THE UNIVERSITY OF YAOUNDE I
UNIVERSITE DE YAOUNDE I

FACULTY OF SCIENCE
FACULTE DES SCIENCES

GRADUATE PROGRAM FOR LIFE SCIENCES, HEALTH AND THE ENVIRONMENT
CENTRE DE RECHERCHE ET FORMATION DOCTORALE- SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT

DEPARTMENT OF BIOCHEMISTRY
DEPARTEMENT DE BIOCHIMIE

LABORATORY FOR PHYTOBIOCHEMISTRY AND MEDICINAL PLANTS STUDIES
LABORATOIRE DE PHYTOBIOCHIMIE ET D'ETUDE DES PLANTES MEDICINALES

ANTIMICROBIAL AND BIOCONTROL AGENTS UNIT
UNITE DES AGENTS ANTIMICROBIENS ET DE BIOCONTROLE

Activity–guided discovery of highly potent antiplasmodial subfractions from *Terminalia mantaly* (*Combretaceae*)

Thesis presented in partial fulfilment of the requirements for the award of a Doctorate – Ph.D. in Biochemistry

By

JIATSA MBOUNA Cedric Derick
Registration Nº 07R326
M. Sc in Biochemistry

Thesis Director

FEKAM BOYOM Fabrice
Professor

2021 – 2022

UNIVERSITY OF YAOUNDE I

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Graduate Program for Life Sciences
Health and the Environment

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Centre de Recherche & de Formation Doctorale,
Science de la Vie, Sante & Environnement

DEPARTMENT OF BIOCHEMISTRY
DEPARTEMENT DE BIOCHIMIE

CERTIFICATE OF CORRECTION OF THE DOCTORAL THESIS
ATTESTATION DE CORRECTION DE LA THESE DE DOCTORAT

We, the undersigned Thesis Director, and Board of Assessors of the Doctorate/PhD thesis in Biochemistry entitled: **Activity – guided discovery of highly potent antiplasmodial subfractions from *Terminalia mantaly* (*Combretaceae*)** defended on January 6, 2022, by **Mr. JIATSA MBOUNA Cedric Derick** - registration number 07R326, hereby certify that the candidate has completed the corrections of the above-mentioned thesis as requested by the Examiners.

We hereby certify that the Board of Assessors is satisfied with the corrections made and recommend that the Doctorate/PhD degree be awarded to the candidate.

Yaounde, the 30/05/2022

Chair Board of Assessors

MBACHAM Wilfred FON
Professor
MS,DE,ScD(Harvard), FASI, FCAS, FAAS
Public Health Biotechnologist

Thesis Director

FEKAM BOYOM Fabrice
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Professor

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Senior Research Officer

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MOUNDIPA FEWOU Paul,
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Division de la Programmation et du Suivi des Activités Académiques

LIST OF PERMANENT TEACHING STAFF **LISTE DES ENSEIGNANTS PERMANENTS**

ACADEMIC YEAR 2021 - 2022

(By Department and by Grade)

Last updated: 22 September 2021

ADMINISTRATION

DEAN: TCHOUANKEU Jean-Claude, Associate Professor

VICE – DEAN / DPSAA: ATCHADE Alex de Théodore, Associate Professor

VICE – DEAN / DSSE: NYEGUE Maximilienne Ascension, Professor

VICE – DEAN / DRC: ABOSSOLO Monique, Associate Professor

Head of Administrative and Financial Division: NDOYE FOE Marie C.F., Associate Professor

Head of Division of Academic affairs, Research, and Corporation: AJEAGAH Gideon AGHAINDUM, Professor

1- DEPARTMENT OF BIOCHEMISTRY (BCH) (38)			
N°	**NAMES AND FIRST – NAMES**	**GRADE**	**OBSERVATIONS**
1	BIGOGA DIAGA Jude	Professor	On duty
2	FEKAM BOYOM Fabrice	Professor	On duty
3	FOKOU Elie	Professor	On duty
4	KANSCI Germain	Professor	On duty
5	MBACHAM FON Wilfried	Professor	On duty
6	**MOUNDIPA FEWOU Paul**	**Professor**	**Head of Department**
7	NINTCHOM PENLAP V. spouse BENG	Professor	On duty
8	OBEN Julius ENYONG	Professor	On duty
9	ACHU Merci BIH	Associate Professor	On duty
10	ATOGHO Barbara Mma	Associate Professor	On duty
11	AZANTSA KINGUE GABIN BORIS	Associate Professor	On duty
12	BELINGA born NDOYE FOE M. C. F.	Associate Professor	Head of AFD FS UY1
13	BOUDJEKO Thaddée	Associate Professor	On duty
14	DJUIDJE NGOUNOUE Marcelline	Associate Professor	On duty
15	EFFA NNOMO Pierre	Associate Professor	On duty
16	EWANE Cécile Anne	Associate Professor	On duty
17	MOFOR spouse TEUGWA Clotilde	Associate Professor	Service Inspector MINESUP
18	NANA Louise spouse WAKAM	Associate Professor	On duty
19	NGONDI Judith Laure	Associate Professor	On duty
20	NGUEFACK Julienne	Associate Professor	On duty
21	NJAYOU Frédéric Nico	Associate Professor	On duty
22	TCHANA KOUATCHOUA Angele	Associate Professor	On duty
23	AKINDEH MBUH NJI	Senior Lecturer	On duty
24	BEBEE Fadimatou	Senior Lecturer	On duty
25	BEBOY EDJENGUELE Sara Nathalie	Senior Lecturer	On duty
26	DAKOLE DABOY Charles	Senior Lecturer	On duty
27	DJUIKWO NKONGA Ruth Viviane	Senior Lecturer	On duty
28	DONGMO LEKAGNE Joseph Blaise	Senior Lecturer	On duty
29	FONKOUA Martin	Senior Lecturer	On duty
30	KOTUE KAPTUE Charles	Senior Lecturer	On duty
31	LUNGA Paul KEILAH	Senior Lecturer	On duty
32	MANANGA Marlyse Josephine	Senior Lecturer	On duty
33	MBONG ANGIE M. Mary Anne	Senior Lecturer	On duty
34	Palmer MASUMBE NETONGO	Senior Lecturer	On duty
35	PECHANGOU NSANGOU Sylvain	Senior Lecturer	On duty
36	MBOUCHE FANMOE Marceline Joëlle	Assistant	On duty
37	OWONA AYISSI Vincent Brice	Assistant	On duty
38	WILFRIED ANGIE Abia	Assistant	On duty
2- DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (ABP) (46)			
1	AJEAGAH Gideon AGHAINDUM	Professor	Head of DAASR FS UY1
2	**BILONG BILONG Charles-Félix**	**Professor**	**Head of Department**
3	DIMO Théophile	Professor	On duty
4	DJIETO LORDON Champlain	Professor	On duty
5	DZEUFIET DJOMENI Paul Désiré	Professor	On duty
6	ESSOMBA born NTSAMA MBALA	Professor	Vice – Dean FMSB UYI
7	FOMENA Abraham	Professor	On duty
8	KAMTCHOUING Pierre	Professor	On duty

9	KEKEUNOU Sévilor	Professor	On duty
10	NJAMEN Dieudonné	Professor	On duty
11	NJIOKOU Flobert	Professor	On duty
12	NOLA Moïse	Professor	On duty
13	TAN Paul VERNYUY	Professor	On duty
14	TCHUEM TCHUENTE Louis Albert	Professor	Program Coordinator MINSANTE
15	ZEBAZE TOGOUET Serge Hubert	Professor	On duty
16	BILANDA Danielle Claude	Associate Professor	On duty
17	DJIOGUE Séfirin	Associate Professor	On duty
18	JATSA BOUKENG Hermine spouse MEGAPTCHE	Associate Professor	On duty
19	LEKEUFACK FOLEFACK Guy B.	Associate Professor	On duty
20	MEGNEKOU Rosette	Associate Professor	On duty
21	MONY Ruth spouse NTONE	Associate Professor	On duty
22	NGUEGUIM TSOFACK Florence	Associate Professor	On duty
23	TOMBI Jeannette	Associate Professor	On duty
24	ALENE Désirée Chantal	Senior Lecturer	On duty
25	ATSAMO Albert Donatien	Senior Lecturer	On duty
26	BELLET EDIMO Oscar Roger	Senior Lecturer	On duty
27	DONFACK Mireille	Senior Lecturer	On duty
28	ETEME ENAMA Serge	Senior Lecturer	On duty
29	GOUNOUE KAMKUMO Raceline	Senior Lecturer	On duty
30	KANDEDA KAVAYE Antoine	Senior Lecturer	On duty
31	MAHOB Raymond Joseph	Senior Lecturer	On duty
32	MBENOUN MASSE Paul Serge	Senior Lecturer	On duty
33	MOUNGANG Luciane Marlyse	Senior Lecturer	On duty
34	MVEYO NDANKEU Yves Patrick	Senior Lecturer	On duty
35	NGOUATEU KENFACK Omer Bébé	Senior Lecturer	On duty
36	NGUEMBOK	Senior Lecturer	On duty
37	NJUA Clarisse Yafi	Senior Lecturer	Chief of Division UBa
38	NOAH EWOTI Olive Vivien	Senior Lecturer	On duty
39	TADU Zephyrin	Senior Lecturer	On duty
40	TAMSA ARFAO Antoine	Senior Lecturer	On duty
41	YEDE	Senior Lecturer	On duty
42	BASSOCK BAYIHA Etienne Didier	Assistant	On duty
43	ESSAMA MBIDA Désirée Sandrine	Assistant	On duty
44	KOGA MANG DOBARA	Assistant	On duty
45	LEME BANOCK Lucie	Assistant	On duty
46	YOUNOUSSA LAME	Assistant	On duty
3- DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (PBP) (33)			
1	AMBANG Zachée	Professor	Chief of Division UYII
2	BELL Joseph Martin	Professor	On duty
3	DJOCGOUE Pierre François	Professor	On duty
4	MBOLO Marie	Professor	On duty
5	MOSSEBO Dominique Claude	Professor	On duty
6	**YOUMBI Emmanuel**	**Professor**	**Head of Department**
7	ZAPFACK Louis	Professor	On duty
8	ANGONI Hyacinthe	Associate Professor	On duty
9	BIYE Elvire Hortense	Associate Professor	On duty

10	KENGNE NOUMSI Ives Magloire	Associate Professor	On duty
11	MALA Armand William	Associate Professor	On duty
12	MBARGA BINDZI Marie Alain	Associate Professor	Technical Adviser MINESUP
13	NDONGO BEKOLO	Associate Professor	Study Officer MINRESI
14	NGODO MELINGUI Jean Baptiste	Associate Professor	On duty
15	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	On duty
16	TONFACK Libert Brice	Associate Professor	On duty
17	TSOATA Esaïe	Associate Professor	On duty
18	DJEUANI Astride Carole	Senior Lecturer	On duty
19	GOMANDJE Christelle	Senior Lecturer	On duty
20	MAFFO MAFFO Nicole Liliane	Senior Lecturer	On duty
21	MAHBOU SOMO TOUKAM. Gabriel	Senior Lecturer	On duty
22	NGALLE Hermine BILLE	Senior Lecturer	On duty
23	NGOUO Lucas Vincent	Senior Lecturer	On duty
24	NNANGA MEBENGA Ruth Laure	Senior Lecturer	On duty
25	NOUKEU KOUAKAM Armelle	Senior Lecturer	On duty
26	ONANA JEAN MICHEL	Senior Lecturer	On duty
27	GODSWILL NTSOMBAH NTSEFONG	Assistant	On duty
28	KABELONG BANAHO Louis-Paul-Roger	Assistant	On duty
29	KONO Léon Dieudonné	Assistant	On duty
30	LIBALAH Moses BAKONCK	Assistant	On duty
31	LIKENG-LI-NGUE Benoit C	Assistant	On duty
32	TAEDOUNG Evariste Hermann	Assistant	On duty
33	TEMEGNE NONO Carine	Assistant	On duty
4- DEPARTMENT OF INORGANIC CHEMISTRY (ICH) (33)			
1	**AGWARA ONDOH Moïse**	**Professor**	**Head of Department**
2	DJOUFAC WOUMFO Emmanuel	Professor	On duty
3	Florence UFI CHINJE spouse MELO	Professor	Rector UNdere
4	GHOGOMU Paul MINGO	Professor	Minister in charge of Missions PR
5	NANSEU Njiki Charles Péguy	Professor	On duty
6	NDIFON Peter TEKE	Professor	Technical Adviser MINRESI
7	NDIKONTAR Maurice KOR	Professor	Vice – Dean UBda
8	NENWA Justin	Professor	On duty
9	NGAMENI Emmanuel	Professor	Dean FS UDS
10	NGOMO Horace MANGA	Professor	Vice – Chancellor UB
11	ACAYANKA Elie	Associate Professor	On duty
12	BABALE born DJAM DOUDOU	Associate Professor	Responsible for Missions PR
13	EMADACK Alphonse	Associate Professor	On duty
14	KAMGANG YOUBI Georges	Associate Professor	On duty
15	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	On duty
16	KONG SAKEO	Associate Professor	On duty
17	NDI NSAMI Julius	Associate Professor	On duty
18	NJIOMOU C. spouse DJANGANG	Associate Professor	On duty
19	NJOYA Dayirou	Associate Professor	On duty
20	TCHAKOUTE KOUAMO Hervé	Associate Professor	On duty
21	BELIBI BELIBI Placide Désiré	Senior Lecturer	Chief Service ENS Bertoua
22	CHEUMANI YONA Arnaud M.	Senior Lecturer	On duty
23	KENNE DEDZO GUSTAVE	Senior Lecturer	On duty
24	KOUOTOU DAOUDA	Senior Lecturer	On duty

25	MAKON Thomas Beauregard	Senior Lecturer	On duty
26	MBEY Jean Aime	Senior Lecturer	On duty
27	NCHIMI NONO KATIA	Senior Lecturer	On duty
28	NEBA born NDOSIRI Bridget NDOYE	Senior Lecturer	Technical Adviser MINFEM
29	NYAMEN Linda Dyorisse	Senior Lecturer	On duty
30	PABOUDAM GBAMBIE A.	Senior Lecturer	On duty
31	NJANKWA NJABONG N. Eric	Assistant	On duty
32	PATOUOSSA ISSOFA	Assistant	On duty
33	SIEWE Jean Mermoz	Assistant	On duty
5- DEPARTMENT OF ORGANIC CHEMISTRY (OCH) (38)			
1	DONGO Etienne	Professor	Vice – Dean FSE UY1
2	GHOGOMU TIH Robert Ralph	Professor	Director IBAF UDs
3	NGOUELA Silvère Augustin	Professor	Head of Department UDs
4	NYASSE Barthélemy	Professor	On duty
5	**PEGNYEMB Dieudonné Emmanuel**	**Professor**	**Director MINESUP / Head of Department**
6	WANDJI Jean	Professor	On duty
7	Alex de Théodore ATCHADE	Associate Professor	Vice – Dean DPSAA
8	AMBASSA Pantaléon	Associate Professor	On duty
9	EYONG Kenneth OBEN	Associate Professor	On duty
10	FOLEFOC Gabriel NGOSONG	Associate Professor	On duty
11	FOTSO WABO Ghislain	Associate Professor	On duty
12	KEUMEDJIO Félix	Associate Professor	On duty
13	KEUMOGNE Marguerite	Associate Professor	On duty
14	KOUAM Jacques	Associate Professor	On duty
15	MBAZOA born DJAMA Céline	Associate Professor	On duty
16	MKOUNGA Pierre	Associate Professor	On duty
17	MVOT AKAK CARINE	Associate Professor	On duty
18	NGO MBING Joséphine	Associate Professor	Deputy Director MINRESI
19	NGONO BIKOBO Dominique Serge	Associate Professor	Study Officer MINESUP
20	NOTE LOUGBOT Olivier Placide	Associate Professor	Chief Service MINESUP
21	NOUNGOUE TCHAMO Diderot	Associate Professor	On duty
22	TABOPDA KUATE Turibio	Associate Professor	On duty
23	TAGATSING FOTSING Maurice	Associate Professor	On duty
24	TCHOUANKEU Jean-Claude	Associate Professor	Dean FS UYI
25	TIH born NGO BILONG E. Anastasie	Associate Professor	On duty
26	YANKEP Emmanuel	Associate Professor	On duty
27	ZONDEGOUMBA Ernestine	Associate Professor	On duty
28	KAMTO Eutrophe Le Doux	Senior Lecturer	On duty
29	NGNINTEDO Dominique	Senior Lecturer	On duty
30	NGOMO Orléans	Senior Lecturer	On duty
31	OUAHOUO WACHE Blandine M.	Senior Lecturer	On duty
32	SIELINOU TEDJON Valérie	Senior Lecturer	On duty
33	MESSI Angélique Nicolas	Assistant	On duty
34	TSEMEUGNE Joseph	Assistant	On duty
35	TCHAMGOUE Joseph	Assistant	On duty
36	TSAFACK Maurice	Assistant	On duty
37	TSAMO Armelle	Assistant	On duty
38	NONO Eric Carly	Assistant	On duty

6- DEPARTMENT COMPUTER SCIENCES (CSC) (25)			
1	ATSA ETOUNDI Roger	Professor	Chief Division MINESUP
2	FOUDA NDJODO Marcel Laurent	Professor	Head of Department ENS / Head IGA MINESUP
3	NDOUNDAM Réné	Associate Professor	On duty
4	ABESSOLO ALO'O Gislain	Senior Lecturer	On duty
5	**AMINOU Halidou**	**Senior Lecturer**	**Head of Department**
6	DJAM Xaviera YOUH - KIMBI	Senior Lecturer	On duty
7	DOMGA KOMGUEM Rodrigue	Senior Lecturer	On duty
8	EBELE Serge Alain	Senior Lecturer	On duty
9	KOUOKAM KOUOKAM E. A.	Senior Lecturer	On duty
10	MELATAGIA YONTA Paulin	Senior Lecturer	On duty
11	MONTHE DJIADEU Valery M.	Senior Lecturer	On duty
12	MOTO MPONG Serge Alain	Senior Lecturer	On duty
13	OLLE OLLE Daniel Claude Delort	Senior Lecturer	Deputy Director – ENSET Ebolowa
14	TAPAMO Hyppolite	Senior Lecturer	On duty
15	TINDO Gilbert	Senior Lecturer	On duty
16	TSOPZE Norbert	Senior Lecturer	On duty
17	WAKU KOUAMOU Jules	Senior Lecturer	On duty
18	BAYEM Jacques Narcisse	Assistant	On duty
19	EKODECK Stéphane Gaël Raymond	Assistant	On duty
20	HAMZA Adamou	Assistant	On duty
21	JIOMEKONG AZANZI Fidel	Assistant	On duty
22	MAKEMBE. S . Oswald	Assistant	On duty
23	MESSI NGUELE Thomas	Assistant	On duty
24	MEYEMDOU Nadège Sylvianne	Assistant	On duty
25	NKONDOCK. MI. BAHANACK.N.	Assistant	On duty
7- DEPARTMENT OF MATHEMATICS (MAT) (30)			
1	**AYISSI Raoult Domingo**	**Professor**	**Head of Department**
2	EMVUDU WONO Yves S.	Professor	Inspector MINESUP
3	KIANPI Maurice	Associate Professor	On duty
4	MBANG Joseph	Associate Professor	On duty
5	MBEHOU Mohamed	Associate Professor	On duty
6	MBELE BIDIMA Martin Ledoux	Associate Professor	On duty
7	NKUIMI JUGNIA Célestin	Associate Professor	On duty
8	NOUNDJEU Pierre	Associate Professor	Chief Service FS UY1
9	TCHAPNDA NJABO Sophonie B.	Associate Professor	Director AIMS Rwanda
10	TCHOUNDJA Edgar Landry	Associate Professor	On duty
11	AGHOUKENG JIOFACK Jean Gérard	Senior Lecturer	Head of Unit MINPLAMAT
12	CHENDJOU Gilbert	Senior Lecturer	On duty
13	DJIADEU NGAHA Michel	Senior Lecturer	On duty
14	DOUANLA YONTA Herman	Senior Lecturer	On duty
15	FOMEKONG Christophe	Senior Lecturer	On duty
16	KIKI Maxime Armand	Senior Lecturer	On duty
17	MBAKOP Guy Merlin	Senior Lecturer	On duty
18	MENGUE MENGUE David Joe	Senior Lecturer	On duty
19	NGUEFACK Bernard	Senior Lecturer	On duty
20	NIMPA PEFOUKEU Romain	Senior Lecturer	On duty
21	POLA DOUNDOU Emmanuel	Senior Lecturer	On duty

22	TAKAM SOH Patrice	Senior Lecturer	On duty
23	TCHANGANG Roger Duclos	Senior Lecturer	On duty
24	TETSADJIO TCHILEPECK M. E.	Senior Lecturer	On duty
25	TIAYA TSAGUE N. Anne-Marie	Senior Lecturer	On duty
26	BITYE MVONDO Esther Claudine	Assistant	On duty
27	MBATAKOU Salomon Joseph	Assistant	On duty
28	MBIAKOP Hilaire George	Assistant	On duty
29	MEFENZA NOUNTU Thiery	Assistant	On duty
30	TCHEUTIA Daniel Duviol	Assistant	On duty
8- DEPARTMENT OF MICROBIOLOGY (MIB) (18)			
1	**ESSIA NGANG Jean Justin**	**Professor**	**Head of Department**
2	NYEGUE Maximilienne Ascension	Professor	Vice – Dean DSSE
3	NWAGA Dieudonné M.	Professor	On duty
4	ASSAM ASSAM Jean Paul	Associate Professor	On duty
5	BOYOMO ONANA	Associate Professor	On duty
6	RIWOM Sara Honorine	Associate Professor	On duty
7	SADO KAMDEM Sylvain Leroy	Associate Professor	On duty
8	BODA Maurice	Senior Lecturer	On duty
9	BOUGNOM Blaise Pascal	Senior Lecturer	On duty
10	ESSONO OBOUGOU Germain G.	Senior Lecturer	On duty
11	NJIKI BIKOÏ Jacky	Senior Lecturer	On duty
12	TCHIKOUA Roger	Senior Lecturer	On duty
13	ESSONO Damien Marie	Assistant	On duty
14	LAMYE Glory MOH	Assistant	On duty
15	MEYIN A EBONG Solange	Assistant	On duty
16	NKOUDOU ZE Nardis	Assistant	On duty
17	SAKE NGANE Carole Stéphanie	Assistant	On duty
18	TOBOLBAÏ Richard	Assistant	On duty
9- DEPARTMENT OF PHYSICS (PHY) (40)			
1	BEN-BOLIE Germain Hubert	Professor	On duty
2	DJUIDJE KENMOE spouse ALOYEM	Professor	On duty
3	EKOBENA FOUDA Henri Paul	Professor	Vice – Rector UNdere
4	ESSIMBI ZOBO Bernard	Professor	On duty
5	KOFANE Timoléon Crépin	Professor	On duty
6	NANA ENGO Serge Guy	Professor	On duty
7	NANA NBENDJO Blaise	Professor	On duty
8	**NDJAKA Jean Marie Bienvenu**	**Professor**	**Head of Department**
9	NJANDJOCK NOUCK Philippe	Professor	On duty
10	NOUAYOU Robert	Professor	On duty
11	PEMHA Elkana	Professor	On duty
12	TABOD Charles TABOD	Professor	Dean FS UBda
13	TCHAWOUA Clément	Professor	On duty
14	WOAFO Paul	Professor	On duty
15	ZEKENG Serge Sylvain	Professor	On duty
16	BIYA MOTTO Frédéric	Associate Professor	Managing Director HYDRO Mekin
17	BODO Bertrand	Associate Professor	On duty
18	ENYEGUE A NYAM spouse BELINGA	Associate Professor	On duty
19	EYEBE FOUDA Jean sire	Associate Professor	On duty

20	FEWO Serge Ibraïd	Associate Professor	On duty
21	HONA Jacques	Associate Professor	On duty
22	MBANE BIOUELE César	Associate Professor	On duty
23	MBINACK Clément	Associate Professor	On duty
24	NDOP Joseph	Associate Professor	On duty
25	SAIDOU	Associate Professor	Deputy Director MINRESI
26	SIEWE SIEWE Martin	Associate Professor	On duty
27	SIMO Elie	Associate Professor	On duty
28	VONDOU Derbetini Appolinaire	Associate Professor	On duty
29	WAKATA born BEYA Annie	Associate Professor	Director ENS UY1
30	ABDOURAHIMI	Senior Lecturer	On duty
31	CHAMANI Roméo	Senior Lecturer	On duty
32	EDONGUE HERVAIS	Senior Lecturer	On duty
33	FOUEDJIO David	Senior Lecturer	Head of Unit MINADER
34	MBONO SAMBA Yves Christian U.	Senior Lecturer	On duty
35	MELI'I Joelle Larissa	Senior Lecturer	On duty
36	MVOGO ALAIN	Senior Lecturer	On duty
37	OBOUNOU Marcel	Senior Lecturer	Deputy Director Université Inter-Etat Sangmélima
38	WOULACHE Rosalie Laure	Senior Lecturer	On duty
39	AYISSI EYEBE Guy François Valérie	Assistant	On duty
40	TEYOU NGOUPOU Ariel	Assistant	On duty
10- DEPARTMENT OF EARTH SCIENCES (ESC) (42)			
1	BITOM Dieudonné	Professor	Dean FASA – UDS
2	FOUATEU Rose spouse YONGUE	Professor	On duty
3	NDAM NGOUPAYOU Jules-Remy	Professor	On duty
4	**NDJIGUI Paul Désiré**	**Professor**	**Head of Department**
5	NGOS III Simon	Professor	On duty
6	NKOUMBOU Charles	Professor	On duty
7	NZENTI Jean-Paul	Professor	On duty
8	ABOSSOLO born ANGUE Monique	Associate Professor	Vice – Dean DRC FS UYI
9	BISSO Dieudonné	Associate Professor	Project Director Memve'ele Barrage
10	EKOMANE Emile	Associate Professor	On duty
11	GANNO Sylvestre	Associate Professor	On duty
12	GHOGOMU Richard TANWI	Associate Professor	Chief Division UMA
13	MOUNDI Amidou	Associate Professor	Technical Advisor MINIMDT
14	NGUEUTCHOUA Gabriel	Associate Professor	Deputy Study Officer MINRESI
15	NJILAH Isaac KONFOR	Associate Professor	On duty
16	NYECK Bruno	Associate Professor	On duty
17	ONANA Vincent Laurent	Associate Professor	Chief Service UYII
18	TCHAKOUNTE J. spouse NOUMBEM	Associate Professor	Head of Unit MINRESI
19	TCHOUANKOUE Jean-Pierre	Associate Professor	On duty
20	TEMDJIM Robert	Associate Professor	On duty
21	YENE ATANGANA Joseph Q.	Associate Professor	Chief Division MINTP
22	ZO'O ZAME Philémon	Associate Professor	Managing Director ART
23	ANABA ONANA Achille Basile	Senior Lecturer	On duty
24	BEKOA Etienne	Senior Lecturer	On duty
25	ELISE SABABA	Senior Lecturer	On duty
26	ESSONO Jean	Senior Lecturer	On duty

27	EYONG JOHN TAKEM	Senior Lecturer	On duty
28	FUH Calistus Gentry	Senior Lecturer	Secretary of State MINMIDT
29	LAMILEN BILLA Daniel	Senior Lecturer	On duty
30	MBESSE CECILE OLIVE	Senior Lecturer	On duty
31	MBIDA YEM	Senior Lecturer	On duty
32	METANG Victor	Senior Lecturer	On duty
33	MINYEM Dieudonné-Lucien	Senior Lecturer	Chief Division UMa
34	NGO BELNOUN Rose Noël	Senior Lecturer	On duty
35	NGO BIDJECK Louise Marie	Senior Lecturer	On duty
36	NOMO NEGUE Emmanuel	Senior Lecturer	On duty
37	NTSAMA ATANGANA Jacqueline	Senior Lecturer	On duty
38	TCHAPTCHET TCHATO De P.	Senior Lecturer	On duty
39	TEHNA Nathanaël	Senior Lecturer	On duty
40	TEMGA Jean Pierre	Senior Lecturer	On duty
41	FEUMBA Roger	Assistant	On duty
42	MBANGA NYOBE Jules	Assistant	On duty

SUMMARY OF DISTRIBUTION OF PERMANENT TEACHING STAFF IN THE FACULTY OF SCIENCE ACCORDING TO DEPARTMENTS

NUMBER OF LECTURERS (WOMEN)					
Departments	**Professors**	**Associate Professors**	**Senior Lecturers**	**Assistants**	**Total**
BCH	8 (1)	14 (10)	13 (5)	3 (1)	**38 (17)**
ABP	15 (1)	8 (5)	18 (5)	5 (2)	**46 (13)**
PBP	7 (1)	10 (1)	9 (6)	7 (1)	**33 (9)**
ICH	10 (1)	10 (2)	10 (3)	3 (0)	**33 (6)**
OCH	6 (0)	21 (6)	5 (2)	6 (1)	**38 (9)**
CSC	2 (0)	1 (0)	14 (0)	8 (1)	**25 (1)**
MAT	2 (0)	8 (0)	15 (1)	5 (1)	**30 (2)**
MIB	3 (1)	4 (1)	5 (1)	6 (2)	**18 (5)**
PHY	15 (1)	14 (2)	9 (2)	2 (0)	**40 (5)**
ESC	7 (1)	15 (2)	18 (4)	2 (0)	**42 (7)**
Total	**75 (7)**	**105 (29)**	**116 (29)**	**47 (9)**	**343 (74)**

A total of 343 (74)

- Professors: 75 (7)
- Associate Professors: 105 (29)
- Senior Lecturers: 116 (29)
- Assistants: 47 (9)

The Dean of the Faculty of Sciences

DEDICATION

I dedicate this work to

My late father **MBOUNA Fidèle**

My mother **TSOLEFACK Colette**

My mother-in-law **DJEUGUE Bernadette**

My brothers and sisters **FOTSING MBOUNA Agnès**, **FEUDJIO NGEAFOUET Borel**, **DJIATSA SOKENG William**, **MOUAFO MBOUNA Kelvin**, **MOUNGUE MBOUNA Nadine**, **DONGMO MBOUNA Arlette**, **DONGOUO MBOUNA Cyrille**, **TOUKAM TENE Phylomène, ESSIYA MBOUNA Rachelle**, **EKO MBOUNA Jacky Flore**, **PAGUI TENE Lionel**.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
µg	: microgram
µM	: micromolar
ACTs	: Artemisinin – based Combination Therapies
AIDS	: Acquired Immuno – Deficiency Syndrome
ATCC	: American Type Culture Collection
CC_{50}	: 50% cell Cytotoxic Concentration
cGMP	: cyclic Guanosine Monophosphate
Chl	: Chloroform
CSP	: Circumsporozoite Protein
DHA	: Dihydroartemisinin
DMEM	: Dulbecco's Modified Eagle's Medium
DMSO	: Dimethyl sulfoxide
EA	: Ethyl Acetate
EDTA	: Ethylene diamine tetra acetate
GC	: Gas Chromatography
H	: Hexane
HEK	: Human Embryogenic Kidney
HEK293T	: Human embryonic kidney cells 293T
HEPES	: 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid
HIV	: Human Immunodeficiency Viruses
HNC	: Herbier National du Cameroun
IC_{50}	: 50% Inhibitory Concentration
IC_{50}*Pf*3D7	: 50% Inhibitory Concentration on *Plasmodium falciparum* 3D7 strain
IC_{50}*Pf*INDO	: 50% Inhibitory Concentration on *Plasmodium falciparum* INDO strain
IMPM	: Institute for Medical Research and Medicinal Plants Studies
l	: leaf
m	: methanol
MFC	: Minimal Fungicidal Concentration
MIC	: Minimal Inhibitory Concentration
mL	: milliliter
mM	: millimolar
MS	: Mass Spectrometry
MSP	: Merozoite Surface Protein
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
P. falciparum	: *Plasmodium falciparum*
*Pf*3D7	: *Plasmodium falciparum* 3D7 strain
*Pf*INDO	: *Plasmodium falciparum* INDO strain
r	: root
R3	: final residue

RDTs : Rapid Diagnostic Tests
RI : Resistance Index
RPMI : Roswell Park Memorial Institute medium
S.P. : Sulfadoxine – Pyrimethamine
sb : stem bark
SD : Standard Deviation
SI : Selectivity Index
SI_{Pf3D7} : Selectivity index on *Plasmodium falciparum* 3D7 strain
SI_{PfINDO} : Selectivity index on *Plasmodium falciparum* INDO strain
T. avicennioides : *Terminalia avicennioides*
T. catappa : *Terminalia catappa*
T. chebula : *Terminalia cheluba*
T. mantaly : *Terminalia mantaly*
T. mollis : *Terminalia mollis*
T. spinosa : *Terminalia spinosa*
T. superba : *Terminalia superba*
TLC : Thin Layer Chromatography
Tm : *Terminalia mantaly*
Tml^{m} : *Terminalia mantaly* leaf methanol extract
Tml^{w} : *Terminalia mantaly* leaf water extract
Tml^{w}Chl : *Terminalia mantaly* leaf water extract Chloroform fraction
Tml^{w}EA : *Terminalia mantaly* leaf water extract Ethyl Acetate fraction
Tml^{w}H : *Terminalia mantaly* leaf water extract Hexane fraction
Tml^{w}M : *Terminalia mantaly* leaf water extract Methanol fraction
Tml^{w}R3 : *Terminalia mantaly* leaf water extract final residue
Tmr^{m} : *Terminalia mantaly* root methanol extract
Tmr^{w} : *Terminalia mantaly* root water extract
Tmsb^{m} : *Terminalia mantaly* stem bark methanol extract
Tmsb^{w} : *Terminalia mantaly* stem bark water extract
Tmsb^{w}Chl : *Terminalia mantaly* stem bark water extract Chloroform fraction
Tmsb^{w}EA : *Terminalia mantaly* stem bark water extract Ethyl Acetate fraction
Tmsb^{w}H : *Terminalia mantaly* stem bark water extract Hexane fraction
Tmsb^{w}M : *Terminalia mantaly* stem bark water extract Methanol fraction
Tmsb^{w}R3 : *Terminalia mantaly* stem bark water extract final residue
Ts : *Terminalia superba*
Tsl^{m} : *Terminalia superba* leaf methanol extract
Tsl^{w} : *Terminalia superba* leaf water extract
Tsl^{w}Chl : *Terminalia superba* leaf water extract Chloroform fraction
Tsl^{w}EA : *Terminalia superba* leaf water extract Ethyl Acetate fraction
Tsl^{w}H : *Terminalia superba* leaf water extract Hexane fraction
Tsl^{w}M : *Terminalia superba* leaf water extract Methanol fraction
Tsl^{w}R3 : *Terminalia superba* leaf water extract final residue
TSR : Thrombospondin Repeat

*Ts*r^{m}	: *Terminalia superba* root methanol extract
*Ts*r^{m}Chl	: *Terminalia superba* root methanol extract Dichloromethane fraction
*Ts*r^{m}EA	: *Terminalia superba* root methanol extract Ethyl Acetate fraction
*Ts*r^{m}H	: *Terminalia superba* root methanol extract Hexane fraction
*Ts*r^{m}R3	: *Terminalia superba* root methanol extract final residue
*Ts*r^{w}	: *Terminalia superba* root water extract
*Ts*sb^{m}	: *Terminalia superba* stem bark methanol extract
*Ts*sb^{w}	: *Terminalia superba* stem bark water extract
w	: water
WHO	: World Health Organization
µL	: microliter

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ABSTRACT

The emergence and spread of malaria parasites resistant to artemisinin-based combination therapy (ACTs) stresses the need for novel drugs against malaria. In our search of new antiplasmodial hits with novel ways to reversely interact with parasite, we investigated extracts from leaves, stem bark and roots of *Terminalia mantaly* (*Tm*) and *Terminalia superba* (*Ts*), antimalarial traditional plants, against the Chloroquine–sensitive 3D7 (*Pf*3D7) and resistant INDO (*Pf*INDO) strains of *Plasmodium (P.) falciparum*. Twelve methanolic (m) and water (w) extracts obtained by maceration of powdered dried leaves (l), stem bark (sb) and root (r) of *Tm* and *Ts* were assessed for their *in vitro* antiplasmodial potential against *Pf*INDO and *Pf*3D7 using the SYBR green I-based fluorescence assay. The cytotoxicity of potent extracts was evaluated *in vitro* using the MTT assay on the human embryonic kidney 239T cells line (HEK239T). Four crudes extracts, the aqueous extracts from leaves (*Tm*l^{w}) and stem bark (*Tm*sb^{w}) of *Tm*; the aqueous extract from leaves (*Ts*l^{w}) and the methanolic extract from roots (*Ts*r^{m}) of *Ts*, having the best antiplasmodial potential (IC_{50}*Pf*INDO, IC_{50}*Pf*3D7: 0.26–2.66 µg/mL) and highly selective (75 < SI_{PfINDO}, SI_{Pf3D7} > 769) as per plant species, were activities–guided fractionated using increasing polarity gradient exhaustion, followed by the column chromatography of the highly potent fraction of each species: the methanolic fraction of *Tm*sb^{w}, (*Tm*sb^{w}M, IC_{50}*Pf*INDO/*Pf*3D7: 0.39/1.28 µg/mL, SI_{PfINDO}/$_{Pf3D7}$ > 512/156) and the ethyl acetate fraction of *Ts*r^{m} (*Ts*r^{m}EA, IC_{50}*Pf*INDO/*Pf*3D7: 1.65/1.82 µg/mL, SI_{PfINDO}/$_{Pf3D7}$ > 109/121). Nine subfractions out of the 61 obtained from both *Tm*sb^{w}M and *Ts*r^{m}EA, viz *Tm*25, *Tm*28-30, *Tm*33-36 and *Tm*38 displayed highly potent antiplasmodial activity (IC_{50}*Pf*INDO: 0.41–0.84 µg/mL, IC_{50}*Pf*3D7: 0.29–1.06 µg/mL) and very high selectivity (50 < SI_{PfINDO}, SI_{Pf3D7} > 344). Of these, *Tm*36 subfraction (IC_{50}*Pf*INDO: 0.41 µg/mL, SI_{PfINDO} > 243; IC_{50}*Pf*3D7: 0.29 µg/mL, SI_{Pf3D7} > 344) showed the highest promise. The GC–MS analysis of actives subfractions led to the identification of 104 different phytochemicals then the multivariate statistical analysis identified 15 of these compounds that could be the base of the strong antiplasmodial activity and selectivity observed with *T. mantaly* stem bark extracts. The blood life cycle stages–specific inhibition studies of the five very promising subfractions showed that *Tm*28, *Tm*29, *Tm*30, *Tm*36 and *Tm*38 inhibited all asexual stages of the intraerythrocytic life cycle of *P. falciparum*, with strong and specific activities on ring stage development, merozoites egress and invasion processes. The results of this study are utmost as they report for the first time the antiplasmodial activity of *Terminalia mantaly*. Meanwhile, in-deep investigations are required to complete this work and characterize new hit compounds from highly potent antiplasmodial subfractions to feed naturally occurring antimalarial drugs discovery pipeline with the purpose to tackle the emergence of drug resistance.

