Pharmacogn. Commn. 2020;10(1):2-6
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

In vitro Antiplasmodial and Cytotoxic activity of Three Medicinal Plants used Traditionally for Treatment of Malaria

Ruth Anyango Omole[1,2]*, Mainen Julius Moshi[1], Muhammad Ilias[3], Walker Larry[3], Hamisi M. Malebo[4], Leonida Kerubo Omosa[5], Jacob O. Midiwo[5]

[1]Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, TANZANIA.
[2]Department of Chemical Science and Technology, Technical University of Kenya, P.O. Box 52428- 00200, Nairobi, KENYA.
[3]Research Institute of Pharmaceutical Sciences, University of Mississippi, P.O Box 1848-38677 USA.
[4]Department of Traditional Medicine Research, National Institute for Medical Research, P.O. Box 9653, Dar es Salaam, TANZANIA.
[5]Department of Chemistry, University of Nairobi, P.O. Box 30197, Chiromo Road, Nairobi, KENYA.

ABSTRACT

Introduction: Reports of emergence of Artemisinin Combination Therapies (ACTs) resistant malaria parasites in Greater Mekong region and Equatorial Guinea, is a strong reason necessitating increased efforts to discover new antimalarial compounds with novel mechanisms of action. Plants have potential to yield new antiplasmodial compounds. This study investigated the safety and efficacy of three plants; *Bersama abyssinica* Fresen, *Rubus keniensis* Standl and *Hypoestes verticillaris* (L.f.) Sol. ex Roem. and Schult that are used by the Ogiek community of Kenya for treatment of malaria. **Methodology:** The crude extracts were tested for *in vitro* antimalarial activity using *Plasmodium falciparum* strains W2 (chloroquine resistant) and D6 (chloroquine sensitive). Safety evaluation was done using monkey kidney Vero cells and the brine shrimp lethality test. **Results:** Dichloromethane: methanol (1:1) and 5% aqueous methanol extracts of the three plants exhibited *in vitro* antiplasmodial activity against the W2 and D6 *Plasmodium falciparum* strains with IC_{50}= 12.11–19.18 µg/mL, 5.46-7.04 µg/mL and 9.82 – 34.52 µg/mL, respectively. *H. verticillaris* extracts were the most active against the two *Plasmodium falciparum* strains. The dichloromethane: methanol extracts of the three plants exhibited lower toxicity on monkey kidney Vero cells relative to antiplasmodial activity as compared to the 5% aqueous methanol extracts. The mean Vero cells: parasite selectivity index of the dichloromethane: methanol extracts was (4.8), *B. abyssinica* (3.75) and *R. keniensis* (1.9), while for the 5% aqueous methanol extracts they were *H. verticillaris* (1.0), *B. abyssinica* (1.95) and R. keniensis (1.75). A similar toxicity profile was exhibited by brine shrimp lethality results. **Conclusion:** The results support the use of the three plants for the treatment of malaria. Therefore, they have potential to yield safe and effective compounds targeting *P. falciparum* malaria.

Key words: Antiplasmodial activity, Cytotoxic activity, *Bersama abyssinica*, *Rubus keniensis, Hypoestes verticillaris*.

Correspondence:
Dr. Ruth Omole
Department of Chemical Science and Technology, Technical University of Kenya, P.O Box 52428 00200, Nairobi, KENYA.
Phone no. +254723310672
E-mail: oruth2002@yahoo.com
DOI: 10.5530/pc.2020.1.2

INTRODUCTION

According to WHO, 219 million malaria cases and 435,000 deaths, respectively were reported in the year 2017 in 83 countries, with 90% of global malaria cases occurring in Africa. Children below five years and pregnant women are the most affected.[1] Malaria in pregnancy leads to abortion, low birth weight, stillbirths and maternal anemia.[2] Malaria is a disease caused by a parasite and transmitted through the bites of female *Anopheles* mosquito. Four main malaria parasites include; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale.* Ten years ago, *Plasmodium knowlesi*, was found to be endemic in humans in parts of Southeast Asia.[3] *Plasmodium falciparum* causes severe and fatal malaria, which accounts for 90% of all cases and deaths worldwide.[4]Malaria can be controlled using vector control methods, vaccines and chemotherapy. Vector control has had challenges like the resistance of the vector to available insecticides and environmental pollution by the residual sprays[5] and likewise the recently developed RTS, S; a pre-erythrocytic vaccine for the prevention of clinical *P. falciparum* malaria in children, does not have a sufficiently high efficacy.[6] Chemotherapy, therefore, remains the main strategy for malaria control, but it has become less effective due to high costs, drug resistance and inadequate armory of drugs. Malaria parasites have developed resistance to many of the available antimalarials.[7] Resistance to the WHO recommended first-line artemisinin combination therapies (ACTs), as of March 2017, has been reported in Southeast Asia.[8] The resistance might spread to other areas. New classes of antimalarial drugs are needed to provide alternative drugs with different modes of action to cope with the developing *P. falciparum* resistance towards current antimalarials.

