In vitro antimalarial activity of selected medicinal plants native to Tigray region of Ethiopia

Gebretsadkan Hintsa Tekulu (gebretsadkan.hintsa@mu.edu.et)
College of Health Sciences, Mekelle University https://orcid.org/0000-0002-2301-1453
Sarah D'Alessandro
Universita di Milano
Silvia Parapini
University of Milan
Nicoletta Basilico
Universita di Milano
Aman Karim
Mekelle University College of Health Sciences

Research

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Abstract

Background

Various medicinal plants are used as alternative remedies for the management of malaria, an important infectious disease responsible for around 228 million cases and 405,000 deaths worldwide in 2018. The worsening of the disease is highly associated to an emergence of drug-resistant parasites. Hence, the search of new alternative antimalarial agents from traditionally used medicinal plants is the most viable approach. The current study was aimed to evaluate the in vitro antiplasmodial property of *Aloe elegans*, *Aloe monticola*, *Capparis tomentosa, Hygrophila schulli* and *Tephrosia gracilipes* extracts.

Methods

Leaf latexes were collected from *Aloe elegans* and *Aloe monticola*, whereas cold maceration with 70% ethanol was used to prepare extracts from roots of *Capparis tomentosa* and *Tephrosia gracilipes* , and leaves of *Hygrophila schulli* . The antiplasmodial activity of the extracts against asexual and gametocyte stages was tested using parasite lactate dehydrogenase assay and luciferase assay, respectively. Cell cytotoxicity was assessed on human microvascular endothelial cells by the MTT assay

Results

Of the five selected medicinal plants, leaf latex of *Aloe monticola* showed the best activity against both asexual stages and stage V gametocytes of *Plasmodium falciparum* . The leaf latex of *Aloe elegans* and ethanolic extract of *Hygrophila schulli* leaves also showed antiplasmodial property against asexual stages. On the contrary, the roots of *Capparis tomentosa* and *Tephrosa gracilipe*s were inactive.

Conclusion

Findings of this study may partly support the acclaimed traditional use of the leaves latexes of both *Aloe elegans* and *Aloe monticola* and the ethanolic extract of *Hygrophila schulli* leaves for the management of mild to moderate malaria.

Background

Beyond 80% of the global population are heavily using traditional medicines, commonly plant-based medicines, for management of numerous types of illnesses including malaria [1]. Malaria is an infectious disease caused by the parasites belonging to *Plasmodium* genus with *P. falciparum* causing the most life-threatening type of malaria [2]. Even though there is a progress in decreasing malaria cases and deaths, the global malaria burden seems to have reached a plateau and above 50% of the global communities are still at risk of malaria. Based on the World Health Organization (WHO) malaria report 2019, 228 million cases and 405,000 deaths from malaria were estimated worldwide in 2018. Beyond 90% of these cases and deaths occur in Sub-Saharan Africa and about 70% of the global deaths from malaria were in children under-five [3]. Malaria has also a great impact on the socio-economic growth of

developing countries, costing to Africa more than 10-12 billion US$ per year and slowing down the economic growth by as much as 1.3% annually [4].

Even though, WHO had planned to eliminate malaria, no significant progress was made in reducing the number of cases worldwide during the period 2015–2017. This can be attributed to fast emergence of drug-resistant strains of the parasites, availability of limited number of antimalarial drugs and emergence of new *Plasmodium* species (*P. knowlesi*) [5]. These hitches strengthened the imperative need to search new inexpensive and harmless antimalarial remedies which have become a critical priority on the global malaria research agenda. Thus, as history had taught us, the traditional medicinal plants can represent a source of alternative remedies for malaria [6-8].