Keywords: Antiplasmodial, *Terminalia mantaly*, *Terminalia superba*, *Plasmodium falciparum*, stages–specific inhibition.

RESUME

L'émergence des résistances aux ACTs souligne l'urgence de la recherche de nouveaux antipaludéens et c'est dans cette logique que la présente étude a été définie. L'activité antiplasmodiale *in vitro* des extraits de *Terminalia mantaly* (*Tm*) et de *Terminalia superba* (*Ts*) a été étudiée contre les souches chloroquine-sensible 3D7 (*Pf*3D7) et chloroquine-résistante INDO (*Pf*INDO) de *P. falciparum*. Douze extraits bruts obtenus par macération méthanolique et aqueuse des poudres de feuilles, d'écorces de tronc et de racines de *Tm* et *Ts* ont été testés *in vitro* sur *Pf*INDO et *Pf*3D7 via le test de fluorescence au SYBR Green–I. L'évaluation de la cytotoxicité des extraits actifs sur les cellules embryonnaires du rein humain (HEK239T) a été faite par la méthode spectrophotométrique utilisant le MTT. Les extraits aqueux de feuilles (*Tm*l^{w}), d'écorces de tronc (*Tm*sb^{w}) de *Tm*, de feuilles (*Ts*l^{w}) et l'extrait méthanolique des racines (*Ts*r^{m}) de *Ts*, ont été sélectionnés puis soumis à un double fractionnement bioguidé par épuisement des extraits avec des solvants de polarité croissante, suivi d'une chromatographie sur colonne de la fraction la plus active (IC_{50} < 2 µg/mL) par espèce. L'étude phytochimique de neuf sous–fractions très actives de *Tm* a été faite par la CPG couplée à la SM. L'étude de l'inhibition des différents stades de croissance intraérythrocytaire de *P. falciparum* a été effectuée avec les cinq sous–fractions les plus actives de *Tm* (IC_{50} < 1 µg/mL), par un examen microscopique des frottis de culture de *Pf*3D7 sous coloration différentielle au Giemsa, suivi d'un dénombrement d'érythrocytes parasités par cytométrie de flux. Des résultats obtenus, le dégrossissement des extraits *Tm*l^{w}, *Tm*sb^{w}, *Ts*l^{w} et *Ts*r^{m} (IC_{50}*Pf*INDO, IC_{50}*Pf*3D7 : 0,26–2,66 µg/mL ; 75 < SI_{PfINDO}, SI_{Pf3D7} > 769) a permis d'obtenir 61 sous–fractions, dont 39 provenant de la fraction *Tm*sb^{w}M (IC_{50}*Pf*INDO/*Pf*3D7 : 0,39/1,28 µg/mL ; SI_{PfINDO}/$_{Pf3D7}$ > 512/156) de *Tm* et 22 de la fraction *Ts*r^{m}EA (IC_{50}*Pf*INDO/*Pf*3D7 : 1,65/1,82 µg/mL ; SI_{PfINDO}/$_{Pf3D7}$ > 109/121) de *Ts*. Neuf sous–fractions toutes de *Tm* et codifiées *Tm*25, *Tm*28-30, *Tm*33-36 et *Tm*38, ont montré une excellente activité (IC_{50}*Pf*INDO, IC_{50}*Pf*3D7 : 0,41–0,84 / 0,29–1,06 µg/mL ; 50 < SI_{PfINDO}, SI_{Pf3D7} > 344). La sous–fraction *Tm*36 (IC_{50}*Pf*INDO/*Pf*3D7 : 0,41/0,29 µg/mL, SI_{PfINDO}/$_{Pf3D7}$ > 243/344) a présenté la meilleure activité biologique. L'étude phytochimique a permis de recenser 104 composés. En outre, une analyse croisée des profiles phytochimiques a permis d'identifier 15 composés, qui seraient à la base de l'excellente activité antiplasmodiale des extraits d'écorces de tronc de *Tm*. Les sous–fractions *Tm*28, *Tm*29, *Tm*30, *Tm*36 et *Tm*38 ont inhibé tous les stades asexués du cycle intraérythrocytaire de *P. falciparum*, avec un effet prononcé sur le stade en anneau, les processus de libération et d'invasion des mérozoïtes. Les résultats de cette étude démontrent pour la première fois le potentiel antiplasmodial *in vitro* de *Terminalia mantaly*. Cependant, des investigations poussées seront nécessaires pour caractériser les molécules aux nouveaux mécanismes d'action, qui serviront de base au développement des nouveaux médicaments antipaludéens.

Mots clés : activité antiplasmodiale, *Terminalia mantaly*, *Terminalia superba*, *Plasmodium falciparum*, inhibition spécifique des stades intraérythrocytaires.

INTRODUCTION

INTRODUCTION

Malaria remains one of the most important infectious diseases (Held *et al*., 2017) and *Plasmodium falciparum* is the species responsible for nearly all reported severe malaria cases and deaths (World Malaria Report, 2018; 2020). In 2019, an estimated 229 million cases and about 409 000 deaths from malaria occurred in 87 malaria endemic countries. Most of the cases (215 million cases ~ 94%) and deaths (384 000 deaths ~ 94%) were registered in WHO African Region, especially in sub–Saharan Africa (World Malaria Report, 2020). In Cameroon, malaria is the major cause of morbidity and mortality among the most vulnerable groups including children aged under 5 years (18%), pregnant women (5%), people living with HIV/AIDS (5.5%) and the poor (40%) (Ndong *et al*., 2014). Nationally, Malaria accounts for 48% of all hospital admissions, 30% of morbidity and 67% of childhood mortality per year (Antonio-Nkondjio *et al*., 2013).

Nowadays, treatment of both uncomplicated and severe malaria cases heavily relies on a single class of compounds, Artemisinin derivatives and except Quinine, no other substances for treatment of severe malaria are available (Held *et al*., 2017). Although Artemisinin–based Combination Therapies (ACTs) have proved to be effective in controlling the disease in many malaria endemic areas (Roberts *et al*., 2017), recently reported increasing parasites clearance half–life followed by the appearance of clinical phenotype of *Plasmodium falciparum* resistant to Artemisinin and its derivatives in the wide areas of Southeast Asia (Dondorp *et al*., 2009; Ashley *et al*., 2014; Fairhurst and Dondorp, 2016), coupled to the reported potential emergence of indigenous Artemisinin–resistant *Plasmodium falciparum* in Central Africa (Lu *et al*., 2017), highlight the fragility of available malaria treatment measures.

To overcome multidrug–resistance issue in malaria, a major goal is to identify the next generation of antiplasmodial lead compounds that act early on parasite's developmental cycle with news mechanisms of actions (Roberts *et al*., 2016). It has become a consensus that, in addition to the standard requirements for any new lead compound in the antimalarial drugs discovery pipeline (safety and efficacy, oral delivery, stability), novel antimalarial drugs to be deployed in areas of endemicity should also present low sensibility to resistance development, as well as target multiple stages in the parasite life cycle (Burrows *et al*., 2013; Erath *et al*., 2015). Of note, recent flow cytometry–based analysis of ten antimalarials widely used clinically showed that only Artemisinin, Artesunate and Cycloheximide have significant effect on parasite's ring stage; moreover, only Artemisinin exhibited significant activity against

schizonts, and none of the antimalarials prevented the invasion of merozoites (Wilson *et al*., 2013). Therefore, the solely available antimalarial drugs that target ring–stage parasites are Artemisinin and Artesunate whereas most antimalarial agents act later in the parasite development cycle on the metabolically more active trophozoites and/or schizonts (Ehrhardt *et al*., 2016). Nevertheless, trophozoite~ and schizont~infected erythrocytes are responsible for severe clinical pathology, by being sequestered in the microvasculature and causing impaired tissue perfusion and endothelial cell activation, which appears to be the main cause of fatal malaria (Hughes *et al*., 2010). Thus, targeting ring stages is therefore crucial and attractive, as it interrupts the development of rings into disease–mediating trophozoite and schizont stages and thereby prevent the progression of the infection to severe forms (Burrows *et al*., 2013; Wilson *et al*., 2013).

Overall, the discovery of new antiplasmodial hit compounds directed against novel targets and with potency against different stages of the asexual life cycle of *Plasmodium falciparum* is of utmost importance in the development of new drugs against malaria. Indeed, numerous recent studies report the antiplasmodial potential of synthetic compounds and plant extracts with activities against blood stages of the malaria parasite. For instance, two synthesized compounds namely UFC501 (4–Nitro styrylquinoline chemical class) and UCF201 (Spirocyclic chromanes family) were reported to be early–acting in blocking parasite development at ring, trophozoite and schizont stages of development as well as merozoites invasion at nanomolar concentrations **(**Roberts *et al*., 2016; 2017). Additionally, Erath *et al*., (2015), who investigated small molecules from Xenomycins chemical class, reported their effectiveness in clearing the liver, the blood asexual and the sexual stages of *P. falciparum*. Plasmodione, a potent antiplasmodial lead molecule with activity at nanomolar concentrations, also exerted strong inhibition of both asexual and sexual stages of blood stage of *P. falciparum* (Ehrhardt *et al*., 2016). Likewise, two fractions from *Parthenium hysterophorus* extracts obtained through activity-guided fractionation of ethanolic extract demonstrated promising antiplasmodial potency and ability to target all cell cycle stages of blood stage malaria parasite (Singh *et al*., 2015).

It is worth noting that Quinine and Artemisinin, the only known drugs with demonstrated effectiveness against malaria parasites are plant-derived specialized secondary metabolites, with their respective origins in the medicinal plants *Cinchona officinalis* and *Artemisia annua* (Dobson, 2001). This has supported the search for new antimalarial chemical pharmacophores from plants. Medicinal plants are considered as an important source of

chemotherapeutic agents, and indigenous healthcare systems play a vital role in the management of community health and the discovery of novel pharmaceuticals against malaria. In Cameroon, pharmacopeia plants are widely used to treat malaria and several other diseases, particularly in areas where access to standard treatments is limited. *Terminalia mantaly* and *Terminalia superba* have been reported in such areas in Cameroon as sources of treatment for various diseases including malaria and/or related symptoms (Titanji *et al*., 2008). However, very little is known about the antiplasmodial activity of extracts from these plants.

In our search of new antiplasmodial natural compounds with novel ways to reversely interact with parasite, we investigated *in vitro* the potency of *Terminalia mantaly* (*Tm*) and *Terminalia superba* (*Ts*) extracts, as source of antiplasmodial hits with activity on stages of *Plasmodium falciparum* intraerythrocytic life cycle.

Specifically, we

- Screen aqueous and methanolic extracts from *Terminalia mantaly* and *Terminalia superba* for *in vitro* antiplasmodial activity and cytotoxicity.
- Perform a double steps activity–guided fractionation of potent crude extracts using increasing polarity gradient solvent exhaustion followed by Column Chromatography.
- Determine the phytochemical profiles of potent antiplasmodial subfractions through Gas Chromatography coupled to Mass Spectrometry (GC–MS).
- Assess intraerythrocytic stages specific inhibition and post–drugs growth suppression activities of highly potent antiplasmodial subfractions.

Chapter 1:

LITERATURE REVIEW

I – LITERATURE REVIEW

1- Generalities on Malaria

1-1- Disease generalities

Malaria is caused by infection with protozoan parasites of the *Plasmodium* species. *Plasmodium falciparum* is widespread in Africa while *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* infections are less common and geographically restricted (Roucher *et al*., 2014; Howes *et al*., 2015). *Plasmodium knowlesi*, the fifth human parasite is essentially a primate malaria species from remote forested areas of Southeast Asian countries (White, 2008; Putaporntip *et al*., 2009).

The parasites are transmitted by *Anopheles* mosquitoes, with *Anopheles gambiae*, *Anopheles funestus*, and *Anopheles Arabiensis* being the most prevalent in Africa (Sinka *et al*., 2010). Patients with non–specific symptoms, including fever, rigors, and chills, and the majority will not require hospital admission. Severe malaria develops in a minority, and in children it may manifest as a fever, impaired consciousness, severe anemia, respiratory distress, convulsions, and hypoglycemia, among other symptoms. The epidemiology of malaria varies geographically depending on the local malaria transmission intensity (Nkumama *et al*., 2017).

1-2- Malaria global trends and burden

In 2019, an estimated 229 million cases of malaria occurred worldwide, compared to 211 million cases in 2015 and 217 million cases in 2016 (World Malaria Report, 2016; 2020). Most malaria cases in 2019 were in the WHO African Region with 94% (215 million cases), followed by the WHO South–East Asia Region accounting for 5% (World Malaria Report, 2020). In 2019, of the 87 countries reporting indigenous malaria cases, 29 countries all in sub-Saharan Africa, except India (carrying about 2% of the global burden) accounted for 95% of the global malaria burden. The incidence rate of malaria is estimated to have decreased by 27% globally, from 80 to 58 cases per 1000 population at risk, between 2000 and 2015. However, the number of cases per 1000 population at risk has slowly decreased by 2% between 2016 and 2019 moving to 57 over 4 years (World Malaria Report, 2015; 2017; 2018; 2020).

Plasmodium falciparum is the most prevalent malaria parasite in sub-Saharan Africa, accounting for 99.0% and 99.7% of estimated malaria cases in 2016 and 2017 respectively (World Malaria Report, 2017; 2018). Outside of Africa, *Plasmodium vivax* is the predominant

parasite in the WHO Region of the Americas, representing 64% of malaria cases, and is above 30% in the WHO South–East Asia and 40% in the Eastern Mediterranean regions (World Malaria Report, 2017).

The WHO recorded an estimated 409,000 deaths from malaria globally in 2019 and the WHO Africa was the leading region with 94% (384,000 deaths) of the overall reported deaths, followed by the WHO South–East Asia Region (World Malaria Report, 2020). Overall, 32 countries with 31 of them located in sub–Saharan Africa accounted for 95% of global malaria deaths in 2019. And, between 2015 and 2019, mortality rates stalled in the WHO region of South–East Asia, the Western Pacific and Africa, and even increased in the Eastern Mediterranean and the Americas (World Malaria Report, 2016; 2017; 2018, 2020). Although malaria case incidence has fallen globally since 2010, the rate of decline has stalled and even reversed in some regions since 2015, and mortality rates have followed a similar trend (World Malaria Report, 2018; 2020).

1-3- Malaria trends and burden in Central Africa

Briefly, malaria infection is still a major public health concern for Africa and the high burden of malaria in Africa is exclusively attribute to *P. falciparum*, which adapts and cospecializes with *Anopheles gambiae*, the most effective and widespread malaria vector. Since 2000, the incidence of malaria has been reduced by 17% and malaria mortality rates by 26%. However, the rate of decline has stalled and even reversed in some regions since 2014, and despite all recent efforts and investment made to reduce malaria burden across the continent, the African region continues to consistently average from 92% to 94% of malaria cases and deaths worldwide year on year (Mbacham *et al*., 2019 in Ariey *et al*., 2019).

Africa has the most substantial burden, especially sub–Saharan African countries, with about 215 million cases of illness and 384,000 deaths in 2019 (World Malaria Report, 2020). In 2019, about 186 million people living in the 10 countries were at high risk to Malaria and the transmission, exclusively due to *Plasmodium falciparum*, occurs throughout the year and was highly seasonal in the north of Cameroon, northern Chad, and the southern part of the Democratic Republic of the Congo. The WHO reported about 51 million estimated cases and almost 90,000 estimated deaths from Central Africa in 2019 (World Malaria Report, 2020).

Five countries in the subregion accounted for 80% of the estimated cases in 2019 *viz.* the Democratic Republic of the Congo with 55.5% of the estimated cases, followed by Angola

(14.9%), Cameroon (12.8%), Burundi (5.8%) and Chad (5.2%). A similar distribution was seen for estimated malaria deaths, the Democratic Republic of the Congo leading with 49% of total subregion reported deaths, followed by Angola (15%), Cameroon (13%) and Chad which registered 10%of the total deaths (World Malaria Report, 2020). More than 48 million cases were reported in the public and private sector, and in the community; of these, 46.8% were in children aged under 5 years and 46.9 million (96.3%) were confirmed (World Malaria Report, 2020).

Five countries saw an increase in estimated malaria incidence between 2015 and 2019; Burundi had the largest increase (54%), followed by Angola (18%), Sao Tome and Principe (10%), the Democratic Republic of the Congo (5%) and the Congo with a 4% increment (World Malaria Report, 2020). Chad and Gabon had an increase of nearly 50% in reported cases between 2016 and 2017 (World Malaria Report, 2018). The subregion registered an increase from 6.3 million in 2010 to 47 million confirmed cases of malaria in 2019 and above 12% (40,400 in 2010 versus 45,400 in 2019) increase of confirmed deaths due to Malaria between 2010 and 2019 (World Malaria Report, 2018; 2020).

1-4- Malaria trends and burden in Cameroon

Cameroon is a malaria endemic country where it is the leading cause of morbidity and mortality among the most vulnerable groups (Ntonifor and Veyufambom, 2016), including children aged under 5 years (18%), pregnant women (5%), people living with HIV/AIDS (5.5%) and the poor (40%) (Ndong *et al*., 2014). Nationally, malaria accounts for 48% of all hospital admissions, 30% of morbidity and 67% of childhood mortality per year (Antonio-Nkondjio *et al*., 2013).

In Central Africa, Cameroon is the second country with the highest estimated malaria cases reported in 2016 (14%) and 2017 (16%) behind the Democratic Republic of Congo (World Malaria Report, 2017; 2018). In 2017, 71% of national population were leaving in area with high transmission to Malaria whereas the remaining 29% were in area with low Malaria transmission. The WHO reported 6,291,256 estimated cases of Malaria and 11,233 estimated deaths due to Malaria (World Malaria Report, 2020). *Plasmodium falciparum* is at 100% the causative agent of all reported cases of malaria and affordable deaths.

1-5- Malaria vector transmission

There are more than 400 different species of *Anopheles* mosquito, with around 30 that are malaria vectors of major importance. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment and transmission is stronger in areas where the mosquito life span is longer, allowing the parasite to complete its development in the mosquito, and especially where human density is high, giving more probability to bite humans rather than animals (Mbacham *et al*., 2019 in Ariey *et al*., 2019).

Plasmodia are spread from one person to another by female mosquitoes of the genus *Anopheles*. The *Anopheles gambiae* complex are the most important vector in Sub–Saharan Africa (World Malaria Report, 2014). In Central Africa, the most predominant species of *Anopheles* transmitting Malaria are *Anopheles arabiensis, Anopheles funestus, Anopheles gambiae, Anopheles hancocki, Anopheles melas, Anopheles moucheti, Anopheles nili and Anopheles pharoensis* (World Malaria Report, 2018; 2020).

In Cameroon, five species of *Anopheles* namely *Anopheles gambiae, Anopheles arabiensis, Anopheles funestus, Anopheles moucheti* and *Anopheles nili* were identified as the most responsible of disease transmission across the country (World Malaria Report, 2018).

1-6- Malaria pathogenesis and disease transmission

Malaria infection starts with the inoculation of a small number of sporozoites by a probing female anopheline mosquito (White, 2017). These motile parasites pass to the liver within an hour. Having invaded hepatocytes, they then begin a period of rapid asexual multiplication (Fairley, 1947; White *et al*., 2014), dividing approximately every 8 h until each infected liver cell contains thousands of merozoites. In *Plasmodium vivax* and *Plasmodium ovale* malaria infections, a subpopulation of sporozoites form dormant liver stages called hypnozoites which awaken weeks or months later to cause relapses of malaria (White *et al*., 2014).

At the completion of pre–erythrocytic development and following hepatic schizont rupture the newly liberated merozoites enter the blood stream and promptly invade erythrocytes. The growing intraerythrocytic malaria parasites begin to consume the red cell contents. The complete life cycle in the red blood cells approximates one day for *Plasmodium knowlesi*, two days for *Plasmodium falciparum, Plasmodium vivax* and *Plasmodium ovale* and three days for *Plasmodium malariae* (White *et al*., 2014a). A small subpopulation of asexual

parasites may stop growing and dividing for days or weeks, staying in dormancy (Cheng *et al.*, 2012) [Figure 1].

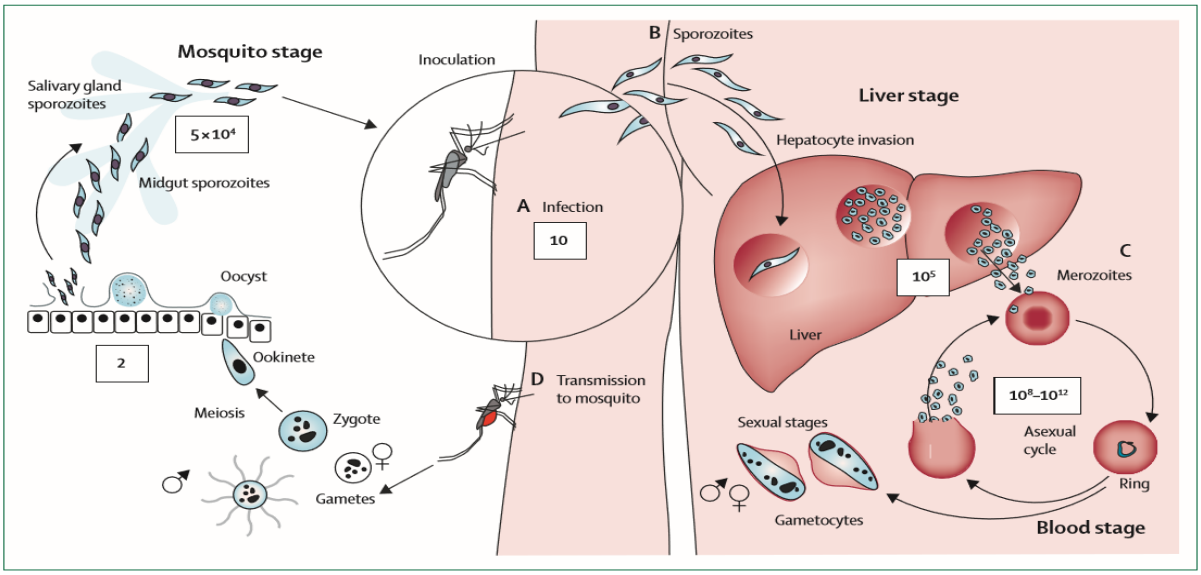


Figure 1: Life cycle of *Plasmodium falciparum* in human and in anopheline mosquito.

The cycle begins with inoculation of motile sporozoites into the dermis [**A**], which then travel to the liver [**B**]; each sporozoite invades a hepatocyte and then multiplies. After about a week, the liver schizonts burst, releasing into the bloodstream thousands of merozoites that invade red blood cells and begin the asexual cycle [**C**]. Illness starts when total asexual parasite numbers in the circulation reach roughly 100 million. Some parasites develop into sexual forms, gametocytes. Gametocytes are taken up by a feeding anopheline mosquito [**D**] and reproduce sexually, forming an ookinete and then an oocyst in the mosquito gut. The oocyst bursts and liberates sporozoites, which migrate to the salivary glands to await inoculation at the next blood feed. The entire cycle can take about 1 month (White *et al.*, 2014b).

1-6-1. *Plasmodium* pre–erythrocytic life cycle

Plasmodium sporozoites are injected into the host dermis during a blood feed. The fate of these sporozoites is not well understood, but they can take 1–3 hours to exit from this site. Here, they rely on gliding motility, a random process enabling a proportion to reach and penetrate a blood vessel to enter the bloodstream. Those remaining in the skin can be destroyed and are drained by the lymphatics, where a host immune response is generated. The protein Trap-like protein plays a role in exit from the dermis, as mutant sporozoites lacking its function display normal gliding motility but cannot enter the circulation. Those that enter the blood stream quickly access the liver by a process known as traversal. This involves crossing the sinusoidal barrier comprising fenestrated endothelial cells and macrophage–like Kupffer cells (Tavares *et al.*, 2013). Proteins required for traversal include sporozoite microneme protein essential for traversal (Ishino *et al.*, 2004), perforin–like protein 1 (Risco-Castillo *et al.*, 2015), cell traversal protein for ookinetes and sporozoites, phospholipase (Bhanot *et al.*, 2005), and

gamete egress and sporozoite traversal protein. The function of these proteins in cell traversal is not yet clearly understood, although perforin–like protein 1 has a membrane attack complex/perforin-like domain, suggesting that it plays a role in punching holes in membranes. Sporozoites traverse through cells by forming a transient vacuole, and perforin–like protein 1 and pH sensing is involved in egress from this structure (Risco-Castillo *et al*., 2015).

It has been suggested that cell traversal through the sinusoidal barrier is important for infectivity by priming the sporozoite for invasion of hepatocytes, the cells in which sporozoites develop. However, the primary role of sporozoite traversal is crossing the sinusoidal barrier (Tavares *et al*., 2013). In fact, sporozoites injected into the dermis are in the migratory mode and upon interaction with hepatocytes, they convert to invasive mode. One signal for this switch is recognition of hepatocytes through binding higher sulfated forms of heparin sulfate proteoglycans activating calcium–dependent protein kinase 6 (Coppi *et al*., 2007). The tetraspanin CD81 and scavenger receptor B1 are human hepatocyte surface proteins required for invasion and formation of a parasitophorous vacuole by *P. falciparum* sporozoites (Rodrigues *et al*., 2008). In contrast, the hepatocyte receptor EphA2 is not required for hepatocyte invasion but for intra-hepatocytic development by establishment of the parasitophorous vacuole through interaction with parasite proteins p52 and p36 (Kaushansky *et al*., 2015).

A dense coat covers the sporozoite, and a key protein is the circumsporozoite protein (CSP), consisting of a highly repetitive region and a type I thrombospondin repeat (TSR). Invasion of hepatocytes requires binding of CSP to highly sulfated proteoglycans, activating processing of CSP and removal of the N terminus exposing the TSR domain (Herrera *et al*., 2015). Subsequent steps involve proteins, including thrombospondin–related anonymous protein and apical membrane antigen-1, with adhesive domains released from the apical organelles (micronemes and rhoptries). Once hepatocyte infection is established, the sporozoite transforms over the subsequent 2–10 days to a liver stage or exo–erythrocytic form, and development culminates in release of up to 40,000 merozoites per hepatocyte into the bloodstream by budding of parasite-filled vesicles called merosomes (Sturm *et al*., 2006).

1-6-2. *Plasmodium* intra–erythrocytic life cycle

Once released from liver, free merozoites invade erythrocytes in a fast, dynamic and multi–steps process including pre–invasion, active invasion, and echinocytosis (Weiss *et al*., 2015) that is complete within 2 minutes. Pre–invasion is the initial interaction of merozoites

with erythrocytes, and little is understood about the molecular details of this step. Merozoite surface protein 1 (MSP1) is the major glycophosphatidylinositol–associated protein on the merozoite surface (Holder, 1994), and its acts as a platform on the merozoite surface for at least three large complexes with different extrinsic proteins that bind erythrocytes (Lin *et al*., 2016). However, merozoites lacking surface MSP1 can invade erythrocytes, suggesting that it is not absolutely required for invasion (Das *et al*., 2015). It is possible that MSP1 is involved in display of proteins involved in evasion of host responses rather than directly in merozoite invasion.

Merozoites actively invade red blood cells using an actin–myosin motor. Invagination of the erythrocyte membrane during invasion contributes to the formation of the parasitophorous vacuole, a compartment within which the parasite develops isolated from the red blood cell cytosol. After invasion, the asexual developmental cycle is initiated. Morphologically defined ring stage parasites mature and grow within the erythrocytes as they degrade the host hemoglobin, to progress into trophozoite stage. Multiple rounds of asynchronous nuclear division occur during the process of schizogony, for trophozoites to mature in schizont stage, followed by a concerted invagination of the plasma membrane, which produces daughter merozoites (Deu, 2017).

Once erythrocyte infection is established, over the subsequent 48 hours, cell division or schizogony results in 16 – 32 merozoites that egress when developed, which results in destruction of the erythrocyte membrane and explosive release of parasites to access new host cells for invasion and the beginning of a new life cycle [Figure 1]. The coordinated process of merozoite egress is tightly regulated and involves several protein kinases, including the plant–like Calcium–dependent protein kinase (Dvorin *et al*., 2010) and cGMP-dependent protein kinase (Collins *et al*., 2013). MSP1 has a role in egress of merozoites from *P. falciparum*–infected cells through subtilisin 1 processing on the merozoite surface that activates its ability to bind the erythrocyte membrane protein spectrin (Das *et al*., 2015).

Parasite multiplication rates in non–immune patients in this early stage of infection, before the symptoms of malaria, range typically from six to ten–fold per cycle, but sometimes can reach twenty–fold (Fairley, 1947; Simpson *et al*., 2002; White, 2002; Dietz *et al*., 2006). As a result, total parasite numbers in the blood rise exponentially from 10^4 to 10^5 in the first asexual cycle to reach 10^8 after 3 to 4 cycles (6 ~ 8 days for *Plasmodium falciparum*). One hundred million parasites in the body of an adult human corresponds with a blood parasite

density of about 50/µL (Fairley, 1947; White, 2002) and this density is usually associated with the onset of fever and illness in non–immune subjects, and known as pyrogenic density (Kitchen *et al*., 1949).

The addition of a pre–erythrocytic liver development of 5.5 to 7 days plus about 8 days of blood stage multiplication results in the usual incubation period of 11 to 15 days in *Plasmodium falciparum* malaria (Kitchen *et al*., 1949). People who have had multiple previous malaria infections acquire an antitoxic immunity called premunition, which results in higher parasite densities being tolerated without symptoms, although densities over 10,000/µL are usually associated with illness even in areas of high malaria transmission (White *et al*., 2014; Smith *et al*., 1994). Immunity slows parasite multiplication and accelerates parasite clearance. In most infections after logarithmic parasite multiplication there is an abrupt reduction in parasite multiplication at high densities, and severe malaria is a consequence of continuous multiplication of infecting parasites (Chotivanich *et al*., 2000).

A subpopulation of the blood stage parasites commits to sexual development forming male and female gametocytes. This reduces the parasite multiplication rate. Commitment or switching to sexual development occurs immediately in *Plasmodium vivax* malaria, which becomes infectious to mosquitoes at/or even below pyrogenic densities, whereas gametocytogenesis is delayed in *Plasmodium falciparum* malaria (Bousema and Drakeley, 2011). The switching increases with duration of infection, anemia, and other stresses to the parasite population such as partially effective anti–malarial treatment.

In *Plasmodium falciparum* infections, the developing sexual stages sequester for about 7 ~ 10 days in venules and capillaries and particularly in the bone marrow before reentering the circulation as immature stage 5 gametocytes. As a result, peak *Plasmodium falciparum* sexual stage densities typically occur approximately 10 days after the peak asexual densities (White, 2017). Gametocytes are cleared relatively slowly from the blood, so they accumulate with respect to asexual parasites and can predominate in chronic infections. The gametocytes of *Plasmodium falciparum* malaria are relatively insensitive to most anti–malarial drugs, except for the 8–aminoquinolines whereas the gametocytes of the other human malaria parasites are considered as drug sensitive (Bousema and Drakeley, 2011; White *et al*., 2014b).

1-6-3. Clinical manifestations of the disease

Malaria symptoms develop from 6 days post – infecting bite. Most of *P. falciparum* present manifestations within a month following infection by female *Anopheles* mosquito, but they can also appear later, usually within 12 months and especially in individuals taking prophylaxis. It is important to notice that *P. vivax, P. malariae* and *P. ovale* infections commonly have a longer incubation period. Furthermore, *P. vivax* and *P. ovale* can relapse or present symptoms after an extended period because of activation of hepatic hypnozoites (Walker, Nadjm and Whitty, 2018). Patients can also undergo recrudescence, in which parasites reappear in the blood system following failed treatment that has not completely cleared them. *P. vivax, P. malariae* and *P. ovale* are much less likely to cause severe disease than *P. falciparum*, but *P. falciparum* and *P. malariae* malaria do not relapse. Therefore, a second episode of malaria due to *P. falciparum* clearly suggests treatment failure or reinfection.

The first symptoms of malaria are non - specific, and include a vague absence of wellbeing, headache, fatigue, muscle aches, and abdominal discomfort, which are followed by irregular fever. Nausea, vomiting, and orthostatic hypotension occur frequently. Generalized seizures are associated specifically with *P. falciparum* malaria and might be followed by coma in case of cerebral malaria. Most patients with uncomplicated infections have few abnormal physical findings other than fever, mild anaemia, and, after several days, a palpable spleen. The liver can become enlarged, especially in young children, whereas mild jaundice is more likely in adults. In young children living in regions in which transmission is stable, recurrent infections cause chronic anaemia and splenomegaly (White *et al*., 2014b).

Other symptoms are also non – specific and can be misleading, commonly resulting in misdiagnose of influenza, hepatitis, gastroenteritis, or meningitis for example. In children with severe *falciparum* malaria, respiratory distress, anemia, convulsion, and hypoglycemia are more common. In cerebral malaria, malarial retinopathy including retinal whitening, white – centered hemorrhages and papilloedema may appear. Globally, pulmonary oedema, acute respiratory distress syndrome and acute kidney injury occur over half of all cases of life – threatening malaria in non – immune adults (Walker, Nadjm and Whitty, 2018).

1-6-4. Malaria transition from pathogenesis to transmission

The malaria parasite is transmitted by female *Anopheles* mosquitoes, which bite mainly between dusk and dawn. During rounds of schizogony in the bloodstream, a proportion of parasites undergo a developmental switch initiating commitment to sexual development to

form male and female gametocytes. The transmission of malaria from humans to mosquitoes is dependent on development of the sexual stages (Cowman *et al*., 2016). Although molecular events around this developmental switch remain elusive, the timing of transition occurs at some point in the previous schizogony cycle, and daughter merozoites from a single schizont-infected cell are committed to develop into either gametocytes or asexual schizonts. Environmental stimuli, such as high parasitemia and exposure to drugs such as chloroquine, are associated with increased conversion to gametocyte production.

P. falciparum gametocyte maturation is an extended process relative to other species. Once commitment has initiated, it takes 11 days for mature gametocytes that are infectious to mosquitoes to develop. During this time, they remain sequestered within bone marrow (Joice *et al*., 2014), avoiding splenic clearance until emerging into the peripheral circulation for an unknown time until uptake by a feeding mosquito. It is worth noting that and early diagnosis and appropriate treatment regimen applied in case of malaria will be surely life – saving.

1-7- Malaria case management

Malaria case management, consisting of early diagnosis and prompt effective treatment, remains a vital component of malaria control and elimination processes. Uncomplicated *P. falciparum* malaria can progress rapidly to severe forms of the disease, especially in patients with no or low immunity. Severe *falciparum* malaria is almost fatal when not proper follow with adequate treatment regimen. Thus, malaria programmes should always ensure access to early diagnosis, prompt and effective treatment within 48 hours following onset of malaria symptoms (WHO – Guidelines for the treatment of malaria, 2015).

With the purpose to reduce the spread of drug resistance, avoid unnecessary use of antimalarial drugs and better identify other febrile illness, antimalarials medicines should only be administered to patients who have been effectively diagnosed malaria positive, and adherence to full treatment course must be highly recommended. In line with the protection of available and future antimalarial medicines, all diagnosed case of malaria should be treated with a combination of at least two effective antimalarial drugs with different mechanisms of action (WHO – Guidelines for the treatment of malaria, 2015).

1-7-1. Malaria diagnosis

Diagnosing malaria is a key component of effective case management and monitoring of antimalarial programs worldwide (Ayong *et al*., 2019 in Ariey *et al*., 2019). Nowadays, access to parasitological diagnosis of malaria is available worldwide with the use of quality–

assured rapid diagnostic tests (RDTs). Prompt and accurate diagnosis of malaria is part of effective disease management, and all patients with suspected malaria should be treated based on a confirmed diagnosis either by microscopy examination or Rapid Diagnosis Test (RTD) testing of blood sample. It is worth noting that pro–active and correct diagnosis in malaria – endemic areas is of utmost importance mainly for the most vulnerable groups such as young children under 5 years old and non–immune individuals, in whom *P. falciparum* malaria is easily fatal (WHO – Guidelines for the treatment of malaria, 2015).

Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood and its efficacy relies on many factors such as the different forms of the five malaria parasites species, the different stages of erythrocytic schizogony, the endemicity of different species, the inter – relation between levels of transmission, population movement, parasitemia, immunity, and signs and symptoms; drug resistance, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, which can all influence the identification and interpretation of malaria parasitemia in a diagnostic test (Tangpukdee *et al*., 2009).

1-7-1.1. Clinical diagnosis

Clinical diagnosis is based on the patient's signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus (Looareesuwan, 1999).

A clinical diagnosis of malaria is still challenging because of the non - specificity of the signs and symptoms, which overlap considerably with other common, as well as potentially life – threatening diseases, like common viral or bacterial infections, and other febrile illnesses (Tangpukdee *et al*., 2009). The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for patients with non-malarial fevers in endemic areas (Mc Morrow *et al*., 2008).

1-7-1.2. Laboratory diagnosis

The non – specific nature of the clinical signs and symptoms of malaria may result in over – treatment of malaria or lack of treatment of other diseases in malaria – endemic areas, and misdiagnosis in non – endemic areas (Bhandari *et al*., 2008). In the laboratory, malaria is diagnosed using different techniques such as conventional microscopic diagnosis by staining

thin and thick peripheral blood smears (Ngasala *et al*., 2008), Quantitative Buffy Coat method (Bhandari *et al*., 2008), Rapid Diagnostic Tests, Serological Tests and molecular diagnostic methods, such as polymerase chain reaction (Holland and Kiechle, 2005; Vo *et al*., 2007).

