

Exploring Medicinal Plants for Better Health: Antiplasmodial properties of *Clerodendrum myricoides* and *Dodonaea angustifolia*

Ethiopia

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Summary

Malaria is still a public health problem in Ethiopia, affecting mainly children under five years of age and the elderly. Anti-malarial drugs are mainly available in major cities and some rural towns and health posts. However, the rural community still uses herbal medicine to control malaria-related illnesses. Therefore, the validation of medicinal plants is a very important engagement of researchers in the biomedical fields. The leaves of the sand olive (*Dodonaea angustifolia*) and *Clerodendrum (Rotheca) myricoides* are used to treat malaria in traditional practices in some parts of Ethiopia. The antiplasmodial activity of both plants was checked using the

rodent parasite *Plasmodium berghei* in Swiss albino mice using a standard Peter's four day suppressive method. Extracts made using different organic solvents (methanol, ethyl acetate and others) from the dried leaves of *D. angustifolia* and *C. myricoides* showed strong activity in suppressing the plasmodial parasite load in the mice. In conclusion, the present study demonstrated significant suppression of *P. berghei* in the infected mice that now needs to be further substantiated using human plasmodium parasites. Establishing the efficacy and safety of the plant extracts is also important to develop herbal preparations against malaria.

Background and Justification

The majority of the rural Ethiopian population relies on traditional herbal remedies for its primary health care needs. However, the scientific merit and health benefits of many of the country's medicinal plants is not yet fully studied.

Malaria, caused by *Plasmodium* parasites, is still a public health problem in Ethiopia, affecting mainly children under five years of age and the elderly. Anti-malarial drugs are available mostly in the major cities and rural towns.

Over-use of antimalarial drugs however, is leading to growing problems with drug resistance. Therefore, development of new drugs is being given due attention. The discovery of artemisinin from *Artemisia annua*, based on traditional Chinese medicine, was a breakthrough in the development of antimalarial drugs. Attempts are now being made to discover antimalarial constituents in other potentially antimalarial medicinal plants such as *Dodonaea angustifolia* and *Clerodendrum (Rotheca) myricoides*.



Figure 1: *Dodonaea angustifolia* (l.) and *Clerodendrum (Rotheca) myricoides* (r.), Ethiopian plants with potential antiplasmodial activities (source Wikipedia).

Dodonaea, commonly called hop-bushes, is a genus with 70 identified species. The sand olive (*Dodonaea angustifolia*) (*Syn: Dodonaea viscosa*) (family Sapindaceae) is a shrub that grows up to 3 metres tall and is indigenous in most Ethiopian regions. Ethnopharmacology reporting in different parts of the world indicates a variety of therapeutic uses. Stem or leaf infusions are used to treat sore throats and root infusions to treat colds. Extracts of leaves of this plant exhibited antibacterial and antioxidant properties (Teffo *et al.*, 2009; Riaz *et al.*, 2012). Furthermore, the crude extract of the leaves (Tekalign *et al.*, 2010) and of the seeds (Mengiste *et al.*, 2012) showed antimalarial activity

The genus *Clerodendrum* L. (family Lamiaceae) is widely distributed in tropical and subtropical regions. Locally, in Amharic, the Ethiopian language, it is called Misrch and is an open shrub reaching 2 to 3 metres tall and 2 metres in diameter with dark green glossy leaves about 10 cm long. It is used for various ailments. For example the bark of the plant is used for abdominal pains, malaria and against snake bites. The roots and leaves of *Clerodendrum myricoides* are also used to treat gonorrhoea, rabies, measles, eye diseases, malaria, swelling in the body, wound dressing, haemorrhoids and asthma.

Anti-malarial drugs are not affordable for the rural poor community and a preference for herbal medicine by many people is driving the search for effective antimalarial plants. In addition, *Plasmodium* species especially *P. falciparum*, are becoming resistant to the majority of today's available anti-malarial drugs and the infection is one of the most serious causes of morbidity and mortality globally, particularly in sub-Saharan Africa. Therefore, there remains a need to develop new and highly efficient antimalarial drugs that are efficacious and affordable. Many plants are claimed to have antimalarial effects but more often than not the claims prove to be incorrect. Our long years of testing medicinal plants with claimed antiplasmodial activity have showed that *D.angustifolia* and *C. myricoides* have good potential for further development.

Thus, the main objective this case study was to come up with the validation (or otherwise) of the claimed antimalarial effects of the two plants by testing them in animal models using a *Plasmodium* parasite that can easily infect mice and use them as test systems for further detailed investigation in human *Plasmodium* parasites.

Description

The research was undertaken in our research laboratory following established laboratory practice. The protocols were set based on our experience and adapting experimental methods already in use.

Both *D. angustifolia* and *C. myricoides* were collected from a hilly area in the eastern part of Addis Ababa in September 2011 and 2012. Identification and authentication of the plant specimens was done at the National Herbarium of Addis Ababa University by a botanist and voucher specimens were deposited as voucher number GG04/2011 and GG04/2012 in the Herbarium.

Plant collection and the preparation of laboratory animals for the experiments were done after ethical approval from the College of Natural & Computational Sciences Ethics Board.

The antiplasmodial activity of the plants was checked in Swiss albino mice using a modified version of the standard Peter's four day suppressive method (Peters *et al.*, 1975).

The leaves of the plants were separately dried and powdered before supplying an oral dose of a known amount to the mice.

For *in vivo* antiplasmodial assay, the plant extracts (fractions, subfractions) and the standard drug chloroquine (CQ) and the mouse infective CQ sensitive strain of *Plasmodium berghei* was used. The parasite was maintained by serial passage of blood obtained from infected mice to non-infected ones on a weekly basis in the Animal House of the College of Natural Sciences, Addis Ababa University.