Indigenous communities have used plants to treat malaria without knowledge of activity and safety. Plants have played an important role in the discovery and development of some of the antimalarials in clinical use, such as artemisinin and quinine.[9] Continuous screening of medicinal plants both *in vitro* and *in vivo* may provide crucial evidence for their use for the treatment of malaria. Consequently, the isolation of active compounds from these medicinal plants could offer a critical lead for the discovery of new antimalarial drugs.[10] *Bersama abyssinica* is used to treat malaria, stomachache, pneumonia, sexually transmitted diseases and tuberculosis by the Ogiek and Sabaot communities in Kenya.[11-13] In Ivory Coast, it is also used to treat malaria, tuberculosis, typhoid fever and stagnation by Bete people.[14,15] It has been reported to treat rheumatism, snake bites, as an aphrodisiac and to cure cancer.[16-18] *Hypoestes verticillaris* is used by the Ogiek community in Kenya to cure tuberculosis, chest pain, malaria and dry coughs. *Rubus keniensis* is used to treat stomachache, sexually transmitted infections and malaria among the Ogiek community.[11,13] Based on the traditional uses of these plants, they were selected for this study to validate the folkloric claims and ascertain their safety. The plants were tested for antimalarial activity *in vitro* using *Plasmodium falciparum* W2 and D6 strains. The extracts were also subjected to cytotoxicity testing to ascertain their safety using mammalian cells and the brine shrimp lethality test.

MATERIALS AND METHODS

Collection and extraction of plant material

The plants were collected in May, 2015 in Mau forest with the help of botanist Patrick Mutiso. The voucher specimens RO2015/01, RO2015/02 and RO2015/03 are deposited in the herbarium of the Department of Botany at the University of Nairobi. The plant samples were air dried for a minimum of two weeks and then ground into powder using a miller. The ground materials were weighed and cold extracted with 1:1 dichloromethane: methanol and 5% aqueous methanol, respectively, to obtain crude extracts. The filtered extracts were combined and solvents removed under reduced pressure. The dried extracts were weighed and stored in a freezer (-4ºC) until needed for biological testing. The % yields are recorded in Table 1.

Materials and chemicals

Dichloromethane and methanol were all obtained from Kobian Kenya Ltd in Nairobi. *Plasmodium falciparum* W2 (CQ resistant) strain and D6 (CQ-susceptible) strain were used for the study. The parasites were obtained from BEI-resources (MR4/ATCC Manassas VA, USA).[^{3}H]-hypoxanthine and Rosewell Park Memorial Institute 1640 (RPMI-1640) powdered medium were purchased from Glico Laboratories California, U.S.A. Anti-coagulant free blood-collecting bags and sterile gloves (Trifex®), 15 and 50 mL centrifuge tubes (Brinkmann Instruments Company, Westbury U.S.A), 50 and 150 mL culture flasks (Corning®, U.S.A), microscopes slides and cover slips (Sigma Chemical Company, U.S.A), 0.45 and 0.22 µm filter units (Naglene®, Naglene Company, U.S.A), serological Pasteur pipettes (Fischer Scientific, Pittsburg, U.S.A) were acquired through Diagnostics, U.S.A. Brine shrimp eggs (*Artemia* nauplii) were purchased from Aquaculture innovations (Grahams town, South Africa).

