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Research Article
Evaluation of the Antiplasmodial Properties of Namibian Medicinal Plant Species, *Moringa ovalifolia*

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Abstract

Background and Objective: Malaria is a major cause of morbidity and mortality in Sub-Saharan Africa but it is on the decline in some Southern African countries including Namibia, which is moving towards elimination of the disease. Despite the availability of effective medicines in Namibia, some communities do not accept allopathic medicines, preferring traditional medicines. This study was conducted to determine the phytochemistry and the efficacy of *Moringa ovalifolia* (*M. ovalifolia*) an ethnomedicinal plant, to provide a basis for their integration into mainstream malaria case management. **Materials and Methods:** *Moringa ovalifolia* was screened for known classes of antimalarial phytochemicals using thin layer chromatography. *In vitro* antiplasmodial activity of aqueous and organic extracts from *Moringa ovalifolia* was measured using parasitaemia post-treatment with plant extracts as well as the IC_{50} values. Data analysis using two-way ANOVA to determine the significant interactions between plant extracts and plasmodic growth. **Results:** Phytochemical screening of *M. ovalifolia* revealed the presence of flavonoids, anthraquinones, coumarin, terpenoids and alkaloids. Against *Plasmodium falciparum* (*P. falciparum*) D10, the leaf extracts of *M. ovalifolia* were the most effective with IC_{50} values of 14.30 and 20.73 μg mL^{-1} for the organic and aqueous extracts, respectively. **Conclusion:** *M. ovalifolia* extracts exhibited moderate antiplasmodial properties *in vitro* and have potential as antimalarials. These findings provide a basis for further investigation into their phytochemistry as well as *in vivo* studies on their safety and efficacy to support their use as an alternative treatment for malaria.

Key words: Antiplasmodial, malaria, medicinal plants, *Moringa ovalifolia*, *Plasmodium falciparum*, phytochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria is one of the most wide spread infections globally[1-3]. In 2013, an estimated 198 million cases of malaria and 584,000 deaths were reported worldwide[4]. In many African countries including Namibia, malaria is a public health concern, where 15,692 cases and 61 deaths were reported in 2014, mostly among pregnant women and children under the age of 5 years[1]. However, much progress has been made in reducing malaria over the past decade and the country is targeting elimination of the disease by 2020[5]. This requires elimination of all transmission foci including at risk communities that do not use conventional treatments for malaria but opt for traditional remedies because of their accessibility and affordability and/or cultural perceptions, beliefs and norms[6]. Therefore, to ensure that malaria elimination in Namibia is achieved whilst respecting cultural norms, studies on traditional medicine should be carried out to determine the efficacy and safety of all antimalarial treatments before the promotion of their use.

Herbal remedies from plants have been used by communities in developing countries to manage or cure many diseases including HIV/AIDS, tuberculosis, sickle cell anaemia, diabetes, mental illnesses and microbial infections. Parasitic, infections such as malaria are also treated with medicinal plants, contributing to lowering the mortality, morbidity caused by the disease[7]. In many African countries, rural people recognize folk medicine as their primary means of healthcare, regardless of the availability and accessibility status of orthodox medical care[8]. Plant secondary metabolites are the major contributing components in extracts that elicits healing effect on the body[9]. These compounds have also been used to inform the synthesis of well-known drugs such as artemisinin, which was isolated from the Chinese herb *Artemisia annua*[10] and quinine from the Cinchona bark[11]. Globally, over 1000 plants are known to treat fever and other malaria-associated symptoms in the traditional setting[12]. Several studies have identified a number of Namibian plant species that are used to treat symptoms of malaria[9,13-16]. However, there is limited data about their antiplasmodial properties and phytochemistry.