People believe that traditional medicines are easily accessible, inexpensive, and sometimes efficacious compared to the conventional medicines [9, 10]. Many potential effective medicinal plants are locally available and some of them are part of the traditional medicine of Kunama ethnic group who are well known for their ethnomedicinal knowledge and practice. *Aloe elegans* [Aloeceae] leaf latex, *Aloe monticola* [Aloeceae] leaf latex, *Hygrophila schulli* leaf [**Acanthaceae],** roots of *Capparis tomentosa* [Capparaceae] and *Tephrosa gracilipes* [Leguminosae] are among the most frequently used medicinal plants by Kunama ethnic group in Ethiopia as traditional remedies for malaria disease [11, 12]. However, the traditional use of these medicinal plants for malaria treatment has so far not been scientifically validated. The current research was conducted to appraise the antiplasmodial property of *A. elegans, A. monticola, C. tomentosa, H. schulli*, and *T. gracilipes* extracts on the basis of the traditional use.

Materials And Methods

Plant materials collection

The leaves latexes of *A. elegans* and *A. monticola* were collected from Axum and Hgumburda, Tigray regional state, Ethiopia, respectively. The root part of *C. tomentosa* and *T. gracilipes* were collected from the locality where the Kunama ethnic group has resided. The leaves of *H. schulli* were collected from Grakahsu, Tigray regional state, Ethiopia. All the samples were collected in November 2018. Each plant was authenticated and specimens were deposited in the National Herbarium, Addis Ababa University, Ethiopia.

Consumables and equipments

Ethanol, chloroquine (CQ), drying oven (Genlab, England), refrigerator, Roswell Park Memorial Institute (RPMI-1640) medium (EuroClone, Celbio), AlbuMax (Invitrogen, Milan, Italy), hypoxanthine, HEPES, glutamine, dimethyl sulfoxide (DMSO), 96-well flat-bottomed microplates, incubator, and UV-VIS spectrophotometer were used to conduct this study.

Preparation of the extracts

The leaf latexes of both *A. elegans* and *A. monticola* were collected and dried as per the method described and were considered as an extract [1, 13]. The leaf of *H. schulli* and the roots of *C. tomentosa* and *T. gracilipes* were dried under shaded open air. These plant materials were pounded separately using grinding mill. The powdered plant materials were then macerated using 70% ethanol for three days separately and filtered using What man's filter paper. The marcs were re-macerated two times and filtered after 72 hours. The filtrates of each plant were combined separately and dried in oven at 40 ºC. The dried latexes and ethanolic extracts were stored in a refrigerator at -4 ºC until used for further investigation.

Antiplasmodial activity

P. falciparum cultures were prepared based on the method described by Trager and Jensen [14] with minor modifications. The CQ-sensitive D10 and CQ-resistant W2 strains were preserved at 5% hematocrit (human type A+ erythrocytes) in RPMI 1640 medium (EuroClone, Celbio) supplemented with 1% AlbuMax (Invitrogen, Milan, Italy), 0.01% hypoxanthine, 20 mM Hepes, and 2 mM glutamine. All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Test samples were dissolved in DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, non-toxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates and serial dilutions made. Asynchronous cultures with parasitaemia 1-1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 hours at 37 °C. Parasite growth was examined spectrophotometrically (OD650) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of the method of Makler [15, 16]. The antiplasmodial activity is expressed as 50% inhibitory concentrations (IC_{50}) as mean ± standard deviation of at least three separate experiments performed in duplicate.

Antimalarial activity against stage IV-V *P. falciparum* gametocytes

The 3D7elo1-pfs16-CBG99 transgenic strain expressing the CBG99 luciferase under the pfs16 gametocyte specific promoter was used and gametocytes cultures were conducted as described [17, 18]. Methylene blue was used as positive control.

