In Vivo Antimalarial Activity of Ethanol Extracts of the Leaves of Three Plant Species Collected From Yayu Coffee Forest Biosphere Reserve, Southwest Ethiopia

Solomon Yeshanew (solarm12@yahoo.com)
Debre Markos University https://orcid.org/0000-0001-8165-4062
Worke Gete
Debre Markos University
Desalegn Chilo
Mettu University

Research

Keywords: Antimalarial activity, In-vivo, Yayu biosphere reserve

Posted Date: February 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-183304/v1

In vivo antimalarial activity of ethanol extracts of the leaves of three plant species collected from Yayu Coffee Forest Biosphere Reserve, Southwest Ethiopia

Solomon Yeshanew,[1*] Worke Gete,[1] Desalegn Chilo[2]

[1]Department of Biology, Debre Markos University, P.O.Box 269, Debre Markos, Ethiopia

[2]Department of Clinical Pharmacy, Mettu University, P.O.Box: 318, Mettu, Ethiopia

[*]Corresponding author e-mail: solarm12@yahoo.com , Tel: +251-913-936-754

Abstract

Introduction: In the era of multidrug resistant Plasmodium parasites, the management and control of malaria infection become complicated. Therefore, Alternative but effective plant based antimalarial treatments need to be discovered. In the search for potential antimalarial medicines, the present study tried to examine *In-vivo* efficacy evaluation of ethanol crude extracts of *Croton macrostachyus*, *Ruta chalepensis,* and *Vernonia amygdalina* against *Plasmodium berghei* in Swiss albino mice.

Methods: Oral acute toxicity assessment of the extracts was done in mice received up to 3000 mg/kg of dosage to see safety level of the plant materials. The standard 4-days antimalarial suppressive test was also employed to determine growth inhibition of parasitaemia at doses of 400, 600, and 800 mg/kg of the extracts. In addition, preliminary phytochemical screenings of the extracts were also carried out according to the standard procedures.

Results: Extracts of the plant materials did not produce serious acute toxic effects on mice received up to a single dose of 3000 mg/kg. Although complete clearance was not recorded, extracts of the plant materials produced dose dependent suppression of the parasitaemia. The highest growth inhibition recorded was by extract of *V. amygdalina* (61.44%) followed by *C.*

macrostachyus (59.3%) at 800 mg/kg of tested doses. Whereas, complete parasitaemia clearance was attributed in mice which got 25 mg/kg of Chloroquine (CQ). In addition, survival time of experimental mice was recorded and the finding revealed mice treated with the extracts lived longer than the negative control groups. The phytochemical screening of the crude extracts was also carried out and revealed the presence of antimalarial active constituents such as alkaloids, saponins, cardiac glycosides, flavonoids, terpenoids, steroids, phenols, and tannins.

Conclusion: The present study therefore, confirms ethanol crude extracts of *C. macrostachyus*, *R. chalepensis,* and *V. amygdalina* are safe and rich with active secondary metabolites which have promising antimalarial effects.

Keywords/phrases: Antimalarial activity, *In-vivo,* Yayu biosphere reserve

Introduction

Malaria remains one of the most significant causes of morbidity and mortality in resource poor countries especially in sub-Sahara African children under the age of five years and pregnant women (1, 2). According to WHO malaria report in 2015, malaria was prevalent in more than 90 countries of the tropical and semitropical world (Africa, Amazon, central and southern America; central, south and south east Asia; Pacific) that are home to more than half of the world's people and it is still a persistent problem in most of these areas (3).

In Ethiopia, the disease is one of the leading public health problems and it is the main causes of morbidity and mortality. *Plasmodium falciparum* and *P. vivax* are the two main species

accounting for 60% and 40% of malaria cases respectively (4). Approximately 75% of the country is malarious with about 68% of its population living in areas at risk of malaria (5).

Drug resistant *Plasmodium* species now a day is a major problem in malaria control campaign (6). Resistance of *Plasmodium* parasites to the currently available antimalarial medicines has been reported from different parts of the planate. It has been documented for *P. falciparum, P. vivax* and *P. malariae*. However, *P. falciparum* resistance has been reported in all available antimalarial drugs (amodiaquine, chloroquine, mefloquine, quinine, sulfadoxine-pyrimethamine and artemisinin derivatives) (7, 8, 9).

Therefore, there is an immediate need of developing new antimalarial drugs hopefully accompanied by the understanding of their mode of actions. And one of the best sources for developing new and efficient antimalarial drugs that can easily affordable by those poor living in the malarious endemic tropical countries is traditional medicinal plants and other natural products (10).