Moringa ovalifolia, a medicinal plant found in the western and central parts of Namibia, near Halali in Etosha and in the "Sprokieswoud" to the west of Okaukuejo[17,18] are used traditionally to treat malaria[16]. The leaves are prepared and drunk as a decoction. Similarly, the leaves are also used to treat symptoms of malaria including vomiting and diarrhoea. In this study, the classes of antimalarial compounds, as well as the antiplasmodial effects against *P. falciparum* of *M. ovalifolia* were determined. *M. ovalifolia* was selected on the basis of its traditional use.

METHODS AND MATERIALS

Plant collection and authentication: *M. ovalifolia* was collected in the Etosha district in 2013. One kilogram of fresh *Moringa* leaves and twigs (stems) were collected from one sampling site. Voucher specimen were prepared and deposited with the herbarium of the National Botanical Research Institute (NBRI) of Namibia, for identity verification. The leaves and twigs were air dried for 4 weeks, ground and stored at -20°C for long term use. All reagents used were of commercial grade and were purchased from local vendors.

Preparation of extracts: Organic extracts were prepared by soaking 10 g of plant material in 100 mL methanol for 48 h at room temperature. Similarly, aqueous extracts were prepared by macerating 10 g of the pulverized plant material in 100 mL double distilled water for 5 h at 66°C in a water bath. All extracts were filtered using Whatman No. 1 filter paper and the filtrates were concentrated using a rotary evaporator, which were kept at -20°C prior to freeze drying. The dry extracts were collected from the round bottom flasks, weighed and stored in airtight sterile tubes at -20°C.

Thin layer chromatography: Thin layer chromatography (TLC) analysis was conducted by resuspending 1 mg of dry organic and aqueous extracts in 1 mL methanol and double distilled water, respectively. Ten microliters of extract was spotted on MERK silica gel 60 F_{254} plates using thin capillary tubes. The TLC plates were developed in the respective solvent systems and the compounds were visualized under a UV lamp, Konrad Benda (Germany) at 366 nm and/or with the appropriate staining reagents as shown in Table 1.

Stock solution preparation: Stock solutions for both aqueous and organic extracts were prepared at a concentration of 500 g mL^{-1}. The lyophilized aqueous and methanol extracts were resuspended in double distilled water and dimethyl sulfoxide (DMSO), respectively. The stock solution for chloroquine (positive control) was similarly prepared in water at a concentration of 500 µg mL^{-1}. All the stock solutions were sterilized by filtration using 0.22 µm syringe filters.

Antiplasmodial assessment: The plant extracts were screened for antimalarial activity using an *in vitro* model. Antiplasmodial activity of plant extracts was determined using the *P. falciparum* D10 strain (chloroquine sensitive), which was obtained from the American Type Culture Collection (ATCC). The parasites were maintained daily with Roswell

Table 1: TLC solvent systems and staining reagents for classes of known antimalarial compounds

Phytochemical compounds	Mobile phases	Staining reagents	Colour change
Alkaloids	Methanol:ammonium hydroxide (200:3)[19]	Dragendorff reagent[20]	Orange-yellow to brown
Terpenoids	Hexane:ethyl acetate (17:3)[19]	Liebermann-Burchards reagent[21]	Pink
Flavonoids	Butanol:acetic acid:water (4:1:5)[22]	1% methanolic aluminium chloride solution[23]	Yellow fluorescence in UV light
Coumarins	Chloroform[19]	10% ethanolic potassium hydroxide solution[24]	Blue fluorescence in UV light
Anthraquinones	Ethyl acetate:methanol:water (100:17:13)[19]	10% methanolic potassium hydroxide solution[25]	Red in UV light, red in visible light