To trigger gametocytogenesis, asexual parasites cultures were diluted to 0.5% parasitaemia and medium was changed daily, to obtain a parasitaemia higher than 5% when the cultures were treated for 48-72h with N-acetylglucosamine (NAG) (Sigma-Aldrich) to clear residual asexual parasites and to obtain virtually pure gametocytes cultures. Stage IV-V gametocytes were obtained and used for the experiments after 12-14 days from the addition of NAG to the culture. The luciferase activity was taken as measure of gametocytes viability [18]. Briefly, 100 µL of culture medium were removed from each well to increase

haematocrit; 70µL of resuspended culture were transferred to a black 96-well plate; 70µL of D-luciferin (1mM in citrate buffer 0·1 M, pH 5·5) were added. Luminescence measurements were performed after 10 min with 500 ms integration time using a microplate reader Synergy4 (BioTek). The results are expressed as 50% inhibitory concentrations (IC_{50}), extrapolated from the non-linear regression analysis of the concentration–response curve. Each IC_{50} value is the mean ± standard deviation of at least three separate experiments performed in duplicate.

Cell cytotoxicity assays

The long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV 40 large T Antigen [19] was maintained in MCDB 131 medium (Invitrogen, Milan, Italy) supplemented with 10% fetal calf serum (HyClone, Celbio, Milan, Italy), 10 ng/ml of epidermal growth factor, 1 µg/mL of hydrocortisone, 2 mM glutamine and 20 mM Hepes buffer (EuroClone). For the cytotoxicity assays, cells were treated with serial dilutions of test compounds and cell proliferation evaluated using the MTT assay already described [20]. Plates were incubated for 72 h at 37°C in 5% CO_2, then 20 µL of a 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (M-2128 Sigma) in PBS was added for an additional 3 h at 37°C. The supernatants were then discarded and the dark blue formazan crystals dissolved in 100 µL of lysing buffer consisting of 20% (w/v) of a solution of SDS (Sigma), 40% of N,N dimethylformamide (Merck) in H_2O, at pH 4.7 adjusted with 80% acetic acid. The plates were then read on a microplate reader (Synergy 4 Bio-Tek Instruments) at a test wavelength of 550 nm and a reference wavelength of 650 nm.

Data analysis

GraphPad Prism version 8 was used to compute the data obtained in this study and results were presented as Mean ± standard deviation (M ± SD). One-way analysis of variance (ANOVA) accompanied by Tukey's honestly significance difference (HSD) *post-hoc* test was also carried out to compare mean of groups to each other. $P<0.05$ was considered as a significant.

Results

The antiplasmodial property of the tested medicinal plants' extracts against the CQ-sensitive D10 and CQ-resistant W2 strains of *P. falciparum* is presented in Fig. 1 and Fig. 2, respectively. The extracts of the selected medicinal plants, except *C. tomentosa* root extract, induced a concentration-dependent *P. falciparum* growth inhibition. Of the five extracts, the leaf latex of *A. monticola* possessed the highest inhibitory effect against both D10 and W2 *P. falciparum* strains. Next to the leaf latex of *A. monticola*, the leaf latex of *A. elegans* and ethanolic extract of *H. schulli* leaves also showed antiplasmodial activity against both strains.

The half-inhibitory concentration (IC_{50} values) of the selected plants extracts is presented in Table 1. The leaf latex of *A. monticola* showed the lowest IC_{50} value when compared with the other extracts against both the CQ-sensitive *P. falciparum* D10 strain (IC_{50} = 16.94 ± 4.64µg/mL) and CQ-resistant *P. falciparum* W2 strain (IC_{50} = 20.69 ± 4.98µg/mL). The leaf latex of *A. elegans* and ethanolic leaf extract of *H. schulli* also showed moderate activity against both the D10 and W2 *P. falciparum* strains. However, the root extracts of *C. tomentosa* and *T. gracilipes* were not active with an IC_{50} value >200 µg/mL against both D10 and W2 strains.

Plant extracts were then tested against stage V *P. falciparum* gametocytes in order to investigate their transmission blocking potential. Only *A. monticola* extract showed activity against gametocytes with an IC_{50} of 93.92 ± 14.18 µg/mL.

None of the extracts was toxic, until 500 µg/mL, when cytotoxicity was assessed against human microvascular endothelial cells (HMEC-1). The selectivity index (SI = IC_{50} on HMEC-1/IC_{50} on *P. falciparum*) of *A. monticola* was > 25 and >5 for asexual and sexual stages, respectively.