Many plant species continue to be used in traditional medicine for the treatment of malaria and many people depend up on such remedies as they cannot afford or do not have access to standard antimalarial drugs (11). Plants have been played a critical role in the history of malaria with Peruvian bark (*Cinchona* spp.), being the first effective treatment for this complications (12). The long established use of quinine and the more recent introduction of artemisinin and their derivatives as highly effective antimalarials demonstrates that plant species are important sources for the discovery of new antimalarial agents (11). Far recently, the WHO estimated 80% of people worldwide rely on herbal medicines for some part of their

primary health care (13); especially developing countries where malaria is endemic depend strongly on traditional medicine as a source for inexpensive treatment of this disease. However, scientific data to validate the antimalarial properties of these herbal remedies are scarce (14).

Since malaria is the number one public health problem in Ethiopia and the majority of people are living in malaria risk rural areas coupled with the inaccessible and unaffordable antimalarial drugs; they strongly depends on traditional medicines particularly herbal remedies to treat the disease and disease related pathologies (15, 16). There is huge number of plant species used by the people to treat malaria in different parts of the country. Consequently, it is important that traditional medicinal plants should be investigated in order to establish their safety and efficacy as well to determine their potential as source of new plant based anti-malarial drugs (17, 18). This study thus aimed to examine the potential antimalarial activity of ethanol crude extracts of the leaves of three plant species namely *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* which are collected from Yayu Coffee Forest Biosphere Reserve *In-vivo* against *Plasmodium berghei* in Swiss albino mice.

Materials and methods

Plant material collection and authentication

Fresh leaves of the plant material were collected from Yayu Coffee Forest Biosphere Reserve; which is located in Ilu Aba Boor zone, Oromia regional state and 550 km southwest from the capital Addis Ababa, Ethiopia during the months of January, 2018. The collected plant specimen (*Croton macrostachyus*, *Ruta chalepensis* and *Vernonia amygdalina*) then identified and authenticated at the Ethiopian National Herbarium, Addis Ababa University, Ethiopia. The

voucher specimens were deposited in collection number of (Meu 01, 02, and 03/2018) respectively for further references.

Crude extracts preparation of the plant material

The fresh leaves of the plant were cleaned, cut into pieces and air dried. The dried leaves then ground in to coarse powder using electrical cross bitter mill (IEC, 158VDE0660, Germany). Crude extracts then prepared by cold maceration technique; soaking the plant powder in 1:12 w/v ratios with ethanol using Erlenmeyer flasks. The flasks containing the plant powders dissolved in ethanol were placed on orbital shaker (Thermoforma, USA) at 120 rotations per minute (rpm) for 72hs. The mixtures were filtered using gauze and then the filtrates were passed through Whatman paper number 1 (Wagtech international Ltd, England). The filtrate of the extract was removed under reduced pressure using rotary evaporator (Buchi type TRE121, Switzerland) at 60 rpm and 30 °C to obtain the crude extract. Then, all the extracts were stored separately in screw caped glass bottle at -4 ^{o}C until used.

Experimental animals

Swiss albino mice of 6 to 8 weeks old weighing 25 to 32g were obtained from Addis Ababa University and Ethiopian Public Heath Institution, Ethiopia. Female mice were used for *In-vivo* acute toxicity test and males were used for *In-vivo* antimalarial assay. Each mouse was used once. They were used in accordance with NIH Guide for the care and use of laboratory animals (19).

Experimental pathogen and infection procedure

The antimalarial evaluation of the extracts performed using CQ-sensitive strain of the rodent malaria parasite; *Plasmodium berghei* ANKA. It was collected from Ethiopian Public Heath Institution and kept alive by continuous intraperitoneal passage in mice on weekly bases. Percent parasitaemia of the donor mouse was first determined; about 20 to 30% parasitaemia and blood was collected through gentle cardiac puncher from the donor mouse using syringe after it has been scarified by chloroform. Then, 1ml of blood was diluted with 4ml of physiological saline (0.9%) and 1ml of the dilution contains 5×10^6 of infected erythrocytes. Therefore, each mouse was infected on day zero (D0) intraperitoneally with 0.2ml dilution of infected blood (standard inoculum) containing approximately $1x10^6$ *P. berghei* parasitized red blood cells (20).

Oral acute toxicity tests

Acute toxicity test for each crude extracts were carried-out using twenty five healthy female mice. The mice were then randomized into five groups of five mice per group. The mice were subjected to fast overnight and the four groups were given 500, 1000, 2000 and 3000 mg/kg of the extract orally dissolving with 0.4ml of 20% of dimethyl sulfoxide (DMSO) for each mouse respectively. The fifth group (which was the control) was provided with same volume of the vehicle for each mouse in the group. Then, the presence and absence of acute toxic signs such as hair erection, lacrimation, reduction in motor and feeding activities, urination, convulsion and mortality within 24 hours were recorded.