Park Memorial Institute Medium (RPMI) 1640 media supplemented with L-glutamine, 25 mM hydroxyethyl piperazine ethane sulfonic acid (HEPES) buffer, 0.02 mg mL^{-1} gentamycin, 4% glucose, 2 mM sodium hydroxide and 10% human heat inactivated serum. Fresh O^+ erythrocytes were added daily to maintain a 2% haematocrit. Antiplasmodial activity was measured using parasitaemia. Stock solutions were diluted to 25, 50 and 100 µg mL^{-1} in the culture medium and added to cell culture flasks containing plasmodial cultures of 1.5% parasitaemia and 2% haematocrit. Positive controls were treated with chloroquine (25 µg mL^{-1}), whilst non-treated flasks were used as negative controls, all assays were done in triplicate. All flasks were then gassed with a gas mixture of 90% N_2, 5% CO_2 and 5% O_2, sealed and were incubated at 37°C for 48 h. Growth inhibition was determined after 48 h on the trophozoite growth stage of the *P. falciparum* as this is the period where the growth of the *P. falciparum* parasites is greatest in the erythrocytes[26].

Statistical analysis: The parasitaemia of all treatments and controls were analyzed at X100 magnification using a compound microscope. Graph Pad Prism, version 6, software was used for data analysis. The average percentage inhibition was expressed as Mean±SE (standard error). Two-way ANOVA was used to determine the significant interactions between plant extracts and concentrations. Furthermore, the Tukey's multiple comparisons test was also performed to determine the significant interactions between concentrations per plant part, within treatments. Values of $p<0.05$ were considered significant.

RESULTS

Phytochemical screening: The *M. ovalifolia* twigs and leaf organic extracts exhibited the presence of all compounds tested for including flavonoids, coumarins terpenoids, anthraquinones and alkaloids (Table 2). The aqueous extracts of the leaves and twigs both had flavonoids, coumarins, anthraquinones and alkaloids. Interesting to note, flavonoids and anthraquinones were present in the aqueous and organic extracts for both leaves and twigs.

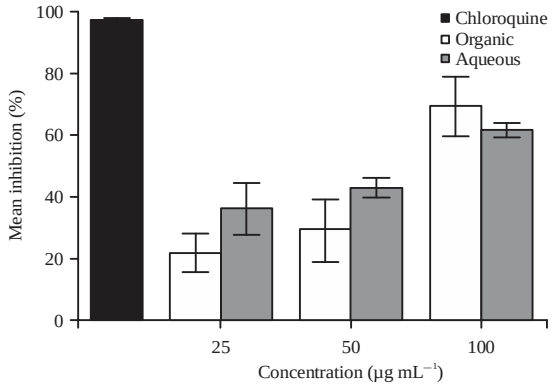

Fig. 1: Mean percentage inhibition (%) of *P. falciparum* D10 for leaf extracts of *M.ovalifolia* after 48 h
Data are presented as Means±SEM at a 5% significance level

***In vitro* antiplasmodial activity:** *M. ovalifolia* (leaves and twigs) were screened against *P. falciparum* D10, based on the wide range of phytochemicals present, as revealed by TLC analysis (Table 2). The leaf extracts of *M. ovalifolia* (LMO) indicated growth inhibition of the *Plasmodium* parasite, for both organic and aqueous extracts (Fig. 1). Maximum growth inhibition (91.1%) was obtained with the positive control (chloroquine) at a concentration of 25 µg mL^{-1}. The LMO showed maximum inhibitory effects (69.5% for the organic extract and 61.7% for the aqueous extract) at a concentration of 100 µg mL^{-1}. There was a concentration dependant effect for organic extracts with significant differences between 25 and 100 µg mL^{-1} ($p = 0.0016$) and 50 and 100 µg mL^{-1} ($p = 0.0055$). For the aqueous extracts there was no significant difference across all the concentrations (25 and 50 µg mL^{-1}, $p = 0.7904$, 25 and 100 µg mL^{-1}, $p = 0.7000$ and 50 and 100 µg mL^{-1}, $p = 0.2064$), hence there was no concentration dependent effect. The aqueous and organic extracts of the twigs of *M. ovalifolia* (TMO) inhibited growth of the *Plasmodium* parasites, with the aqueous extracts indicating a concentration dependent effect (Fig. 2). The organic extracts of TMO showed maximum activity (67%) at the highest concentration (100 µg mL^{-1}), whereas, the aqueous extracts of TMO exhibited maximum activity (44.3%) at 100 µg mL^{-1}. Furthermore, there was no significant difference between the concentrations for the TMO organic extracts, except between