Table 1: *In vitro* antiplasmodial and cytotoxic activity of the selected medicinal plants

Plant extract	IC_{50} (µg/mL)			
	D10	W2	3D7 gametocytes	HMEC-1
AE	122.06 ± 16.02	100.21 ± 5.46	>200	>500
AM	16.94 ± 4.64	20.69 ± 4.99	93.92 ± 14.86	>500
CT	>200	>200	>200	>500
HS	103.11 ± 24.75	115.76 ± 42.99	>200	>500
TG	>200	>200	>200	>500
CQ (ng/mL)	11.25 ± 1.96	150.14 ± 18.10		
Methylene blue	NT	NT	0.05 ± 0.01	

Results are presented as mean ± standard deviation of three independent experiments each performed in duplicate; AE, leaf latex of *A. elegans*; AM, leaf latex of *A. monticola*; CT, ethanolic extract of *C. tomentosa* roots; HS, ethanolic extract of *H. schulli* leaves; TG, ethanolic extract of *T. gracilipes* roots; CQ, chloroquine.

Discussion

History has taught us searching effective antimalarial agents from traditional medicines particularly medicinal plants [6, 7]. In the current study, the antiplasmodial property of five medicinal plants' extracts was screened against the asexual stages of a CQ-sensitive D10 and CQ-resistant W2 strains and against gametocytes of a 3D7 transgenic strain of *P. falciparum*. Among the five selected plants used for the traditional treatment of malaria in Ethiopia, *Aloe elegans* leaf latex, *Aloe monticola* leaf latex, *Hygrophila schulli* leaf exhibited a moderate *in vitro* activity against asexual forms of *P. falciparum***,** while roots of *Capparis tomentosa* and *Tephrosa gracilipes* were inactive. The latex of *A. monticola* leaves showed the lowest IC_{50} value against both strains of *P. falciparum* used. The antiplasmodial activity of the leaf latexes of both *A. monticola* and *A. elegans* is consistent with the reported *in vitro* antiplasmodial and *in vivo* antimalarial properties of the extracts of different *Aloe* species [1, 21-25]. *Aloe monticola* leaf latex showed activity against late stage gametocytes, the sexual stage circulating in the blood stream responsible for malaria transmission. Malaria eradication needs drugs that prevent transmission of the parasite from humans to mosquitoes and thus it is important to found transmission blocking compounds. The activity of *A. monticola* against gametocytes represent an interesting result since mature stage gametocytes are refractory to most antimalarial drugs, with the exception of primaquine. Artemisinins, the mainstay of recommended malaria treatment, are quite active against immature (I-III) stage gametocytes, but exert low inhibition on mature stage gametocytes [26].

The root extract of *C. tomentosa* did not inhibit the parasite growth at all concentrations used in this study. However, other studies reported that the dichloromethane extracts of root, stem and leaf of *C. tomentosa* exhibited a promising *in vitro* antiplasmodial property [21, 27]. Additionally, the methanolic extract of roots of *C. tomentosa* exerted a statistically significant ($P<0.05$) antimalarial activity against *P. berghei* in mice [28]. These controversies needs further study to explore the effectiveness of the root extracts of *C. tomentosa* using different chemical and bio-assays.

Various studies on the antimalarial activity of the genus *Tephrosa* have been reported. In one such study, the dicloromethane:methanol (1:1) roots extract of *Tephrosia villosa*, and stem extract of *T. purpurea* showed good to moderate antiplasmodial activity against the CQ-sensitive (D6) and CQ-resistant (W2) strains of *P. falciparum* [29, 30]. Similarly, the flavonoid compounds and crude extract obtained from *T. elata* seedpods have been shown to exhibit good antiplasmodial activities (IC_{50} = 2.8 - 9.6 µg/mL) [31]. Another study on the dicloromethane:methanol (50:50) extract of the stem of *T. purpurea*, the aerial parts of *T. subtriflora,* and isolated flavonoids showed *in vitro* chemo-suppressive effect against the CQ-sensitive strains of *P. falciparum* (D6) [32, 33]. Conversely, in this study, the ethanolic root extract of *T. gracilipes* was inactive (IC_{50} >200 µg/mL), which could be due to interspecies, environmental and/or growth condition differences, though the plant is on use as a traditional medicine. Also *T. gracilipes* might have inactive molecule/s that need/s biological transformation to an active molecule. Thus, studying the *in vivo* antimalarial property of this plant extract is necessary.