In vivo antimalarial suppressive tests

For the antimalarial suppressive tests of the plant extracts, a standard 4-day suppressive test was employed against CQ-sensitive strain of *P. berghei* infection in mice (20). For each extract, twenty (20) mice randomly in to four (4) groups were used. At day zero (D_0), each mouse in all groups were infected by *P. berghei* parasitaemia with standard inoculums (1x106 *P. berghei* infected RBCs) intraperitoneally (21). Three hours after parasite inoculation, the three groups administered with 400, 600 and 800 mg/kg of extract by dissolving the extract with 0.2 ml of the respective vehicle for each mouse orally using a standard oral needle for four consecutive days starting from D_0 in a 24hr schedule. The rest two groups were used as a negative control and positive control which were given 0.2 ml of the same vehicle and 25 mg/kg of CQ respectively.

Parasitological study

On the 5thday (D_4) of the experiment, fresh blood samples were collected from the tail snip of each mouse and thin smear on to a microscope slide were prepared (21). Parasitaemia then examined under light microscopy starting from power of 10 and then in to power 100 (22). Finally, percentage parasitaemia and parasite suppression were calculated using the following two formulas as Fidock et al has described respectively (23).

$$\%\text{Parasitaemia} = \frac{\text{Numbere of Infected RBCs x 100}}{\text{Total number of RBCs}}$$

$$\%\text{Suppression} = 100 - \left(\frac{\text{Mean parasitaemia in the study group x 100}}{\text{Mean parasitaemia in negative control group}}\right)$$

Determination of mean survival time of mice

Mortality rate were supervised daily and the number of days from the time of parasite inoculation up to death were recorded for each mouse in treatment and control groups throughout the follow up observation period. Then, the mean survival time (MST) for each group was calculated using a mathematical formula as described by Mengiste et al(24).

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

Phytochemical screening

The preliminary phytochemical screening of the ethanol extracts of the leaves of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* were made for the identification of selected antimalarial active secondary metabolities such as alkaloids, saponins, glycosides, flavonoids, terpenoids, steroids, phenols and tannins according to the standard procedures and operation manual [25, 26].

Statistical analysis

SPSS version 20 statistical software was used for analysis of the data. One sample t-test was employed to calculate the mean value of parasitaemia and survival time for all variables and one way analysis of variance (ANOVA) was undertaken to compare the level of parasitaemia and survival time of *P. berghei* infected mice between the control and extract treated groups at D_4 of the study respectively. All results were presented as the Mean ± SEM (Standard Error Mean) and statistical significance was considered when $P<0.05$ at the 95% of confidence interval.

Results

Extract yields

Percentage yield of ethanol crude extracts of the leaves of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* is presented in Table 1. A 1:12 w/v ratio of cold maceration technique of extraction was employed and yields comprised of 14 to 22% of the total plant material used.

Table 1. Percentage yield of ethanol crude extracts of the leaves of *C. macrostachyus, R. chalepensis,* and *V. amygdalina*

Plant species	Dry powder (g)	Solvent volume (ml)	Ratio (w/v)	Yield (g)	Yield (%)
R. chalepensis	80	1000	1:12	17.76	22.2
C. macrostachyus	80	1000	1:12	11.20	14
V. amygdalina	80	1000	1:12	12.08	15.1

Acute toxicity

According to the data from Table 2, ethanol crude extracts of the leaves of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* showed no mortality of mice within 24 hours after being administered with 500, 1000, 2000 and 3000 mg/kg of the extracts. Minor gross physical and behavioral changes such as depression, decreased feeding activities and hair erection of mice were seen for about half a day at 2000 and 3000 mg/kg dose of the extracts received mice. However, they returned back to their normal physical and motility conditions and have been active during the follow up five days of period.

Table 2: Acute toxic manifestation seen after administration of ethanol extracts of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* in female mice.

Response	Extract treatment (mg/kg)		
	C. macrostachyus	***R. chalepensis***	***V. amygdalina***

	500	1000	2000	3000	500	1000	2000	3000	500	1000	2000	3000
Hair erection	A	A	P	P	A	A	P	P	A	A	A	P
Lacrimation	A	A	A	A	A	A	A	P	A	A	P	P
Decreased activity	A	A	A	A	A	A	A	P	A	A	A	P
Decreased appetite	A	P	P	P	A	A	P	P	A	P	P	P
Depression	A	P	P	P	A	P	P	P	P	P	P	P
Mortality	A	A	A	A	A	A	A	A	A	A	A	A

Note. A: Absent, P: present

Antimalarial suppressive tests

Extract treated *P. berghei* infected mice produced dose dependent and varying degree of antimalarial suppressive effects although complete clearance of parasitaemia was not recorded. However, the positive control group treated with 25 mg/kg of CQ showed no parasitaemia at D_4 of post infection (Table 3).