In vitro antiplasmodial assay

The semi-automated micro-dilution technique for assessing *in vitro* antiplasmodial activity was adopted for the drug sensitivity studies for the various plant extracts against *P. falciparum* isolates.[19,20] The 96 flat-bottom well micro-titre plates (8 rows ×12 columns) were set such that all wells except the controls contain 25 µL of doubling concentrations of drug solutions. Parasitized red blood cells (200 µL) were added so that the total volume per well is 225 µL. The dry plant extract samples were retrieved from the freezer (4ºC) and dissolved in 50 µL of dimethylsulphoxide (DMSO) (solvent concentration in tests did not exceed 0.02%) and the volume adjusted to 20 mL with distilled water.[21] The test culture at ring stage, having a percentage parasiatemia (%P) ≥ 4% and growth rates (GR) ≥ 3% were used for sensitivity tests. After examining the parasites under microscope, the % P of the test culture to be added to the wells of pre-dosed plates were adjusted to 0.4% and haemactocrit (hct) adjusted to 1.5% with 50% RBC. The mixture (200 µL) was then added into each well except for H_7 to H_{12}. The micro-titre plates were then incubated at 37°C. After 48 h, [G-^{3}H] hypoxanthine was pulsed in aliquots of 25 µL into each well and the plates incubated for further 48 h. The cells were harvested using a multiple semi-automatic cell harvester onto glass fibre filters for each row from A to H. The filters were then dried at 37ºC overnight (18 h), introduced into scintillation vials, 1 mL of scintillation fluid (ecolume) added and the vials were loaded into a liquid scintillation β-counter. Disintegrations per minute were calculated for each sample representing the incorporation of [G-^{3}H] hypoxanthine into the parasite nucleic acids. The IC_{50} values were calculated using the XL fit curve fitting software. Chloroquine and artemisinin were used as positive control. Extracts with IC_{50} = <50 µg/mL were considered as active[22] and classified as highly active (IC_{50} = <5 µg/mL), promising activity (IC_{50} = 5-15 µg/mL), moderately active (IC_{50} = 15-50 µg/mL) and inactive (IC_{50} = >50 µg/mL).

In vitro cytotoxicity assay

In vitro cytotoxicity assay of the extracts was carried out following a colometric assay using Vero type 199 kidney epithelial monkey cells.[23] Cells were maintained in minimum essential medium (MEM), (GIBCO, Grand Island, New York) containing 10% fetal bovine serum (FBS). Cell suspension (2×10^5 cells/mL) was seeded onto 96 well micro-titer plates row (A-H) and incubated at 37ºC under 5% CO_2 for 12 h to allow cells to attach after which drugs were added. Row H carried the highest drug concentration and serial dilution was carried out to row B. Row A wells 3, 6, 9 and 12 served as blanks while the remaining eight wells were negative controls (cells without drugs). The cells were incubated for 48 h at 37ºC under 5% CO_2, after which 10 µL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye was added and the mixture incubated for another 4 h. The media was removed from the wells using a micro-pipette and 100 µL of DMSO added. The plates were read on a scanning multi-well spectrophotometer (Multiscan Exlassystems, U.K) at 562 and 620 nm as reference. Podophylotoxin, a natural product isolated from *Podophyllum* species and its semisynthetic derivative etopoxide, were used as positive standards.

Data was analysed as follows:

$$\text{Cell viability (\%)} = \frac{OD\ sample\ 562 - OD\ sample\ 620}{OD\ control\ 562 - OD620} \times 100$$

Where OD = optical density

Data was transferred onto a graphic programme (EXCEL) and expressed as percentage of the untreated controls. The 50% cytotoxicity concentration (CC_{50}) was calculated from linear regression analysis.

Brine shrimp lethality test

Plant extracts were dissolved in DMSO to make stock solutions (40 mg/mL) and different volumes of extracts from the stock solutions were added into different vials each containing artificial sea water (3.8 g of sea salt in 1 L of distilled water) followed by addition of ten brine shrimp larvae. The volume in each vial was adjusted to 5 mL by adding more artificial sea water to achieve the final concentrations of 240, 120, 80, 40, 24 and 8 µg/mL. The maximum concentration of DMSO in the final volume was restricted to 0.6%. Each level of concentration was tested in duplicate. The negative controls contained brine shrimp larvae, artificial sea water and 0.6% DMSO. The vials were incubated under light for 24 h and the live and dead larvae were counted after this period of incubation.