- **Microscopic diagnosis of Malaria**

Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites. Briefly, the patient's finger is cleaned with 70% ethyl alcohol, allowed to dry and then the side of fingertip is picked with a sharp sterile lancet and two drops of blood are placed on a glass slide. A blood spot is stirred in a circular motion with the corner of the slide to prepare a thick blood film, taking care to not make the preparation too thick, and allowed to dry without fixative. After drying, the spot is stained with Giemsa, and washed by placing the film in buffered water. The slide is then air – dry in a vertical position and examination is done using a light microscope (Chotivanich *et al*., 2006; Tangpukdee *et al*., 2009).

- **Quantitative Buffy Coat method for Malaria diagnosis**

This method involves staining parasite deoxyribonucleic acid in micro – hematocrit tubes with fluorescent dyes like acridine orange, and its subsequent detection by epi – fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope (Chotivanich *et al*., 2006). The parasite nuclei fluoresce bright green, while cytoplasm appears yellow – orange.

- **Rapid Diagnostic Tests (RDTs)**

The RDTs are based on the detection of malaria antigen in blood flowing along a membrane containing specific anti–malaria antibodies. Most products target a *Plasmodium falciparum* – specific protein such as histidine-rich protein II or lactate dehydrogenase. Some tests detect *P. falciparum* specific and distinguish non – *P. falciparum* infections from mixed malaria infections. Although most RDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria; a new RDT method were developed recently for detecting *P. knowlesi* malaria (McCutchan *et al*., 2008; Lee *et al*., 2008).

- **Serological Tests**

The diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites and immunofluorescence antibody testing as example were reliable serologic test for malaria in recent decades (She *et al*., 2007). Malaria antibody detection is performed using the indirect fluorescent antibody

- **Molecular diagnosis**

The new molecular biological technologies such as PCR, loop – mediated isothermal amplification, microarray, mass spectrometry, and flow cytometric assay techniques, have permitted extensive characterization of the malaria parasite and are generating new strategies for malaria diagnosis (Tangpukdee *et al*., 2009).

1-7-2. Malaria treatment

The successful chemotherapy in the treatment of malaria is effective by identification of the infecting malarial species, exploiting metabolic differences between the host and the parasite and blocking the parasite from proliferating and the complexities occurring after it. It is also noticeable that geographical conditions and the clinical manifestation of the infected individual are very important in the selection of appropriate drug as because some *Plasmodium* species, particularly in some areas are more resistant to certain antimalarials as compared to other species of the different geographical area (Dondorp *et al*., 2009; Wongsrichanalai and Sibley, 2013; Severini and Menegon, 2015).

The World Health Organization usually recommends the combination therapy for antimalarials because this will halt the appearance of drug resistance (WHO – World Malaria Report, 2014). Moreover, the combination of two or more active drugs with different mechanism of action is more effective than a single drug against the *Plasmodium* species. There is a backlog of molecules belonging to various chemical family and available to treat malaria infection either as single treatment regimen or in combination of partner drugs, that constitute the core of malaria therapy.

- **Quinine**

Quinine is an alkaloid derived from the bark of the C*inchona officinalis* tree and was the first chemical used in the treatment of malaria. Quinine is widely used in treating uncomplicated malaria and it acts on the blood schizonticidal stage of *P. falciparum, P. vivax, P. ovale, P. malariae* and gametocytocidal stage of *P. vivax* and *P. malariae*.

- **Chloroquine**

Chloroquine acts in the erythrocyte stage of *Plasmodium*, by interfering with the hemoglobin digestion by the parasite (Lehane and Kirk, 2008). The development of resistant strains throughout the world made chloroquine to not be considered anymore as an effective antimalarial medicine.

- **Amodiaquine**

Amodiaquine belongs to 4-aminoquinoline chemical family and its plasmodicidal action is not completely understood. Amodiaquine can be used as partner drug with Artesunate and Sulfadoxine – Pyrimethamine (Olliaro and Mussano, 2003), in combination against malaria infections.

- **Artemisinin and derivatives compounds**

Artemisinin, a chemical from *Artemisia annua* plant is a 1,2,4-trioxane compound, incorporating an endoperoxide bridge essential for antimalarial activity. Artemisinin is a highly potent and effective medicine in the treatment of *Plasmodium falciparum* resistant strains (Meshnick, 2002). Artemisinins get activated in the ring stage of the parasite during the opening of the ring, which then generates free radical species that gets linked to specific protein target site, thereby inhibiting its function (Wang *et al*., 2010). Its fast action and less side effects led the World Health Organization (WHO) to recommend Artemisinins as the first choice in treating drug–resistant strains worldwide. Artemisinin derivatives include dihydroartemisinin, artemether, arteether and artesunate. Artemisinin and derivates are the only antimalarials with required efficacy, minor toxicity, and less side effects available to treat severe malaria. The WHO has recommended the use of artemisinin derivatives in combination with other antimalarials (partner drugs) with the purpose to delay the emergence of resistance (World Malaria Report, 2014). The current useful antimalarials drugs are listed in the Table 1 below.

Table 1: Antimalarial drugs, chemical classes, mechanisms of action, advantages, drawbacks, and indications

Antimalarials	Chemical classes	Mechanisms of action	Advantages	Drawbacks and adverse effects	Clinical indications	References
Chloroquine	4 - Aminoquinoline	Prevents heme detoxification into nontoxic hemozoin into food vacuole of *Plasmodium* parasites	high bio – availability and well tolerated at therapeutic doses	Pruritus, hepatitis, skin eruptions, headache, and drug resistances. Long half – life	Uncomplicated malaria caused by *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*.	Winstanley and Ward, 2006; WHO – Guidelines for the treatment of malaria, 2015; Baruah *et al.*, 2017; Pan *et al.*, 2018
Amodiaquine			Effective against Chloroquine – resistant malaria parasite	Hemolytic anemia, peripheral neuropathy		
Piperaquine				Potential cardiotoxicity		
Primaquine	8 – Aminoquinoline	Produces intermediates reactive that disrupt metabolic processes of plasmodial mitochondria and interfere with its electron transport	The only hypnozoitocidal and gametocytocidal drug for *Plasmodium vivax* and *ovale*	Abdominal pain, nausea, vomiting, hypertension, and cardiac arrhythmia	*Plasmodium vivax* and *Plasmodium ovale* malaria infection	
Quinine	Arylamino – alcohol	Prevents heme detoxification into nontoxic hemozoin into food vacuole of *Plasmodium* parasites	High oral bio – availability	Hypoglycemia, hematologic disorders, neurotoxic. Small therapeutic index	Uncomplicated and severe malaria	
Mefloquine			Potent antiplasmodial activity against erythrocytic large ring and trophozoites asexual stages	Anxiety, irritability, dizziness, paranoia, suicidal ideation, depression, hallucinations	Malaria chemoprophylaxis, Uncomplicated malaria	
Proguanil	Biguanide	Acts by inhibiting dihydrofolate reductase thus folic acid synthesis via its active principle cycloguanil	Active against all stages of *Plasmodium falciparum* life cycle	Variations in metabolism and parasites resistance	Malaria chemoprophylaxis, uncomplicated malaria treatment in travelers outside malaria – endemic areas.	
Atovaquone	Naphthoquinone	Inhibits the transport of several enzymes and interferes with the cytochrome electron transport system in *Plasmodium* mitochondria	Effective antimalarial activity against all stages of all *Plasmodium* species	High – grade resistance, long elimination half – life, poor and variable absorption		

Sulfadoxine (S)	Sulfonamide	Inhibitor of dihydropteroate synthetase, key enzyme of folic acid synthesis	Well tolerated and good bio – availability	Gastrointestinal disturbances, headache, dizziness, redness, Photosensitivity, leukopenia, haemolityc anemia	Malaria chemoprophylaxis, Uncomplicated malaria treatment in travelers outside malaria – endemic areas.	Winstanley and Ward, 2006; WHO – Guidelines for the treatment of malaria, 2015; Baruah *et al.*, 2017; Pan *et al.*, 2018
Pyrimethamine (P)	Glycosylamine	Inhibitor of dihydrofolate reductase, thus prevent folic acid synthesis	Well absorbed after oral administration			
Artemether	Sesquiterpene lactone	Generation of cation – mediated reactive intermediates and reduction of the peroxide bridge that kill parasite through series of molecular oxidations	Well tolerated and good bio – availability. Fast action against all plasmodia blood stages	Neurotoxicity, bradycardia, poor water solubility, resistances	*Plasmodium falciparum* Chloroquine resistant malaria	
Artesunate				Low oral bio – availability, parasites resistances		
Dihydroartemisinin (DHA)				Very short half – life, parasites resistances		
Artemether – Lumefantrine	Artemisinin – based Combination Therapy	Generation of cation – mediated reactive intermediates and reduction of the peroxide bridge. Prevents heme detoxification into non – toxic hemozoin. Inhibition of dihydropteroate synthetase and dihydrofolate reductase.	Wide therapeutic index, well tolerated, good bioavailability, fast action against blood stages. Effective against Chloroquine – resistant malaria parasite	Nausea, dizziness, headache, hepatotoxicity, agranulocytosis, abdominal pain, anorexia, insomnia, fatigue, bradycardia, diarrhea and vomiting, anaemia, cough, parasites resistances	Uncomplicated and severe malaria. *Plasmodium falciparum* Multi – drugs resistant malaria	
Artesunate – Amodiaquine						
Artesunate – Mefloquine						
DHA – Piperaquine						
Artesunate – S.P.						
Sulfadoxine – Pyrimethamine	Other Combination Therapy	Inhibitors of dihydropteroate synthetase and dihydrofolate reductase	Well tolerated and good bio – availability.	Development of resistance, headache, dizziness, redness, photosensitivity, leukopenia	Malaria chemoprophylaxis, Uncomplicated malaria treatment in travelers outside malaria – endemic areas.	
Atovaquone – Proguanil		Dihydrofolate reductase inhibitor through cycloguanil; synergistic interaction that lower effective concentration of Atovaquone	Effective antimalarial activity against all stages of all *Plasmodium* species	Cough, headache, diarrhea and vomiting, abdominal pain, oral ulceration, hepatic failure		

DHA: Dihydroartemisinin, **S.P.:** Sulfadoxine – Pyrimethamine

The main disadvantages of Artemisinins and their combination therapies are the high incidence of recrudescence and relapses along with the high cost and dosing complexity (Chen *et al*., 2007; Douglas *et al*., 2010). Besides, there are numerous relevant reports recently populated on the emergence and the development of resistance to artemisinin derivatives and their various therapeutics combinations in the Thai – Cambodian border (Dondorp *et al*., 2009; Ashley *et al*., 2014; Fairhurst and Dondorp, 2016). Moreover, Lu *et al*. (2017) reported the potential emergence of indigenous Artemisinin – resistant *Plasmodium falciparum* strain in Central Africa.

Early diagnosis and treatment together with vector control comprise the cornerstone of effective malaria control. Of note, anticipated effective treatment of *P. falciparum* infections prevents progression of the disease to severe malaria, which still takes more than 400,000 lives every year (World Malaria Report, 2020). The first-line treatment of uncomplicated *falciparum* malaria in all malaria endemic countries is still with artemisinin combination therapies (ACTs). It is therefore of great concern to regional and global malaria elimination initiatives that artemisinin resistant *P. falciparum* has emerged and spread in the Greater Mekong Subregion and, more recently, has emerged independently in Guyana, Papua New Guinea, Central Africa, Ethiopia, Uganda and particularly in Rwanda, where its prevalence has recently increased (Lu *et al*., 2017; Mathieu *et al*., 2020; World Health Organization, 2020; Uwimana *et al*., 2020; Rosenthal, 2021; Uwimana *et al*., 2021). In artemisinin resistant *P. falciparum* infections, the malaria parasites are still cleared after ACTs treatment but, because of the loss of ring stage susceptibility, parasite killing is reduced and clearance is slower. As a result, the artemisinin component of the ACTs contributes less to the antimalarial effect and efficacy becomes more dependent upon the partner drug (Imwong *et al*., 2021). There are currently six ACTs recommended *viz* artesunate-sulfadoxine-pyrimethamine, artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, dihydroartemisinin-piperaquine and recently artesunate-pyronaridine. When susceptibility to the partner drug declines, ACT efficacy drops significantly, and the proportion of recrudescent infections increases.

Besides concerning drugs resistances, reliable molecular markers are available for *P. falciparum* resistance to almost all currently deployed antimalarial medicines including artemisinins [mutations in the propeller region of *PfKelch*], sulfadoxine-pyrimethamine [mutations in dihydrofolate reductase, *Pf*DHFR, and dihydropteroate synthase, *Pf*DHPS], mefloquine [amplification of multidrug resistance-1, *Pf*MDR1], and piperaquine [amplification of *Pf*Plasmepsin2/3] and specific *P. falciparum* chloroquine resistance transporter, *Pf*CRT,

mutations (Uwimana *et al*., 2021). Molecular markers explaining most of the variance in susceptibility for the other partner drugs, lumefantrine, amodiaquine and pyronaridine are not well established (Imwong *et al*., 2021).

Artemisinin resistance is defined by a delayed parasite clearance half-life ($PCt_{½} > 5$ h) *in vivo* following an artemisinin-based treatment (Noedl *et al*., 2008), or by an increased parasite survival rate following a brief exposure to a high dose of dihydroartemisinin in the ring-stage survival assay (RSA^{0-3h}) *in vitro* (Witkowski *et al*., 2013). The first artemisinin-resistant laboratory strain (F32-ART5) was selected through *in vitro* exposure to a dose-escalating sequential artemisinin regimen and led to the identification of the M476I mutation in the parasite's *Pf*k13 gene as the main driver of resistance (Ariey *et al*., 2014). Propeller polymorphisms of the *kelch13* are useful molecular markers for monitoring the emergence and spread of artemisinins resistance (Ariey *et al*., 2014; Ashley *et al*., 2014). To date, 10 non-synonymous mutations in *kelch13* domain have been validated as polymorphisms for artemisinins resistance, these include F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, and C580Y (World Malaria Report, 2020). Predictive analytics estimated that by 2040, African malaria-endemic countries, accounting for about 94% of both malaria cases and deaths, might face widespread artemisinins resistance (Scott *et al*., 2018); and such a scenario without appropriate countermeasures in term of effective drugs availability would be a major public health disaster.

More recently, the World Health Organization recommended the widespread immunization of children across sub-Saharan Africa and elsewhere with the world's first RTS, S vaccine against malaria. However, it is important to note that this vaccine does not offer full protection against malaria as it reduces the risk of contracting malaria by only 40% and the risk of hospital admission with severe malaria by around 30% when at least three doses are administered. Hence the WHO is right in saying "At the same time, we acknowledge that this is not a silver bullet solution, and we must continue to develop new more effective therapies, vaccines, and preventive tools against malaria (World Health Organization, 2021- https://www.who.int/news/item/06-10-2021-who-recommends-groundbreaking-malaria-vaccine-for-children-at-risk. Accessed on November 5, 2021).

There are numerous chemical regimens for chemoprophylaxis in malaria–endemic countries. Also, several drugs are available to deal with pathogenesis and clinical manifestations of the disease (Mehlhorn, 2008; Pan *et al*., 2018). However, extensively

reported parasite resistances to almost all chemicals available to cure malaria and related symptoms, coupled to the backlog of physiological, socio–economic and pharmaceutical drawbacks associated with the current malaria therapy strategy underline the fragility of the whole malaria control and elimination program.

The above–stated facts are of great concern for the global malaria control strategies, as they clearly highlight the urgent need to develop newer antimalarial medicines to fight against the disease and its various etiological parasites.

1-8- Traditional medicine as a baseline to support malaria control and elimination

In Africa and many other developing countries, traditional medicine is generally used as the first choice to treat diseases as they are obtained most affordable and accessible from locally available plants or other natural sources (Tarkang *et al*., 2016). Plants are the major resource for the treatment of malaria infections in sub – Saharan Africa, and mainly where health care facilities are limited (De Ridder *et al*., 2008).

Plant materials and their extracts continue to make a huge contribution to malaria chemotherapy, either directly as antimalarial phytomedicine, or as important source of lead compounds for the discovery of new and potent antimalarial drugs (Boyom *et al*., 2011; Chinsembu, 2015). For instances, the most potent drugs currently utilized to fight against malaria namely quinine and artemisinin stem respectively from *Cinchona officinalis* and *Artemisia annua* plants extracts (Castellanos *et al*., 2009; Tu, 2011). In Cameroon, plant extracts are still widely used to combat malaria in daily practices, especially in areas where access to standard treatments is limited (Tsabang *et al*., 2012; Tchokouaha *et al*., 2015).

The rich and diversify Cameroonian flora is a potential reservoir of potent antiplasmodial natural compounds and its exploration based on ethnopharmacological approach is still promising in the fight against malaria. *In vitro* screening of such plants extracts for inhibitory activity against malaria parasites is the first step in the search for new plant – derived antimalarial leads.

2- Generalities on studied plants

Combretaceae is a plant family of the Cameroonian pharmacopeia, whose extracts are extensively used in Central and West Africa to treat various diseases including malaria and/or related symptoms (Ngemenya *et al*., 2004; Titanji *et al*., 2008; Zirihi *et al*., 2012).

2-1- Generalities on *Combretaceae* family

This family consists of erect or sinuous trees, shrubs and lianas (Satabié, 1983). *Combretaceae* family include 20 genera and up to 600 tropical and subtropical species. In Africa there are 11 genera and 250 species; and in Cameroon, we have 9 genera and 61 species. Among other genera belonging to the *Combretaceae* family and present in Cameroon, there is the *Terminalia* genus (Satabié, 1983).

2-2- General characteristics of *Terminalia* genus

The genus *Terminalia* is recognized by its trunk which is strongly fissured, its alternate leaves and especially by its apetalous, hermaphrodite or unisexual male flowers. The fruits are flattened with two wings (Thiombiano *et al*., 2006). They are tall trees, savannah shrubs or monoecious. This genus is composed of 100 to 150 tropical species, including about 30 in Africa and 11 species in Cameroon (Satabié, 1983).

2-3- *Terminalia mantaly* H. Perrier

- **Botanic description**

It is a tree of 10 to 20 meters, its branches are storied and have the shape of an umbrella. The flowers are small and greenish, in ears erect 5 cm long [Figure 2]. The fruits are small, oval and the seeds can reach 1.5 cm long (Satabié, 1983; Arbonnier, 2004; Zirihi *et al*., 2012).

Figure 2: Some plant parts of *Terminalia mantaly* (**Photography Jiatsa, 2019**)

- **Ecology and distribution**

Terminalia mantaly is a tree of dense, dry forests in western Madagascar, especially along streams and in depressions. *Terminalia mantaly* needs a fertile soil to grow better. The plant is distributed in Central, Eastern and Southern Africa, from Senegal to Cameroon, till Uganda (Arbonnier, 2004).

- **Ethnobotanical Uses**

The wood from plant is used in ordinary interior carpentry, in the manufacture of doors, windows, stairs, partitions, claddings, mounting tool handles and as firewood (Arbonnier, 2004).

The plant is also very popular in traditional pharmacopoeia. Indeed, bark and wood are used in Madagascar to cure dysentery. Traditional healers use decoctions and infusions of

leaves in the treatment of several pathologies, including gastroenteritis, arterial hypertension, diabetes, oral, skin and genital diseases (Coulibaly, 2006). Leaves and stem bark from *Terminalia mantaly* are used in African folk medicine as antimalarial remedies (N'Guessan *et al*., 2009; Kamo *et al*., 2015).

- **Previous works**

Yapi *et al*. (2011) evaluated the antifungal activity of aqueous extracts, hydroethanolic and bark fractions on *Candida albicans*. The non – soluble residue in hexane was found to be very active with MFC of 24.37 µg/mL whereas the fraction with methylene chloride was less active with MFC of 780 µg/mL.

Ngouana *et al*. (2015) assessed the antifungal potential of crude extracts and fractions from leaves and stem bark against some *Candida* species. Their investigation reported high antifungal activity of Terminalia mantaly leaf and stem bark extracts and fraction both in single dose with their MIC values ranging from 40 µg/mL to 160 µg/mL, and in combination displaying synergistic interaction with other plants extracts.

Tchuenmogne *et al*. (2017) set up a baseline report on phytochemistry of *Terminalia mantaly* stem bark. They isolated seven compounds and reported their activity against *Candida albicans, Candida parapsilosis* and *Candida krusei* with MIC values lower than 4 µM.

Toghueo *et al*. (2018) studied antiplasmodial potential of ten Cameroonian medicinal plants including *Terminalia mantaly* and report the anti – *Plasmodium falciparum* activity of water decoction extracts from leaves and stem bark against strains 3D7 and INDO with the IC_{50} values of 2.49/1.90 µg/mL and 3.70/2.80 µg/mL respectively.

2-4- *Terminalia superba* Eng. et Diels

- **Botanic description**

Terminalia superba is a large tree, from 30 to 50 m tall and 5 m in girth, bole cylindrical, long and straight with large, flat buttresses. The root system is frequently shallow, and as the tree ages the taproot disappears. Leaves are simple, alternate, in tufts at the ends of the branches [Figure 3]. The Fruit is a small, transversely winged, sessile, golden-brown smooth nut and usually contains one seed (Satabié, 1983; Orwa *et al*., 2009).

- **Ecology and distribution**

Terminalia superba is essentially a tree of deciduous forest and sheds its leaves in the dry season. It is characteristic of tropical high secondary forest areas with a dry season of about

4 months. The specie is especially plentiful at some distance from the coast, but it gains at the expense of the rainforest following clearances (Satabié, 1983; Orwa *et al*., 2009).

The plant grows best on rich, well – drained alluvial soils, but is also found on other types such as lateritic sands, gravel and clays, lava, black basaltic clays and crystalline soils. *Terminalia superba* is native of West and Central Africa, mainly found in Cameroon, Central African Republic, Cote d'Ivoire, Congo, Equatorial Guinea, Gabon, Ghana, Nigeria and has been exported overtime in several countries of South America where the tree is exotic (Satabié, 1983; Orwa *et al*., 2009).

Figure 3: Some plant parts of *Terminalia superba* (**Photography Jiatsa, 2019**)

- **Ethnobotanical Uses**

Terminalia superba has great importance in paper making, offering the capability of producing a relatively wide range of pulps (Orwa *et al*., 2009). The leaves and stem bark from *Terminalia superba* are widely used locally and particularly in the western region traditional medicine to cure malaria and related symptoms (Ngemenya *et al*., 2004; Titanji *et al*., 2008).

- **Previous works**

Adewunmi *et al*. (2001) investigated the activity of root and stem of *Terminalia superba* against *Trypanosoma congolense* IL 1180 and reported IC_{50} values of 56.1 µg/mL for root ethanolic extract, 91.73 and 55.26 µg/ mL for stem hexane and ethanolic extracts.

Nguemenya *et al*. (2004) investigated and reported antiplasmodial activity of methanolic extract from leaves of *Terminalia superba*, with a 50% inhibitory concentration of 19.5 µg/mL on *Plasmodium falciparum* F32 strain.

Antia *et al*. (2009) evaluated *in vitro* anti – *Trypanosoma brucei brucei* activity of root and stem extracts from *Terminalia superba* and reported minimal inhibitory concentration of 3.1 mg/mL for each extract.

Kuete *et al*. (2010) reported the antimycobacterial, antibacterial and antifungal activities of stem bark extracts, fractions and two – afforded compounds from *Terminalia superba*, with minimal inhibitory concentration values ranging from 19.53 to 78.12 µg/mL on all tested mycobacteria, bacteria and fungi.

Keumoe *et al*. (2016) studied antimycobacterial activity of aqueous and methanolic extracts from leaf, stem bark and root of *Terminalia superba*, and reported inhibition of *Mycobacterium ulcerans* with minimal inhibitory concentrations from 62.5 to 250 µg/mL.

Chapter 2:

MATERIAL & METHODS

II- MATERIAL AND METHODS

1- Material

1-1- Plant material

The leaf, stem bark, and root samples were collected from *Terminalia mantaly* H. Perrier de la Bâthie and *Terminalia superba* Engl. & Diels during August to September 2014 in Nkolbisson and Ngoa – Ekelle, Yaoundé, Cameroon; plants samples were harvested in the afternoon. These plants were identified at the National Herbarium of Cameroon, Yaoundé where voucher specimens were deposited under the reference numbers 64212/HNC for *Terminalia mantaly* (*Tm*) and 55030/HNC for *Terminalia superba* (*Ts*) respectively.

1-2- *Plasmodium falciparum* strains

The *Plasmodium falciparum* strains 3D7 (Chloroquine–sensitive) and INDO (Chloroquine–resistant) were obtained from Malaria Research and Reference Reagent Resource (MR4) center, BEI Resources, 10801 University Blvd. Fax: 703-365-2898 Manassas, VA 20110-2209 USA (www.beiresources.org).

1-3- Mammalian cells line Human Embryogenic Kidney 239T (HEK 239T)

Mammalian Cell lines HEK 239T were obtained from American Type Culture Collection (ATCC), BEI Resources, 10801 University Blvd. Fax: 703-365-2898 Manassas, VA 20110-2209 USA (www.beiresources.org).

1-4- Reagents, Chemicals and Materials suppliers

Chloroquine phosphate, Glucose, HEPES, Giemsa, Hypoxanthine, RPMI-1640, Sodium bicarbonate, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), RNAse, Sorbitol, DMSO and flat-bottomed 96-well plates were sourced from Sigma Chemicals (Sigma-Aldrich, New Delhi, India). Phosphate buffered saline (PBS), Fetal bovine serum (FBS), Albumax I, Dulbecco's Modified Eagle Medium (DMEM), EDTA, Saponin, Triton X-100, YOYO-1 dye, and Gentamicin were purchased from Gibco (Gibco, Waltham, MA, USA). SYBR Green I from Invitrogen was supplied by Merck (Merck, New Delhi, India). Silica gel for column chromatography (60–230 mesh) was supplied by Merck (Merck, Darmstadt, Germany). Dichloromethane and methanol were procured from Brenntag (Brenntag, Essen, Germany) while ethyl acetate was delivered by Solventis (Solventis, Guildford, United Kingdom).

2- Methods

2-1- Plants crude extraction and fractionation

The various organs of each plant sample were air – dried and ground into fine powder using an electric mill (Hammer Mill, Leabon 9FQ). One kilogram of each plant part powder was separately macerated in 10 L of distilled water and methanol respectively for 72 hours at room temperature (27 – 29°C). The methanol macerates were filtered and filtrates evaporated using a rotary evaporator (Rotavapor, BUCHI 071) at 65°C. The aqueous extracts were lyophilized at the laboratory of Phytochemistry, Institute for Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon using a Virtis Wizard 2.0 Freeze Dryer Lyophilizer, Model: XLS-70. The dried crude extracts were subsequently subjected to antiplasmodial screening *in vitro* against the resistant *Pf*INDO and sensitive *Pf*3D7 strains, and cytotoxicity assessment. The crude extracts with potent antiplasmodial activity and high selectivity index were selected and subjected to successive solid – liquid solvent extractions at increasing polarity gradient.

Precisely, the dried aqueous and methanolic crude extracts [100 g of *Tm*l^{w} (water extract from leaves of *Tm*), *Tm*sb^{w} (water extract from stem bark of *Tm*), and *Ts*l^{w} (water extract from leaves of *Ts*), and 20 g *Ts*r^{m} (methanolic extract from roots of *Ts*)], selected based on their antiplasmodial activity and selectivity (IC_{50} < 3 µg/mL; SI > 75 on both strains) were successively extracted with n-hexane (1L), methylene chloride (1L), ethyl acetate (1L), and methanol (1L) to afford n-hexane (H), methylene chloride (Chl), ethyl acetate (EA), and methanol (M) fractions respectively, together with an insoluble final residues (R4) as described in Figure 4 below.

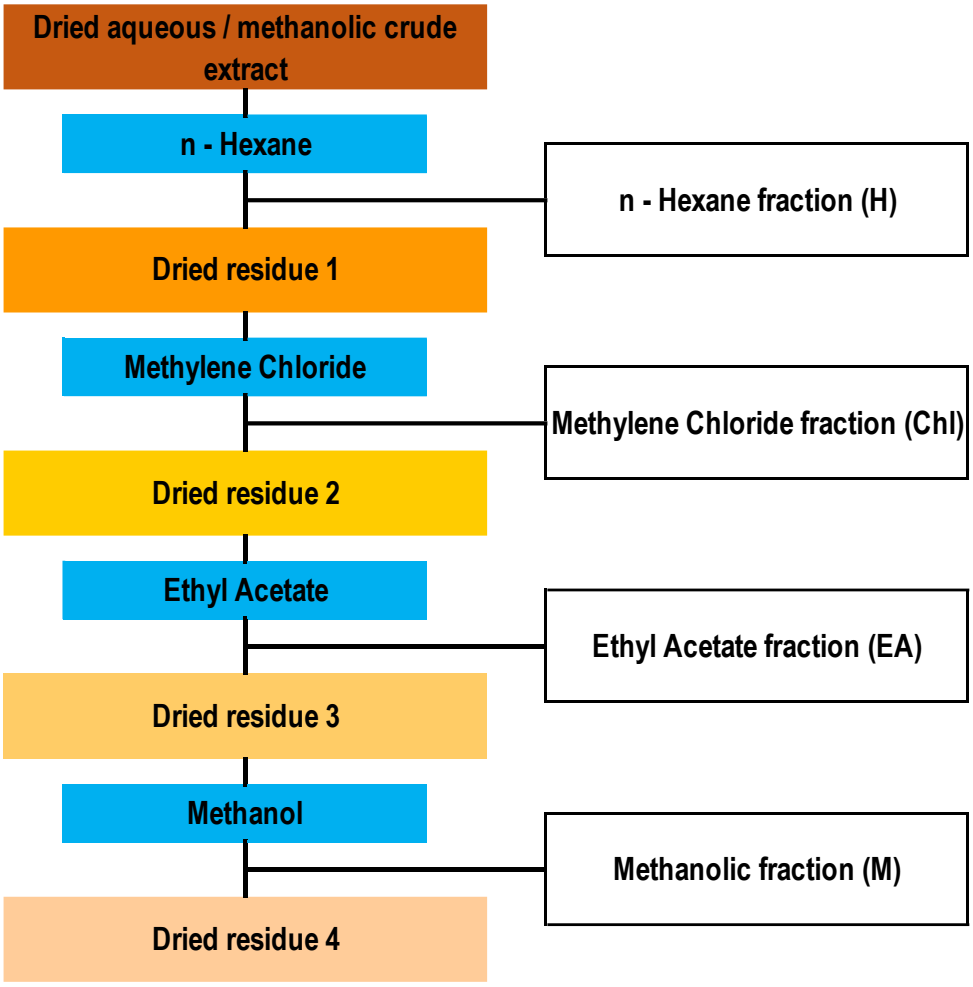


Figure 4: Scheme of successive solid – liquid extractions of the crude extracts

The afforded fractions were filtered separately through Whatman N° 4-filter paper and were then concentrated using rotary evaporator at 70°C (n – hexane), 40°C (methylene chloride), 78°C (ethyl acetate), and 65°C (methanol) respectively. The dried fractions and final residues were weighed, and the first step fractionation yields calculated relative to the weight of the starting crude extracts.

2-2- Fractionation of promising fractions by Column Chromatography

The two fractions abbreviated *Tms*b^{w}M for the methanolic fraction from aqueous extract from stem bark of *Terminalia mantaly* (*Tms*b^{w}), and *Ts*r^{m}EA for the ethyl acetate fraction from methanolic extract from roots of *Terminalia superba* (*Ts*r^{m}), presenting the higher fractionation yields and the best biological parameters for each respective species were selected and submitted to second step fractionation through Column Chromatography.

An amount of 25 g of fraction *Tm*sb^{w}M was subjected to silica gel column chromatography (Merck, 60–230 mesh), eluted with the mixtures of methylene chloride (DCM) – methanol (Brenntag, Germany – Chemicals, Germany) at increasing polarity [(100:0) – (55:45)], resulting in the collection of 352 subfractions of 125 mL each, which were combined on the basis of Thin Layer Chromatography (TLC) analysis into 39 sub–fractions codified *Tm*01 to *Tm*39.

A portion of 81 g of *Ts*r^{m}EA was also submitted to silica gel column chromatography (Merck, 60 – 230 mesh) using hexane – ethyl acetate (Brenntag, Belgium – Solventis, England) and ethyl acetate – methanol (Solventis, England – Chemicals, Germany) at increasing polarity [(100:0) – (0:100)] for each solvents mixture; 721 subfractions of 125 mL were collected and combined on the basis of Thin Layer Chromatography analysis into 22 subfractions codified *Ts*01 to *Ts*22.

In both cases, the fractionation yields of various subfractions were calculated with respect to the fractions starting mass and expressed as percentage of such. The 61 subfractions obtained from both fractions were screened for antiplasmodial activity *in vitro* against *Pf*INDO and *Pf*3D7 and the cytotoxicity of actives subfractions was subsequently evaluated.

2-3- *In vitro* cultivation of *Plasmodium falciparum*

Chloroquine – sensitive strain 3D7 (*Pf*3D7) and Chloroquine – resistant strain INDO (*Pf*INDO) of *Plasmodium falciparum* were maintained at the Malaria Research Laboratory, International Centre for Genetic Engineering and Biotechnology, New Delhi, India, and used for *in vitro* blood stage testing of antiplasmodial activity of extracts. *P. falciparum* culture was maintained according to the method described by Trager and Jensen (1976) with minor modifications (Kaushik *et al*., 2015), in fresh O positive human erythrocytes suspended at 4% hematocrit in RPMI 1640 16.2 g/L (Sigma – Aldrich, New Delhi, India) containing 25 mM HEPES, 11.11 mM Glucose, 0.2% Sodium bicarbonate (Sigma – Aldrich, New Delhi, India), 0.5% Albumax I (Gibco, Waltham, MA USA), 45 µg/L Hypoxanthine (Sigma – Aldrich, New Delhi, India), and 50 µg/L Gentamicin (Gibco, Waltham, MA USA) then incubated at 37°C under a gas mixture of 5% O_2, 5% CO_2, and 90% N_2. Every day, infected erythrocytes were transferred into fresh complete medium to propagate the culture. At parasitaemia > 10%, the cultures were diluted into healthy red blood cells to reduce % parasitaemia to 1-2 % to maintain the cultures in stress free conditions.

2-4- *In vitro* antiplasmodial assays of crude extracts, fractions, and subfractions

2-4-1. Dilution of crude extracts, fractions, subfractions and positive control

The stock solutions of plant extracts, fractions, and subfractions were prepared at 100 µg/mL in Dimethyl sulfoxide (DMSO) while Chloroquine, the positive control (Sigma–Aldrich, New Delhi, India) stock solution was prepared in water (Milli – Q grade, Netherlands) at 1 mM. All stocks solutions were then diluted in 96–well round bottom tissue culture grade plates (Corning, USA) with fresh RPMI 1640 culture medium to achieve the required concentrations for testing. In all cases, except for Chloroquine (positive control), the final solution contained 0.4% DMSO, which was found to be non-toxic to both parasite and erythrocytes. The extracts and fractions were tested at concentrations ranging from 0.10 to 25 µg/ml, subfractions from 0.03 to 8 µg/mL, and Chloroquine at 1 µM as highest concentration.

2-4-2. Parasite synchronization

The ring stage synchronized parasite were obtained by 5% sorbitol serial treatment as described by Lambros and Vanderberg, 1979. In fact, sorbitol works on the principle of selective lysis of erythrocytes infected with mature stages of the parasite *viz* trophozoites and schizonts, keeping rings and uninfected erythrocytes unaffected.

Precisely, *P. falciparum* culture was harvested by spinning at 1500 RPM for 5 min and the supernatant removed. Further, 5 times the volume of the pellet in term of 5% sorbitol were added to the pellet, gently mix, and incubate at 37°C for 5 min. The sorbitol was therefore removed by spin washing (1500 RPM for 5 min) with incomplete medium. The synchronized parasites were re – suspended at 4% hematocrit in fresh complete medium and incubate under standard culture conditions. The smears were prepared and observed to confirm the synchronization status.

2-4-3. *In vitro* antiplasmodial assays

The extracts, fractions, subfractions and positive control (Chloroquine) were evaluated for antiplasmodial activity against *Pf*INDO and *Pf*3D7 strains. For drug screening, the SYBR Green I–based fluorescence assay was set up as described by Smilkstein *et al.* (2004). Precisely, 100 µL of Sorbitol–synchronized parasites (Lambros and Vanderberg, 1979) were incubated under normal culture conditions (37°C, 5% CO_2, 5% O_2, 90% N_2) at 1% parasitaemia and 2% hematocrit in flat–bottomed 96–well plates (Corning, USA) in the absence or presence of increasing concentrations of extracts, fractions (0.1 to 25 µg/mL) or subfractions (0.03 to 8.00

µg/mL) for 48 h. Chloroquine (Sigma – Aldrich, New Delhi, India) was used as positive control and tested at 1 µM highest concentration, while 0.4% (v/v) DMSO was used as the negative control. Upon incubation, 100 µL of SYBR Green I lysis buffer [Tris (20 mM; pH 7.5), EDTA (5mM), Saponin (0.008%, w/v), and Triton X-100 (0.08%, v/v)] were added to each well and mixed twice gently, then transfer into black microplates and incubated in dark at 37°C for 1 h. Fluorescence was then measured with a Victor fluorescence multiwell plate reader (Perkin Elmer, Waltham, MA USA) with excitation and emission wavelength bands centered at 485 and 530 nm respectively. The fluorescence counts were plotted against extracts concentrations and the 50% inhibitory concentration (IC_{50}) was determined by analysis of dose–response curves using non–linear regression, with the IC Estimator–version 1.2 software, http://www.antimalarial-icestimator.net/MethodIntro.htm (Free Software Foundation, Boston, MA, USA). The results were validated microscopically by examination of Giemsa-stained smears of extracts, fractions, or subfractions treated cultures versus untreated parasite cultures. The resistance indexes (RI) were calculated as followed

$$\text{Resistance Index (RI)} = \frac{\text{Inhibitory Concentration 50\% } Pf\text{INDO } (\text{IC}_{50}Pf\text{INDO})}{\text{Inhibitory Concentration 50\% } Pf\text{3D7 } (\text{IC}_{50}Pf\text{3D7})}$$

2-5- Assessment of the cytotoxicity of active crude extracts, fractions, and subfractions on HEK239T cells

The cytotoxicity of antiplasmodial extracts, fractions, and subfractions was assessed using the MTT assay as previously described by Mosmann (1983), targeting HEK239T cells (ATCC, Manassas USA) cultured in complete medium containing 13.5 g/l DMEM (Gibco, Waltham, MA USA), 10% foetal bovine serum (Gibco, Waltham, MA USA), 0.21% Sodium bicarbonate (Sigma – Aldrich, New Delhi, India) and 50 µg/mL Gentamicin (Gibco, Waltham, MA USA). Essentially, HEK293T cells at $5x10^3$cells/200 µL/well were seeded into 96 – well flat bottom tissue culture plates (Corning, USA) in complete medium. 50 µL of serially diluted extracts, fractions, or subfractions solutions were added after 24 h of seeding then incubated for 48 h in a humidified atmosphere at 37°C and 5% CO_2. DMSO was added as positive inhibitor at 10% *v/v*. Twenty microliters of a stock solution of MTT (5 mg/mL in 1X phosphate buffered saline) were added to each well, gently mixed and incubated for additional 4 h. After spinning the plate at 1,500 rpm for 5 min, the supernatant was carefully removed and 100 µL of DMSO at 0.4% (stop agent) was added. Formazan formation was read on a microtiter plate reader (Versa Max Microplate Reader, Molecular Devices, USA) at 570 nm. The 50%

cytotoxic concentrations (CC_{50}) of actives extracts, fractions, and subfractions were determined by analysis of dose–response curves.