Compared to the negative control, extract treated *P. berghei* infected mice showed significant suppressive effects. It was detected that the level of *P. berghei* parasitaemia in the negative control group was 46.53±1.23 while the level of parasitaemia in mice administered with extracts of *V. amygdalina* at the highest dose was 17.94±0.31 at the fifth day of post infection. Similarly, the respective crude extracts of *R. chalepensis* and *C. macrostachyus* at a dose of 800 mg/kg produced 56.72% and 59.30% parasitaemia suppressive effects respectively when comparison were made with the negative control group. However, among the three plant crude extracts, the highest parasitaemia suppression was recorded in *V. amygdalina* extract treated groups followed by *C. macrostachyus*.

Table 3. *In-vovo* antimalarial suppressive effect of ethanol crude extracts of *C. macrostachyus, R. chalepensis* and *V. amygdalina* in mice.

Treatments	Dose (mg/kg)	%Parasitaemia	%Suppression	p-value

DMSO (5%)	0.2ml	46.53±1.23	0.00	---
	400	25.47±0.61	45.26	0.74
V. amygdalina	600	21.84±0.74	53.06	0.81
	800	17.94±0.31	61.44	0.18
	400	25.17±0.94	45.90	0.78
R. chalepensis	600	23.26±0.59	50.01	0.85
	800	20.14±0.28	56.72	0.63
	400	24.51±0.37	47.32	0.32
C. macrostachyus	600	20.43±0.25	56.10	0.04
	800	18.94±0.32	59.30	0.10
CQ	25	0.00±0.00	100.00	<0.01

CQ: chloroquine, ND: note done, DMSO: dimethylsulphoxide

Survival time determination

Treatment with extracts of all the three species of plant material were improved the life expectancy of *P. berghei* infected mice while the corresponding negative control (0.2ml of 5% DMSO) treated mice died shorter (Table 4). Dose of the extracts were found associated significantly with the survival time of mice in the treatment groups (Figure 1).

Table 4. Survival time of ethanol crude extracts of *C. macrostachyus, R. chalepensis* and *V. amygdalina* treated *P. berghei* infected and mice

Treatments	**Dose (mg/kg)**	**%Suppression**	**Survival time/day**	**p-value**
DMSO (5%)	0.2ml	0.00	6.20±0.85	---
	400	45.26	9.20±0.58	0.66
V. amygdalina	600	53.06	11.4±1.12	0.32
	800	61.44	14.60±0.68	0.19
	400	45.90	7.00±0.32	0.39
R. chalepensis	600	50.01	9.00±0.84	0.02
	800	56.72	10.60±0.68	0.19
	400	47.32	9.80±0.58	0.66
C. macrostachyus	600	56.10	10.60±0.68	0.19
	800	59.30	12.40±0.68	0.19
CQ	25	100.00	ND	ND

In mice, the higher parasitaemia suppression recorded, the longer they lived. Thus, parasitaemia infected mice treated with 800 mg/kg of plant material crude extracts for the

three species of study plant produced the highest percent suppression of parasitaemia and lived the longest time when compared with lower dose treatments and the negative control groups.

Preliminary phytochemical screenings

As shown from (Table 4), the presence and absence of antimalarial active secondary metabolities among extracts of the three plant materials are demonstrated. Alkaloids, Tannins and Terpinoids were found from all the three extracts whereas steroids from *V. amygdalina,* and flavonoids and saponins from *R. chalepensis* were absent.

Table 4. Secondary metabolities identified from the ethanol crude extracts of *C. macrostachyus, R. chalepensis* and *V. amygdalina*

Phytochemicals	Results		
	C. macrostachyus	***R. chalepensis***	***V. amygdalina***
Alkaloids	+	+	+
Flavonoids	+	-	+
Cardiac glycosides	+	+	-
Phenols	-	+	+
Saponins	+	-	+
Steroids	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+