Table 2: Phytochemical screening of *M. ovalifolia* for classes of antiplasmodial compounds

			Classes of compounds				
Plant species	Extract type	Plant part	F	C	T	An	Al
Moringa ovalifolia	Aqueous	Leaves	+	+	-	+	+
		Twigs	+	+	-	+	+
	Organic	Leaves	+	+	+	+	+
		Twigs	+	+	+	+	+

+: Present, -: Absent, F: Flavonoids, C: Coumarins, T: Terpenoids, An: Anthraquinones, Al: Alkaloids

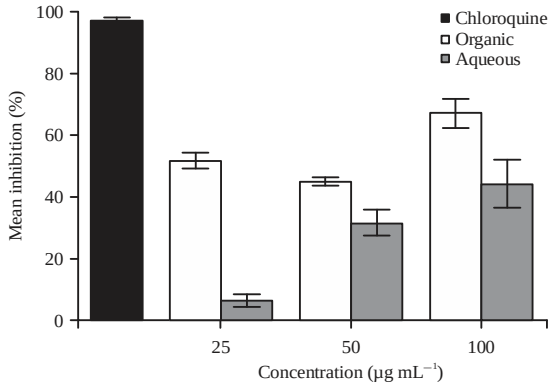

Fig. 2: Mean percentage inhibition (%) of *Plasmodium falciparum* D10 for twig extracts of *M. ovalifolia* after 48 h

Data are presented as Means±SEM at a 5% significance level

50 and 100 µg mL^{-1} (p = 0.015). On the other hand, for the aqueous extracts there was a significant difference between concentrations (25 and 100 µg mL^{-1}, p = 0.019, 50 and 100 µg mL^{-1}, p = 0.012). The IC_{50} values of the plants were calculated and found to be 14.30 and 20.73 µg mL^{-1} for organic and aqueous extracts of LMO, respectively and 26.85 and 94.92 µg mL^{-1} for organic and aqueous extracts of TMO, respectively.

DISCUSSION

M. ovalifolia leaf extracts contained classes of antiplasmodial compounds, flavonoids, anthraquinones, coumarins and alkaloids. They also showed antiplasmodial activity against *P. falciparum* D10, with the leaf extracts exhibiting the highest activity (IC_{50} = 14.30 µg mL^{-1}, organic) and (IC_{50} = 20.73 µg mL^{-1}, aqueous).

The compounds tested for this study are classes of antiplasmodial compounds[27], their presence in the plant extracts can be correlated to biological activities[28], such as the antiplasmodial activities observed in this study. The following compounds were previously identified in the leaves of *M. ovalifolia*: kaempferol, quercetin and myrietin. The presence of these flavonoids corroborates the findings of this study[29]. Variations in the phytochemicals in the plant parts have been reported and may be influenced by growth conditions which may include climate, geographic location affecting production of secondary metabolites. It was report finding the same phytochemicals in the leaf and twig extracts. It has been reported that different plant parts can produce the same active compounds, thus exhibiting similar biological activities, the same phytochemicals were found in leaf and twig extracts[30]. Therefore, the use of non-destructive harvesting of plant parts such as leaves by herbalists or traditional healers should be encouraged to increase plant conservation.