Conclusion

The leaf latexes of the two *Aloe* species (*A. monticola* and *A. elegans*) and the ethanolic extract of *H. schulli* showed a promising *in vitro* antiplasmodial property against the CQ-sensitive (D10) and CQ-resistant (W2) strains of *P. falciparum*. *A. monticola* showed activity also against 3D7 gametocytes. Thus, the antiplasmodial property of the three plants partly justifies and may support the traditional acclaimed use against malaria. However, the ethanolic root extracts of *C. tomentosa* and *T. gracilipes* did not exert suppressive property on the growth of *P. falciparum*. Further *in vivo* antimalarial property and phytochemical analysis of the promising plants extracts seems important to confirm their antimalarial activity. The root extracts of *C. tomentosa* and *T. gracilipes* may also be examined for their *in vivo* antimalarial activity to determine if there is/are reactionary drug/s that needs biological transformation to act.

Abbreviations

CQ: Chloroquine

DMSO: Dimethyl Sulfoxide

GDP: Gross Domestic Product

HSD: Honestly Significance Difference

IC_{50}: Half inhibitory amount of a substance

pLDH: parasite Lactate dehydrogenase

RPMI: Roswell Park Memorial Institute

WHO: World Health organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The data used and analysed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GHT has designed and developed the concept, prepared the extracts, and wrote first draft of the manuscript. NB, SP and SD performed the experiments on *P. falciparum* and human cells. AK has involved in designing and developing the concept. All the authors have revised the manuscript for its intellectual content, approved the final draft, and agreed to be accountable with regard to this work.

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Authors' information

[1]Department of Pharmacognosy, School of Pharmacy, College of Health Sciences, Mekelle University, P.O.Box 1871, Mekelle, Ethiopia. [2]Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università di Milano, Via Pascal 36, 20133 Milano, Italy. [3]Centro Interuniversitario di ricerca sulla malaria-Italian Malaria Network (CIRM-IMN). [4]Dipartimento di Scienze Biomediche per la salute, Università di Milano, Via Pascal 36, 20133 Milano, Italy.