Discussion

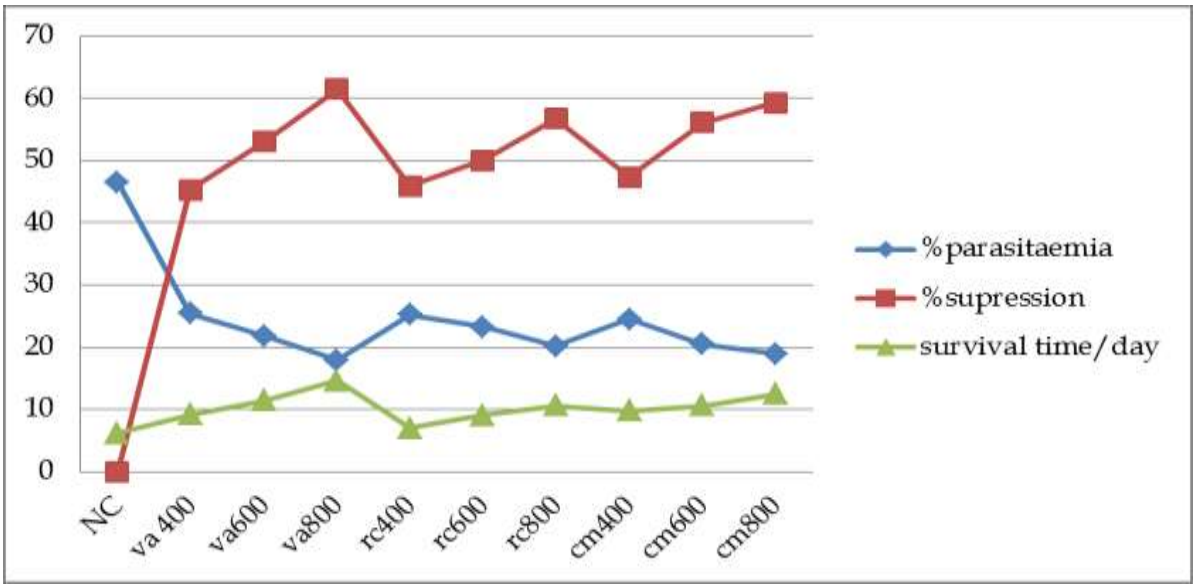
Although medicinal plants are assumed to be safe, potentially toxic species were discovered and therefore, determination of the safety level of plant extracts need to be considered before used for treatment purposes (27, 28). Thus, acute toxicity tests of the ethanol crude extracts of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* were carried out among healthy mice up to the highest tested dose of 3000 mg/kg of the extracts orally. It is then found slight physical

and behavioral changes such as depression and hair erection for about half a day. However, absence of serious acute toxic symptoms such as mortality, impaired movement, listlessness and reduced motor activity within 24 hours and survival of mice during the follow up period justifies the extracts are safe and do not produce oral toxicity at the tested dosages. It is also indication of the estimated oral median lethal dose (LD_{50}) is not toxic and the extracts at 6000 mg/kg body weight could be non-lethal (28). This is one ground justification why these plant species are widely used in traditional treatment of malaria in Ethiopian folk medicines (29).

In the present study, all the three crude extracts established statistically significant ($P<0.05$) and dose dependent *P. berghei* parasitaemia suppression when compared with the respective negative control. These antimalarial activity of ethanol crude extracts of the plant materials might be implicated with the presence of phytochemical constituents such as alkaloids, saponins, cardiac glycosides, flavonoids, terpenoids, steroids, phenols and tannins (31, 32, 33) which are attributed in the antiplasmodial activities alone or the synergetic effects of them. Many other antimalarial active plants species including the potent source of artimesinin, *Artemisia annua* (wormwood) also possess the above mentioned secondary metabolities (34, 35).

Krettli et al in his review demonstrated that a plant based compound considered as an active antimalarial when it produces 30% or more inhibition against parasitaemia of *P. berghei* in mice. The above generalization supports the result of the present study in which all plant extracts produced significant parasite suppression by more than 45% although complete clearance of the parasite yet not detected (21). Similar studies on other plant species such as *Acacia nilotica* (36), Mo*rinda lucida* (37), *Otostegia integrefolia* (38), *Clerodendrum myricoides,*

Dodonea angustifolia and *Aloe debrana* (37) and *Asparagus africanus* (40) also reported that extracts of the plant species produced dose dependent and significant parasitaemia growth inhibition against *P. berghei* in mice. The present study therefore, is evidences demonstrating the relevance of these herbal medicines are promising in the search for new antimalarial medicines.



NC: negative control, va: *V. amygdalina,* rc: *R. chalepensis,* cm: *C. macrostachyus*

Figure 1. Percent parasitaemia, suppression and survival time of extract treated *P. berghei* infected mice.

Several plant based medicinal derivatives have identified antiplasmodial mode of actions to exert the antimalarial activity by interfering with the parasites ability to eliminate the toxic byproduct of hemoglobin digestion or directly killing young intraerythrocytic malaria parasites or inhibiting protein synthesis of the parasite or by other unknown mode of action or using synergetic mechanisms of actions of the two or more (41, 42, 43). However, plant extracts of the present study are crude; the antimalarial action could be the result of

synergetic effects of different phytochemical constituents although the mechanisms of action have not been yet explained in the study.

Mean survival time of mice treated with extracts of the plant materials were lived longer compared to the negative controls. This confirms that the ethanol crude extracts of the plant materials suppressed *P. berghei* parasitaemia and probably reduced the overall pathogenic effect of the parasite in the experimental mice. A justification therefore can be provided that the plant material could probably use to inhibit further multiplication of *Plasmodium* parasitaemia until the infected individuals get modern medical treatments (44).