The selectivity Indices (SI) were calculated for each active extracts, fractions, and subfractions using the following formula

$$\text{Selectivity Index (SI)} = \frac{\text{Cytotoxic Concentration 50\% } (CC_{50})}{\text{Inhibitory Concentration 50\% } (IC_{50})}$$

2-6- Chemical profiling of promising subfractions by Gas Chromatography – Mass Spectrometry (GC – MS)

The potent subfractions (IC_{50} < 2 µg/mL, SI > 80 – fold, both on *Pf*INDO and *Pf*3D7) were submitted to GC – MS analysis using a GC-MS-QP2010 Ultra system (Shimadzu Co., Japan), constitute of an auto – sampler (AOC – 20i) and a Gas Chromatograph interfaced to a Mass Spectrometer instrument (Column Rtx-5MS: 30 m length × 0.25 mm I. d. × 0.25 µm film thickness), composed of 100% dimethylpolysiloxane. The analysis was performed in the electron impact (EI) mode with 70 eV as ionization energy. Helium (99.99%) was used as carrier gas at 1 mL/min constant flow rate.

The experiment was performed by injecting 1 µL of sub–fractions with a split ratio of 20:1, at maintained injector temperature of 260 °C and ion – source temperature of 280 °C. The oven temperature was maintained at 110 °C (isothermal for 2 min), then programmed at 10 °C/min increment to 200 °C, followed by another increment of 5 °C/min to 280 °C, and finally isothermal for 9 minutes at 280 °C. Data was evaluated using total ion count (TIC) for compound identification and quantification. Mass spectra were taken at 70eV, a scan interval of 0.5s and fragments from 40 to 550Da. Interpretation and identity of chemo – types from Mass – Spectra were done using available mass spectral database NIST (National Institute of Standard and Technology) and WILEY libraries, by comparing spectrums of phyto – compounds with those from libraries (Swamy *et al.*, 2017). The relationship between antiplasmodial subfractions and their chemical compositions was assessed using the Hierarchal Cluster Analysis (HCA) with the Euclidean distances as the similarity measure and the Ward's linkage as the clustering algorithm; results were expressed in the form of dendrogram. Structures of identified phytochemicals were drawn using ChemDraw Ultra 12.0.2, Cambridge Software.

2-7- Intraerythrocytic stages specific inhibition assays and post – drugs exposure growth suppression of highly potent subfractions

2-7-1. Stage Enrichment by Density Gradient Separation

The ring, trophozoite and schizont – stages of parasites *Pf*3D7 were enriched by 60% percoll gradient density synchronization as described by Rivadeneira *et al*. (1983). Percoll consists of colloidal silica particles of 15 – 30 nm diameter coated with polyvinylpyrrolidone prepared at 23% w/w in distilled water. The percoll is well suited for density gradient experiments because it possesses a low viscosity compared to alternatives, a low osmolarity and no toxicity towards cells and their constituents.

Briefly, the culture with up to 5% parasitaemia of trophozoites and schizonts were harvest by spinning (1500 RPM or ~300 g, 5min), the supernatant was removed and the pellet re–suspended at 20% hematocrit in incomplete medium. To separate trophozoites and schizonts from rings and uninfected red blood cells (URBCs), the cell suspension was overlay onto equal volume of 60% percoll solution already aliquoted in microfuge tube and spin [2000 RPM (514 g), 20 min, 4^0C] in swing bucket centrifuge. Post centrifugation, three layers appeared: the upper layer had culture medium, the interphase had trophozoites and schizonts, the lower layer had percoll, and pellet had rings and uninfected RBCs (Red blood cells).

The interphase (trophozoites and schizonts layer) was then transferred into fresh tubes and spin wash (1500 RPM or ~300 g, 5min) with fresh complete medium. To separate trophozoites from schizonts, the interphase was re–suspended at 20% hematocrit in incomplete medium and overlay onto equal volume of 45% percoll gradient already aliquoted in microfuge tube and spin (2000 RPM or 514 g, 20 min, 4^0C) in swing bucket centrifuge. The infected RBCs were split into three layers as described above, with interphase layer containing schizonts and pellet containing trophozoites stage infected RBCs. The interphase for pure schizonts and pellet for pure trophozoites were pipetted out into fresh tubes and spin wash (1500 RPM or ~300 g, 5 min) with fresh complete medium.

The previous pellet containing rings and URBCs was re–suspended at 20% hematocrit in incomplete medium and overlay onto equal volume of 66 % percoll gradient already aliquoted in microfuge tube and spin (2000 RPM or 514 g, 20 min, 4^0C) in swing bucket centrifuge to obtain pure rings stage. The RBCs were then separated in layers, with interphase layer containing rings stage and the pellet rich of URBCs. The interphase for pure rings was pipetted and transferred into fresh tubes, and spin wash (1500 RPM or ~300 g, 5 min) with

complete medium. The respective pure ring, trophozoite and schizont stages were used at 2% parasitaemia for stages – specific inhibition experiments.

2-7-2. Stage – specific inhibition and post – drugs exposure growth suppression assays

Highly potent antiplasmodial subfractions (IC_{50} < 1 µg/mL; SI > 145–fold, both on *Pf*INDO and *Pf*3D7 and fractionation yield ≥ 0.1%) were assessed for their intraerythrocytic stage specific inhibitory activities. *P. falciparum* 3D7 strain cultures were highly synchronized using a combination of Percoll density gradient separation of ring, trophozoite and schizont as described above (Rivadeneira *et al*., 1983).

For stage – specific inhibition assay, synchronized cultures of ring, trophozoite and schizonts stages at 2% parasitaemia and 2% hematocrit, were treated with sub – fractions at their 99% Inhibitory Concentration (IC_{99}) for respective durations of 48 h (rings), 24 h (trophozoites) and 12 h (schizonts) under regular culture conditions. Giemsa – stained thin smears were prepared at 6 h and 12 h intervals for microscopic evaluations of the intraerythrocytic maturation of parasite and to determine the effect of subfractions on parasite growth and stage transition (Roberts *et al*., 2016). The samples were also collected at these time intervals fixed in a solution containing 0.04% glutaraldehyde in PBS, permeabilized with 0.25% Triton X-100, treated with RNAse (50 mg/ml) and stained with 10.24 mM YOYO-1 (Bouillon *et al*., 2013). Flow cytometry acquisition was then performed in Thermo Fisher Attune NxT at a voltage of 260 with excitation wavelength of 488 nm and an optical filter of 530/30, with the aim to determine the cultures samples parasitaemia.

To assess the post–drug exposure effect of subfractions on parasite growth and life cycle, the remaining wells in the plates at the end of stage–specific inhibition testing, 12, 24- and 48-hours post–infection and treatment for schizont, trophozoite and rings assays respectively, were centrifuged using a swinging bucket rotor and extracts containing media were removed. The cell pellets were then spined washed three times with 200 µL of fresh medium, re–suspended in 100 µL of inhibitor free RPMI complete medium, and therefore incubated in culture conditions for 48 hours for rings stage, and 24 hours for trophozoites and 12 hours schizonts stages (Singh *et al*., 2015).

The percentages of stage–specific inhibition and post–extract growth suppression of each subfraction were calculated in comparison to negative controls (inhibitor free parasite culture of each stage), by microscopic counting of about 3000 cells (10 – 12 fields) of each

parasite stage, to appreciate stage–specific growth inhibition, parasiticidal or parasitostatic effect, and the post – extracts exposure growth suppression of highly potent subfractions on *Plasmodium falciparum* 3D7.

2-8- Statistical analysis

All data were analyzed using Microsoft Excel software, for table setting and graphs plotting. The IC_{50} were determined by analysis of dose–response curves using non – linear regression with the IC Estimator–version 1.2 software, http://www.antimalarial-icestimator.net/MethodIntro.htm. The Hierarchal Cluster Analysis and the Principal Component Analysis were completed using XLSTAT 2019.3.2 by Addinsoft for Excel.

Chapter 3:

RESULTS & DISCUSSION

III- RESULTS AND DISCUSSION

The present study assessed the biological properties of *Terminalia mantaly* and *Terminalia superba* extracts as sources of antiplasmodial agents.

1- Antiplasmodial activity and cytotoxicity of crude extracts from *Terminalia mantaly* and *Terminalia superba*

A total of 12 crude extracts were prepared from the leaves, stem bark and roots of *T. mantaly* and *T. superba* by maceration in distilled water and methanol and further assessed for biological activities. Table 2 summarizes the results of crude extracts preparation and biological screening against *P. falciparum* INDO (*Pf*INDO) and 3D7 (*Pf*3D7) strains and HEK239T mammalian cells.

Table 2: Crude extracts preparation yields and antiplasmodial activity parameters

Plant	Part	Solvent	Extract code	Extraction yield (%)	IC_{50} ± SD (µg/mL)		RI	CC_{50} (µg/mL)	Selectivity Index	
					*Pf*INDO	*Pf*3D7			*Pf*INDO	*Pf*3D7
T. mantaly	Leaf	Water	*Tm*l^{w}	35.50	2.09±0.06	2.66±0.31	0.79	> 200	> 95.69	> 75.19
		Methanol	*Tm*l^{m}	19.54	2.69±0.04	2.61±0.43	1.03	> 200	> 74.35	> 76.63
	Stem bark	Water	*Tm*sb^{w}	19.93	0.26±0.02	1.03±0.04	0.25	> 200	> 769.23	> 194.17
		Methanol	*Tm*sb^{m}	19.32	3.63±0.50	2.91±0.12	1.25	> 200	> 55.10	> 68.73
	Root	Water	*Tm*r^{w}	07.45	> 25	> 25	-	-	-	-
		Methanol	*Tm*r^{m}	09.01	7.01±0.82	5.04±0.68	1.39	> 200	> 28.53	> 39.68
T. superba	Leaf	Water	*Ts*l^{w}	17.18	0.57±0.06	1.26±0.12	0.45	> 200	> 350.88	> 158.73
		Methanol	*Ts*l^{m}	12.07	3.38±0.54	2.13±0.13	1.59	> 200	> 59.17	> 93.90
	Stem bark	Water	*Ts*sb^{w}	14.15	3.70±0.03	1.42±0.09	2.61	> 200	> 54.05	> 140.85
		Methanol	*Ts*sb^{m}	10.35	2.68±0.20	1.85±0.13	1.44	> 200	> 74.63	> 108.11
	Root	Water	*Ts*r^{w}	04.54	18.88±0.14	16.43±0.14	1.15	-	-	-
		Methanol	*Ts*r^{m}	02.27	2.38±0.08	2.28±0.20	1.04	> 200	> 84.03	> 87.72
Reference drug		Chloroquine (µM)		-	0.40±0.00	0.04±0.01	10.00	-	-	-

Activity data are presented as means of triplicate experiments. **IC_{50}**: 50% Inhibitory Concentration; **CC_{50}**: 50% Cell Cytotoxic Concentration; SD: Standard deviation from triplicate experiments; **RI**: Resistance Index; resistance index was calculated as the ratio of IC_{50} *Pf*$INDO_{resistant}$/$IC50$*Pf*$3D7_{sensitive}$; **SI**: Selectivity Index, ***Tm***: *Terminalia mantaly*; ***Ts***: *Terminalia superba*; **l**: leaf; **sb**: stem bark; **r**: root; **^{w}**: Water; **^{m}**: Methanol.

The dose – response curves schematizing the antiplasmodial activity of crude extracts are presented in Figure 5.

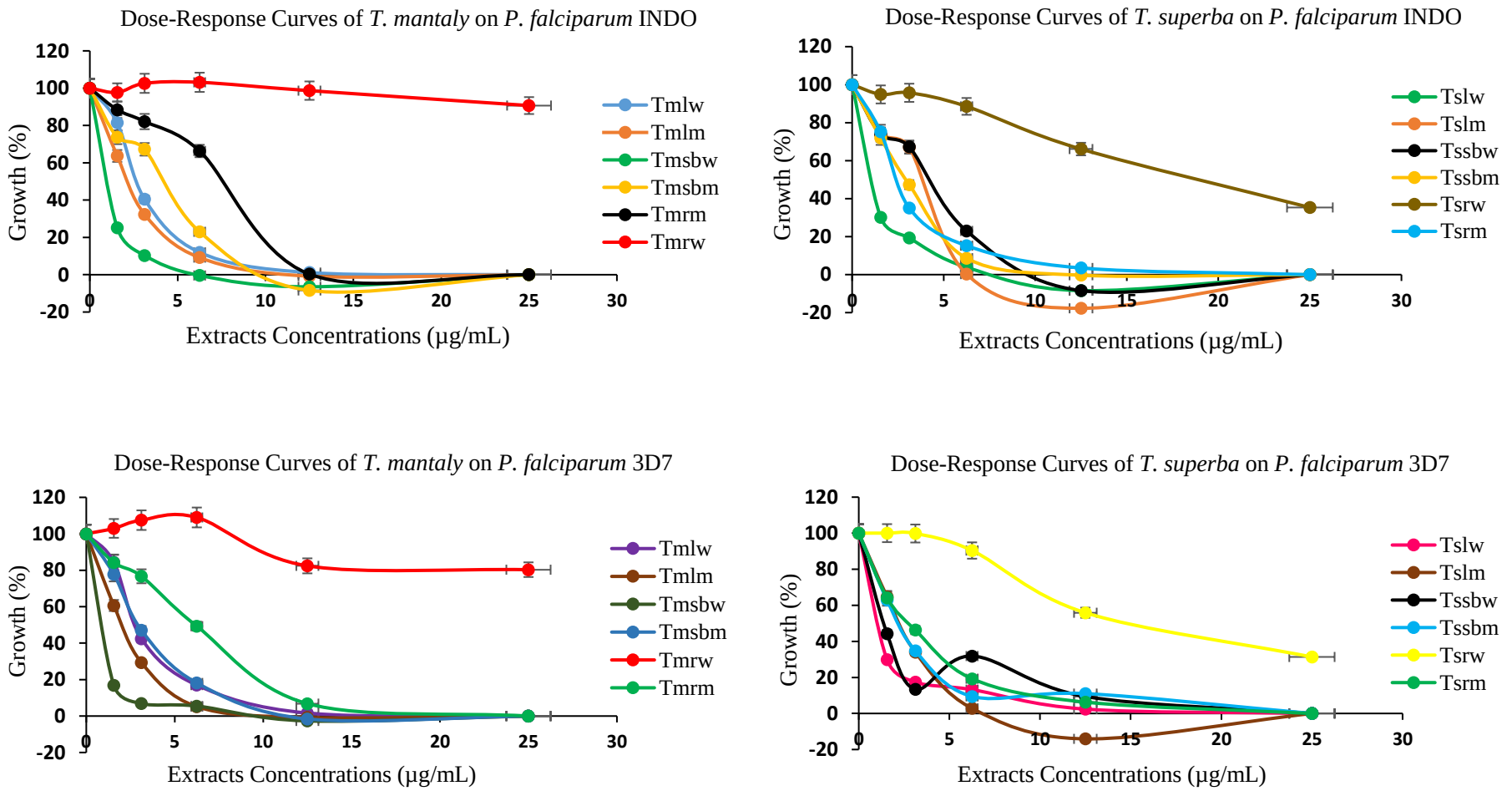


Figure 5: Dose – response curves of *Terminalia mantaly* and *Terminalia superba* crude extracts on *P. falciparum* INDO and 3D7 strains (***Tm:*** *Terminalia mantaly*, ***Ts:*** *Terminalia superba*, **l***:* leaf, **sb***:* stem bark, **r***:* roots, **w***:* Water, **m***:* Methanol).

The results from Table 2 shows that the extraction yields varied from 7.45% to 35.50% for *T. mantaly* and from 2.27 to 17.18% for *T. superba*. The extracts yields varied according to the plant species, the plant parts, and the solvent of extraction. In general, aqueous extracts from leaves were higher than that of methanol extracts. Water is environment – friendly and cheap solvent, that is ideal for the extraction of bioactive principles from any source.

The antiplasmodial activity (expressed as IC_{50}) of crude extracts ranged from 0.26 to > 25 µg/mL with 9 extracts showing very promising potency (IC_{50} < 5 µg/mL). The aqueous extract from *T. mantaly* stem bark (*Tm*sb^{w}) showed the best antiplasmodial activity and selectivity respectively on *Pf*INDO and *Pf*3D7 strains with IC_{50} values of 0.26 µg/mL (SI_{PfINDO} > 769.23) and 1.03 µg/mL (SI_{Pf3D7} > 194.17). For *T. superba*, the aqueous extract from leaves (*Ts*l^{w}) also showed high antiplasmodial activity and selectivity with IC_{50} values of 0.57 µg/mL (SI_{PfINDO} > 350.88) and 1.26 µg/mL (SI_{Pf3D7} > 158.73) on *Pf*INDO and *Pf*3D7 strains respectively.

The fact that most values hovering around one, resistance index data on crude samples [Table 2] suggests that the antiplasmodial metabolites present in the plant extracts are potent against both chloroquine sensitive and resistant strains of *P. falciparum*. Meanwhile, *Tm*sb^{w} (RI: 0.25), and *Ts*l^{w} (RI: 0.45) were 3- to 4-fold respectively more active against *Pf*INDO than *Pf*3D7 suggesting that they act through a unique drug target in the resistant strain. The selectivity indices of crude extracts ranging from > 28 to > 769 suggest that the crude extracts not only exhibit potent antiplasmodial potency but also possess great selectivity in their action. The thresholds for the *in vitro* antiplasmodial activity of the plant extracts were classified according to Gessler *et al.* (1994), as very good potency for IC_{50} < 10 µg/ml, as moderate potency for 10 µg/ml ≤ IC_{50} ≤ 50 µg/ml, and low antiplasmodial potency for IC_{50} > 50 µg/ml. Based on this classification, results obtained indicate that most of the tested extracts exerted very good activities against both sensitive and resistant strains of *P. falciparum.*

Very few reports are available in the literature on the activity of *Terminalia* plants against *P. falciparum*. Ngemenya *et al.* (2004) previously reported the antiplasmodial activity (IC_{50} of 19.5 µg/mL) of methanolic extract from the leaf of *T. superba* against the chloroquine – sensitive *P. falciparum* F32 strain. This value is about five to nine – fold greater than that of similar extract tested in this study against *Pf*INDO and *Pf*3D7. This activity discrepancy might be explained by the difference in parasite strains and the specific features of parasites relating to drug susceptibility. Indeed, there might be a relationship between *in vitro* adaptation to

culture of *P. falciparum* and drug resistant characteristic of the strain. There is also the possibility of changes in the metabolic pathways during *in vitro* routine culture maintenance (Le Bras *et al*., 1983). Likewise, Adewunmi *et al.* (2001) investigated the activity of root and stem of *T. superba* against a related protozoan parasite, *Trypanosoma congolense* IL 1180 and reported IC_{50} values of 56.1 µg/mL (root ethanolic extract), 91.73 µg/mL and 55.26 µg/mL for stem hexane and ethanolic extracts.

2- Antiplasmodial activity and cytotoxicity of fractions from the most promising extracts

The four crude extracts showing promising antiplasmodial activity profile were selected and fractionated via successive solid – liquid extractions [Figure 4] using four different organic solvents (n – Hexane, Methylene Chloride, Ethyl Acetate, and Methanol). They included the aqueous extracts from leaf and stem bark of *Terminalia mantaly* (*Tm*l^{w}: IC_{50}*Pf*INDO: 2.09 µg/mL, SI_{PfINDO} > 95; IC_{50}*Pf*3D7: 2.66 µg/mL, SI_{Pf3D7} > 75; and *Tm*sb^{w}: IC_{50}*Pf*INDO: 0.26 µg/mL, SI_{PfINDO} > 769; IC_{50}*Pf*3D7: 1.03 µg/mL, SI_{Pf3D7} > 194), and the aqueous and methanolic extracts from leaf and root of *Terminalia superba* (*Ts*l^{w}: IC_{50}*Pf*INDO: 0.57 µg/mL, SI_{PfINDO} > 350; IC_{50}*Pf*3D7: 1.26 µg/mL, SI_{Pf3D7}> 158; and *Ts*r^{m}: IC_{50}*Pf*INDO: 2.38 µg/mL, SI_{PfINDO} > 84; IC_{50}*Pf*3D7: 2.28 µg/mL, SI_{Pf3D7}> 87).

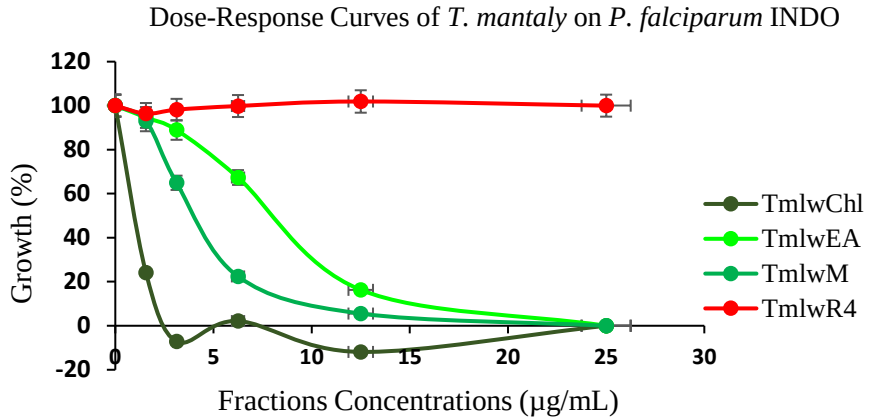
The fractionation of each aqueous crude extract (*Tm*l^{w}, *Tm*sb^{w}, and *Ts*l^{w}) led to 4 fractions and 1 final residue (R4) for each aqueous crude extract. Whereas, the fractionation of *Ts*r^{m} yielded 3 fractions and a final residue (R3) as after Ethyl Acetate exhaustion, the final residue was fully dissolvable into methanol. A total of 15 fractions and 4 final residues (19 fractions) were obtained from these promising crude extracts splitting and tested for biological activities as reported in Table 3. The dose–response curves of the tested fractions against *P. falciparum* INDO and 3D7 are given in Figure 6A, B.

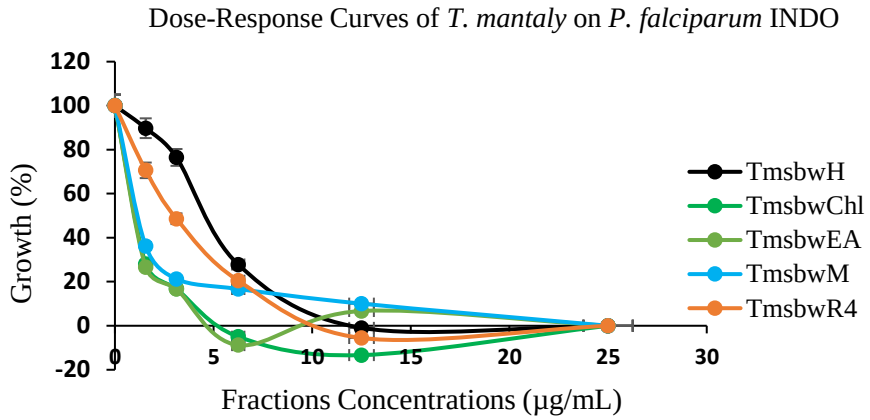
Table 3: Yields of fractions, their antiplasmodial and cytotoxicity activities

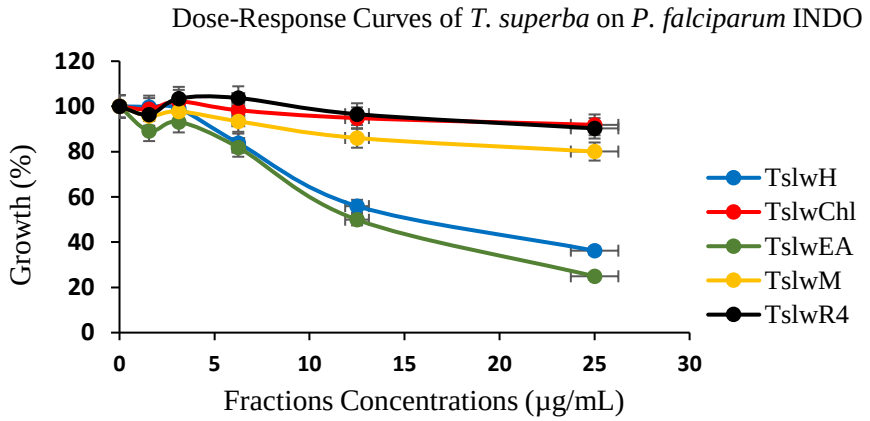
Plant	Crude Extract	Solvent	Fraction code	Fraction Yield (%)	IC_{50} ± SD (µg/mL)		RI	CC_{50} (µg/mL)	Selectivity Index	
					*Pf*INDO	*Pf*3D7			*Pf*INDO	*Pf*3D7
T. mantaly	*Tml*^{w}	Hexane	*Tml*^{w}H	00.00	-	-	-	-	-	-
		DCM	*Tml*^{w}Chl	20.78	0.36±0.01	0.96±0.01	0.37	> 200	> 555.55	> 208.33
		EA	*Tml*^{w}EA	02.54	6.74±0.49	6.88±0.58	0.98	> 200	> 29.67	> 29.07
		Methanol	*Tml*^{w}M	31.48	3.71±0.07	3.29±0.13	1.13	> 200	> 53.91	> 60.79
		-	*Tml*^{w}R4	43.79	> 25	> 25	-	-	-	-
	Tmsb^{w}	Hexane	*Tmsb*^{w}H	00.16	4.50±0.24	4.40±0.27	1.02	> 200	> 44.44	> 45.45
		DCM	*Tmsb*^{w}Chl	00.67	0.56±0.05	1.12±0.07	0.50	> 200	> 357.14	> 178.57
		EA	*Tmsb*^{w}EA	01.26	0.68±0.14	1.35±0.42	0.50	> 200	> 294.11	> 148.15
		Methanol	*Tmsb*^{w}M	63.65	0.39±0.03	1.28±0.08	0.30	> 200	> 512.82	> 156.25
		-	*Tmsb*^{w}R4	31.35	2.80±0.21	2.44±0.16	1.15	> 200	> 71.43	> 8.97
T. superba	*Tsl*^{w}	Hexane	*Tsl*^{w}H	00.70	14.09±1.88	6.99±0.68	2.02	126.03	> 14.19	> 28.61
		DCM	*Tsl*^{w}Chl	04.74	> 25	> 25	-	-	-	-
		EA	*Tsl*^{w}EA	04.47	6.89±0.94	7.06±0.76	0.97	> 200	> 29.03	> 28.33
		Methanol	*Tsl*^{w}M	26.34	> 25	> 25	-	-	-	-
		-	*Tsl*^{w}R4	61.90	> 25	> 25	-	-	-	-
	Tsr^{m}	Hexane	*Tsr*^{m}H	19.36	> 25	> 25	-	-	-	-
		DCM	*Tsr*^{m}Chl	05.54	2.26±0.09	4.93±0.73	0.46	> 200	> 88.49	> 40.57
		EA	*Tsr*^{m}EA	06.18	1.82±0.04	1.65±0.24	1.10	> 200	> 109.89	> 121.21
		-	*Tsr*^{m}R3	63.82	5.22±0.26	4.70±1.47	1.11	> 200	> 38.31	> 42.55
Reference drug		Chloroquine (µM)		-	0.40±0.00	0.04±0.00	10.00	-	-	-

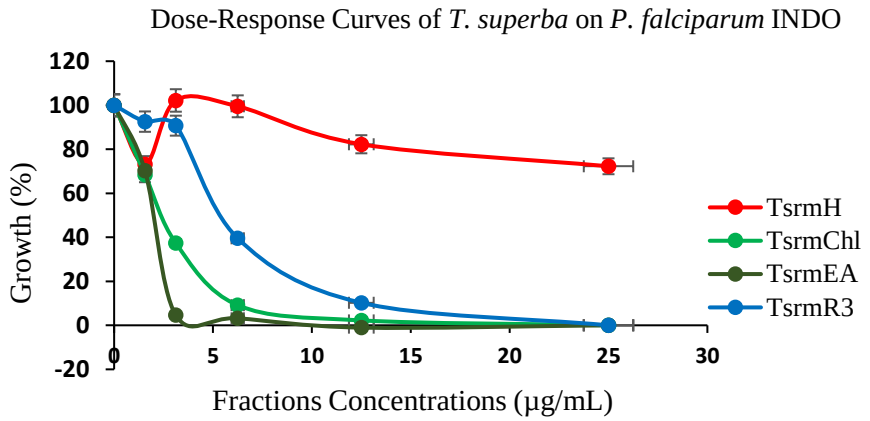
Activity data are presented as means of triplicate experiments. **IC_{50}**: 50% Inhibitory Concentration, **CC_{50}**: 50% Cell Cytotoxic Concentration, SD: Standard deviation from triplicate experiments, **RI**: Resistance Index; resistance index was calculated as the ratio of IC_{50} *Pf*INDO$_{resistant}$/IC_{50} *Pf*3D7$_{sensitive}$, **SI**: Selectivity Index, ***Tm***: *Terminalia mantaly*, ***Ts***: *Terminalia superba*, *Tml*^{w}: *Terminalia mantaly* leaf water extract, *Tmsb*^{w}: *Terminalia mantaly* stem bark water extract, *Tsl*^{w}: *Terminalia superba* leaf water extract, *Tsr*^{m}: *Terminalia superba* root methanol extract, **H**: Hexane, **DCM / Chl**: Methylene Chloride, **EA**: Ethyl Acetate **M**: Methanol, **R3 / R4**: final residue.

A


Dose-Response Curves of T. mantaly on P. falciparum INDO
Growth (%)
Fractions Concentrations (µg/mL)
TmlwChl
TmlwEA
TmlwM
TmlwR4


Dose-Response Curves of T. mantaly on P. falciparum INDO
Growth (%)
Fractions Concentrations (µg/mL)
TmsbwH
TmsbwChl
TmsbwEA
TmsbwM
TmsbwR4


Dose-Response Curves of T. superba on P. falciparum INDO
Growth (%)
Fractions Concentrations (µg/mL)
TslwH
TslwChl
TslwEA
TslwM
TslwR4


Dose-Response Curves of T. superba on P. falciparum INDO
Growth (%)
Fractions Concentrations (µg/mL)
TsrmH
TsrmChl
TsrmEA
TsrmR3

B

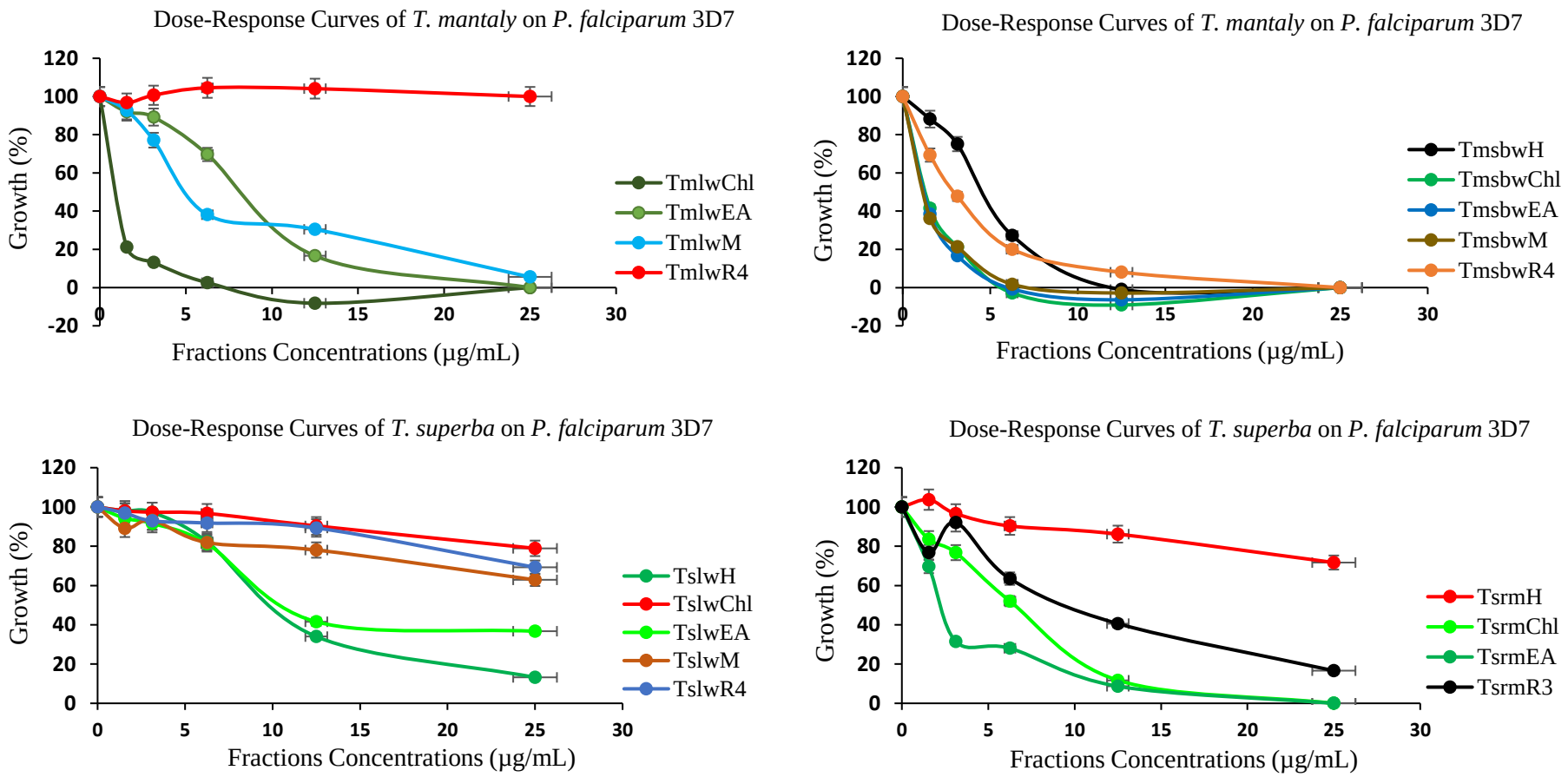


Figure 6: Dose – response curves of fractions from *Terminalia mantaly* and *Terminalia superba* on *P. falciparum* INDO [A] and 3D7 [B] strains (***Tm****: Terminalia mantaly,* ***Ts****: Terminalia superba,* ***Tmlw****: Terminalia mantaly* leaf water extract, ***Tmsbw****: Terminalia mantaly* stem bark water extract, ***Tslw****: Terminalia superba* leaf water extract, ***Tsrm****: Terminalia superba* root methanol extract, **H***:* Hexane, **Chl**: Methylene Chloride, **EA**: Ethyl Acetate, **M**: Methanol, **R3 / R4**: final residue).

Overall, twelve fractions showed antiplasmodial potencies against both resistant and sensitive strains of *P. falciparum*, with IC_{50} values ranging from 0.36 to 14.09 µg/mL. Five out of those twelve fractions codified *Tm*l^{w}Chl, *Tm*sb^{w}Chl, *Tm*sb^{w}EA, *Tm*sb^{w}M, and *Ts*r^{m}EA exhibited potent antiplasmodial activity with IC_{50} values below 2 µg/mL on both strains, and high selectivity (SI > 109).

The dichloromethane fraction (*Tm*l^{w}Chl) from the leaf aqueous extract of *Terminalia mantaly* (*Tm*l^{w}) showed the highest antiplasmodial activity and selectivity, with IC_{50}*Pf*INDO = 0.36 µg/mL, IC_{50}*Pf*3D7= 0.96 µg/mL and SI > 208 – fold. Also, 3 fractions from the stem bark aqueous extract of *T. mantaly* (*Tm*sb^{w}) exerted high activity mainly against the resistant *Pf*INDO strain, namely the dichloromethane fraction (*Tm*sb^{w}Chl, IC_{50}*Pf*INDO: 0.56 µg/mL vs IC_{50}*Pf*3D7: 1.12 µg/mL), the ethyl acetate fraction (*Tm*sb^{w}EA, IC_{50}*Pf*INDO: 0.68 µg/mL vs IC_{50}*Pf*3D7: 1.35 µg/mL), and the methanol fraction (*Tm*sb^{w}M, IC_{50}*Pf*INDO: 0.39 µg/mL vs IC_{50}*Pf*3D7: 1.28 µg/mL). Overall, these 3 fractions were found to be at least two – fold more active against the resistant *Pf*INDO strain than the sensitive *Pf*3D7 and showed very good selectivity with SI > 148. Besides, the ethyl acetate fraction of the root methanolic extract of *T. superba* also exerted potent antiplasmodial activity (*Ts*r^{m}EA, IC_{50}*Pf*INDO: 1.82 µg/mL, IC_{50}*Pf*3D7: 1.65 µg/mL, SI > 109).

Four other fractions from both plants (*T. mantaly* and *T. superba*) showed significant activity profiles with IC_{50} values ranging from 2.26 to 4.93 µg/mL, *viz. Tm*l^{w}M (IC_{50}*Pf*INDO: 3.71, IC_{50}*Pf*3D7: 3.29 µg/mL), *Tm*sb^{w}H (IC_{50}*Pf*INDO: 4.50, IC_{50}*Pf*3D7: 4.40 µg/mL), *Tm*sb^{w}R4 (IC_{50}*Pf*INDO: 2.80, IC_{50}*Pf*3D7: 2.44 µg/mL), and *Ts*r^{m}Chl (IC_{50}*Pf*INDO: 2.26, IC_{50}*Pf*3D7: 4.93 µg/mL). However, these latter fractions showed moderate selectivity (8 < SI > 71).