The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC_{50}) determined from the graph.[24] The brine shrimp results are interpreted as follows LC_{50} < 1.0 µg/mL - Highly toxic; LC_{50} 1.0 - 10.0 µg/mL – toxic; LC_{50} 10-30 µg/mL – moderately toxic; LC_{50}> 30 < 100 µg/mL – mildly toxic and >100 µg/mL as non-toxic.[25]

RESULTS

Antiplasmodial activity

Table 2 shows that all the extracts of the three plants exhibited *in vitro* antiplasmodial activity against *P. falciparum* W2 and W6 strains. *Hypoestes verticillaris* dichloromethane/methanol extract gave an IC_{50} of 5.51 µg/mL against D6 strain and 5.48 µg/mL against W2 strain; while the 5% aqueous ethanol extract gave IC_{50} = 5.46 µg/mL against D6 strain and 7.04 µg/mL against W2 strain, respectively. *Rubus keniensis* dichloromethane/methanol extract gave IC_{50}= 34.52 µg/mL against D6 strain and 19.45µg/mL against W2 strain; while the 5% aqueous methanol extract gave IC_{50} = 12.52 µg/mL against D6 strain and 9.82 µg/mL against W2 strain. *Bersama abyssinica* dichloromethane/methanol extract gave IC_{50} = 12.85 µg/mL against D6 strain and 12.48 µg/mL against W2 strain;

while the 5% aqueous methanol extract gave IC_{50} = 19.18 μg/mL against D6 strain and 12.11 μg/mL against W2 strain.

Cytotoxic activity against Monkey Kidney cells

Table 2 shows that none of the tested extracts was highly toxic against the mammalian cells. The toxicity results indicate that both the dichloromethane: methanol (1:1) and 5% aqueous methanol extracts of *R. keniensis* exhibited moderate toxicity on the monkey kidney cells with CC_{50}> 47.60 μg/mL and CC_{50} = 11.25 μg/mL, respectively. Similarly, both the extracts of *B. abyssinica* (CC_{50} = 38.43 and 28.97 μg/mL) and *H. verticillaris* (CC_{50} = 26.22 and 10.75 μg/mL) exhibited moderate toxicity against the mammalian cell lines. The mean selectivity index for the three plant extracts was *R. Keniensis* (1.90; 1.0); *B. abyssinica* (3.75; 1.95) and *H. verticillaris* (4.8; 1.75) for dichloromethane: methanol (1:1) and 5% aqueous methanol extracts, respectively.

Brine shrimp toxicity

The brine shrimp test results (Table 3) show that both the 1:1dichloromethane: methanol and 5% aqueous methanol extracts of *B. abyssinica* extracts were not toxic, as their LC_{50} values > 1000 and 282.52 μg/mL, respectively. The 1:1dichloromethane: methanol and 5% aqueous methanol extracts of *H. verticillaris* exhibited moderate toxicity, with LC_{50} values of 85.64 and 88.22 μg/mL, respectively. According to our set classification, the 1:1 dichloromethane: methanol of *R. keniensis* with an LC_{50} of 177.68 is determined to be non-toxic, while the 5% aqueous methanol extract with an LC_{50} of 85.06 μg/mL was moderately toxic to brine shrimp larvae.

Table 1: Percentage yield of the plant extracts.

Plant	Quantity of dry sample (kg)	DCM: MeOH (g)	% Yield	5% H_2O/ MeOH (g)	% Yield
B. abyssinica (stem bark)	5.7	597.5	10.5	192.1	3.4
R. keniensis (root bark)	2.1	221.1	10.5	50.4	2.8
H. verticillaris (whole plant)	3.9	324.1	8.3	99.1	2.7

$$\% \text{ yield} = \frac{\text{weight of the crude}}{\text{weight of dried sample}} \text{ x } 100$$

Table 2: Antiplasmodial and cytotoxicity activity.

Plant Extract	IC_{50} D6 (μg/mL)	D6 SI	IC_{50} W2 (μg/mL)	W2 SI	Vero CC_{50}(μg/mL)
R. keniensis (1:1)	34.52	>1.4	19.45	>2.4	>47.60
*R. keniensis*5% H_2O/ MeOH	12.52	0.9	9.82	1.1	11.25
B. abyssinica (1:1)	12.85	3	8.48	4.5	38.43
B. abyssinica 5% H_2O/ MeOH	19.18	1.5	12.11	2.4	28.97
H. verticillaris (1:1)	5.51	4.8	5.48	4.8	26.22
H. verticillaris 5% H_2O/ MeOH	5.46	2	7.04	1.5	10.75
Chloroquine	0.00124		0.00153		
Artemisinin	0.00708		0.0000492		

SI: selectivity index (CC_{50}vero cells/IC_{50} parasites), MeOH: methanol, H_2O: water

Table 3: Brine shrimp lethality test.