Even though, extract treated mice lived longer time than the ones fed with vehicle, the highest survival time at a dose of 800 mg/kg body weight were recorded in all the three cases. However, it was not significantly longer than the negative controls. It is probably because of the plant extracts did not cleared all parasitaemia from the mice. Furthermore, ethanol used for dissolving plants material justifying the traditional usage of these plant species as malaria remedy and the use of water and ethanol (local alcohols) as common solvents in traditional medicine.

Conclusion

Ethanol crude extracts of the leaves of *C. macrostachyus*, *R. chalepensis* and *V. amygdalina* produced significant dose dependent antimalarial activity *In vivo* against *P. berghei* in Swiss albino mice. The extracts were found safe in mice received up to the highest single dose of 3000 mg/kg. The phytochemical screenings of the extracts also revealed the presence of

potent antimalarial secondary metabolities. Thus, these plant species could potentially be used as alternative source for the development of new plant based antimalarial agent.

Acknowledgments

Mettu University owes the gratitude for the materials and reagents support to carry out this study. Phytochemical screenings of the extracts were kindly done at Addis Ababa University.

Authors' contributions

SY conceived the study; SY, WG and DC carried out the field and laboratory work; SY analysed the data; SY and WG wrote the paper. All authors critically read, revised and approved the final manuscript.

Competing interest

We authors declare no competing interest exist between us.

Ethical considerations

The study was carried out after having ethical clearance endorsement from Research Ethical and Technical Review Clearance Committee (College of Medical Sciences, Mettu University, Ethiopia). The committee approved the experimental protocols using the NIH Guideline for the care and use of laboratory animals (19).

Funding/Support

This study was financially supported by Research and Technology Transfer Office of Mettu University through annual staff research fund opportunity (grant number: RTTD2009-28).

References

1. Gaston RT, Ramroop S. Prevalence of and factors associated with malaria in children under five years of age in Malawi, using malaria indicator survey data. Heliyon. 2020; 6(5): e03946. doi: 10.1016/j.heliyon.2020.e03946.
2. WHO. World malaria report: World Health Organization, Geneva; 2016. Available at: https://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. Accessed 17 February 2019.
3. WHO. World malaria report: World Health Organization, Geneva; 2015. Available at: https://www.who.int/malaria/publications/world-malaria-report-2015/report/en/. Accessed 14 June 2019.
4. FMOH. Ethiopia national malaria indicator survey 2015. Ethiopian Public Health Institute, Addis Ababa, Ethiopia. 2016. Available at: https://www.ephi.gov.et/images/pictures/download2009/MIS-2015-Final-Report-December-_2016.pdf. Accessed 14 June 2019.
5. Girum T, Shumbej T, Shewangizaw M. Burden of malaria in Ethiopia, 2000-2016: Findings from the global health estimates 2016. Trop Dis Travel Med Vaccines. 2019; 5: 11. doi: 10.1186/s40794-019-0090-z.
6. WHO. Tackling antimalarial drug resistance: Launch of the WHO report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010–2019). World Health Organization. 2020; WHO/UCN/GMP/2020.07. Available at: https://www.who.int/news-room/events/detail/2020/11/19/default-calendar/report-on-antimalarial-drug-efficacy-resistance-and-response-10-years-of-surveillance-(2010-2019). Accessed 21 September 2020.

7. WHO. Guidelines for the treatments of malaria. Third edition. Geneva, Switzerland: 2015. Available at: https://www.who.int/publications/i/item/9789241549127. Accessed 23 November 2020.
8. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in Western Cambodia. N Engl J Med. 2008; 359: 2619-2620. doi: 10.1056/NEJMc0805011.
9. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009; 361: 455-467. doi: 10.1056/NEJMoa0808859.
10. Ginsburg H, Deharo E. A call for using natural compounds in the development of new antimalarial treatments: an introduction. Malar J. 2011; 10: 1-7. doi.org/10.1186/1475-2875-10-S1-S1.
11. Wright CW. Plant derived antimalarial agents: New leads and challenges. Phytochem Rev. 2005; 4: 55-61. doi: 10.1007/s11101-005-3261-7.
12. Yarnell E, Abascal K. Botanical prevention and treatment of malaria. Alt Comp Therapy. 2004; 1: 206-210. doi.org/10.1089/1076280041580332.
13. WHO. Traditional herbal remedies for primary health care. Regional office for South East Asia, Indraprastha Estate, Mahatma Gandhi Marg, New Delhi, India: 2010. Available at: https://apps.who.int/iris/handle/10665/206024. Accessed 12 March 2020.

