *in vitro* models of epilepsy. In our study, leaves and stem bark of *F. thonningii* at 200 mg/kg, the smallest dose tested, significantly delayed the onset of seizures in general and tonicoclonic seizures in particular. At this dose, the stem bark extract entirely protected the animals, while 66.66% of mice were protected by leaf extract.

An ethnobotanical survey of epilepsy treatment in Fongo-Tongo village, Western region of Cameroon by Noumi and Fozi (2003), revealed the use of *D. adescendes* leaves, *V. doniana* and *A. senegalensis* stem bark as drugs against epilepsy. They were effective when given to some epileptics who

claimed to be cured after an average period of 18 months. The ethanolic extract of *D. adescendes* tested at 300 mg/kg, was effective against clonic convulsions induced by PTZ as reported by N'gouemo et al. (1997). In our study, the ethanolic extract of *D. adescendes* was able to protect mice from death at all doses tested, but was neither effective against seizure onset nor tonicoclonic seizures. Concerning *A. senegalensis*, Konate et al. (2012) reported the anticonvulsant property of the methanolic extract and aqueous fraction of the stem bark of *A. senegalensis* at 400 mg/kg. This result is different from what we obtained at the same dose and could be explained by the plant part used in our study or extraction solvent used. However, the activity observed at 800 mg/kg, particularly the onset seizure in our study confirmed the anticonvulsant property of this plant which did reduce the convulsion duration at all doses tested.

A. muricata stem extract at 200 and 400 mg/kg significantly delayed seizures, while the onset of TC seizures was significantly delayed at 200 and 800 mg/kg for the stem and seeds respectively. Our findings are similar to those obtained by N'gouemo et al. (1997) with the ethanolic extract of leaves of this plant at the dose of 300 mg/kg. Twigs and seeds significantly reduced seizure duration and conveyed entire protection at 200 mg/kg body weight in mice.

A. cordifolia leaves did not significantly modify the time to seizure onset, nor the onset of tonicoclonic (TC) convulsions, but significantly reduced the duration of convulsions. In the contrary Pedersen et al. (2009) in a previuous study showed inhibition of spontaneous epileptic form discharges by the ethanolic extract of *A. cordifolia* leaves from Mali. This difference could be due to the difference in solvents used, or in the *in vitro* model compared to the *in vivo* approach used in our study. In fact, the *in vitro* active compounds could have been metabolized to non-active derivatives in the animal's organism or did not cross the blood brain barrier. Otherwise, the active compounds present in the extract do not show effect on the PTZ model (Pedersen et al., 2009).

A comparison between the different extracts, show that the extract *A. Muricata* stem bark at 200 and 400 mg/kg, *A. senegalensis* stem bark and leaves at 800 mg/kg, *F. thonningii* leaves at 200 and 400 mg/kg and *V. doniana* at 400 and 800 mg/kg were able to delay seizure onset with the best activity observed for the *A. muricata* stem bark extract.

The protection offered by plant extracts might be dependent upon synergistic effects of constituents. Indeed, phytochemical analysis of organs of these plants revealed the presence of alkaloids, saponins, tanins, anthraquinones, terpenoids, and flavonoids in the ethanolic and aqueous leaf extracts of *V. doniana* (Agbafor and Nwachukwu, 2011). Ayodele et al., (2007) working on the ethanolic extract of the leaves of *A. cordifolia*, found alkaloids, tannins, saponins, flavonoids and steroidal ring. The authors also stated a relationship between *A. cordifolia* intake and the prevention of neurodegenerative disease. A literature review, conducted by Rastogi et al. (2011), revealed that *D. adscendens* contains triterpenoids, saponins, tetrahydroisoquinolones, phenyle thylamines and indole-3-alkyl amines isolated from the leaves. Three active components were identified as the known triterpenoid glycosides dehydrosoya saponin I (DHS-I), soya saponin I and soya saponin III. Dehydrosoya saponin, the major saponin compound of the plant was the most potent potassium channel opener. As in the case with plants of the Annonaceae family, tannins, steroids and cardiac glycosides were the major compounds found in *A. muricata* (Gajalakshmi et al., 2012), while Arthur et al. (2011) working with the leaf aqueous extract of *A. muricata* found that saponins and flavonoids are also present in the plant. Another report by Boyom et al. (1996) revealed the presence of sesquiterpernes, particularly β- caryophyllene. As concerns *A.*

senegalensis, alkaloids, terpenoids, particularly monoterpenes were reported by Boyom et al. (1996). Saponins, another compound present in *A. senegalensis* was found in the aqueous extract of root bark (Konate et al., 2012). Added to this, resins, alkaloids, proteins, anthraquinones, sterols, glycosides and terpenes were also present in *A. senegalensis* (Adzu et al., 2005).

Some extracts showed no effects in our study despite traditional claims, particularly concerning the onset of seizures, but they were all able to reduce the convulsion duration. Differences in metabolism between rodents and humans might explain such differences and justify the use in traditional medicine despite the lack of effect in the animal model (Pedersen et al., 2009).

As far as the mechanism of action are concerned, the extract either increases the threshold of PTZ- induced convulsion or protects mice against convulsions induced by PTZ. Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents and drugs that enhance inhibitory neuro-transmission by GABAA receptors (benzodiazepine, phenobarbital and valproate) (Chindo et al., 2009). The fact that the extracts protected animal against PTZ-induced seizures may suggest that they contain compounds that facilitate GABAergic transmission (Ayanniyi and Wannang, 2008).

Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, PTZ-induced acute seizures remains 'Gold standard' in the early stages of testing. The PTZ model is assumed to identify anticonvulsant drugs effective against petit mal seizures. The effects of extracts against PTZ-induced seizures thus suggest anticonvulsant efficacy against petit mal epilepsy in man (Chindo et al., 2009).

Conclusion

The pharmacological screening of the extracts of Cameroonian plants traditionally used against epilepsy, led to the identification of several extracts with potential anticonvulsant properties. However further investigation using other models of epilepsy are required to characterize their anticonvulsant and antiepileptic effects.

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