Plant extract	LC_{50} μg/mL	95% CI LL - UL	Regression Equations	Regression coefficient
R. keniensis (1:1)	177.68	124.10 - 254.40	Y=60.419logx - 85.921	0.9876
R. keniensis (5% H_2O/MeOH	85.06	55.58 - 130.17	Y=65.808logx - 76.992	0.9644
B. abyssinica (1:1)	1000	-	Y= 42.77logx - 82.374	0.9829
B. abyssinica(5% H_2O/MeOH	282.52	191.69 – 416.39	Y=62.508logx - 103.2	0.943
H. verticillaris (1:1)	85.64	70.22 - 104.34	Y=109.65logx - 161.92	0.9178
H. verticillaris (5% H_2O/MeOH	88.22	69.57 - 111.86	Y= 91.321logx - 127.67	0.9931

DISCUSSION

This study has provided evidence that the three plants *B. abyssinica*, *R. keniensis* and *H. verticillaris* that are by used the Ogiek community and Sabaots in Kenya have antiplasmodial activity against both chloroquine sensitive and chloroquine resistant *Plasmodium falciparum* D6 and W2 strains, respectively. Furthermore, two independent tests were used to interrogate the safety of two extracts of the three plants, cytotoxicity on Monkey Kidney vero cells and the brine shrimp lethality test. The 1:1 dichloromethane: methanol extracts of all the three plants showed high selectivity against *Plasmodium falciparum* as compared to the mammalian cells with selectivity index (SI) ranging from 1.4 to 4.8. The 1:1 dichloromethane: methanol extract of *H. verticillaris* exhibited the highest selectivity (SI = 4.8). Generally, the 5% aqueous methanol extracts of the three plants exhibited low selectivity against the parasite cells, thus they are judged to have higher toxicity. The brine shrimp results gave a similar safety indicator profile with the 1:1 dichloromethane: methanol extracts of *R. keniensis* and *B. abyssinica* being non-toxic, according to the set criteria. The 5% aqueous methanol extract of *B. abyssinica* was also none toxic. The 5% aqueous methanol extracts of *R. keniensis* and *H. verticillaris* and the 1:1dichloromethane: methanol extract of *H. verticillaris* all showed moderate toxicity against brine shrimp larvae. The two tests predict safety of the extracts and point to the 1:1 dichloromethane: methanol extracts as having a good potential for isolation of active antimalarial compounds against both chloroquine sensitive D6 and chloroquine resistant *Plasmodium falciparum* W2 strains.

Previous phytochemical screening on *Rubus keniensis* indicated the presence of flavonoids, anthraquinones, saponins, steroids, reducing sugars and polyoses. From our literature such no antimalarial activity has been done on this plant. However, anti-microbial screening of the methanol extract of this plant showed that it had a good anti-bacterial activity.[11] Phytochemical investigation on *Bersama abyssinica* revealed the presence of steroids, steroidal gylcosides, sugars, saponins and xanthones. Hellebrigenin acetates isolated from this plant showed anti-tumor activity.[26] Previous studies have indicated that aqueous extract have a good antibacterial activity.[14,27] Phytochemical research on *Hypoestes verticillaris* revealed the presence of alkaloids and terpenoids. Alkaloids like hypoestatin 1 and hypoestatin 2 isolated from this plant were found to inhibit growth of murine P-388 cell line ED_{50} = 10(-5) μg/mL.[28] *Seco*-fusicoccane type diterpeniods have been isolated from this plant.[29] The results provide evidence to support claims of safety and efficacy for treatment of malaria by the Ogiek community in Kenya. Antimalarial plants

used traditionally by the Ogiek community in Kenya should be investigated for their antimalarial potential to increase the knowledge of the useful flora of this community and provide active extracts. The documentation of antiplasmodial and cytotoxic profile of traditionally used species can be useful as an initial and important step in pharmacological evaluation that can lead to more rational use. Furthermore, antiplasmodial plant extracts provide for the starting point for bioassay-guided isolation of new antimalarial chemical constituents.