The crude extracts fractionation led to fractions and final residues with varied activities against *P. falciparum* parasites, with respect to species, plant part, extraction, and fractionation solvents. The overall more potent fraction *Tm*l^{w}Chl (IC_{50}*Pf*INDO: 0.36 µg/mL and IC_{50}*Pf*3D7: 0.96 µg/mL; SI > 208) with an average 4.3 – fold activity magnification derived from the crude extract *Tm*l^{w} (IC_{50}*Pf*INDO: 2.09 µg/mL and IC_{50}*Pf*3D7: 2.66 µg/mL; SI > 75); the fractionation of *Ts*r^{m} led to no significant change in activity. Besides, fractionation of *Tm*sb^{w} (IC_{50}*Pf*INDO: 0.26 µg/mL and IC_{50}*Pf*3D7: 1.03 µg/mL; SI > 194) slightly reduced the antiplasmodial potency, but led to very promising and selective fractions with IC_{50}*Pf*INDO: 0.39 – 4.50µg/mL and IC_{50}*Pf*3D7: 1.12–4.40µg/mL, whereas fractionation of extract *Ts*l^{w} (IC_{50}*Pf*INDO:

0.57µg/mL and IC_{50}*Pf*3D7: 1.26µg/mL) negatively impacted its potency, leading to only 2 moderately active fractions (*Ts*l^{w}H and *Ts*l^{w}EA with IC_{50}: 6.89 – 14.09 µg/mL). Generally, except the hexane fraction from the leaf extract (*Ts*l^{w}H, CC_{50} = 126.03 µg/mL) that showed moderate cytotoxicity effect to a certain extent, all the tested fractions displayed very good potencies and selectivities against both sensitive and resistant strains of *P. falciparum.*

The antiplasmodial activities of extracts, fractions and isolated compounds from many *Terminalia* species have been previously reported. However, to the best of our knowledge, we are reporting for the first time the antiplasmodial activity of extracts from *Terminalia mantaly*. Moreover, it is the first report on the antiplasmodial activity of *Terminalia* species against *Plasmodium falciparum* Chloroquine – resistant INDO strain.

Muganga *et al.* (2014) reported the antiplasmodial activity of *Terminalia mollis* crude methanolic extract (IC_{50}: 3.84 µg/mL), aqueous extract (IC_{50}: 4.66 µg/mL), ethyl acetate fraction (IC_{50}: 2.10 µg/mL), aqueous fraction (IC_{50}: 19.72 µg/mL) and isolated ellagic acid (IC_{50}: 0.17 µg/mL) against *Plasmodium falciparum* 3D7 strain. Mohd Abd Razak *et al.* (2014) reported the antiplasmodial activity of *Terminalia catappa* aqueous extract (IC_{50}: 4.28 µg/mL), methanolic extract (IC_{50}: 5.19 µg/mL) and dichloromethane extract (IC_{50}: 5.29 µg/mL) on *Plasmodium falciparum* K1, using the *in vitro P. falciparum* Histidine – rich protein II assay. Abiodun *et al.* (2011) also reported the antiplasmodial activity of hexane, ethyl acetate and methanolic extracts from *Terminalia catappa* on *Plasmodium falciparum* K1 (IC_{50}: 10.10, 3.05 and 7.42 µg/mL respectively) and *Plasmodium falciparum* NF54 (IC_{50}: 21.93, 6.68 and 9.40 µg/mL respectively) using the [^{3}H]-hypoxanthine incorporation assay. Sanon *et al.* (2013) used the *P. falciparum* Lactate Dehydrogenase assay to evaluate then report the antiplasmodial activity of *Terminalia avicennioides* aqueous, methanolic and dichloromethane extracts from leaf and stem bark with IC_{50} values ranging from 1.60 to 7.40 µg/mL on *P. falciparum* K1. Likewise, Ouattara *et al.*, (2014) reported the activity of *T. avicennioides* ethyl acetate and butanol crude extracts against *P. falciparum* K1 while ellagic acid isolated from the leaf showed potent antiplasmodial activity with an IC_{50} of 0.52 µM, using the fluorescence – based SYBR Green I assay. Bavagan *et al.* (2010) previously used the candle – jar cultivation method to investigate and report the antiplasmodial activity of *Terminalia chebula* hexane, ethyl acetate, acetone, and methanolic extracts on *P. falciparum* 3D7 with IC_{50} values of 51.91, 67.45, 4.76, 42.98 µg/mL respectively. They equally highlighted the antiplasmodial potential of aqueous extracts from stem bark and stem wood of *Terminalia spinosa* on *P. falciparum*

Chloroquine – resistant ENT36 (IC_{50}: 29.50 and 49.20 µg/mL) and Chloroquine – sensitive K67 (IC_{50}: 9.90 and 35.90 µg/mL) strains.

Two fractions abbreviated *Tm*sb^{w}M (IC_{50}*Pf*INDO: 0.39 µg/mL, SI_{PfINDO} > 512-fold; IC_{50}*Pf*3D7: 1.28 µg/mL, SI_{Pf3D7} > 156-fold) for the methanolic fraction from *Tm*sb^{w}, and *Ts*r^{m}EA (IC_{50}*Pf*INDO: 1.82 µg/mL, SI_{PfINDO} > 109-fold; IC_{50}*Pf*3D7: 1.65 µg/mL, SI_{Pf3D7} > 121-fold) for the ethyl acetate fraction from *Ts*r^{m} presenting the higher fractionation yields and potent antiplasmodial activities for each respective species were selected and submitted to the second step bioguided fractionation using Column Chromatography.

3- Antiplasmodial activity and cytotoxicity of subfractions

The column chromatography (CC) of fractions *Tm*sb^{w}M and *Ts*r^{m}EA yield respectively 39 subfractions and 22 subfractions, which showed antiplasmodial profile with their IC_{50} ranged from highly potent activity (0.29 µg/mL) to no activity (> 8 µg/mL). The antiplasmodial potency varied with respect to plant species, plant part as well as the elution solvents.

A total of 21 subfractions out of the 39 prepared from *Tm*sb^{w}M displayed very good antiplasmodial activity having their IC_{50} from 0.29 µg/mL to 4.74 µg/mL [Table 4]. Moreover, 15 actives subfractions (71.43% of actives extracts) exhibited potent antiplasmodial activity (IC_{50} < 2 µg/mL) on both *Pf*INDO and *Pf*3D7 strains. Nine subfractions codified *Tm*25, *Tm*28 – 30, *Tm*33–36, *Tm*38 exerted highly potent antiplasmodial activity (IC_{50} < 1 µg/mL) on both Chloroquine–resistant INDO and Chloroquine–sensitive 3D7 strains of *P. falciparum* (IC_{50}*Pf*INDO: 0.41–0.84 µg/mL, SI_{PfINDO} > 50–243; IC_{50}*Pf*3D7: 0.29–1.06 µg/mL, SI_{Pf3D7} > 95 – 344). The subfraction *Tm*36 displayed the best antiplasmodial potency and the highest selectivity on the two *P. falciparum* strains of the assays (IC_{50}*Pf*INDO: 0.41 µg/mL, SI_{PfINDO} > 243-fold; IC_{50}*Pf*3D7: 0.29 µg/mL, SI_{Pf3D7} > 344-fold). The fractionation of *Ts*r^{m}EA led to 22 subfractions from which 11 exhibited antiplasmodial activity, with IC_{50} values from 1.42 µg/mL to 5.14 µg/mL. Seven actives subfractions namely *Ts*03–04, *Ts*06, *Ts*09 –10, *Ts*12–13 displayed very good antiplasmodial activity (IC_{50} < 5 µg/mL) on the two *P. falciparum* strains of the essay (IC_{50}*Pf*INDO: 1.53 – 3.69 µg/mL; IC_{50}*Pf*3D7: 1.42 – 4.77 µg/mL). Overall, *Ts*13 (IC_{50}*Pf*INDO: 1.53 µg/mL, SI_{PfINDO} > 65-fold; IC_{50}*Pf*3D7: 1.42 µg/mL, SI_{Pf3D7} > 70-fold) presented the highest antiplasmodial activity and selectivity amongst subfractions from *T. superba* *Ts*r^{m}EA.

Table 4: Column chromatography yields and activity parameters of subfractions from *T. mantaly* (*Tm*sb^{w}M) and *T. superba* (*Ts*r^{m}EA)

Plants	Extracts	Fractions	Subfractions	Yields (%)	IC_{50} ± SD (µg/mL)		RI	CC_{50} ± SD (µg/mL)	Selectivity Index (SI)	
					*Pf*INDO	*Pf*3D7			*Pf*INDO	*Pf*3D7
T. mantaly	*Tm*sb^{w}	*Tm*sb^{w}M	*Tm*01	3.728	> 8.00	> 8.00	-	-	-	-
			*Tm*02	0.022	> 8.00	> 8.00	-	-	-	-
			*Tm*03	0.009	> 8.00	> 8.00	-	-	-	-
			*Tm*04	0.011	> 8.00	> 8.00	-	-	-	-
			*Tm*05	0.011	> 8.00	> 8.00	-	-	-	-
			*Tm*06	0.010	> 8.00	> 8.00	-	-	-	-
			*Tm*07	0.004	> 8.00	> 8.00	-	-	-	-
			*Tm*08	0.005	4.44 ± 0.50	4.74 ± 1.30	0.94	20.96 ± 0.42	4.72	4.42
			*Tm*09	0.004	> 8.00	> 8.00	-	-	-	-
			*Tm*10	0.004	> 8.00	> 8.00	-	-	-	-
			*Tm*11	0.018	2.22 ± 0.16	> 8.00	< 0.28	50.10 ± 0.85	22.57	< 6.26
			*Tm*12	5.508	3.44 ± 0.19	4.47 ± 0.49	0.77	37.88 ± 1.17	11.01	8.47
			*Tm*13	0.002	> 8.00	> 8.00	-	-	-	-
			*Tm*14	0.124	> 8.00	> 8.00	-	-	-	-
			*Tm*15	0.004	> 8.00	> 8.00	-	-	-	-
			*Tm*16	0.060	> 8.00	> 8.00	-	-	-	-
			*Tm*17	0.476	> 8.00	> 8.00	-	-	-	-
			*Tm*18	0.256	> 8.00	> 8.00	-	-	-	-
			*Tm*19	0.005	4.43 ± 0.40	4.65 ± 1.30	0.95	22.95 ± 0.35	5.18	4.94
			*Tm*20	13.12	2.37 ± 0.07	2.79 ± 0.26	0.85	23.36 ± 0.01	9.86	8.37
			*Tm*21	0.026	> 8.00	> 8.00	-	-	-	-
			*Tm*22	0.074	1.11 ± 0.06	0.67 ± 0.01	1.66	> 100.00	> 90.09	> 149.25
			*Tm*23	0.018	1.30 ± 0.00	0.72 ± 0.04	1.81	> 100.00	> 76.92	> 138.88
			*Tm*24	0.012	1.41 ± 0.00	1.45 ± 0.01	0.97	> 100.00	> 70.92	> 68.96
			*Tm*25	0.012	0.76 ± 0.01	0.52 ± 0.00	1.46	> 100.00	> 131.58	> 192.31
			*Tm*26	0.540	3.71 ± 0.32	4.47 ± 0.47	0.83	> 100.00	> 26.95	> 22.37
			*Tm*27*	0.106	0.64 ± 0.01	1.18 ± 0.01	0.54	> 100.00	> 156.25	> 84.75
			*Tm*28*δ	0.262	0.60 ± 0.04	0.61 ± 0.01	0.98	> 100.00	> 166.67	> 163.93
			*Tm*29*δ	0.235	0.48 ± 0.02	0.60 ± 0.01	0.80	> 100.00	> 208.33	> 166.67
			*Tm*30*δ	0.155	0.45 ± 0.00	0.39 ± 0.01	1.15	> 100.00	> 222.22	> 256.41
			*Tm*31	34.56	3.91 ± 0.36	3.15 ± 0.63	1.24	25.47 ± 0.01	6.51	8.08
			*Tm*32*	0.271	0.55 ± 0.02	1.21 ± 0.01	0.45	> 100.00	> 181.82	> 82.64

			*Tm*33*	0.891	0.84 ± 0.01	1.06 ± 0.12	0.79	> 100.00	> 119.05	> 94.34
			*Tm*34*	0.074	0.59 ± 0.01	0.68 ± 0.01	0.87	> 100.00	> 169.49	> 147.06
			*Tm*35	0.092	0.58 ± 0.02	0.37 ± 0.00	1.57	29.09 ± 0.88	50.15	78.62
			*Tm*36*δ	0.482	0.41 ± 0.00	0.29 ± 0.00	1.43	> 100.00	> 243.90	> 344.83
			*Tm*37	0.002	> 8.00	> 8.00	-	-	-	-
			*Tm*38*δ	0.131	0.67 ± 0.02	0.45 ± 0.01	1.48	> 100.00	> 149.25	> 222.22
			*Tm*39	9.316	> 8.00	> 8.00	-	-	-	-
T. superba	*Tsr*^{m}	*Tsr*^{m}EA	*Ts*01	2.746	> 8.00	> 8.00	-	-	-	-
			*Ts*02	7.046	> 8.00	> 8.00	-	-	-	-
			*Ts*03	0.002	2.47 ± 0.14	5.14 ± 0.01	0.48	98.65 ± 0.65	39.94	19.19
			*Ts*04	0.002	2.01 ± 0.13	4.51 ± 0.00	0.45	19.82 ± 0.00	9.86	4.39
			*Ts*05	5.741	> 8.00	> 8.00	-	-	-	-
			*Ts*06	0.001	1.83 ± 0.2	4.77 ± 0.01	0.38	20.29 ± 0.00	11.09	4.25
			*Ts*07	0.003	> 8.00	4.58 ± 0.57	> 1.74	6.25 ± 0.98	< 0.78	1.36
			*Ts*08	0.004	> 8.00	3.41 ± 0.42	> 2.35	12.5 ± 0.25	< 1.56	3.66
			*Ts*09	0.002	2.17 ± 0.2	2.53 ± 0.21	0.86	30.34 ± 0.13	13.98	11.99
			*Ts*10	0.001	3.69 ± 0.61	4.51 ± 0.52	0.82	> 100.00	> 27.10	> 22.17
			*Ts*11	0.004	> 8.00	3.83 ± 0.23	> 2.08	22.77 ± 0.00	< 2.85	5.95
			*Ts*12	0.002	2.03 ± 0.45	4.86 ± 0.54	0.42	> 100.00	> 49.26	> 20.58
			*Ts*13	0.002	1.53 ± 0.07	1.42 ± 0.22	1.08	> 100.00	> 65.36	> 70.42
			*Ts*14	0.002	> 8.00	> 8.00	-	-	-	-
			*Ts*15	0.003	> 8.00	> 8.00	-	-	-	-
			*Ts*16	0.016	> 8.00	> 8.00	-	-	-	-
			*Ts*17	0.001	> 8.00	> 8.00	-	-	-	-
			*Ts*18	0.001	3.68 ± 0.21	> 8.00	< 0.46	81.25 ± 0.52	22.08	< 10.16
			*Ts*19	0.001	> 8.00	> 8.00	-	-	-	-
			*Ts*20	2.469	> 8.00	> 8.00	-	-	-	-
			*Ts*21	0.011	> 8.00	> 8.00	-	-	-	-
			*Ts*22	11.00	> 8.00	> 8.00	-	-	-	-

Data are presented as means of triplicate experiments. **IC$_{50}$**: 50% Inhibitory Concentration, **CC$_{50}$**: 50% Cytotoxic Concentration, **SD**: Standard deviation from triplicate experiments, **RI**: resistance index, ***Tm***: *Terminalia mantaly*, ***Ts***: *Terminalia superba*, **sb**: stem bark, **r**: roots, **^{w}**: Water, **^{m}/M**: Methanol, **EA**: Ethyl acetate, *****: was selected for GC – MS chemical profiling, **δ**: was selected for intraerythrocytic stages specific inhibition and post – drugs effects analysis

Besides, some actives antiplasmodial subfractions *Tm*27 (RI: 0.54), *Tm*29 (RI: 0.80), *Tm*32 (RI: 0.45), *Tm*33 (RI: 0.79), *Tm*34 (RI: 0.87) for *T. mantaly* and *Ts*03 (RI: 0.48), *Ts*04 (RI: 0.45), *Ts*06 (RI: 0.38), *Ts*09 (RI: 0.86), *Ts*10 (RI: 0.82), *Ts*12 (RI: 0.42) for *T. superba* demonstrated better antiplasmodial activity on *Pf*INDO than *Pf*3D7 given their RI below 1 [Table 4]. These results are in the same line with those obtained by Mbouna *et al.* (2018) with crude extracts and fractions from *T. mantaly* and *T. superba*, highlighting the possibility of unique selective and novel drugs target into resistant strains INDO of *Plasmodium falciparum*.

The cytotoxic profile of antiplasmodial subfractions revealed CC_{50} values ranging from 20.96 µg/mL to >100 µg/mL for *T. mantaly* extracts, and except *Tm*35 (CC_{50}HEK239T: 29.09 µg/mL) which exerted cytotoxic effect on HEK239 T cells, all the others promising sub – fractions *Tm*22–30, *Tm*32–34, *Tm*36 and *Tm*38, displayed no cytotoxicity with all their CC_{50} values above the highest tested concentration 100 µg/mL (Mbouna *et al.*, 2018). Antiplasmodial actives subfractions from *Ts*r^{m}EA demonstrated substantial toxicity on HEK239 T cell line with CC_{50} from 6.25 µg/mL to 98.65 µg/mL, except *Ts*10, *Ts*12 and *Ts*13 which showed CC_{50} above 100 µg/mL.

The fractionation of *Tms*b^{w}M (IC_{50}*Pf*INDO: 0.39 µg/mL, IC_{50}*Pf*3D7: 1.28 µg/mL) afforded subfraction *Tm*36 (IC_{50}*Pf*INDO: 0.41 µg/mL, IC_{50}*Pf*3D7: 0.29 µg/mL) with the antiplasmodial potency higher than to the sourced fraction antiplasmodial potency, whereas 25 others subfractions obtained from *Tms*b^{w}M showed antiplasmodial activity 5 to 20-fold less effective than *Tms*b^{w}M activity. Likewise, fractionation of *Ts*r^{m}EA (IC_{50}*Pf*INDO: 1.82 µg/mL, IC_{50}*Pf*3D7: 1.65 µg/mL) released subfractions with antiplasmodial potencies globally less than the mother fraction; as except *Ts*13 (IC_{50}*Pf*INDO: 1.53 µg/mL, IC_{50}*Pf*3D7: 1.42 µg/mL) which displayed potency not significantly different from those of *Ts*r^{m}EA, all the others subfractions possessed poor antiplasmodial activity compared to their sourced fraction. The variability observed in antiplasmodial potencies of subfractions from both fractions could be due to the fact that fractionation of promising extracts either concentrated active ingredients into a sub – fraction or shared them between the afforded subfractions (Nwodo *et al.*, 2010). The results achieved from the fractionation clearly underline the fact that high antiplasmodial potency of *T. mantaly* and *T. superba* could be related to the synergistic interaction or multi-factorial effects between phytochemicals present in their extracts (Rasoanaivo *et al.*, 2011).

4- Chemical profiling of potent subfractions from *T. mantaly* fraction *Tm*sb^{w}M

Nine promising subfractions *Tm*27–30, *Tm*32–34, *Tm*36 and *Tm*38, all belonging to *Terminalia mantaly* methanolic fraction *Tm*sb^{w}M with good fractionation yields, potent antiplasmodial activity and very selective were selected and analyzed for their phytochemical composition using Gas Chromatography coupled to Mass Spectrometry (GC–MS). The following Table 5 summarized the results obtained.

Plants produce a huge variety of secondary metabolites as natural protection against microbial and insect attack. They are the natural reservoir of many antiplasmodial chemicals as well as various other therapeutic activities (Fakruddin *et al*., 2012), and their used as an alternative form of health care especially against malaria is increasing as they are promising sources of novel and safe antibiotic prototypes. The GC – MS profiling of highly potent sub – fractions led to the identification of 104 different chemical structures [Table 5], compounds distribution varying with respect to subfraction. *Tm*29 (58 chemical patterns) was the richest and most diversify subfraction whereas *Tm*32 (7 compounds) and *Tm*27 (8 compounds) had less families and numbers of chemicals.

It is worth noting that antiplasmodial potency and selectivity were not directly linked to the numbers of chemicals contained in the potent subfractions. In fact, *Tm*34 (IC_{50}*Pf*INDO: 0.59 µg/mL, IC_{50}*Pf*3D7: 0.68 µg/mL, 18 phytochemicals), *Tm*36 (IC_{50}*Pf*INDO: 0.41 µg/mL, IC_{50}*Pf*3D7: 0.29 µg/mL, 18 phytochemicals), *Tm*38 (IC_{50}*Pf*INDO: 0.67 µg/mL, IC_{50}*Pf*3D7: 0.45 µg/mL, 13 phytochemicals), *Tm*29 (IC_{50}*Pf*INDO: 0.48 µg/mL, IC_{50}*Pf*3D7: 0.60 µg/mL, 58 phytochemicals), and *Tm*30 (IC_{50}*Pf*INDO: 0.45 µg/mL, IC_{50}*Pf*3D7: 0.39 µg/mL, 26 phytochemicals) displayed all highly potent (IC_{50} < 1 µg/mL) and highly selective (SI > 100-fold) antiplasmodial activity with IC_{50} ranging 0.29–0.68 µg/mL and, 147-fold < SI < 344-fold selectivity on both *Pf*INDO and *Pf*3D7, no matter the number of phytochemicals ingredients constituting them. Besides, most of the phytocompounds identified from *Tm*sb^{w}M subfractions are made-up of chemical functional groups such as Oxide, Carbonyl, Carboxylic acid, alcohol, phenolic ring, naphthalene, alkene link that are regularly encountered in antimalarial hits and leads pharmacophores, and even more in currently deployed antimalarial drugs such as Quinine, Chloroquine, Mefloquine, Artemisinin and Dihydroartemisin.

Table 5: Phytochemical's composition of potent subfractions from *Terminalia mantaly* fraction *Tm*sb^{W}M

№	Compounds	Retention [min]	Abundance in sub - fraction [%]								
			*Tm*27	*Tm*28	*Tm*29	*Tm*30	*Tm*32	*Tm*33	*Tm*34	*Tm*36	*Tm*38
1	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.93	-	-	1.40	1.00	-	-	1.10	-	-
2	Cyclohexene, 1-methyl-4-(1-methyethenyl)	7.09	-	-	-	-	-	-	1.00	-	-
3	D-Limonene	7.14	-	-	1.50	1.20	-	-	-	-	-
4	Eucalyptol	7.22	-	-	0.80	0.30	-	-	-	-	-
5	Acetophenone	8.03	-	-	-	0.90	-	-	1.00	-	-
6	Benzenemathanol,. alpha.,.alpha.-dimethyl	8.60	-	-	-	0.40	-	-	-	-	-
7	Bicyclo [2.2.1] heptan-2-one,, 5,5,6-trimethyl-, Endo	9.09	-	-	0.30	-	-	-	-	-	-
8	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)-	10.05	-	-	-	-	-	-	1.10	-	-
9	(+)-2-Bornanone	10.06	-	0.20	0.90	-	-	-	-	-	-
10	**Cyclohexanone, 5-methyl-2-(1-methylethyl)-,**	**10.25**	**0.40**	**0.10**	**1.60**	**-**	**-**	**-**	**0.60**	**-**	**-**
11	(+)-Neoisomenthol	10.65	-	-	-	-	-	-	-	0.70	-
12	**Cyclohexanol, 5-methyl-2-(1-methylethyl)-,**	**10.81**	**0.40**	**0.10**	**1.30**	**-**	**-**	**-**	**-**	**15.30**	**-**
13	Cyclohexanone, 2-methyl-5-Isopropenyl-,	11.29	-	0.10	0.30	-	-	-	-	-	-
14	**Benzaldehyde, 2,4-dimethyl-**	**11.78**	**0.70**	**2.90**	**2.00**	**11.30**	**34.60**	**4.60**	**6.40**	**18.80**	**25.70**
15	**(-)-Carvone**	**12.40**	**0.50**	**0.50**	**3.80**	**-**	**-**	**-**	**1.50**	**0.90**	**-**
16	Linalyl acetate	12.48	-	0.20	0.30	-	-	-	-	-	-
17	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-,	12.50	-	-	-	0.20	-	-	-	-	-
18	Phenol, 5-methyl-2-(1-methylethyl)-,	13.52	-	-	0.60	-	-	-	-	-	-
19	Naphthalene, 1-methyl-	13.69	-	-	1.70	-	-	-	-	-	-
20	Oxalic acid, 6-ethyloct-3-yl heptyl ester	13.78	-	-	-	0.20	-	-	-	-	-
21	Naphthalene, 2-methyl-	14.04	-	-	0.70	-	-	-	-	-	-
22	**4-(dimethoxy methyl)-1,2-dimethylbenzene**	**14.55**	**-**	**0.30**	**-**	**0.20**	**9.30**	**0.40**	**2.60**	**5.00**	**4.10**
23	Phenol, 2-methoxy-4-(2-propenyl)-	14.90	-	0.10	-	-	-	-	-	-	-
24	Phenol, 2-methoxy-3-(2-propenyl)-	14.91	-	-	2.60	-	-	-	-	-	-
25	Benzene, (2,3-dimethyldecyl)-	15.15	-	-	0.40	-	-	-	-	-	-
26	Furan, tetrahydro-2,2,4,4-tetramethyl-	15.21	-	-	-	-	-	-	-	-	0.50
27	4-Heptanone, 2,3:5,6-diepoxy-2,6-dimethyl-	15.23	-	-	-	-	-	-	-	0.80	-
28	Naphthalene, 2-ethyl-	15.91	-	-	1.40	-	-	-	-	-	-
29	Tetradecane	15.95	-	-	1.20	-	-	-	-	-	-
30	1,5-Dimethylnaphthalene	16.16	-	-	2.00	-	-	-	-	-	-
31	Cyclopentane, 1,1'-ethylidene bis-	16.19	-	-	-	-	-	-	0.60	-	-
32	Naphthalene, 2,6-dimethyl-	16.20	-	-	1.80	-	-	-	-	-	-
33	Cyclohexane, 1-methyl-4-(2-hydroxyethyl)-	16.28	-	-	-	-	-	0.40	-	-	-

34	Cyclopentane, 1,1'-ethylidenebis-	16.29	-	-	-	0.30	-	-	-	-	-
35	1,15-Pentadecanediol	16.37	-	-	-	-	-	-	0.70	-	-
36	1-Oxacyclopentadecan-2-one, 15-isopropenyl	16.46	-	-	-	-	-	0.40	-	-	-
37	Naphthalene, 1,3-dimethyl-	16.47	-	-	4.60	-	-	-	-	-	-
38	1,15-Pentadecanediol	16.48	-	-	-	0.30	-	-	-	-	-
39	Caryophyllene	16.51	-	-	-	-	-	-	-	1.00	-
40	Naphthalene, 1,7-dimethyl-	16.56	-	-	1.90	-	-	-	-	-	-
41	1,4-Dimethylnaphthalene	17.20	-	-	0.90	-	-	-	-	-	-
42	Tetradecane	17.23	-	-	-	0.50	-	-	-	-	-
43	Naphthalene, 1-(2-propenyl)-	17.83	-	-	1.10	-	-	-	-	-	-
44	Naphthalene, 1-propyl-	17.93	-	-	0.90	-	-	-	-	-	-
45	Pentadecane	18.14	-	-	0.90	0.40	-	-	-	-	-
46	**Phenol, 2,4-bis(1,1-dimethylethyl)-**	**18.29**	**0.30**	**4.40**	**-**	**4.20**	**42.00**	**1.30**	**3.60**	**23.50**	**30.90**
47	Naphthalene, (1-methylethyl)-	18.40	-	-	4.50	-	-	-	-	-	-
48	Naphthalene, 2-(1-methylethyl)-	18.42	-	-	0.80	-	-	-	-	-	-
49	Naphthalene, 1,4,6-trimethyl-	18.44	-	-	0.60	-	-	-	-	-	-
50	Naphthalene, 2,3,6-trimethyl-	19.12	-	-	8.50	-	-	-	-	-	-
51	4,6,8-Trimethylazulene	19.45	-	-	0.30	-	-	-	-	-	-
52	Naphthalene, 1,4,6-trimethyl-	19.77	-	-	0.60	-	-	-	-	-	-
53	**Phenol, 2,4,6-tri-tert-butyl-**	**19.92**	**0.30**	**-**	**1.80**	**3.00**	**-**	**2.70**	**4.40**	**-**	**-**
54	D-Mannitol, 1,2:5,6-bis-O-(1-methylethylidene)-	20.06	-	-	-	-	-	-	-	2.20	1.10
55	Hexadecane	20.11	-	-	2.10	-	-	-	-	-	-
56	Naphthalene, 2-methyl-1-propyl-	20.17	-	-	1.10	-	-	-	-	-	-
57	1,1'-Biphenyl, 2-methyl	20.23	-	-	0.40	-	-	-	-	-	-
58	Naphthalene, 1-methyl-7-(1-methylethyl)-	20.48	-	-	1.10	-	-	-	-	-	-
59	Naphthalene, 1-methyl-2-(1-methylethyl)-	20.65	-	-	0.30	-	-	-	-	-	-
60	Chamazulene	20.94	-	-	1.70	-	-	-	-	-	-
61	9H-Fluoren-9-ol	21.05	-	-	0.50	-	-	-	-	-	-
62	Naphthalene, 1,2,3,4-tetramethyl-	21.12	-	-	2.50	-	-	-	-	-	-
63	1,1'-Biphenyl, 2,2'-dimethyl-	21.23	-	-	0.20	-	-	-	-	-	-
64	Heptadecane	21.99	-	-	1.50	-	-	-	-	-	-
65	9H-Fluorene, 9-methyl-	22.20	-	-	1.40	-	-	-	-	-	-
66	Methyl tetradecanoate	22.50	-	-	-	0.40	-	0.30	-	-	-
67	1,1'-Biphenyl, 2,4'-dimethyl-	22.62	-	-	2.00	-	-	-	-	-	-
68	(2-Methyl-3-biphenylyl) methanol	22.92	-	-	0.40	-	-	-	-	-	-
69	Tetradecanoic acid	23.13	-	-	-	-	-	0.70	0.20	-	-
70	9H-Fluorene, 2,3-dimethyl-	23.25	-	-	1.30	-	-	-	-	-	-
71	Benzoic acid, phenylmethyl ester	23.38	-	-	-	0.40	-	0.20	-	-	-
72	Phenanthrene	23.62	-	-	1.70	-	-	-	-	-	-

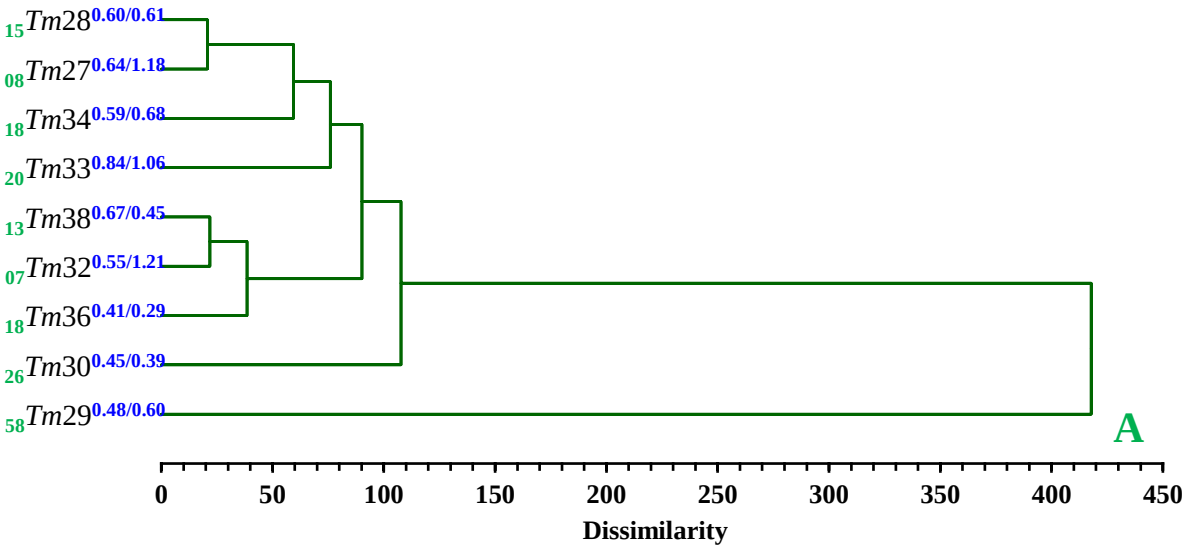
73	Octadecane	23.80	-	-	1.90	-	-	-	-	-	-
74	Hexadecane	23.89	-	-	-	0.30	-	-	-	-	-
75	Dibenzothiophene, 4-methyl-	23.90	-	-	0.70	-	-	-	-	-	-
76	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	24.91	-	-	-	0.30	-	-	-	-	-
77	Nonadecane	25.52	-	-	1.40	-	-	-	-	-	-
78	**Hexadecanoic acid, methyl ester**	**25.96**	**-**	**0.20**	**-**	**0.80**	**1.90**	**0.40**	**0.50**	**1.40**	**1.10**
79	1H-Cyclopropa[l]phenanthrene,1a,9b-dihydro-	25.99	-	-	0.60	-	-	-	-	-	-
80	1,2,5,6-Tetramethylacenaphthylene	26.18	-	-	0.30	-	-	-	-	-	-
81	n-Hexadecanoic acid	26.61	-	0.20	-	-	-	-	-	-	-
82	Phenanthrene, 2,5-dimethyl-	27.74	-	-	1.30	-	-	-	-	-	-
83	Phenanthrene, 2,3-dimethyl-	27.83	-	-	1.50	-	-	-	-	-	-
84	n-Nonadecanol-1	28.55	-	-	-	-	8.50	-	-	3.20	5.40
85	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**28.83**	**-**	**89.70**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
86	**9-Octadecenoic acid, methyl ester, (E)-**	**28.78**	**96.30**	**-**	**-**	**3.90**	**2.00**	**1.70**	**1.80**	**0.50**	**0.60**
87	**Methyl stearate**	**29.16**	**-**	**0.20**	**-**	**0.30**	**1.70**	**-**	**-**	**1.50**	**1.20**
88	Phenanthrene, 2,3,5-trimethyl-	29.59	-	-	1.20	-	-	-	-	-	-
89	**Heneicosane**	**30.34**	**-**	**0.80**	**4.50**	**2.90**	**-**	**3.90**	**17.40**	**-**	**-**
90	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	31.04	-	-	-	0.60	-	-	-	-	-
91	Pentacosane	34.62	-	-	-	22.30	-	18.10	-	-	-
92	**1-Dodecyn-4-ol**	**35.37**	**1.20**	**-**	**10.80**	**44.30**	**-**	**21.10**	**51.70**	**-**	**-**
93	Hexatriacontane	38.53	-	-	-	-	-	-	3.80	-	-
94	Tetratriacontane	40.90	-	-	-	-	-	0.40	-	-	-
95	3-Methyloctacosane	41.01	-	-	-	-	-	0.50	-	-	-
96	2-nitro-1,3-bis(octyloxy)benzene	41.85	-	-	-	-	-	0.40	-	-	-
97	Tetracontane	42.62	-	-	-	-	-	37.90	-	-	-
98	1H-1,3-Benzimidazole, 1,2-bis[4-(1-methylethyl) phenyl]	43.76	-	-	-	-	-	-	-	-	-
99	Hexatriacontane	45.68	-	-	-	-	-	2.80	-	-	-
100	Tetradecanoic acid, octadecyl ester	46.58	-	-	-	-	-	-	-	1.20	-
101	Tetrapentacontane	47.23	-	-	-	-	-	1.70	-	-	-
102	Hexadecanoic acid, octadecyl ester	50.35	-	-	-	-	-	-	-	5.10	5.50
103	**Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy**	**51.06**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**10.50**	**16.30**
104	Octadecanoic acid, octadecyl ester	55.83	-	-	-	-	-	-	-	3.70	6.00

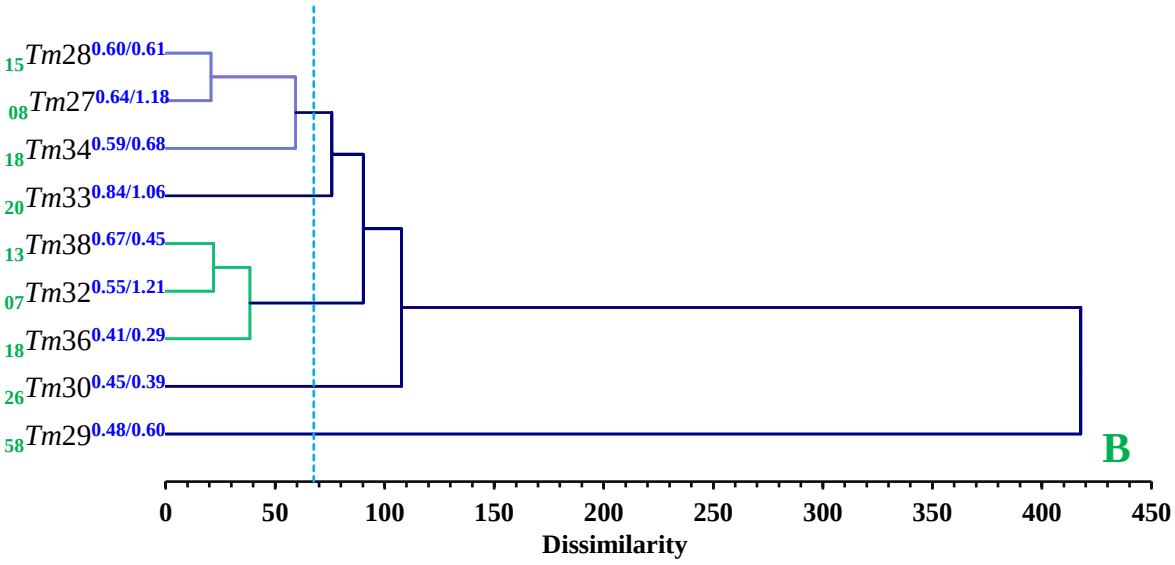
Tm: *Terminalia mantaly;* Potent subfractions (IC50 < 2 µg/mL, SI > 80, against both PfINDO and *Pf*3D7) were analyzed for their phytochemical constituents; RT= retention time. Major specialized metabolites in each subfraction have been shown in bold.