14. Jurga A, Tomása T, Pividalb J. Antimalarial activity of some plant remedies in use in Marracuene, southern Mozambique. J Ethnopharmacol. 1991;32(1-2):79-83. doi.org/10.1016/0378-8741(91)90165-A.
15. Bekele E. Study on actual situation of medicinal plants in Ethiopia. Prepared for JAICAF (Japan Association for International Collaboration of Agriculture and Forestry): 2007. Available at: http://www.endashaw.com. Accessed 15 January 2018.
16. Kassaye KD, Amberbir A, Getachew B, Mussema YA. Historical overview of traditional medicine practices and policy in Ethiopia. Eth J Health Dev. 2006; 20(2): 127-134. doi: 10.4314/ejhd.v20i2.10023.
17. Bandaranayake MW. Quality control, screening, toxicity, and regulation of herbal drugs. In: Modern phytomedicine. Turning medicinal plants into drugs. Pp. 25-57. (Ahmad, I., Aqil, F., and Owais, M. (Eds)). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim: 2006. doi.org/10.1002/9783527609987.ch2.
18. Soetan KO, Aiyelaagbe OO. The need for bioactivity safety evaluation and conservation of medicinal plants. J Med Plants Res. 2009; 3: 324-328.
19. NRC (National Research Council). (1996). Guide for the care and use of laboratory animals. Institute of laboratory animal resources commission on life sciences, National Academy Press, Washington, D.C: 1996. Available at: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf. Accessed 19 July 2018.
20. Peters W. (1967). Rational methods in the search for antimalarial drugs. Trans R Soc Trop Med Hyg. 1967; 61:400-410. doi.org/10.1016/0035-9203(67)90015-6.

21. Krettli AU, Adebayo JO, Krettli LG. Testing of natural products and synthetic molecules aiming at new antimalarials. Curr Drug Targets. 2009; 10: 261-270. 10.2174/138945009787581203.

22. WHO. Basic laboratory methods in medical parasitology. Parasitology laboratory manual. World health Organization, Geneva, Switzerland: 1991. Available at: https://www.who.int/malaria/publications/atoz/9241544104_part1/en/. Accessed 23 April 2019.

23. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery efficacy models for compound screening. Drug Discov. 2004; 3(6):509–520. doi: 10.1038/nrd1416.

24. Mengiste B, Makonnen E, Urga K. *In vivo* antimalarial activity of *Dodonaea Angustifolia* seed extracts against *Plasmodium berghei* in mice model. Momona Eth J Sci. 2012; 4(1):47–63. doi: 10.4314/mejs.v4i1.74056.

25. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3:10–4.

26. Beroa J, Fre´de´richb M, Quetin-Leclercqa J. Antimalarial compounds isolated from plants used in traditional medicine. J Pharm Pharmacol. 2009; 61(11): 1401–1433. doi: 10.1211/jpp/61.11.0001.

27. Ajaiyeoba E, Falade M, Ogbole O, Okpako L, Akinboye D. *In vivo* antimalarial and cytotoxic properties of *Annona senegalensis* extract. Afr J Tradit Complement Altern Med. 2006; 3: 137-141. doi: 10.4314/ajtcam.v3i1.31148.

28. Verma S, Singh SP. Current and future status of herbal medicines. Vet World. 2008; 11: 347-350. doi: 10.5455/vetworld.2008.347-350.

29. CDER. Single dose acute toxicity testing for pharmaceuticals. U.S. Food and Drug Administration. 1996. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/single-dose-acute-toxicity-testing-pharmaceuticals. Accessed 27 October 2020.

30. Giday M, Teklehaymanot T, Animut A, Mekonnen Y. Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. J Ethnopharmacol. 2007; 110(3): 516–525. doi: 10.1016/j.jep.2006.10.011.

31. Offor CE. Comparative Chemical Analyses of *Vernonia amygdalina* and *Azadirachta indica* Leaves. IOSR J Pharm Biol Sci. 2014; 9(5): 73-77.

32. Abeje A, Fikre M, Belayhun K. Isolation and characterization of terpene from leaves of *Croton macrostachyus* (Bissana). J Med Plants Res. 2016; 10(19): 256-260. doi: 10.5897/JMPR2016.6082.

33. Kacem M, Kacem I, Simon G, Ben MA, Chaabouni S, Elfeki AB. Phytochemicals and biological activities of *Ruta chalepensis* L. growing in Tunisia. Food Biosci. 2015; 12:73-83. doi: 10.1016/j.fbio.2015.08.001.

34. Tesso H. Isolation and structure elucidation of natural products from plants. Dissertation. Institute of Organic Chemistry, University of Hamburg, Hamburg: 2004. Available at: https://ediss.sub.uni-hamburg.de/bitstream/ediss/882/1/Dissertation_final_version.PDF. Accessed 07 July 2012.

35. Zhang L, Demain AL. Natural products: drug discovery and therapeutic medicines. Humana press Inc, United States of America: 2005.

36. Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Antiplasmodial activity of aqueous root extract of *Acacia nilotica.* Afr. J. Biochem. Res. 2011; 5: 214-219.