CONCLUSION

Extracts of the plants *Hypoestes verticillaris*, *Bersama abyssinica*, *Rubus keniensis* used by the Ogiek community of Kenya exhibited antiplasmodial activity against both chloroquine sensitive (D6) and chloroquine resistant (W2) *Plasmodium falciparum* strains and extracts of *Hypoestes verticillaris* exhibited the highest activity. Most of the extracts also exhibited high selectivity towards *Plasmodium falciparum* compared to monkey kidney Vero cells. The results show that the plants have potential to yield safe and efficacious compounds to target drug-resistant *P. falciparum malaria* and support their use by the Ogiek community for the treatment of malaria.

AKNOWLEDGEMENT

We wish to thank German Academic Exchange Programme (DAAD) and ISP through KEN-02 project funding this research. Abdul Waziri Kindukuli is appreciated for his technical support in doing BST test. Much thanks to Patrick Mutiso for identifying the plants.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

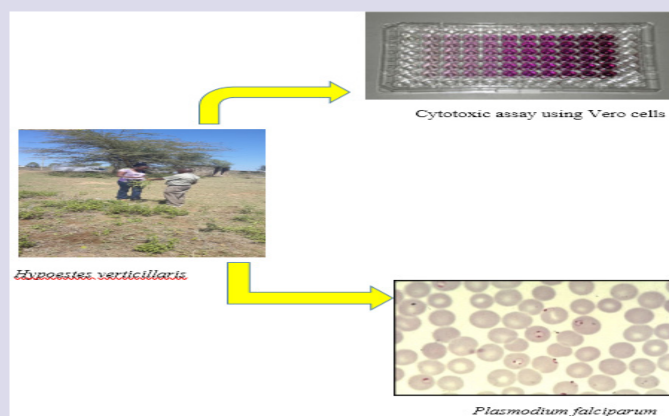
ABBREVIATIONS

ACTs: Artemisinin Combination Therapies; **WHO:** World Health Organization; **CC50:** Cytotoxic concentration fifty; **IC_{50}:** Inhibitory concentration fifty; **DMSO:** Dimethyl Sulfoxide; **CQ:** Chloroquine; **ED50:** Effective Dose fifty; **CI:** Confident Interval; **LL:** Lower Limit; **UL:** Upper Limit.