Relative to their natural based etiology, numerous reviews and investigations reports have been published on plant, fungi, marine sponge and microbial extracts containing some of the chemicals identified in the promising subfractions and others natural compounds sharing the same chemical family, and to a certain extend the same active pharmacophores than *Tm*sb^{w}M sub – fractions active ingredients (Tchokouaha *et al*., 2017; Toghueo *et al*., 2019; Toghueo and Boyom, 2019; Tajuddeen and Van Heerden, 2019). More interestingly, GC – MS analysis led to the selection of 13 phytochemotypes out of the 104 identified structures [Figure 8], present in significant quantities (2.9 – 96.3% of subfractions) and represented in at least four subfractions out of the nine analyzed.

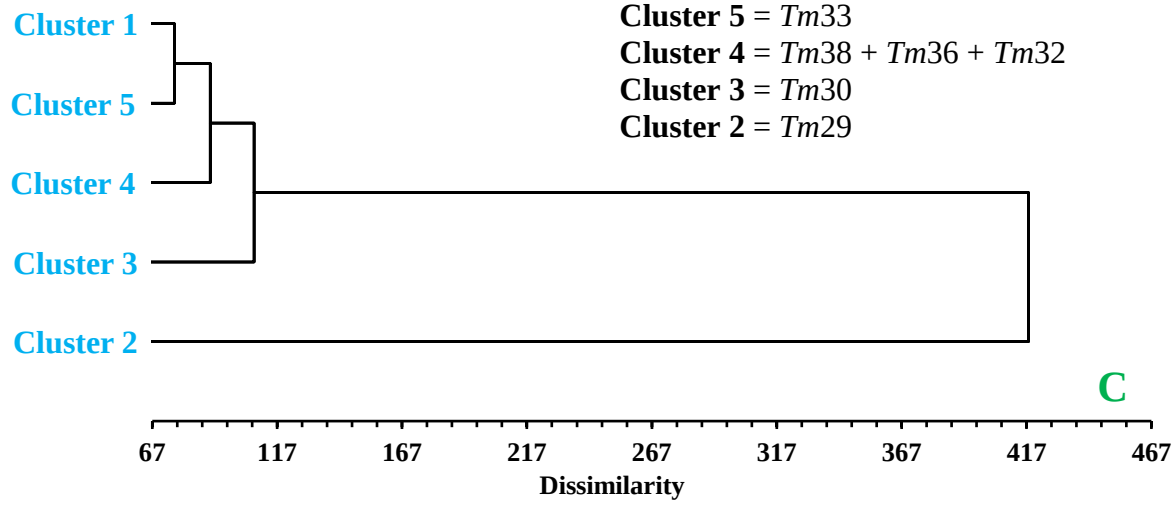
The Hierarchical Cluster Analysis (HCA) and the Principal Component Analysis (PCA) were performed on the nine antiplasmodial subfractions based on their various chemical content and with the purpose to understand the correlation between extracts relative to their chemical uniqueness and the antiplasmodial activity [Figure 7]. The analysis was performed with subfractions phytochemicals composition from GC – MS chemical profiling.

When examining the dendrogram and the biplot of the Figure 7, it is noticeable that subfractions *Tm*27 and *Tm*28 were the closest extracts despite the high discrepancies both in the number of chemicals composing them [±7 compounds difference] and in their antiplasmodial activity against *Pf*3D7 strain [IC_{50}*Pf*3D7 ± 0.57 µg/mL difference]. Likewise, *Tm*32 and *Tm*38 were also two very similar subfractions regardless their respective numbers of compounds [±6 compounds difference] and antiplasmodial activity on *Pf*3D7 [IC_{50}*Pf*3D7 ± 0.76 µg/mL difference]. Obviously, *Tm*29 was isolated from the others eight promising subfractions due to its high content in polycyclic phenolic chemicals such as anthracene, naphthalene and phenanthrene classes. It is also worth noting that *Tm*29, *Tm*30 and *Tm*36 were very dissimilar in their phytochemical fingerprints, notwithstanding the analogous antiplasmodial activity demonstrated by these three extracts, specifically on *Pf*INDO strain [IC_{50}*Pf*INDO = 0.41–0.48 µg/mL, IC_{50}*Pf*3D7 = 0.29–0.60 µg/mL]. The interrelationship between subfractions based on their phytochemical composition is represented in the following Figure 7.





Cluster 1 = *Tm*34 +*Tm*28 + *Tm*27
Cluster 5 = *Tm*33
Cluster 4 = *Tm*38 + *Tm*36 + *Tm*32
Cluster 3 = *Tm*30
Cluster 2 = *Tm*29



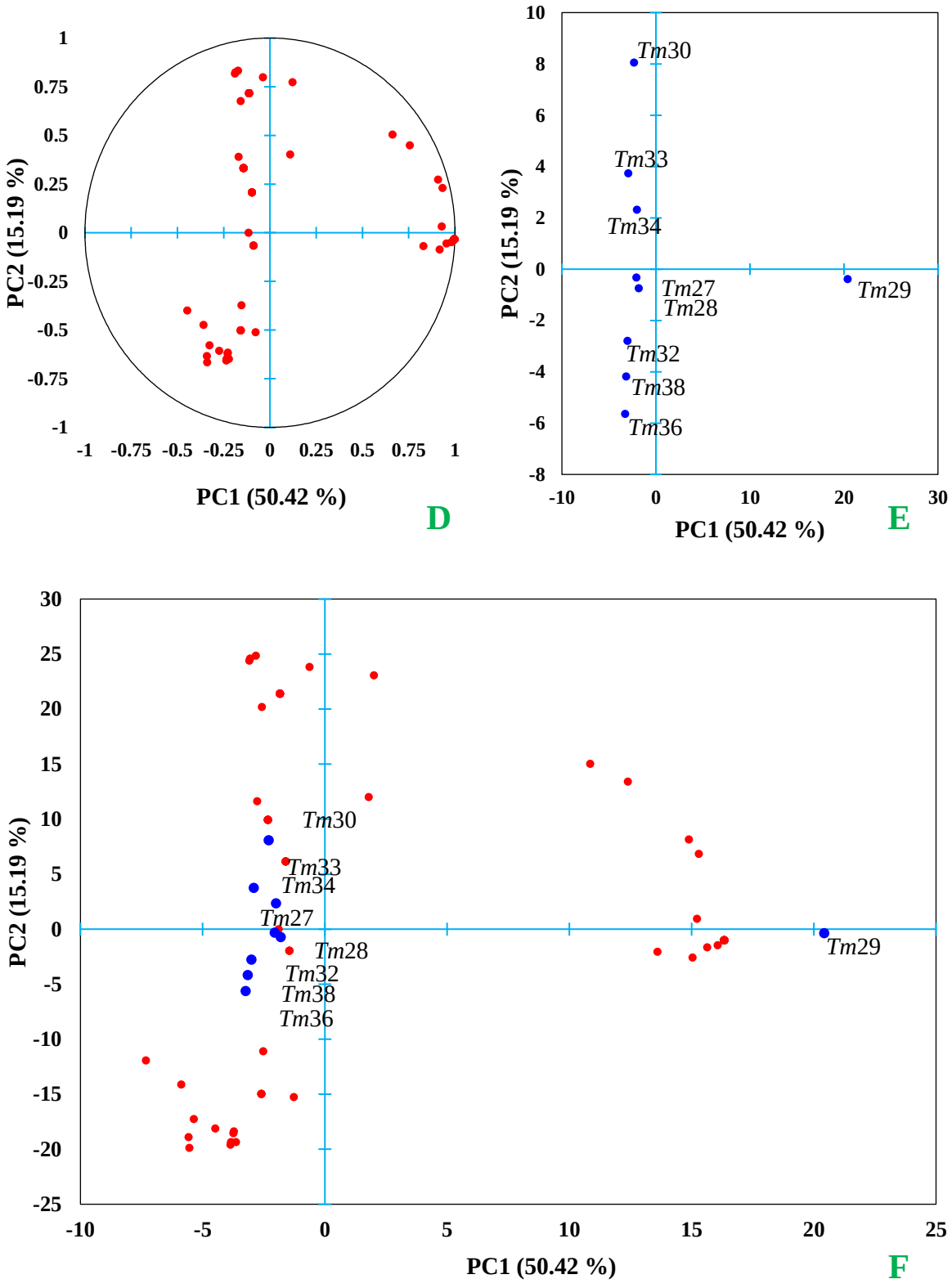


Figure 7. Hierarchical cluster analysis (HCA) [**A, B** and **C**] and Principal component analysis (PCA) [**D, E** and **F**] for relationship amongst actives subfractions based on their phytochemical profiles.

The **Euclidean distances** was used for the similarity measurement and the **Ward's linkage** as the clustering algorithm. Number of compounds*Tm*27-38IC50*Pf*INDO/IC50*Pf*3D7

The HCA and PCA are useful and simple statistical methods for seeking relatively homogeneous clusters based on measured characteristics like quantitative chemical composition, and samples with high similarity can be clustered into the homogenous groups, then correlate them to their principal ingredients. These statistical methods widely used in the origin discrimination, identification, quality assessment of extracts, drugs, and is diffusely applied to the fingerprint analysis (Tan *et al*., 2018). Recently, hierarchical cluster analysis and principal component analysis have been associated to usual fingerprints analysis like GC – MS, UPLC, HPLC to ascertain the interrelationship between extracts based on their chemical profiles and to correlate the clustering to the biological activities observed (Kicel *et al*., 2016; Xu *et al*., 2017; Wang *et al*., 2017; Zeng *et al*., 2018; Gan *et al*., 2019). The HCA regrouped the subfractions in different clusters of one, two and even three promising extracts based on their respective chemical contents. More interestingly, the results obtained from the deepening of GC – MS data by chemicals-based clustering of the promising subfractions showed to a certain extent clear dissimilarity amongst subfractions, despite the vicinity in their antiplasmodial activity. These observations led us to conclude that the highly potent antiplasmodial activity obtained with the subfractions could be due to specific compounds, present in significant quantities and represented in almost all actives subfractions. This group of chemicals might be the core antiplasmodial constituent of *Tm*sb^{w}M. Indeed, their appearance in almost all the highly potent subfractions support their involvement in the excellent antiplasmodial activity demonstrated by *Terminalia mantaly* stem bark extracts. The structures of chemicals present in more than 4 subfractions are represented in the Figure 8 below.

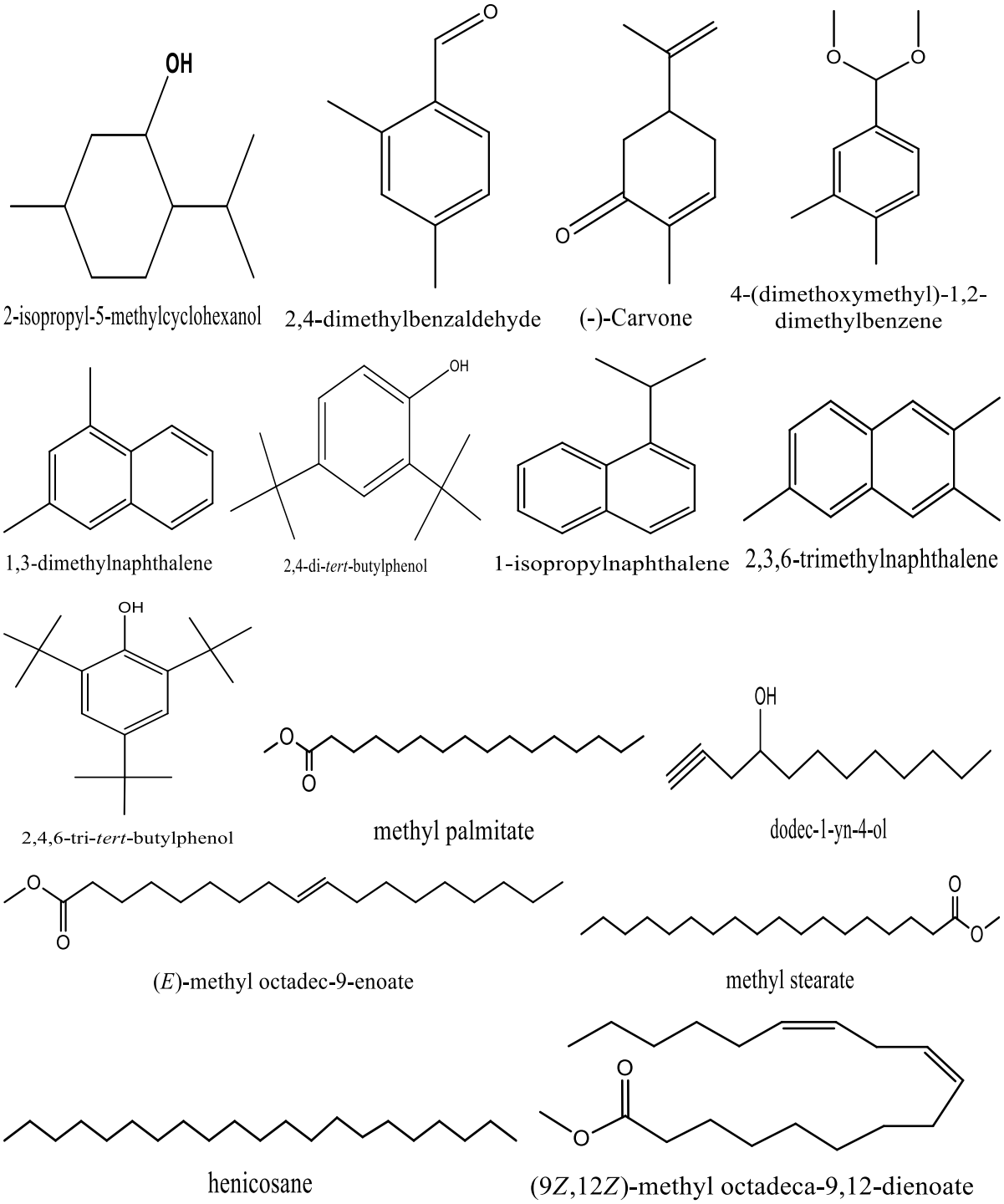


Figure 8: Core antiplasmodial constituents from *Terminalia mantaly* fraction *Tm*sb^{w}M

To the best of our knowledges, none of the compounds from *Tm*sb^{w}M core antiplasmodial chemicals [Figure 8] were reported for its antiplasmodial activity. Though, some plant extracts containing these phytochemicals, and natural compounds derived from plants and sharing the same chemical family, and to a certain extend the same active pharmacophores than *Tm*sb^{w}M subfractions active ingredients, were investigated and published for their antiplasmodial potency. For instance, Boyom *et al.* (2011) assessed

antiplasmodial potential of essenial oil from *Cleistopholis patens* (IC_{50}*Pf*W2: 9.19–15.19 µg/mL) and *Uvariastrum pierreanum* (IC_{50}*Pf*W2: 6.08–13.96 µg/mL) and identify D-Limonene (**3**) and Caryophyllene (**39**) as part of chemical constituents of antiplasmodial volatile extracts. Durant *et al.* (2014) also reported antimalarial activity of volatile extract from leaves of *Plinia cerrocampanensis* (IC_{50}*Pf*W2: 7.3 µg/mL, IC_{50}*Pf*HB3: 10.2 µg/mL) and the GC – MS profiling of this extract revealed Benzaldehyde (**14**) and Caryophyllene (**39**) in its chemical composition. Kpoviessi *et al.* (2013) reported the presence of Limonene (**3**), Carvone (**15**), and Caryophyllene into antiplasmodial (IC_{50}*Pf*3D7: 11.22–52.61 µg/mL) and antitrypanosomal (IC_{50}*Tbb*: 0.25–5.71 µg/mL) volatile extracts from four *Cymbopogon* species. Recently, Okokon *et al.* (2017a, b) assessed and reported antiplasmodial activity of crude ethanolic extract and fractions from *Zea mays* (IC_{50}*Pf*3D7: 8.46–47.88 µg/mL, IC_{50}*Pf*INDO: 3.69 – 49.1 µg/mL), and from roots of *Alchornea laxiflora* (IC_{50}*Pf*3D7: 38.44 – 81.20 µg/mL, IC_{50}*Pf*INDO: 40.17–98.99 µg/mL); the phytochemical analysis of active fractions by GC – MS revealed the presence of Hexadecanoic acid, methyl ester (**78**) and 9-Octadecenoic acid, methyl ester (**86**) as constituents of antiplasmodial extracts.

Moreover, other studies reported antiplasmodial activity of natural chemical patterns isolated from *Terminalia* species or other plants families, sharing the same main functional groups, and belonging to the same chemicals classes than compounds identified in *Tms*b^{w}M subfractions. Just to highlight certain relevant ones, Muganga *et al.* (2014) and Ouattara *et al.* (2014) each isolated Ellagic acid a phenolic compound respectively from *Terminalia mollis* from leaves of *Terminalia avicennioides*, and reported its antiplasmodial potential on *P. falciparum* 3D7 with an IC_{50} of 0.17 µg/mL and on *P. falciparum* K1 with an IC_{50} value of 0.52 µM. Machumi *et al.* (2013) isolated two galloyl derivatives from stem bark of *Terminalia brownii* namely 23-galloylarjunic acid and 4-*O*-(3″,4″-di-*O*-galloyl-α-L-rhamnopyranosyl) ellagic acid, and reported their antiplasmodial potential against *Pf*D6 (IC_{50}: 4.5 µg/mL and 4.7 µg/mL respectively) and *Pf* W2 (IC_{50}: 2.8 µg/mL and 4.7 µg/mL respectively) strains. Shuaibu *et al.* (2008) equally investigated antiplasmodial potential of *Terminalia avicennioides* and activity – guided purification of the butanolic fraction led to the isolation of hydrolysable tannins, Castalagin, Ellagic acid, Flavogallonic acid, Punicalagin and Terchebulin as main bioactive ingredients. Recently, Haidara *et al.* (2018) identified Ellagic acid and its derivatives, Terminalin, oleaterminaloic B and C as potential antiplasmodial actives compounds of *Terminalia macroptera*.

Concerning isolated chemotypes from other plants families, Kankumo *et al.* (2012) reported antiplasmodial activity of 2,3,6-trihydroxy benzoic acid (IC_{50}*Pf*W2: 16.47 µM) and 2,3,6-trihydroxy methyl benzoate (IC_{50}*Pf*W2: 13.04 µM), isolated from fruits of *Sorindeia juglandifolia*; and Tchokouaha *et al.* (2015) isolated Gallic acid from stem bark of *Annona muricata* and reported its antiplasmodial potency against *Pf*W2 with an IC_{50}: 3.32 µg/mL. de Andrade-Neto *et al.* (2007) investigated Amazonian antimalarial plants and isolated an antiplasmodial terpene – phenylpropanoid namely 4-Nerolidylcatechol (IC_{50}*Pf*K1: 0.67 µM) from roots of *Piper peltatum*. Later, Rocha e Silva *et al.* (2015) confirm its antiplasmodial potency *in vitro* with IC_{50}*Pf*K1: 0.67 µM and IC_{50}*Pf*3D7: 0.59 µM, respectively on resistant (K1) and sensitive (3D7) strains of *P. falciparum*. The activity–guided isolation of potent antiplasmodial leads from stems and leaves of *Rhaphidophora decursiva* led to the discovery of 4 phenolics compounds Rhaphidecursinols A and B, grandisin, epigrandisin and decursivine with potency against *P. falciparum* D6 and W2 strains, at IC_{50} values of 3.4 to12.9 µM (Zhang *et al.*, 2001; 2002). 2,5,2′,6′-tetrahydroxybenzophenone, a benzophenone isolated from the stem bark of *Hypericum lanceolatum* displayed antiplasmodial potential against the multidrug-resistant strain W2 of *P. falciparum*, with an IC_{50} of 13.56 µg/mL (Zofou *et al.*, 2011). The phytochemicals studies of seeds extract from *Symphonia globulifera* led to the isolation of an antiplasmodial natural product namely guttiferone A with potent activity against *P. falciparum* W2 strain, with an IC_{50} value of 3.17 µM (Ngouela *et al.*, 2006).

Our findings, together with all the previous published data emphasize the potential of *Terminalia* species to produce secondary metabolites with potent antiplasmodial activity. Furthermore, recent reports on phytochemical studies of *Terminalia mantaly* mainly showed the presence of phenols, flavonoids, tannins, saponins, terpenoids, and steroids (Ngouana *et al.*, 2015; Tchuenmogne *et al.*, 2017). Many compounds belonging to these classes of phytochemicals were found to be highly potent against several sensitive and resistant strains of *P. falciparum* (Zofou *et al.*, 2011; Kamkumo *et al.*, 2012; Pham *et al.*, 2013; Muganga *et al.*, 2014; Su *et al.*, 2015; Malterud, 2017). Moreover, studies on their potential mechanisms of action revealed that phenolic compounds and derivatives are very active as enzymes inhibitors. Examples of such enzymes inhibited by phenolic compounds and derivatives are aspartic proteases, xanthine oxidase, 15-lipoxygenase, α-glucosidase, glucose-6-phosphate dehydrogenase, carbonic anhydrase, and glutathione-S-transferase (Meyers *et al.*, 2015; Malterud, 2017; Tchuenmogne *et al.*, 2017). It is noteworthy that nowadays some of the above–

mentioned enzymes including aspartic proteases, glutathione-S-transferase are clearly identified as potential new targets for drug discovery against malaria.

Following the elucidation of chemical patterns potentially responsible of the high antiplasmodial potency observed in *T. mantaly* subfractions, the next steps in the study aimed to localize potential specific stage in *Plasmodium falciparum* where extracts focus to exert their activity during intraerythrocytic life cycle of the parasite.

5- Intraerythrocytic stages specific inhibition and post – drugs growth suppression of subfractions with excellent potency from *Tm*sb^{w}M

Five highly potent and very selective subfractions (IC_{50} < 1 µg/mL; SI > 145 times, both on *Pf*INDO and *Pf*3D7) namely *Tm*28, *Tm*29, *Tm*30, *Tm*36, and *Tm*38 were selected and assessed for the inhibition of the three main stages of *P. falciparum* asexual life cycle (ring, trophozoite and schizont) in red blood cells at 99% inhibitory concentration (IC_{99}). The stages growth inhibition, the parasitostatic and parasiticidal effects of subfractions, and the post – extract exposure growth suppression, are summarized in the Table 6 below.

Table 6: Stage – specific inhibition and post – extracts exposure growth suppression of subfractions

Subfractions $^{IC_{99}}$	Stage – specific growth inhibition (%)			Post – extracts growth suppression (%)		
	Ring	Trophozoite	Schizont	Ring	Trophozoite	Schizont
*Tm*28 $^{3.83}$	86.78	38.82	37.47	61.34	62.60	64.26
*Tm*29 $^{1.24}$	71.79	53.44	34.22	88.11	41.44	60.20
*Tm*30 $^{0.89}$	85.74	67.60	21.16	82.03	50.80	63.48
*Tm*36 $^{0.94}$	83.26	0.00	0.00	89.17	51.25	43.45
*Tm*38 $^{1.79}$	84.28	49.06	25.38	70.27	0.00	67.69

***Tm**: T. mantaly*, ^{IC99}: 99% inhibitory concentration of each subfraction by exponent

We investigated the effects of highly potent subfractions on *P. falciparum* ring, trophozoite and schizont stages development. For the experiment, we treated highly synchronized cultures of the above-mentioned stages of the intraerythrocytic developmental cycle of *Pf*3D7 with subfractions at their respective IC_{99}. Parasite growth and maturation into various other stages was appreciated by quantifying inhibitors – treated and negative controls (NC or inhibitor free cultures) parasitaemia microscopically. Overall, active subfractions exerted various growth inhibition on the different *P. falciparum* developmental stages, with pronounced inhibition on ring stage [Table 6]. In fact, all subfractions showed strong growth inhibition of ring stage after 48 hours extracts exposure with 71.79% (*Tm*29, parasitaemia: 4.13% vs 14.63% NC) to 86.78% (*Tm*28, parasitaemia: 1.93% vs 14.63% NC) growth

inhibition of *Pf*3D7 rings when compared to drug free culture of rings [Figure 8A, 11]. Obviously, *Tm*28 displayed the highest direct rings growth inhibition percent, it is worth noting that *Tm*30 (parasitaemia: 2.09% vs 14.63% NC), *Tm*36 (parasitaemia: 2.45% vs 14.63% NC) and *Tm*38 (parasitaemia: 2.30% vs 14.63% NC) were also potent inhibitors of the ring stage with 85.74%, 83.26% and 84.28% growth inhibition respectively [Figure 8A, 11]. Parasitemia of parasitized erythrocytes treated with each subfractions are presented Figure 8A.

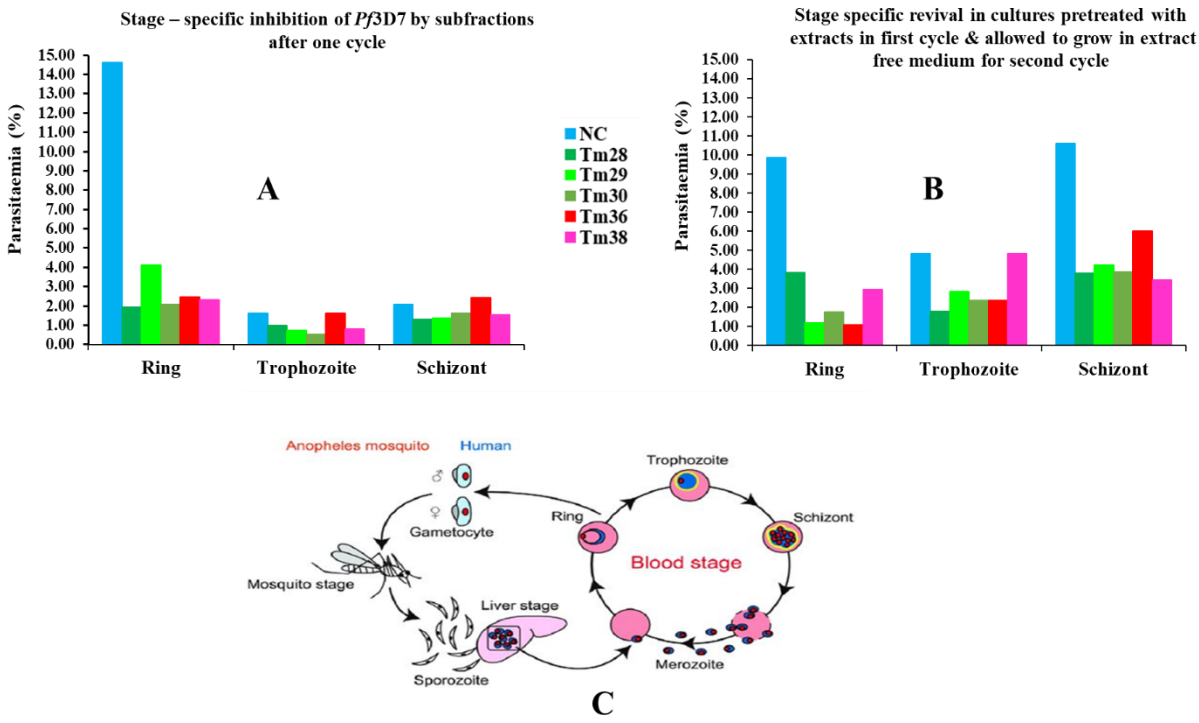


Figure 8: Percent parasitaemia (%P) and stage-specific inhibition of *Pf*3D7 cultures treated with IC_{99} of subfractions.

% P was estimated after microscopic evaluation of 3000 Giemsa-stained cells. Tightly synchronized cultures of rings, trophozoites and schizonts stages were tested each at 2% as starting parasitaemia. (**A**) **%P** in culture stages after 48 hours of treatment with SFs. Note the substantial inhibition of growth by each of the SFs. (**B**): **%P** of cultures taken from A and allowed to grow in SF-free medium for 48 hrs. While revival in inhibitor free medium is curtailed for all 3 stages, the effect is more pronounced for rings and trophozoites than for schizonts. (**C**): Life cycle of malaria parasite across mosquito, liver, and blood stages. Note the exponential increase in %P during the pathological blood stage. ***Tm*№**: *Terminalia mantaly* subfraction code, ***Pf*3D7**: *Plasmodium falciparum* chloroquine-sensitive strain 3D7, **NC** (untreated negative control).

Promising subfractions equally achieved direct trophozoites and schizonts growth inhibition but not as well as ring–to–ring life cycle inhibition. In fact, only *Tm*29 (53.44%, parasitaemia 0.75% vs 1.61% NC) and *Tm*30 (67.60%, parasitaemia 0.52% vs 1.61% NC) exhibited acute trophozoite growth inhibition above 50% at IC_{99}, while the others subfractions showed direct growth inhibition impact less than 50%, on maturation and progress of both

trophozoites and schizonts with percent of maturation inactivation from 29.86% to 49.06 and from 19.87% to 37.47% respectively [Table 6, Figure 8A, 11].

With the purpose to assess parasitostatic, parasiticidal and subsequent post–drugs exposure effect on parasite growth, Giemsa – stained thin smears were prepared at 6 h and 12 h intervals for parasitaemia counting and microscopic evaluations of the intraerythrocytic maturation and transition from stage–to–stage of the parasite life cycle. Later, 48 hours subfractions exposed parasites cultures were 3-fold washed by spin centrifugation to remove inhibitory substances. Parasites free of drugs were then incubated into inhibitors free and fresh complete culture medium for additional 48 hours. Micrographs of synchronized rings stage *Pf*3D7 cultures treated with subfractions are shown in Figure 9.

It was noticed that extracts exposed ring stage growth stopped at trophozoite stage, and *Tm*28 and *Tm*36~treated rings were not able to progress into trophozoite stage [Figure 9]. Moreover, trophozoites from subfractions *Tm*29, *Tm*30 and *Tm*38~exposed rings were not alive, as they were unable to progress into schizont stage and even their subculture in inhibitor free fresh medium did not show any growth, [Table 6, Figure 8B] resulting in pyknotic parasites [Figure 9]. Actives antiplasmodial subfractions *Tm*28, *Tm*29, *Tm*30, *Tm*36 and *Tm*38 were classified as parasiticidal on ring stage of *Plasmodium falciparum* 3D7strain, since no growth was observed in inhibitor free, fresh, and complete medium.

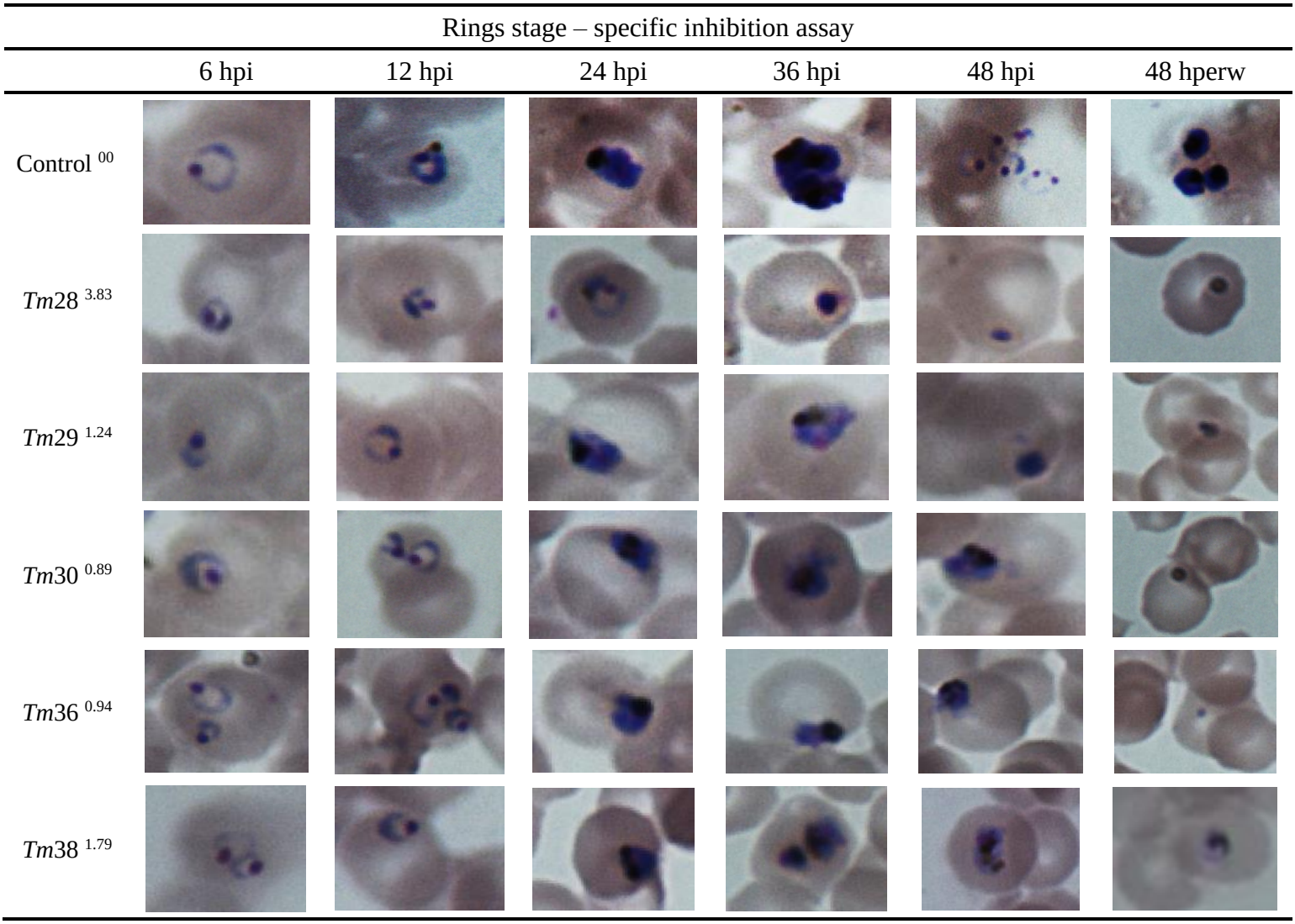


Figure 9: Rings specific inhibition of highly potent antiplasmodial subfractions.
Micrographs of synchronized rings stage *Pf*3D7 cultures treated with subfractions at their respective IC_{99} for 48hrs followed by removal of extracts by spin washing and incubation in inhibitors free medium for an additional 48hrs. ***Tm***: *Terminalia mantaly*, **hpi**: hours post infection, **hperw**: hours post extract removal by spin washing.

Although all tested subfractions displayed low direct inhibitory effect on *Plasmodium falciparum* trophozoites and schizonts [Figure 10], they subsequently demonstrated significant growth suppression on trophozoite and schizont development after subculture in inhibitor ~ free culture medium. In fact, except *Tm*36 which did not display any trophozoite maturation or growth suppression activity, all others antiplasmodial subfractions *Tm*28–30, and *Tm*38 scored high both by blocking parasites transition into next stage of life cycle and suppressing growth of trophozoite (41.44% - 78.62%) and schizont (43.45% - 67.69%) stages [Table 6] when compared to negative controls subcultures. Moreover, the subculture of all subfractions treated trophozoites progressed into schizonts but showed significant merozoites egress delay

characterized by the presence of large number of schizont compared to negative control parasites (NC) cultures which displayed merozoites egress, invasion, and progression into ring stage [Figure 10]. These findings corroborated the significant reduction in parasitaemia [Figure 8B] and the high growth suppression [Table 6] observed on both trophozoites and schizonts. It was also noted that all trophozoites treated with subfractions, progressed into schizonts then merozoites after subculture, but with late egress and incompetent merozoites that failed to invade new erythrocytes for life cycle continuity resulting in no increment in parasitaemia [Figure 10].

Based on the above–mentioned findings and observations, *Tm*28, *Tm*29, *Tm*30, *Tm*36 and *Tm*38, promising subfractions from *Terminalia mantaly* fraction *Tm*sb^{w}M exert their antiplasmodial activity by acting on all intraerythrocytic stages of *Plasmodium falciparum*, with a strong and specific activity on rings stage development, merozoites egress and merozoites invasion processes. Though, further mechanism of action studies at molecular level are required to identify and characterize their targets in rings stage and merozoites. The reported findings are of utmost scientific interest as they spotlight different ways for phytochemicals and extracts to inversely interact with malaria parasite, by targeting *Plasmodium* stages (rings) and biological phenomena (merozoites egress and ingress) different from those usually attack by most of the currently deployed antimalarial medicines. Those usually targeted parasite stages, cellular organelles or biological processes are the food vacuole of late – ring and trophozoite stages parasites, the biosynthesis of folic acid in trophozoites, the mitochondrion electron transport or the apicoplast translation (Dahl and Rosenthal, 2007; Goodman *et al*., 2007; Wilson *et al*., 2013; Roberts *et al*., 2017). Evolution of parasitaemia with respect to time in subfractions ~ treated parasitized cells versus negative controls at the rings and trophozoites stages are displayed in Figure 11 and Figure 12.