37. Unekwuojo GE, James O, Olubunmi AR. Suppressive, curative and prophylactic potentials of Morinda lucida (Benth) against erythrocytic stage of mice infective chloroquine sensitive *Plasmodium berghei* NK-65. Br J Appl Sci Technol. 2011; 1:131–40. doi: 10.9734/BJAST/2011/273.

38. Yeshanew S, Mekonnen Y. Antimalarial activity of *Otostegia integrefolia* leaf extracts against Chloroquine sensitive strain of *Plasmodium berghei* in mice. Pharmacologyonline. 2013; (2): 84-89.

39. Deressa T, Mekonnen Y, Animut A. *In vivo* antimalarial activities of *Clerodendrum myricoides, Dodonea angustifolia* and *Aloe debrana* against *Plasmodium berghei.* Eth J Health Dev. 2010; 24: 25- 29. doi: 10.4314/ejhd.v24i1.62941.

40. Dikasso D, Makonnen E, Debella A, Animut A, Urga K, Makonnen W, Melaku D, Kassa M, Guta M. Antimalarial activity of *Withania somnifera* L. Dunal in mice. Eth Med J. 2006; 44: 279-285.

41. White NJ. Qinghaosu (artemisinin): the price of success. Science. 2008; 18;320(5874):330-334. doi: 10.1126/science.1155165.

42. Bassy AS, Okokon JE, Etim EI, Umoh FU, Bessy E. Evaluation of the *in vivo* antimalarial activity of ethanolic leaf and stem bark extracts of *Anthocleista djalonensis*. Ind J Pharmacol. 2009; 41(6): 258-261. doi: 10.4103/0253-7613.59924.

43. Hobbs C, Duffy P. Drugs for malaria: something old, something new, something borrowed. F1000 Biol Rep. 2011; 3: 24. doi: 10.3410/B3-24.
44. Yeshanew S, Mekonnen Y. The effect of *Otostegia integrefolia* leaf extracts on the packed cell volume, body weight and survival time of *Plasmodium berghei* infected mice. Int J Trop Med. 2013; 8(6): 129-134.

Figures

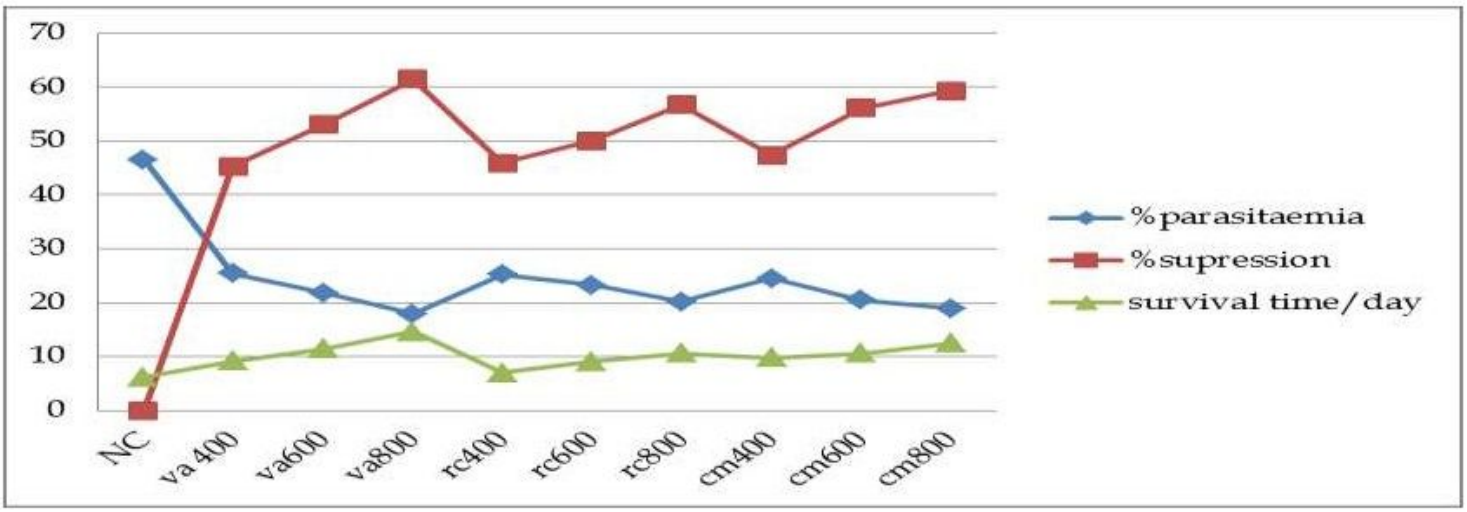


Figure 1

Percent parasitaemia, suppression and survival time of extract treated P. berghei infected mice. NC: negative control, va: V. amygdalina, rc: R. chalepensis, cm: C. macrostachyus

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Discription.pdf