REFERENCES

1. WHO. World malaria report 2018. WHO. World Health Organization. 2018.
2. WHO. World Malaria Report 2016. World Heal Organ Geneva. 2016.
3. Cox-Singh J, Singh B. Knowlesi malaria: Newly emergent and of public health importance?. Trends Parasitol. 2008;24(9):406-10.
4. Guerra CA, Gikandi PW, Tatem AJ, Noor AM, Smith DL, Hay SI, *et al.* The limits and intensity of *Plasmodium falciparum* transmission: Implications for malaria control and elimination worldwide. PLoS Med. 2008;5(2):0300-11.
5. Karunamoorthi K. Vector control a cornerstone in the malaria elimination campaign. Clin Microbiol Infect. 2011;17(11):1608-16.
6. Hemingway J, Shretta R, Wells TNC, Bell D, Djim AA, Achee N, *et al.* Tools and Strategies for Malaria Control and Elimination: What Do We Need to Achieve a Grand Convergence in Malaria?. PLoS Biol. 2016;14(3):e1002380.
7. Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan C, *et al.* A major genome region underlying artemisinin resistance in malaria. Science (80-). 2012;336(6077):79-82.
8. WHO. Responding to antimalarial drug resistance. WHO. World Health Organization. 2017.
9. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep. 2000;17(3):215-34.
10. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov. 2015;14(2):111-29.
11. Amuka O, Machocho AK, Okemo PO, Mbugua PK. Profiling of antimicrobial properties of *Rubus keniensis* Standl. Crude methanol root bark extracts against selected human pathogenic bacteria. J Pharmacogn Phytochem. 2014;2(5):20-3.
12. Njoroge GN, Bussmann RW. Ethnotherapeutic management of Sexually Transmitted Diseases (STDs) and reproductive health conditions in central province of Kenya. Indian J Tradit Knowl. 2009;8:255-61.
13. Okello SV, Nyunja RO, Netondo GW, Onyango JC. Ethnobotanical Study of Medicinal Plants used by Sabaots of Mt. Elgon Kenya. African J Tradit Complement Altern Med. 2008;5:263-70.
14. Bolou GEK, Bagré I, Ouattara K, Djaman AJ. Evaluation of the antibacterial activity of 14 medicinal plants in côte d'ivoire. Trop J Pharm Res. 2011;10(3):335-40.
15. Zirihi GN, N'guessan K, Dibié TE, Grellier P. Ethnopharmacological study of plants used to treat malaria, in traditional medicine, by Bete Populations of Issia (Côte d'Ivoire). J Pharm Sci Res. 2010;2(4):216-27.
16. Amit L, Vikas G, Vaibhav T, Vikash K, Siddhartha G. Phytochemistry and pharmacological activities of *Bersama engleriana* Guerke- An overview. Int Res J Pharm. 2010;1:89-94.
17. Tadesse M, Hunde D, Getachew Y. Survey of medicinal plants used to treat human diseases in Seka Chekorsa, Jimma zone, Ethiopia. Ethiop J Health Sci. 2005;15(2):89-106.
18. Yineger H, Yewhalaw D, Teketay D. Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. J Ethnobiol Ethnomed. 2008;4(1):11.
19. Deloron P, Bras JL. *In vitro* Study of Drug Sensitivity of *Plasmodium falciparum*: Evaluation of a New Semi-Micro Test. Am J Trop Med Hyg. 1983;32(3):447-51.
20. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative Activity Semiautomated Technique. Antimicrob Agents Chemother. 1979;16(6):710-8.
21. Elueze EI, Croft SL, Warhurst DC. Activity of pyronaridine and mepacrine against twelve strains of *Plasmodium falciparum in vitro*. J Antimicrob Chemother. 1996;37(3):511-8.
22. Jonville MC, Kodja H, Humeau L, Fournel J, DeMol P, Cao M, *et al.* Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol. 2008;120(3):382-6.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.
24. Litchfield JT. A method for rapid graphic solution of time-per cent effect curves. J Pharmacol Exp Ther. 1949;97:399-408.
25. Nondo RSO, Moshi MJ, Erasto P, Zofou D, Njouendou AJ, Wanji S, *et al.* Evaluation of the cytotoxic activity of extracts from medicinal plants used for the treatment of malaria in Kagera and Lindi regions, Tanzania. J Appl Pharm Sci. 2015;5(04):7-12.
26. Bowen I, Jackson B, Motawe H. An Investigation of the Stem Bark of *Bersama abyssinica*. Planta Med. 1985;51(6):483-7.
27. Mwambela NZ, Kilambo D, Shahada F, Chacha M. Antifungal efficacy of *Bersama abyssinica* extracts against coffee pathogenic fungus *Gibberella xylarioides*. Asian J Plant Sci Res. 2014;4(4):40-3.
28. Pettit GR, Goswami A, Cragg GM, Schmidt JM, Zou JC. Antineoplastic Agents, 103. The Isolation and Structure of Hypoestestatins 1 and 2 From the East African *Hypoëstes verticillaris*. J Nat Prod. 1984;47(6):913-9.
29. Al-Rehaily AJ, Al-Yahya MA, Mirza HH, Ahmed B. Verticillarone: A new seco- Fusicoccane Diterpenoid Ketonepoxide from *Hypoestes verticillaris*. J Asian Nat Prod Res. 2002;4(2):117-22.

PICTORIAL ABSTRACT



SUMMARY

- Extracts of *B. abyssinica*, *R. keniensis* and *B. abyssinica* were tested for antiplasmodial activity using *P. falciparum* W2 and D6 strains
- The extracts were tested for cytotoxicity using brine shrimp and Vero cells
- *H. verticillaris* showed the highest antiplasmodial activity
- All the extracts showed a moderate toxicity against mammalian cell and *Artemia nauplii*.

ABOUT AUTHORS

Ruth Omole, is a lecturer in the School of Chemistry and Material Science at Technical University of Kenya. She has Master of Science in Natural products and medicinal chemistry. She has done her PhD at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Dares Salaam. Her research interest are mainly phytochemistry, drug discovery and design and organic synthesis.