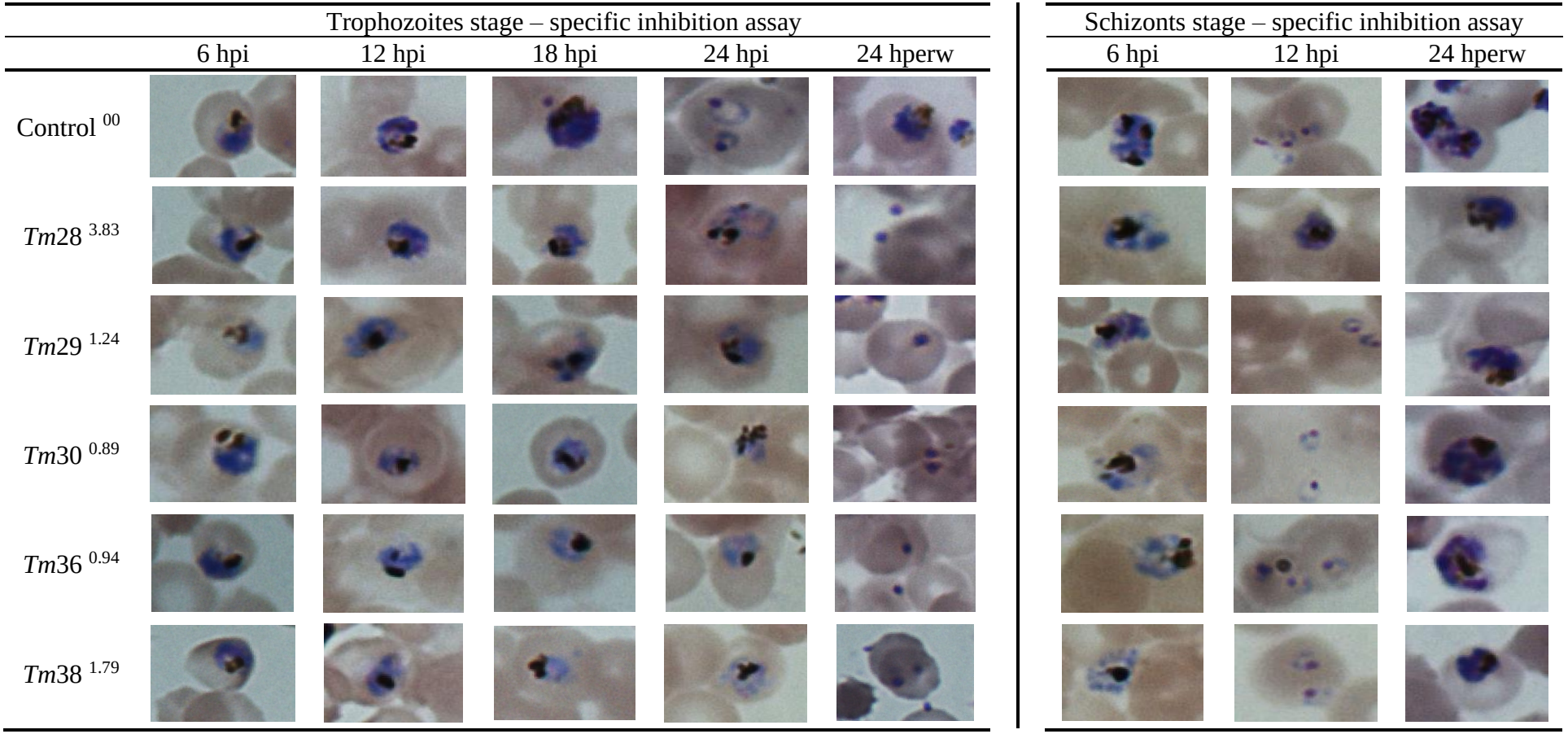
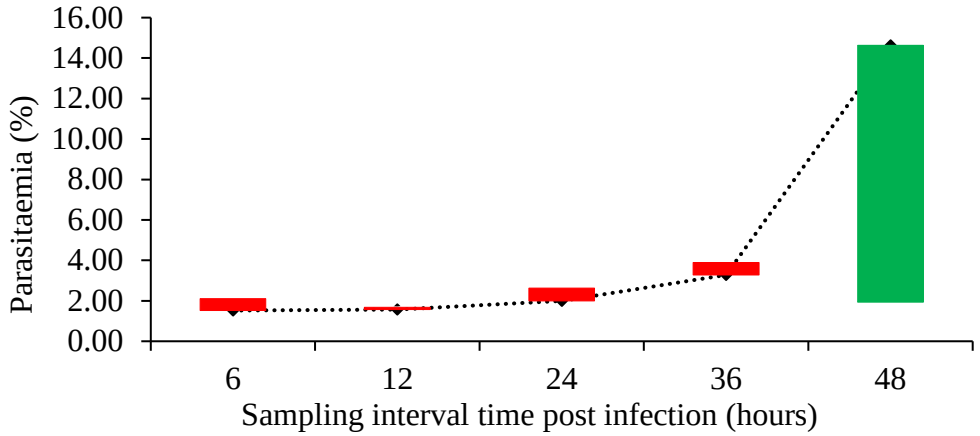


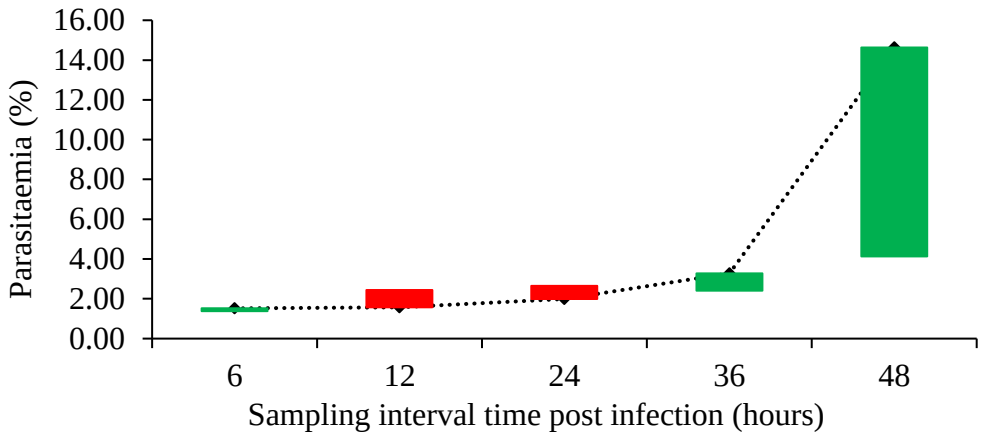
Figure 10: Trophozoites and schizonts specific inhibition of antiplasmodial subfractions.

Micrographs of synchronized trophozoite and schizont stage *Pf*3D7 cultures treated with subfractions at their IC_{99} for 48hrs followed by removal of extracts by spin washing and incubation in inhibitors free medium for an additional 48hrs. ***Tm***: *Terminalia mantaly*, **hpi**: hours post infection, **hperw**: hours post extract removal by spin washing.

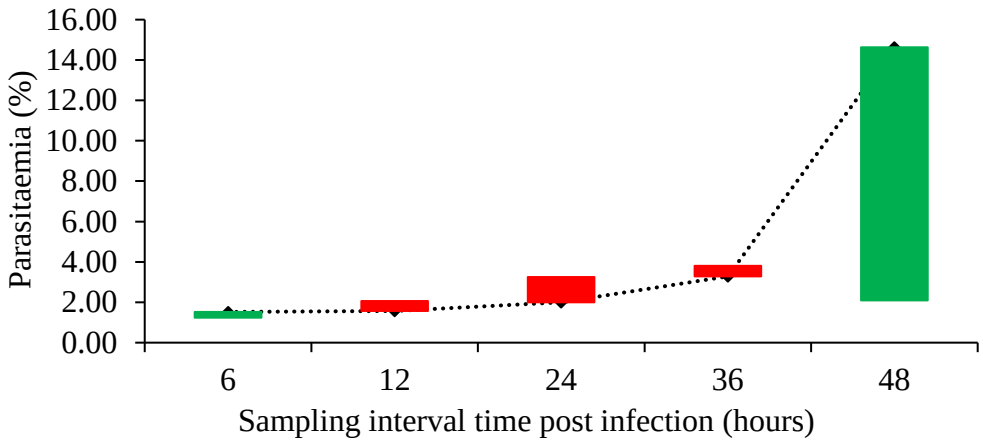
Inhibitory effect of *Tm*28 on ring stage - *Pf*3D7 **86.78%**



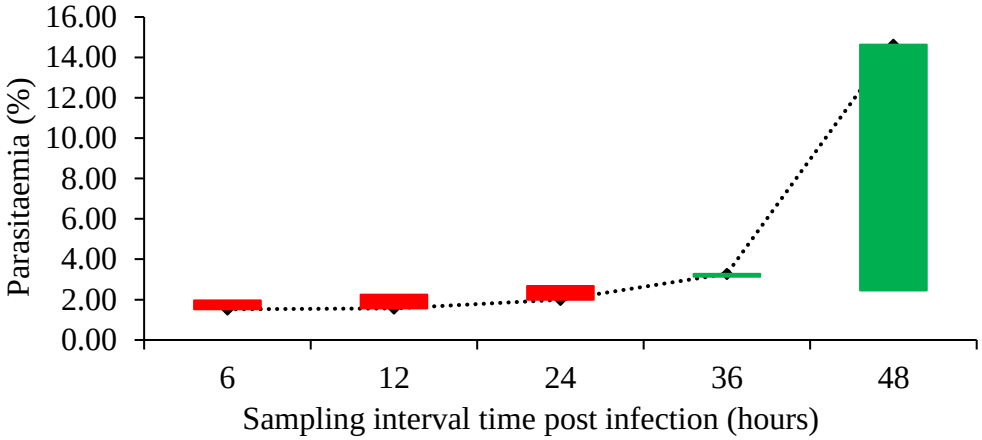
Inhibitory effect of *Tm*29 on ring stage - *Pf*3D7 **71.79%**



Inhibitory effect of *Tm*30 on ring stage - *Pf*3D7 **85.74%**



Inhibitory effect of *Tm*36 on ring stage - *Pf*3D7 **83.26%**



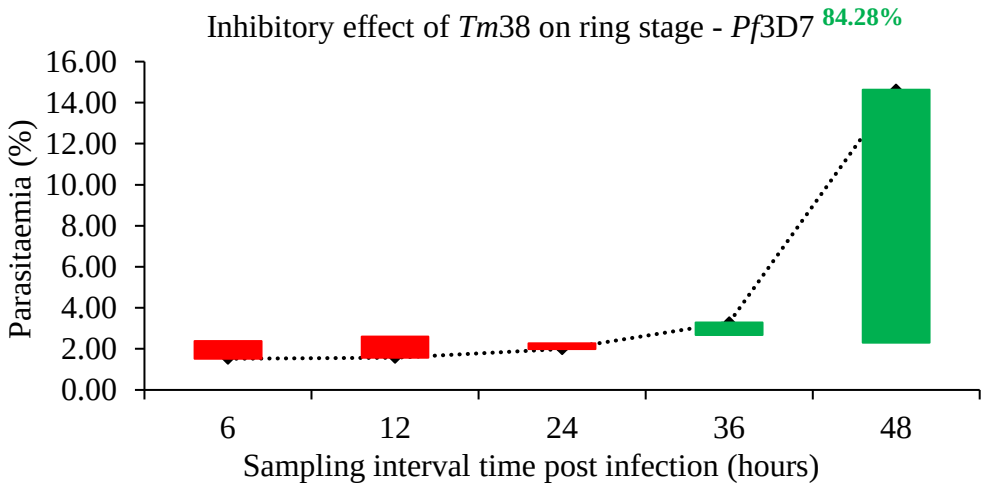
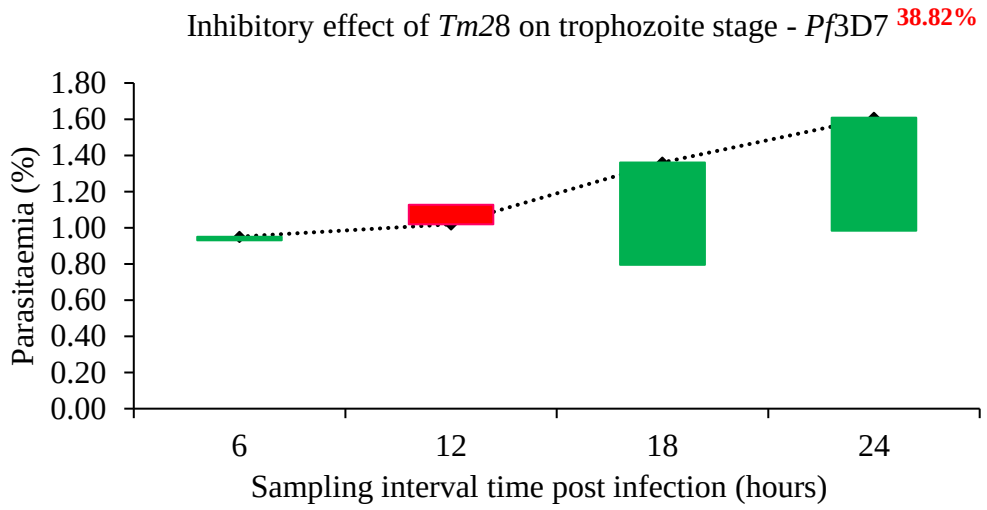
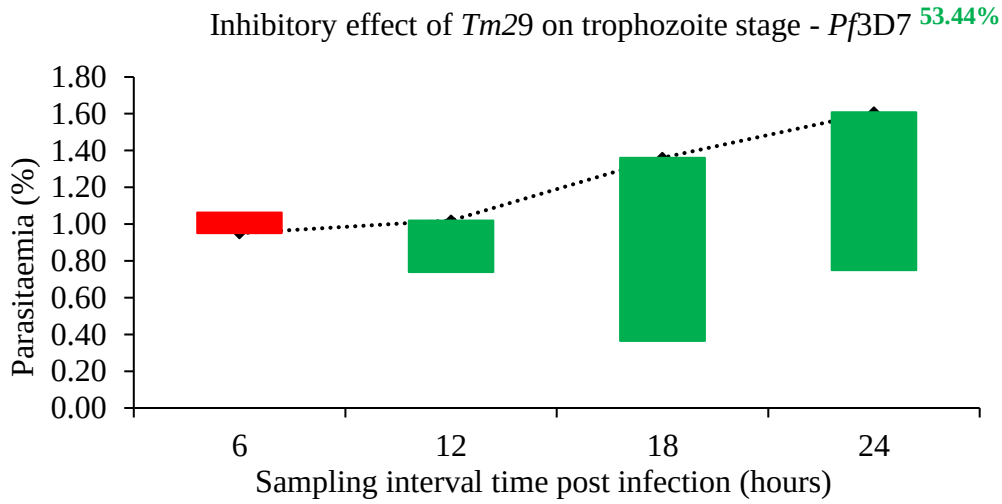
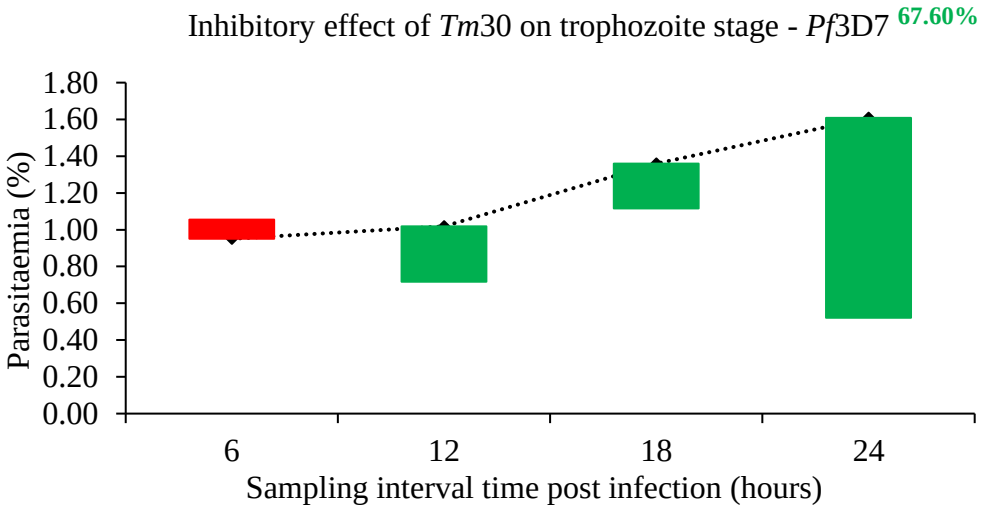
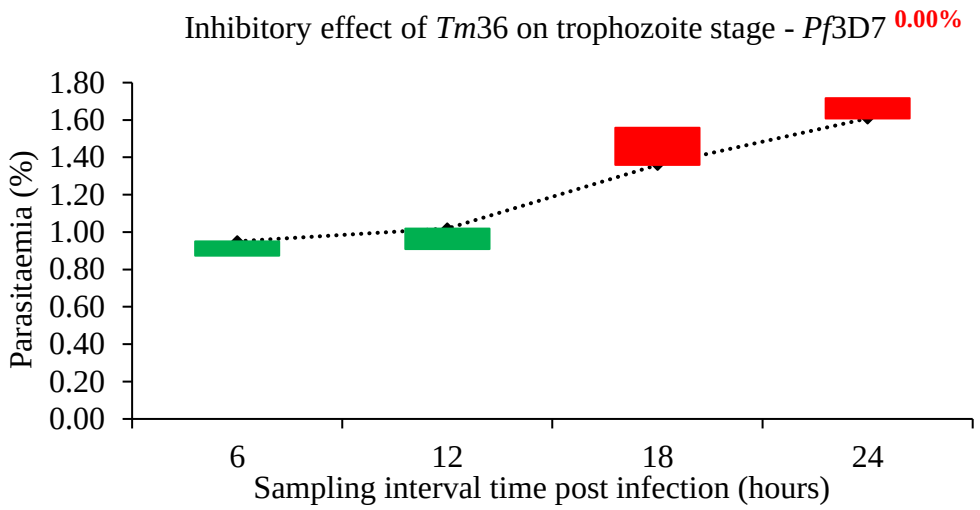


Figure 11: Inhibitory effect of *Terminalia mantaly* highly potent subfractions on *Plasmodium falciparum* ring stage development.
Parasitemia of synchronized rings stage *Pf*3D7 cultures treated with subfractions at their respective IC_{99} for 48 hours plotted in Up- and Down-Bars versus the negative control parasitaemia in dotted line. ***Tm***: *Terminalia mantaly*.









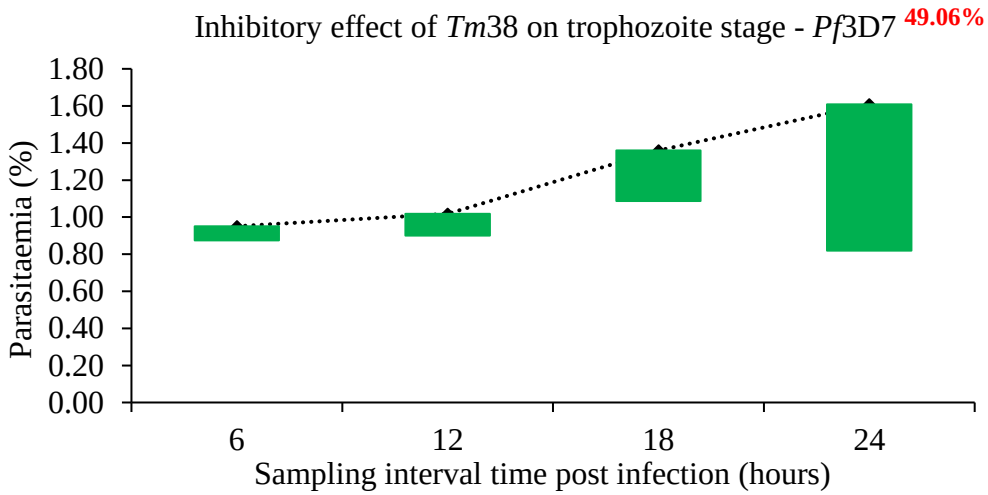


Figure 12: Inhibitory effect of *Terminalia mantaly* highly potent subfractions on *Plasmodium falciparum* trophozoite stage development.
Parasitemia of synchronized trophozoite stage *Pf*3D7 cultures treated with subfractions at their respective IC_{99} for 48 hours plotted in Up- and Down-Bars versus the negative control parasitaemia in dotted line. ***Tm***: *Terminalia mantaly*.

The discovery of new antimalarial drugs is an important ongoing challenge (Pakosz *et al*., 2021), as the treatment of acute malaria currently relies heavily on a single class of compounds *viz*. artemisinins and partners drugs. Besides, extensively reported treatment failures and available evidence of resistance to ACTs including dihydroartemisinin–piperaquine (Saunders *et al*., 2014; Thanh *et al*., 2017), have emphasized the need to discover new antimalarial medicines with novel mechanisms of action. To illustrate, several new drugs candidates have been going through different phases of clinical trials against malaria with some of them showing promise (https://www.mmv.org/research-development/mmv-supported-projects). We identified highly potent antiplasmodial subfractions *Tm*28, *Tm*29, *Tm*30, *Tm*36, and *Tm*38 as having potential to inhibit all asexual blood-stages of *P. falciparum* with preferential killing of rings and merozoites. Our results are in line with those obtained by Singh *et al*. (2015) who reported two fractions from *Parthenium hysterophorus* extract with the ability to target all stages of intraerythrocytic malaria parasite cell cycle. Together with the above-mentioned findings, our study sets the basis for discovery of potential alternative therapies to mitigate the limitations of most of the current drugs deployed to fight against malaria. As additional valid facts on the antimalarial potential of studied plants, Tchatat Tali *et al*. (2020) validated the antimalarial activity and safety of the aqueous extract from the stem bark of *T. mantaly* (IC_{50}*Pf*W2: 0.809 µg/mL) in rodent malaria infection model caused by *Plasmodium berghei*. In fact, this extract exerted a good antimalarial efficacy *in vivo* with ED_{50} of 69.50 mg/kg with no significant effect on biochemical, hematological, and histological parameters. Further, it was safe in mice with a median lethal dose (LD_{50}) > 2000 mg/kg of body weight.

To overcome multidrug – resistance issue in malaria, a major goal is to identify the next generation of antiplasmodial lead compounds that are acting early on parasite's developmental cycle with news ways to interact with parasites (Roberts *et al*., 2016). It has become a consensus that, in addition to the standard requirements for any new lead compound in the antimalarial drugs discovery pipeline (safety and efficacy, oral delivery, stability), novel antimalarial drugs to be deployed in areas of endemicity should also present low sensibility to resistance development, as well as target multiple stages in parasite life cycle (Burrows *et al*., 2013; Erath *et al*., 2015). Currently, the solely available antimalarial drugs that target ring – stage parasites are Artemisinin and Artesunate whereas most antimalarial agents act later in the parasite development cycle on the metabolically more active trophozoites and/or schizonts (Ehrhardt *et al*., 2016). Nevertheless, Trophozoite- and schizont – infected erythrocytes are responsible for severe clinical pathology, by being sequestered in the microvasculature and

causing impaired tissue perfusion and endothelial cell activation, which appears to be the main cause of fatal malaria (Hughes *et al*., 2010). Thus, targeting ring stages is therefore crucial and attractive, as it interrupts the development of rings into disease – mediating trophozoite and schizont stages and thereby prevent the progression of the infection to severe forms (Burrows *et al*., 2013; Wilson *et al*., 2013). For instance, recent flow cytometry – based analysis of ten antimalarials widely used clinically show that only Artemisinin, Artesunate and Cycloheximide have significant effect on parasite's ring stage; moreover, only artemisinin exhibited significant activity against schizonts, and none of the antimalarials prevented the invasion of merozoites (Wilson *et al*., 2013).

Besides, numerous studies recently conducted report antiplasmodial potential of chemicals and extracts with activities against blood stages of parasite. As example, two synthesized compounds namely UFC501 (4 – Nitro styrylquinoline chemical class) and UCF201 (Spirocyclic chromanes family) were reported to be early – acting in blocking parasite development at ring, trophozoite and schizont stages of development as well as merozoites invasion at nanomolar concentrations **(**Roberts *et al*., 2016; 2017). Embedding the same line, Erath *et al*., (2015) investigated small molecules from Xenomycins chemical class and report their effectiveness in clearing liver, blood asexual and sexual stages of *P. falciparum*. Plasmodione, a potent antiplasmodial lead with activity at nanomolar concentrations also exerted strong inhibition of sexual as well as intraerythrocytic stages of *P. falciparum* (Ehrhardt *et al*., 2016).

CONCLUSIONS & PERSPECTIVES

CONCLUSIONS AND PERSPECTIVES

This work was designed to evaluate the antiplasmodial activity of *Terminalia mantaly* and *Terminalia superba* and their stage–specific action. The following conclusions are drawn:

- Four crude extracts *Tm*l^{w}, *Tm*sb^{w}, *Ts*l^{w}, and *Ts*r^{m} out of the twelve prepared from *T. mantaly* and *T. superba* displayed from excellent to potent antiplasmodial activities (IC_{50}: 0.26 – 2.66 µg/mL on both *Pf*INDO and *Pf*3D7) with very high selectivity (75 < SI_{PfINDO}, SI_{Pf3D7} > 769).
- Dual steps activity – guided fractionation of the above – stated promising crude extracts afforded 61 subfractions, 39 subfractions from *T. mantaly* methanolic fraction *Tm*sb^{w}M, and 22 subfractions from the *T. superba* ethyl acetate fraction *Ts*r^{m}EA. Nine of those subfractions all belonging to *T. mantaly* and namely *Tm*25, *Tm*28, *Tm*29, *Tm*30, *Tm*33, *Tm*34, *Tm*35, *Tm*36 and *Tm*38 displayed highly potent antiplasmodial activity having their respective IC_{50}*Pf*INDO: 0.41 – 0.84 µg/mL and IC_{50}*Pf*3D7: 0.29 – 1.06 µg/mL on both strains with very high selectivity (50 < SI_{PfINDO}, SI_{Pf3D7} > 344).
- The GC – MS phytochemical analysis of potent sub – fractions led to the identification of 104 different plant–originated compounds. The analysis of promising subfractions phytochemical content led to the selection of 15 phytochemotypes out of the 104 identified structures, that could represent the core antiplasmodial ingredients of *T. mantaly* stem bark extracts.
- The blood life cycle stages – specific inhibition of highly potent subfractions showed that *Tm*28, *Tm*29, *Tm*30, *Tm*36 and *Tm*38, antiplasmodial subfractions from *Terminalia mantaly* fraction *Tm*sb^{w}M inhibited all asexual stages of the intraerythrocytic life cycle of *Plasmodium falciparum*, with strong and specific activities on ring stage development, merozoites egress and merozoites invasion processes.

Overall, outputs from this work clearly demonstrate the activity of *Terminalia mantaly* and *Terminalia superba* plants extracts, fractions and subfractions against *P. falciparum*, the causative agent of severe malaria cases. Interestingly we report for the first time the antiplasmodial activity of *Terminalia mantaly*. They also support the traditional uses of these plants in ethnomedicine to treat malaria and related symptoms. However, the full validation of these findings requires detailed toxicological studies of the active extracts, fractions, subfractions and compounds. In addition, further deep and detailed investigations are required

to complete this work and identified new hit compounds to feed naturally occurring anti – malarial drugs discovery pipeline to tackle the emergence of drug resistance.

Therefore, we propose to:

- Evaluate the antiplasmodial potential and complete stage – specific inhibition activity of phytochemicals identified as core constituents into highly potent subfractions.
- Determine molecular mechanism of action of actives compounds on rings stage, merozoites egress and merozoites progress to identify at molecular level the targets of *T. mantaly* stem bark pharmacophores.
- Determine the toxicological profile *in vivo* of highly potent chemicals on rodent study models.
- Evaluate antimalarial activity *in vivo* of promising hits on rodent malaria model.

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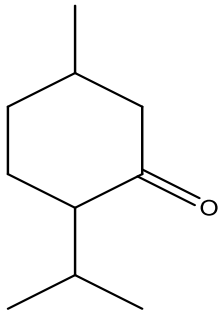
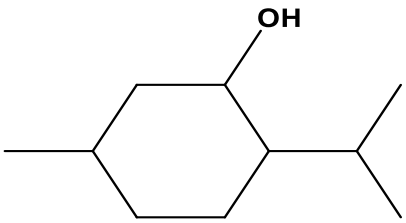
APPENDICES

APPENDICES

Appendix 1: GC – MS phytochemical composition of *Tm*27



Cis-5-methyl-2-(1-methylethyl)-cyclohexanone



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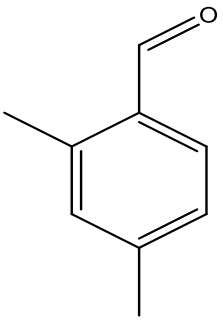
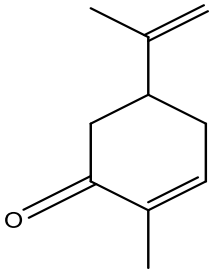
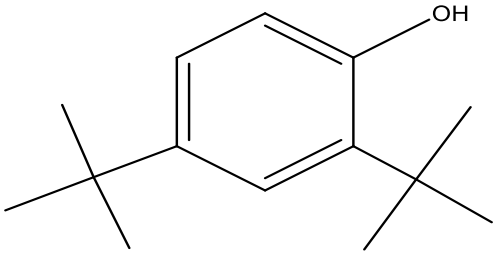


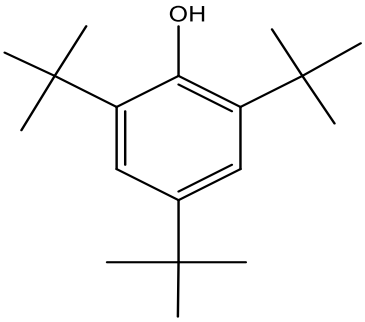
2,4-Dimethylbenzaldehyde



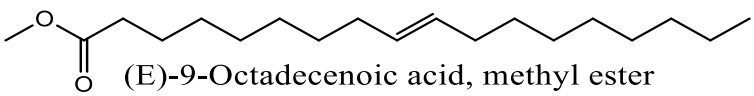
(-)-Carvone



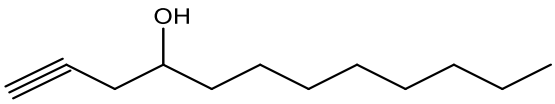
2,4-bis(1,1-dimethylethyl)-Phenol



2,4,6-tri-tert-butyl-Phenol

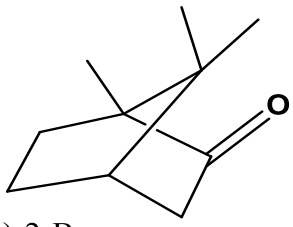


(E)-9-Octadecenoic acid, methyl ester

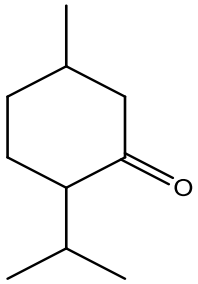


1-Dodecyn-4-ol

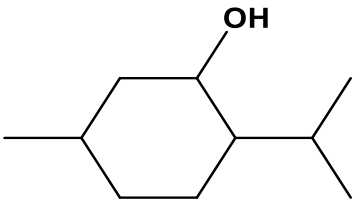
Appendix 2: GC – MS phytochemical composition of *Tm*28



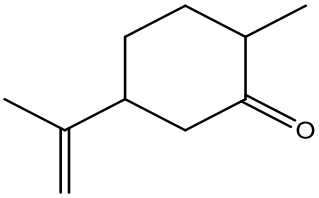
(+)-2-Bornanone



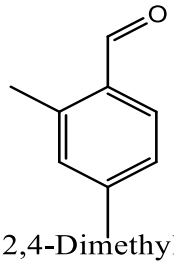
Cis-5-methyl-2-(1-methylethyl)-cyclohexanone



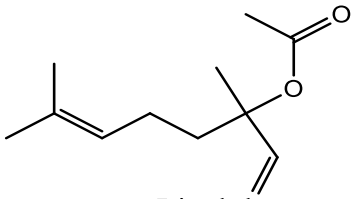
5-methyl-2-(1-methylethyl)-cyclohexanol



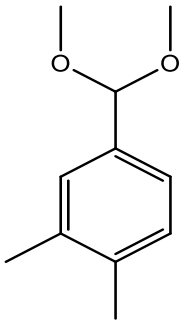
2-methyl-5-(1-methylethenyl)-Cyclohexanone



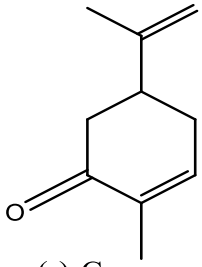
2,4-Dimethylbenzaldehyde



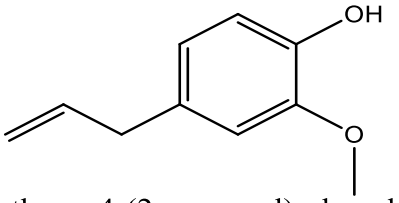
Linalyl acetate



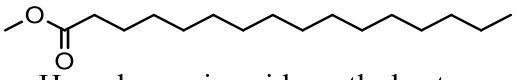
4-(Dimethoxymethyl)-1,2-dimethylbenzene



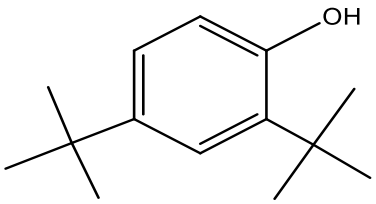
(-)-Carvone



2-Methoxy-4-(2-propenyl)-phenol



Hexadecanoic acid, methyl ester



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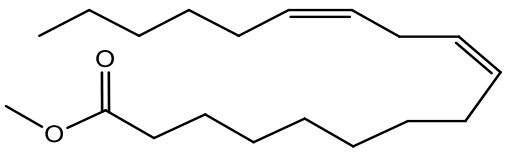
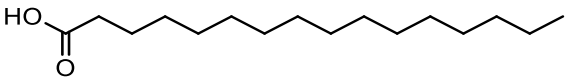
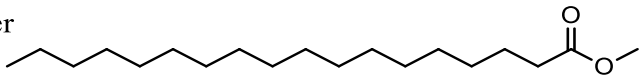


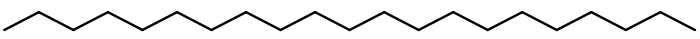
(Z,Z)-9,12-Octadecadienoic acid, methyl ester



Hexadecanoic acid

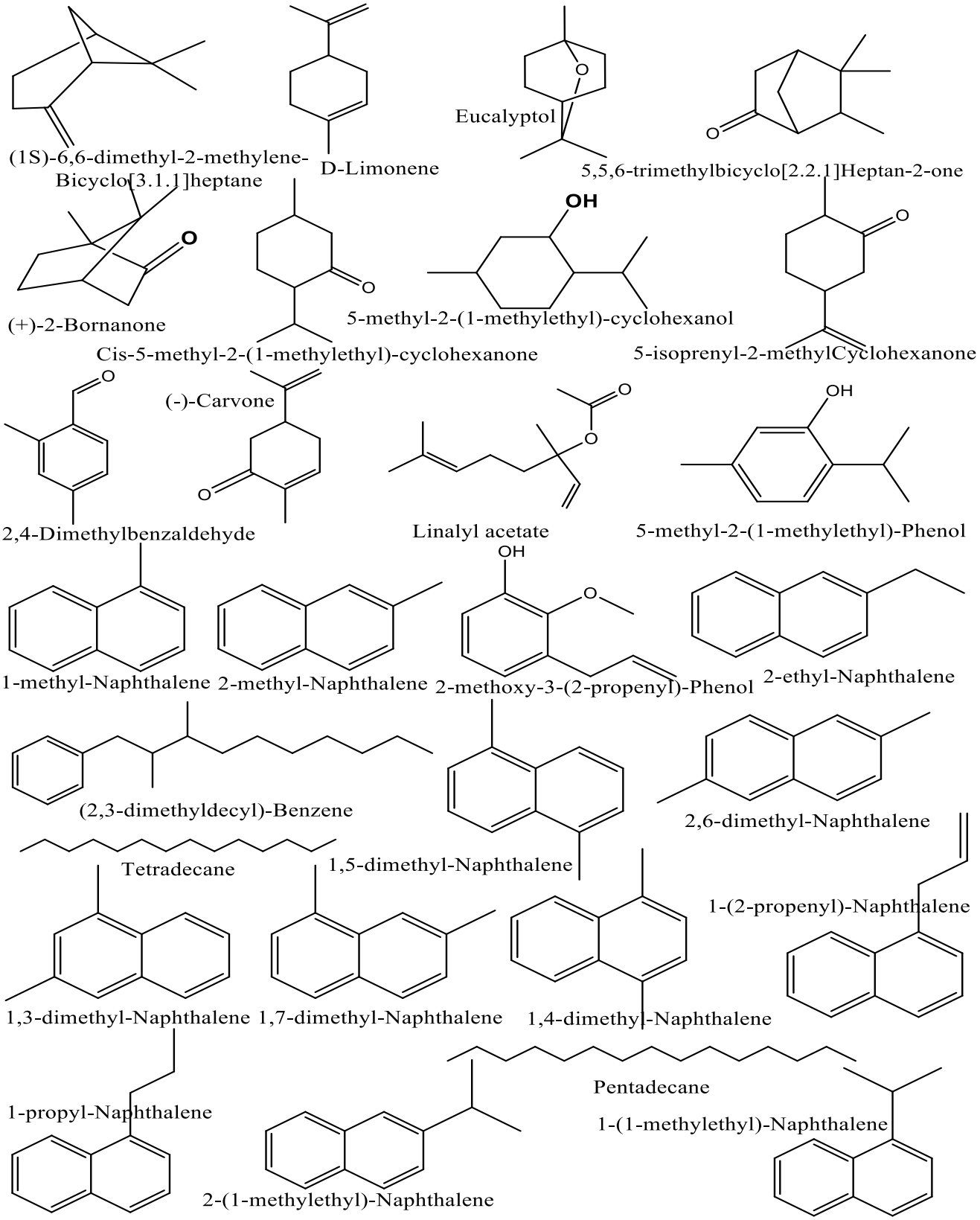


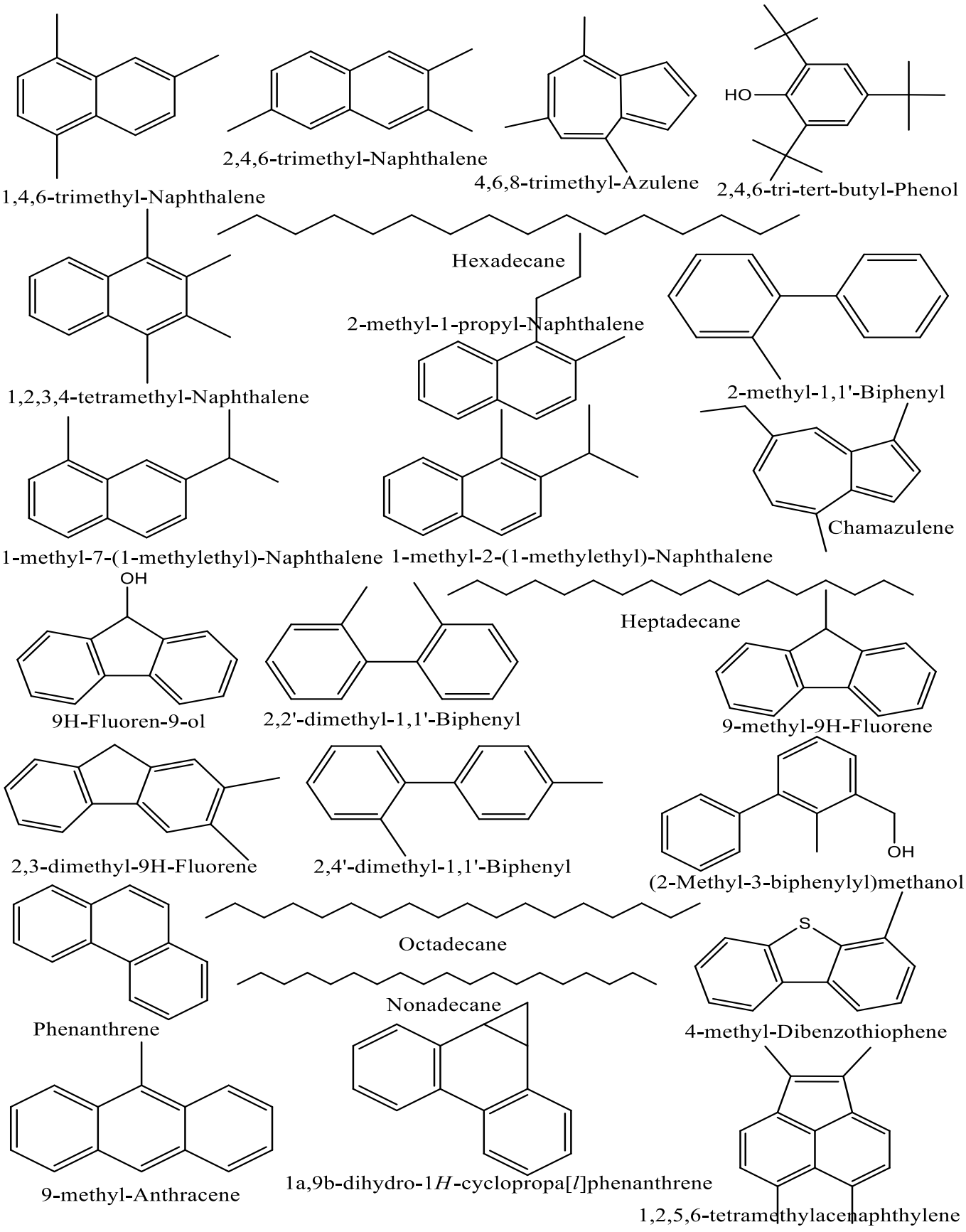
Methyl stearate

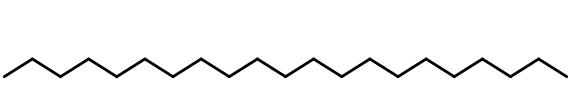


Heneicosane

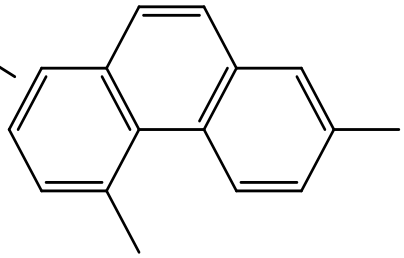
Appendix 3: GC – MS phytochemical composition of *Tm*29