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Figures

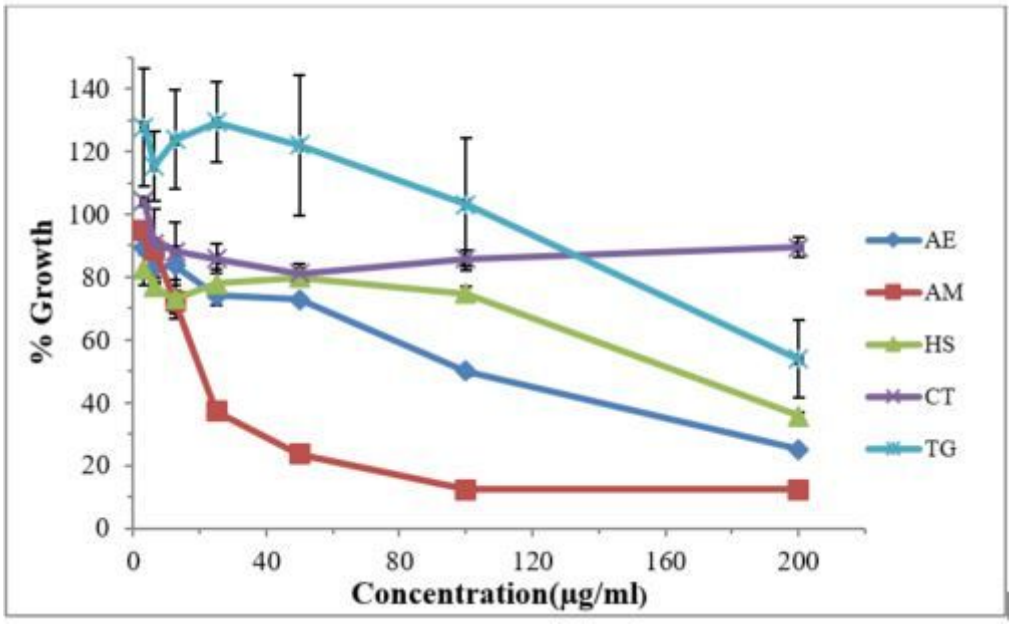

Figure 1

Plot of percentage growth of the CQ-sensitive P. falciparum D10 strain versus concentration of the plant extracts. The error bars indicates the SD of the triplicate results from a representative experiment out of three ; AE, leaf latex of A. elegans; AM, leaf latex of A. monticola; CT, ethanolic extract of C. tomentosa roots; HS, ethanolic extract of H. schulli leaves; TG, ethanolic extract of T. gracilipes roots.

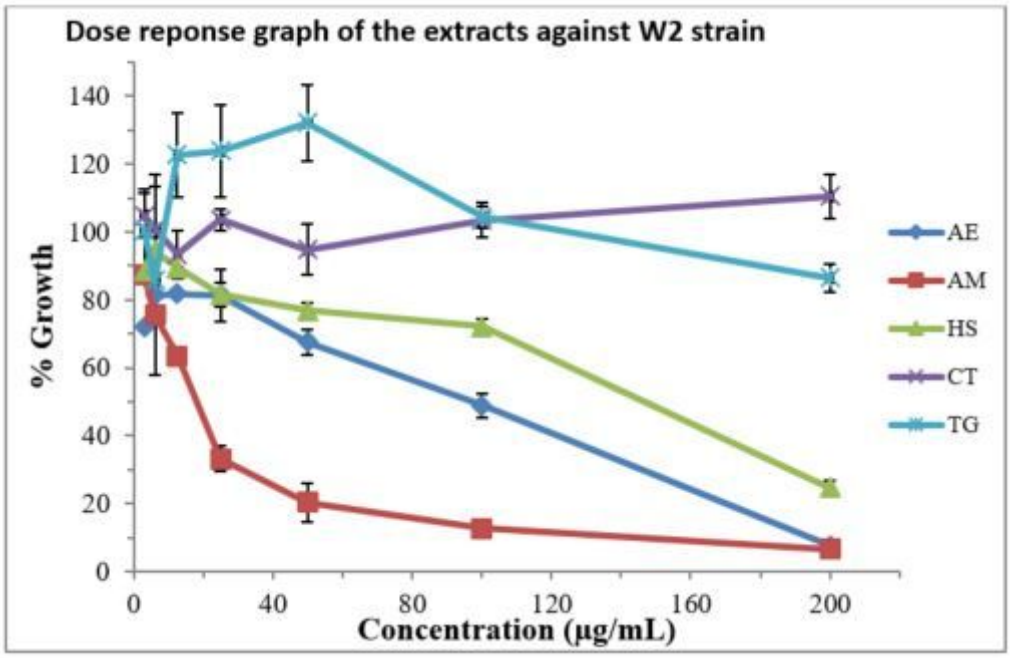

Figure 2

Plot of percentage growth of the CQ-resistant P. falciparum W2 strain versus dose of the plants' extracts. The error bars indicates the SD of the triplicate results from a representative experiment out of; AE, leaf

latex of A. elegans; AM, leaf latex of A. monticola; CT, ethanolic extract of C. tomentosa roots; HS, ethanolic extract of H. schulli leaves; TG, ethanolic extract of T. gracilipes roots.