There are no previous reports of antiplasmodial activity of *M. ovalifolia*. In this account, the *in vitro* antiplasmodial activity of *M. ovalifolia* extracts against the D10 strain of *P. falciparum* was defined according to the inhibitory concentration at 50% (IC_{50}). An extract showing an IC_{50} value <10 µg mL^{-1} indicates good activity, $10 \leq IC_{50} \leq 50$ µg mL^{-1} indicates moderate activity, $50 < IC_{50} \leq 100$ µg mL^{-1} indicates low activity and or $IC_{50} > 100$ µg mL^{-1} is classified as inactive[31]. The leaf extracts of *M. ovalifolia* exhibited moderate activity both organic (14.30 µg mL^{-1}) and aqueous (20.73 µg mL^{-1}), as well as those of the organic twigs extract (26.85 µg mL^{-1}). The aqueous twig extract showed low activity (94.92 µg mL^{-1}). *M. ovalifolia* is used ethnomedicinally to treat malaria but there has only been anecdotal evidence to support this. A related species *Moringa oleifera* has been reported to exhibit *in vivo* antiplasmodial activities with growth inhibition of 97 and 100% at a concentration of 200 mL kg^{-1} [32]. This corroborates the findings of Kott *et al.*[33], that plant species from one genus can have the same bioactive compounds and thus exhibit similar biological activities. This study provides evidence for the antiplasmodial properties of extracts from *Moringa ovalifolia*.

Antiplasmodial activities of the aqueous leaf extracts and the organic extracts of the twigs of *M. ovalifolia* extracts were independent of concentration. This may be as a result of saturated receptors or drug targets. Therapeutic effects are normally produced when pharmacophores bind to receptors. At elevated concentrations of a drug, the therapeutic response reaches a maximum due to saturation of available receptors[34].

Phytoconstituents with low or no activity can also competitively bind to the drug targets, since extracts are made up of a mixture of compounds. Furthermore, the observed antimalarial activities of the extracts at the highest concentration were not significantly high. This may be as a result of low levels of bioactive compounds in extracts or the compounds in extracts may be partial agonists. These compounds produce only a partial response regardless of complete saturation of receptors[34].

The leaf extracts, both aqueous and organic, exhibited higher antiplasmodial activities (lower IC_{50} values) than the twigs. The data of this study therefore, supports the traditional use of the leaves[16]. Overall, the organic extracts had higher activity than the aqueous extracts and this is consistent with findings by other researchers. The organic solvent is superior in extracting bioactive compounds due to its polar properties[35]. Although the antiplasmodial activity of the aqueous extracts was lower, the activity of the leaf extracts was still moderate indicating the traditional choice of solvent (water) is rational. It should also be taken into consideration that low antiplasmodial activity *in vitro* can translate into significant antiplasmodial activities *in vivo*. The route of administration of any pharmaceutical including herbal remedies is of critical importance for activity[36]. Depending on the solubility and bioavailability, active constituents can easily be absorbed into the bloodstream and be transported to the active site. The reported route of administration of *M. ovalifolia* is oral[16]. In a closed system, the compounds as a result can be broken down or metabolized into biologically active compounds, hence exhibiting biological activities *in vivo*, in this instance antiplasmodial activity. This warrants further investigation of *M. ovalifolia* for their antiplasmodial activities *in vivo*.

CONCLUSION

The use of *M. ovalifolia* as treatment for malaria and its symptoms in traditional settings is rational based on the presence on antimalarial compounds flavonoids, anthraquinones, coumarins and alkaloids. Furthermore, extracts of *M. ovalifolia* also showed moderate anti-plasmodial properties *in vitro*. Hence, the extracts can be used in the management of malaria, this the first such report with evidence to support such a use for *M. ovalifolia*. The findings are an important step in the evaluation of the plant as alternative medicines for malaria. Future studies should include *in vitro* and *in vivo* antiplasmodial and toxicity studies to evaluate the safety of *M. ovalifolia*.

SIGNIFICANCE STATEMENTS

This study provides evidence for the possible antiplasmodial activities of extracts from *Moringa ovalifolia*, a plant species indigenous to Namibia. This is the first time scientific data on its antiplasmodial activity has been reported and this warrants further research for its development as an alternative treatment for malaria. *Moringa ovalifolia* extracts can be beneficial for treatment of malaria in communities that do not readily have access to allopathic medicines or prefer to use alternative medicines. This study will help the researcher to validate the usefulness of extracts from *M. ovalifolia* and novel chemical entities from the plant similar to Artemisia.

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