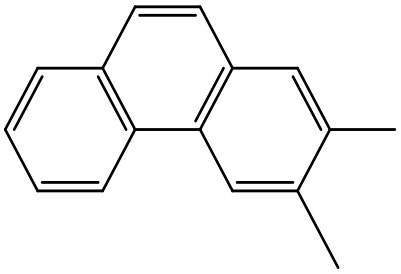

1,4,6-trimethyl-Naphthalene
2,4,6-trimethyl-Naphthalene
4,6,8-trimethyl-Azulene
HO
2,4,6-tri-tert-butyl-Phenol
Hexadecane
1,2,3,4-tetramethyl-Naphthalene
2-methyl-1-propyl-Naphthalene
2-methyl-1,1'-Biphenyl
1-methyl-7-(1-methylethyl)-Naphthalene
1-methyl-2-(1-methylethyl)-Naphthalene
Chamazulene
OH
9H-Fluoren-9-ol
2,2'-dimethyl-1,1'-Biphenyl
Heptadecane
9-methyl-9H-Fluorene
2,3-dimethyl-9H-Fluorene
2,4'-dimethyl-1,1'-Biphenyl
OH
(2-Methyl-3-biphenylyl)methanol
Octadecane
S
Phenanthrene
Nonadecane
4-methyl-Dibenzothiophene
9-methyl-Anthracene
1a,9b-dihydro-1*H*-cyclopropa[*l*]phenanthrene
1,2,5,6-tetramethylacenaphthylene



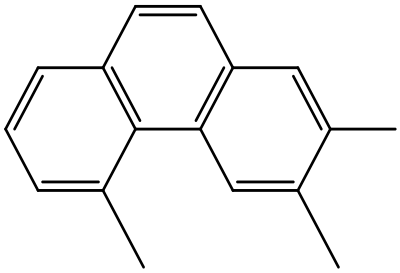
Heneicosane



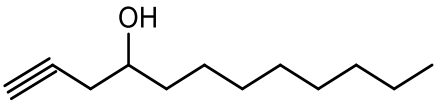
2,5-Dimethylphenanthrene



2,3-Dimethylphenanthrene

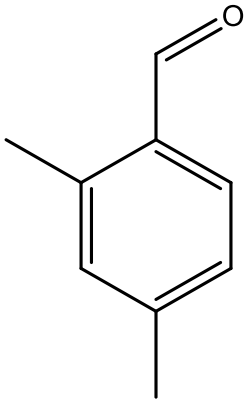


2,3,5-Trimethylphenanthrene

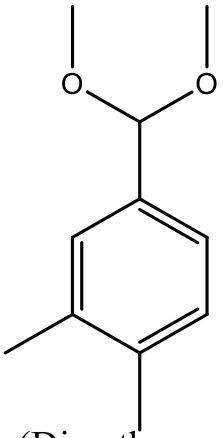


1-Dodecyn-4-ol

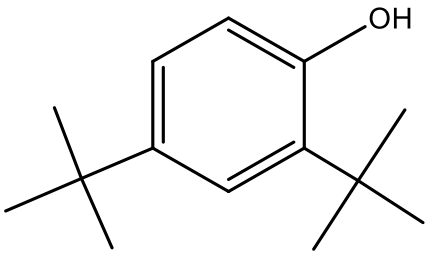
Appendix 4: GC – MS phytochemical composition of *Tm*32



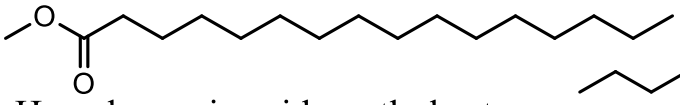
2,4-Dimethylbenzaldehyde



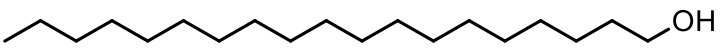
4-(Dimethoxymethyl)-1,2-dimethylbenzene



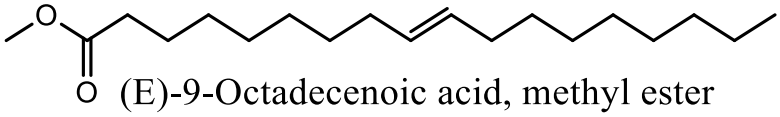
2,4-bis(1,1-dimethylethyl)-Phenol



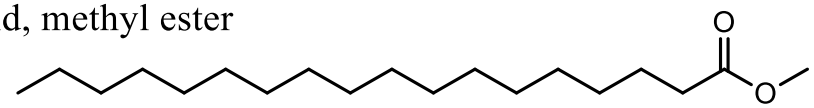
Hexadecanoic acid, methyl ester



n-Nonadecanol-1

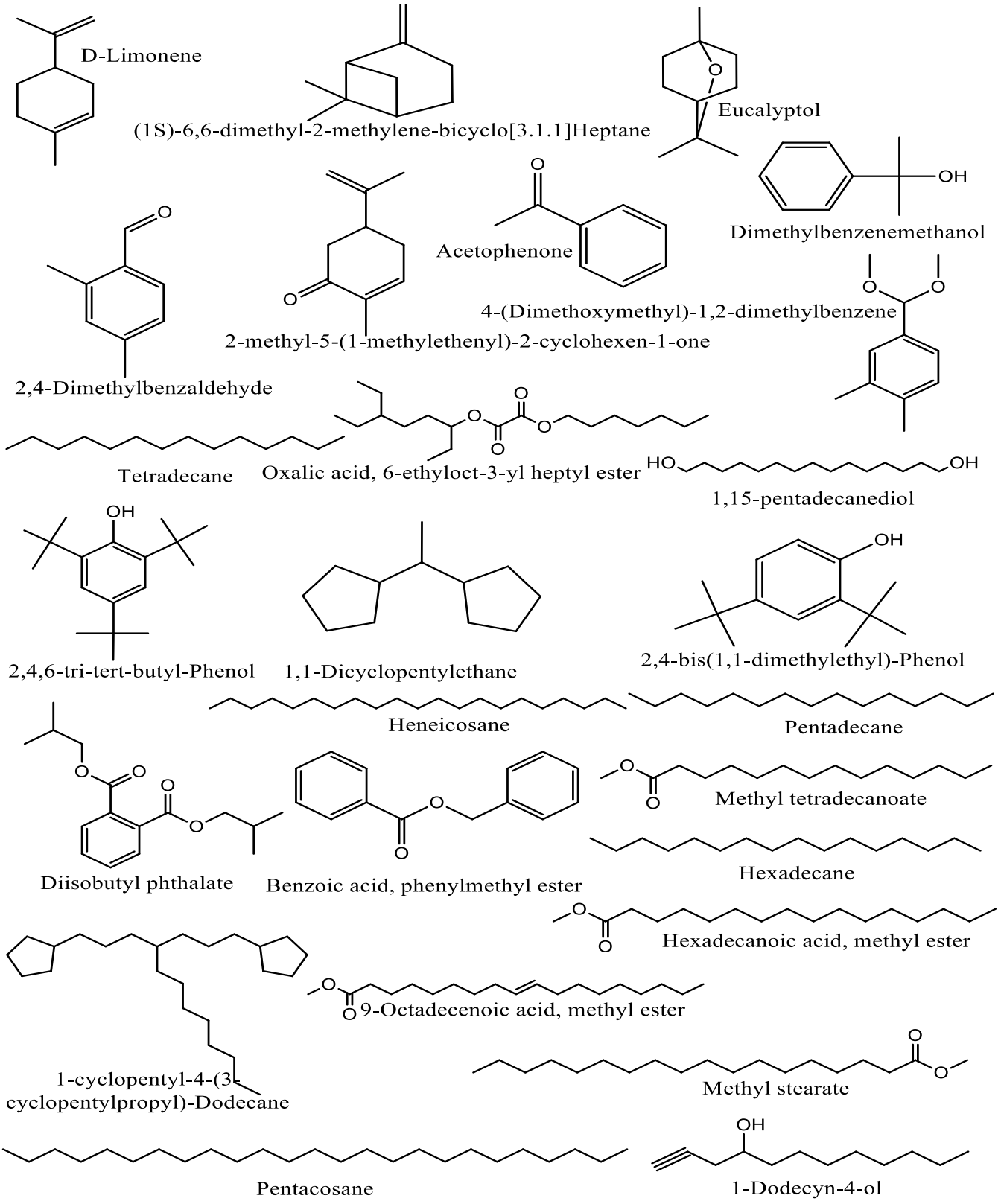


(E)-9-Octadecenoic acid, methyl ester

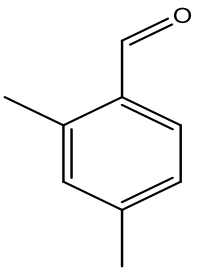


Methyl stearate

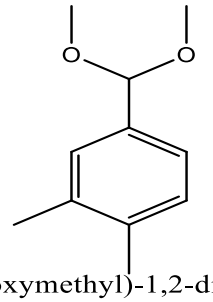
Appendix 5: GC – MS phytochemical composition of *Tm*30



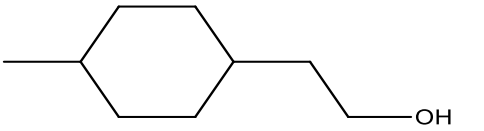
Appendix 6: GC – MS phytochemical composition of *Tm*33



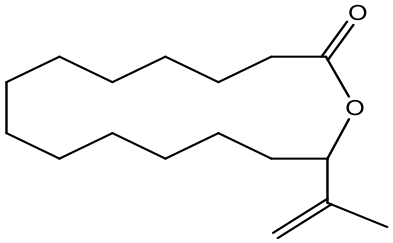
2,4-Dimethylbenzaldehyde



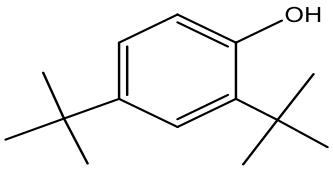
4-(Dimethoxymethyl)-1,2-dimethylbenzene



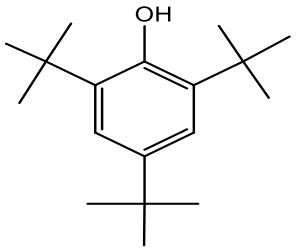
1-methyl-4-(2-hydroxyethyl)-Cyclohexane



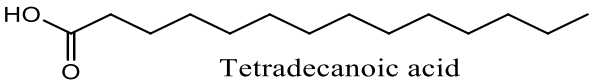
15-isoprenyl-1-Oxacyclopentadecan-2-one



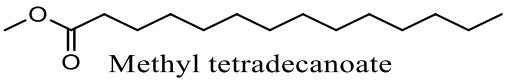
2,4-bis(1,1-dimethylethyl)-Phenol



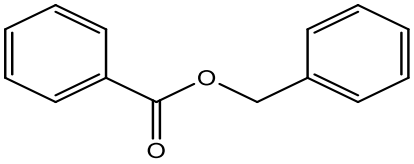
2,4,6-tri-tert-butyl-Phenol



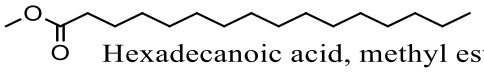
Tetradecanoic acid



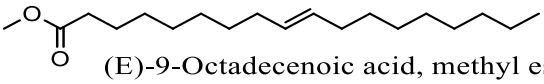
Methyl tetradecanoate



Benzoic acid, phenylmethyl ester



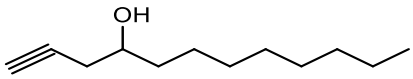
Hexadecanoic acid, methyl ester



(E)-9-Octadecenoic acid, methyl ester

Heneicosane

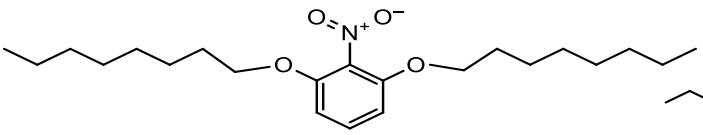
Pentacosane



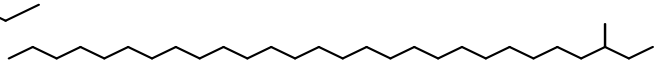
1-Dodecyn-4-ol

Tetracontane

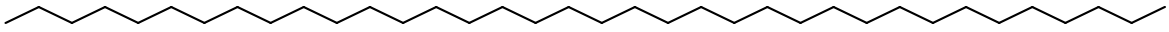
Tetratriacontane



2-nitro-1,3-bis(octyloxy)-Benzene



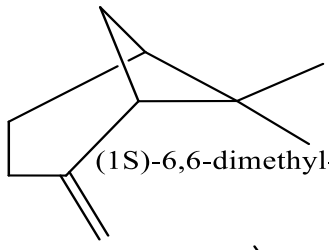
3-methylOctacosane

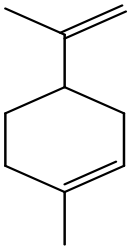


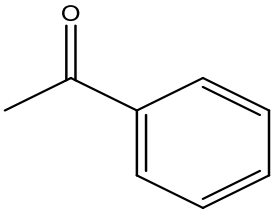
Hexatriacontane

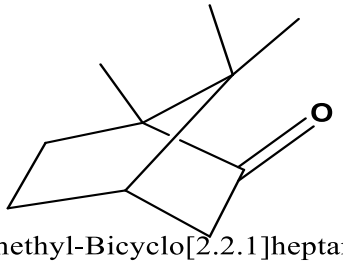
Tetrapentacontane

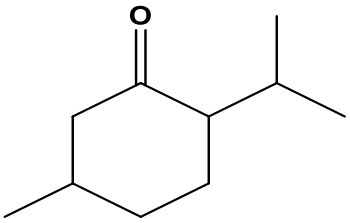
Appendix 7: GC – MS phytochemical composition of *Tm*34

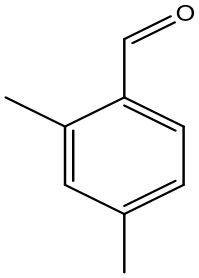

(1S)-6,6-dimethyl-2-methylene-Bicyclo[3.1.1]heptane

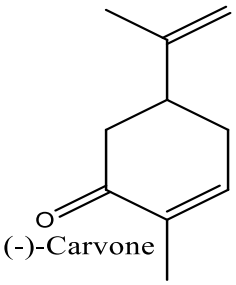

(S)-1-methyl-4-(1-methylethenyl)-Cyclohexene

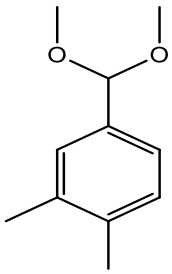

Acetophenone

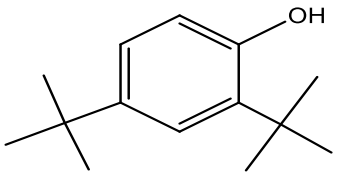

(S)-1,7,7-trimethyl-Bicyclo[2.2.1]heptan-2-one

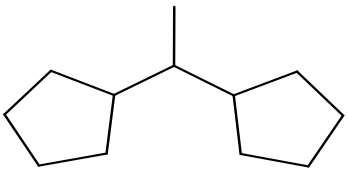

1-Menthone

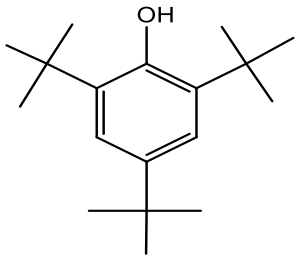

2,4-Dimethylbenzaldehyde

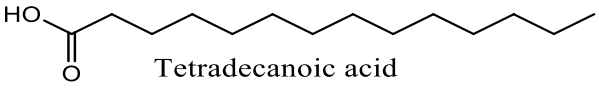

(-)-Carvone

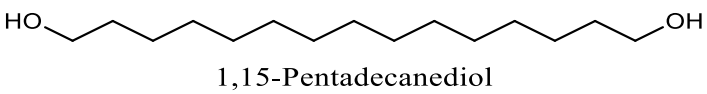

4-(Dimethoxymethyl)-1,2-dimethylbenzene

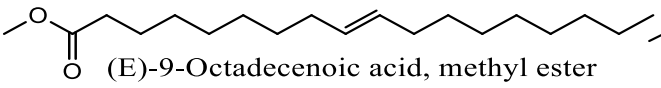

2,4-bis(1,1-dimethylethyl)-Phenol

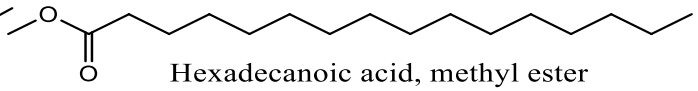

1,1-Dicyclopentylethane


2,4,6-tri-tert-butyl-Phenol

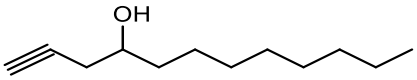

Tetradecanoic acid


1,15-Pentadecanediol


(E)-9-Octadecenoic acid, methyl ester

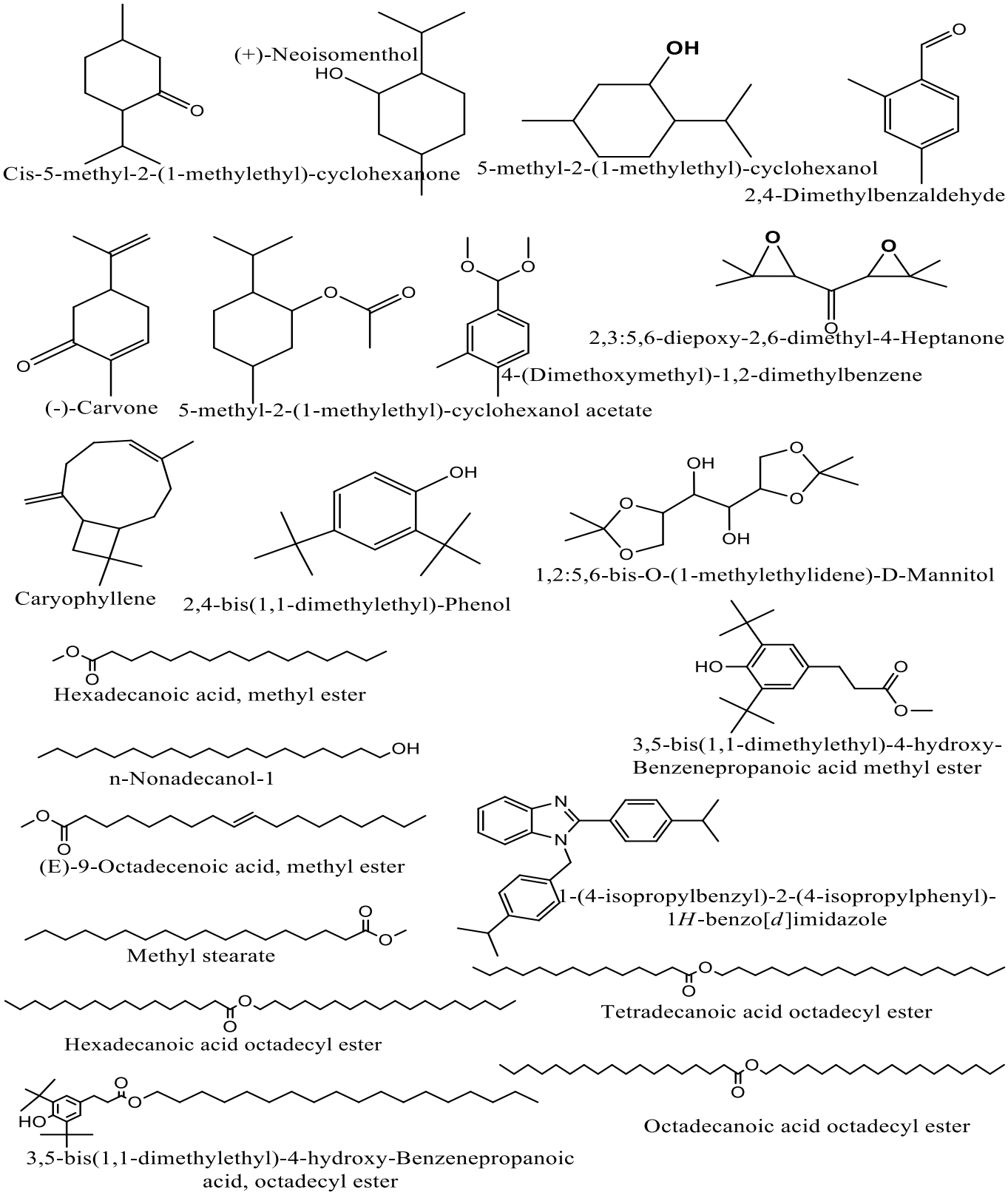

Hexadecanoic acid, methyl ester

Heneicosane

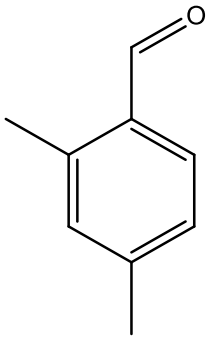

1-Dodecyn-4-ol


Hexatriacontane

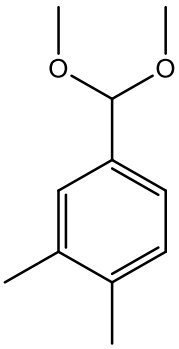
Appendix 8: GC – MS phytochemical composition of *Tm*36



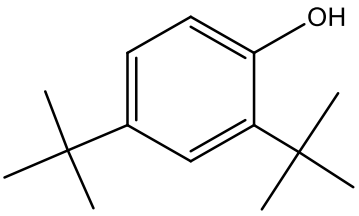
Appendix 9: GC – MS phytochemical composition of *Tm*38



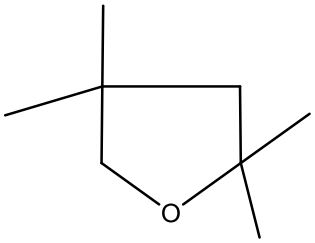
2,4-Dimethylbenzaldehyde



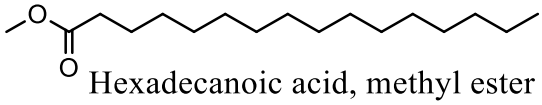
4-(Dimethoxymethyl)-1,2-dimethylbenzene



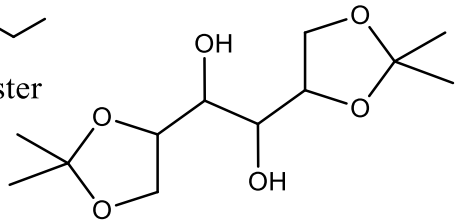
2,4-bis(1,1-dimethylethyl)-Phenol



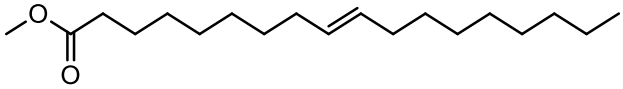
tetrahydro-2,2,4,4-tetramethyl-Furan



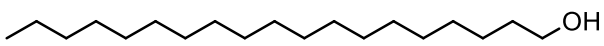
Hexadecanoic acid, methyl ester



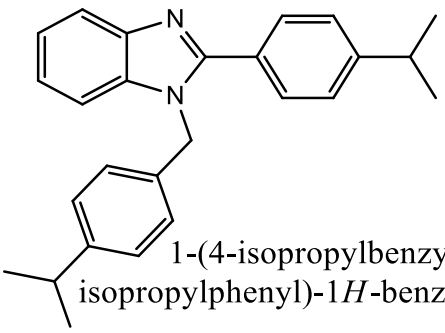
1,2:5,6-bis-O-(1-methylethylidene)-D-Mannitol



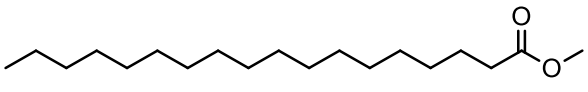
(E)-9-Octadecenoic acid, methyl ester



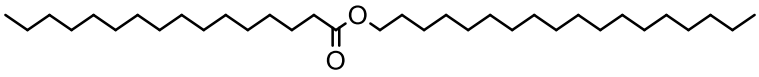
n-Nonadecanol-1



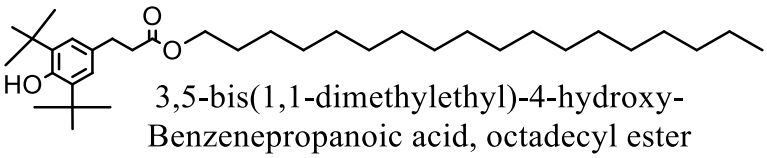
1-(4-isopropylbenzyl)-2-(4-isopropylphenyl)-1*H*-benzo[*d*]imidazole



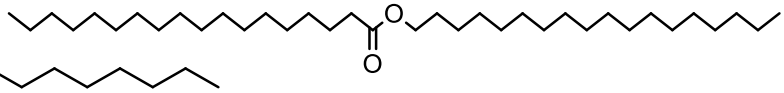
Methyl stearate



Hexadecanoic acid octadecyl ester



3,5-bis(1,1-dimethylethyl)-4-hydroxy-Benzenepropanoic acid, octadecyl ester



Octadecanoic acid octadecyl ester

Appendix 10: Parasitaemia during subfractions intraerythrocytic stage specific inhibition assays

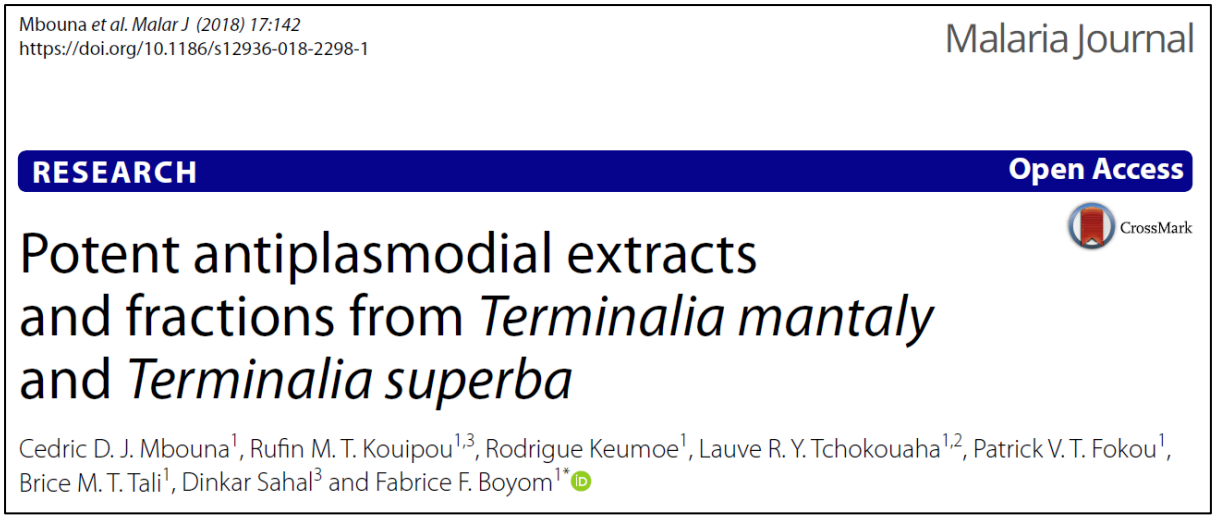
Stages		Ring					Trophozoite				Schizont	
Time (hours)		6	12	24	36	48	6	12	18	24	6	12
PRBC	Red blood cells	2038	1082	2210	2012	1367	1893	1669	1691	1430	2061	1937
	Parasites	31	17	44	66	200	18	17	23	23	29	40
	Parasitaemia (%)	1.52	1.57	1.99	3.28	14.63	0.95	1.02	1.36	1.61	1.41	2.07
Tm 28	Red blood cells	1471	1855	1789	1667	2275	1612	1711	1761	1931	1955	1936
	Parasites	31	31	47	65	44	15	21	14	19	19	25
	Parasitaemia (%)	2.11	1.67	2.63	3.90	1.93	0.93	1.23	0.80	0.98	0.97	1.29
Tm 29	Red blood cells	1595	1639	1661	1789	1817	1630	2028	1371	1736	1704	1914
	Parasites	22	40	44	43	75	32	15	5	13	14	26
	Parasitaemia (%)	1.38	2.44	2.65	2.40	4.13	1.96	0.74	0.36	0.75	0.82	1.36
Tm 30	Red blood cells	1859	1555	1752	1865	1917	1802	2093	1614	1535	1863	2027
	Parasites	23	32	57	71	40	19	15	18	8	19	33
	Parasitaemia (%)	1.24	2.06	3.25	3.81	2.09	1.05	0.72	1.12	0.52	1.02	1.63
Tm 33	Red blood cells	1726	1833	1822	1932	1965	1629	1769	1473	1507	1600	1609
	Parasites	51	55	47	82	259	23	25	15	17	10	47
	Parasitaemia (%)	2.95	3.00	2.58	4.24	13.18	1.41	1.41	1.02	1.13	0.63	2.92
Tm 34	Red blood cells	1720	1801	1816	1825	1759	1696	1844	1321	1982	1655	2236
	Parasites	35	30	41	119	213	25	27	13	17	15	37
	Parasitaemia (%)	2.03	1.67	2.26	6.52	12.11	1.47	1.46	0.98	0.86	0.91	1.65
Tm 36	Red blood cells	1731	1426	1987	1532	1797	1266	1609	1604	2103	1942	1604
	Parasites	34	32	53	48	44	6	9	25	34	18	39
	Parasitaemia (%)	1.96	2.24	2.67	3.13	2.45	0.47	0.56	1.56	1.62	0.93	2.43
Tm 38	Red blood cells	1807	1727	2114	1979	1565	1631	2164	1931	1953	2217	1817
	Parasites	43	45	48	53	36	11	13	21	16	20	28
	Parasitaemia (%)	2.38	2.61	2.27	2.68	2.30	0.67	0.60	1.09	0.82	0.90	1.54

Appendix 11: Parasitaemia during subcultures for subfractions post – drug effects assessment

Stages		Ring					Trophozoite				Schizont	
Time (hours)		6	12	24	36	48	6	12	18	24	6	12
PRBC	Red blood cells	1489	1489	1693	1180	1389	827	1176	926	852	1054	990
	Parasites	166	166	221	123	137	59	69	54	41	76	105
	Parasitaemia (%)	11.15	11.15	13.05	10.42	9.86	7.13	5.87	5.83	4.81	7.21	10.61
Tm 28	Red blood cells	1296	1296	1181	817	708	1121	1121	1170	1389	850	765
	Parasites	184	184	147	18	27	39	39	26	25	71	29
	Parasitaemia (%)	14.20	14.20	12.45	2.20	3.81	3.48	3.48	2.22	1.80	8.35	3.79
Tm 29	Red blood cells	1125	1125	1212	663	1023	1080	1080	1452	1313	894	924
	Parasites	110	110	115	51	12	61	61	21	37	36	39
	Parasitaemia (%)	9.78	9.78	9.49	7.69	1.17	5.65	5.65	1.45	2.82	4.03	4.22
Tm 30	Red blood cells	1199	1199	966	1046	959	929	929	1245	1267	894	1291
	Parasites	120	120	80	83	17	26	26	27	30	46	50
	Parasitaemia (%)	10.01	10.01	8.28	7.93	1.77	2.80	2.80	2.17	2.37	5.15	3.87
Tm 33	Red blood cells	1099	1099	1180	939	1050	869	1104	1041	1252	894	1212
	Parasites	133	133	164	16	53	53	41	26	24	53	54
	Parasitaemia (%)	12.10	12.10	13.90	1.70	5.05	6.10	3.71	2.50	1.92	5.93	4.46
Tm 34	Red blood cells	1428	1428	1279	792	929	951	717	1007	972	1246	876
	Parasites	181	181	187	18	28	32	31	18	10	76	35
	Parasitaemia (%)	12.68	12.68	14.62	2.27	3.01	3.36	4.32	1.79	1.03	6.10	4.00
Tm 36	Red blood cells	1523	1523	1064	979	1123	1129	1129	1096	1023	930	967
	Parasites	193	193	130	70	12	35	35	46	24	43	58
	Parasitaemia (%)	12.67	12.67	12.22	7.15	1.07	3.10	3.10	4.20	2.35	4.62	6.00
Tm 38	Red blood cells	1379	1379	1072	814	989	1019	1019	910	1092	1056	1138
	Parasites	158	158	104	129	29	44	44	37	81	53	39
	Parasitaemia (%)	11.46	11.46	9.70	15.85	2.93	4.32	4.32	4.07	7.42	5.02	3.43

PUBLICATIONS

1. Mbouna, C., Kouipou, R., Keumoe, R., Tchokouaha, L., Fokou, P., Tali, B., Sahal, D. and Boyom, F., **2018**. Potent antiplasmodial extracts and fractions from *Terminalia mantaly* and *Terminalia superba*. *Malaria Journal*, 17(1). https://doi.org/10.1186/s12936-018-2298-1



Mbouna *et al. Malar J (2018) 17:142*
https://doi.org/10.1186/s12936-018-2298-1

Malaria Journal

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Potent antiplasmodial extracts and fractions from *Terminalia mantaly* and *Terminalia superba*

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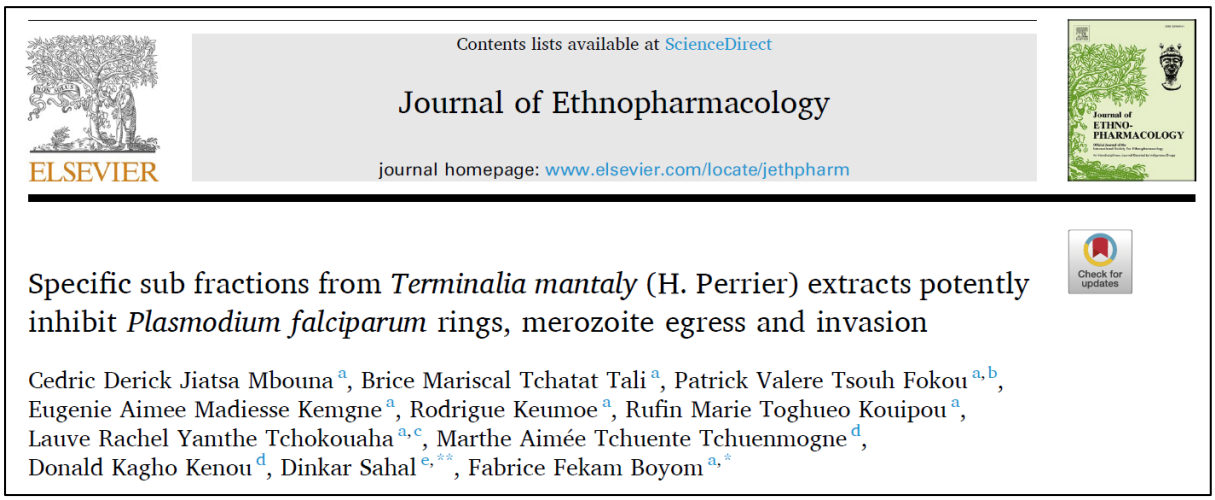
2. Jiatsa Mbouna, C., Tchatat Tali, B., Tsouh Fokou, P., Madiesse Kemgne, E., Keumoe, R., Toghueo Kouipou, R., Yamthe Tchokouaha, L., Tchuente Tchuenmogne, M., Kenou, D., Sahal, D. and Boyom, F., **2021**. Specific sub fractions from *Terminalia mantaly* (H. Perrier) extracts potently inhibit *Plasmodium falciparum* rings, merozoite egress and invasion. *Journal of Ethnopharmacology*, 285, p.114909. https://doi.org/10.1016/j.jep.2021.114909



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Specific sub fractions from *Terminalia mantaly* (H. Perrier) extracts potently inhibit *Plasmodium falciparum* rings, merozoite egress and invasion

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