Each mouse used in the experiment was infected intraperitoneally with 0.2ml of infected blood containing about 1×10^6 - 10^7 *P. berghei*-parasitized erythrocytes. For each experiment about 1 ml of *P. berghei*-infected blood sample was obtained by a gentle cardiac puncture of the donor mice with rising parasitaemia of about 25-35% in such a way that 1 ml blood contains 5×10^6 - 10^7 *P. berghei*-parasitized erythrocytes per ml. This was prepared by determining the percentage of parasitaemia and diluting 1 ml of blood in 4 ml of physiological saline solution (0.9% NaCl).

After the fourth day, blood was taken from the tail end of each mouse and parasitized red blood cells were counted to determine the parasitaemia (parasite load). The percentage parasitaemia and percentage suppression in control and treated mice were calculated using the formulae below.

Percentage parasitaemia in each field is calculated as:

$$\frac{\text{Total number of PRBC} \times 100}{\text{Total number of RBC}}$$

Where, PRBC= Parasitized Red Blood Cells
RBC= Red Blood Cells.

Percentage suppression is calculated as:

$$\frac{\text{Parasitaemia in control (\%)} - \text{Parasitaemia in treated group (\%)} \times 100}{\text{Parasitaemia in control (\%)}}$$

Results

***Dodonaea angustifolia*:** The methanol crude extract of the dried leaves of *D. angustifolia* significantly inhibited parasitaemia in the mice. Further fractionations resulted in subfractions that showed significant inhibition of the percentage parasitaemia and percentage suppression of the parasite load. The aqueous phase (AP) was found to be inactive. The non-polar hexane fraction (HF) of the crude extract was inactive since it only reduces the parasitaemia by 19%. The detailed results are given in Table 1.

Table 1: *In vivo* antiplasmodial effect of ethyl acetate, hexane fraction and aqueous phase extracts of *D. angustifolia* leaves against *P. berghei* in mice.

<i>D. angustifolia</i> 80% methanol extract	Doses mg/kg	Antiplasmodial activity		MST±SEM
		% Parasitaemia ±SEM	% Suppression	
Aqueous phase of extract (AP)	NC	27.48±0.68 ^a	0.00 ^a	7.20±0.37
	150	20.32±0.57 ^b	26.05 ^b	8.20±0.45
	300	19.60±0.81 ^b	28.68 ^b	8.60±0.51
Ethyl acetate (EA) soluble phase	NC	33.00±1.87 ^a	0.00 ^a	7.20±0.37
	150	7.00±0.71 ^b	78.80 ^b	8.10±0.45
	300	8.60±0.51 ^b	74.00 ^b	8.40±0.40
Hexane fraction (HF)	NC	58.90±0.84 ^a	0.00±0.00 ^a	8.20±0.58
	150	50.80±0.97 ^a	14.00±0.81 ^c	8.40±0.51
	300	49.00±0.71 ^a	19.00±0.51 ^c	8.60±0.40
Chloroquine	25	0.00±00 ^b	100.0 ^d	ND

NC = Negative control.

SEM = Standard Error of the Mean. a, b, c indicate values that do not differ significantly ($P < 0.05$)

Values are presented as mean plus or minus standard error of mean ($M \pm SEM$), $n=5$. ND = Not done, NC = Negative control, MST = Mean survival time, %Para = Percentage Parasitaemia, %Supp = Percentage Suppression; a, b, c indicate values in the same column that do not differ significantly ($P < 0.05$).

Most test groups have a statistically significant increase in mean survival time (MST) compared to their corresponding negative control, the mice with no administration of plant extract and chloroquine. All the test mice died between 7- 12 days, except the group of mice treated with CQ that survived longer.

***Clerodendrum myricoides*:** Methanol fraction (MF) and ethyl acetate fraction (EF) obtained from successive fractionation of the ethanol crude extract of *C. myricoides* showed highest activity with suppression of 77.24% and 65.21% parasitaemia at an oral dose of 300 mg/kg/day respectively. Further bioactivity guided fractionation of the ethanol extract provided some fractions which exhibited good antiplasmodial activity. On the other hand, the hexane fraction did not suppress the parasitaemia significantly (Table 2).

The ethyl acetate fraction obtained from ethanol extract of the dried leaves of *C. myricoides*, did not show much difference in its percentage suppression at different doses and it therefore proved better to treat the mice with lower dose as to avoid potential toxicity at higher doses.

Table 2: *In vivo* effects of methanol, ethyl acetate and hexane fractions of ethanol extract of *C. myricoides* leaves against *P. berghei* in mice.

Fractions of <i>C. myricoides</i> ethanol extract	Dose (mg/kg/day)	Antiplasmodial activity	
		% Parasitaemia \pm SEM	% Suppression
Methanol fraction (MF)	NC	35.47 \pm 0.46 ^a	0.00
	150	9.61 \pm 0.38 ^b	72.90
	300	8.07 \pm 0.39 ^b	77.24
Ethyl acetate, fraction (EF)	NC	35.47 \pm 0.46 ^a	0.00
	150	14.23 \pm 0.34 ^b	59.88
	300	12.34 \pm 0.45 ^b	65.21
Hexane, fraction (HF)	NC	28.48 \pm 0.41 ^a	0.00
	150	24.60 \pm 0.84 ^a	15.77
	300	23.51 \pm 0.64 ^a	17.45
Chloroquine (CQ)	25	0.00 ^b	100.00

NC = Negative control.

SEM = Standard Error of the Mean. a, b indicate values that do not differ significantly ($P < 0.05$)

Partnership

The partnership is within our own college, the Addis Ababa University (AAU), College of Natural and Computational sciences, Departments of Biology and Chemistry.

Sustainability

Currently the importance of using medicinal plants in the form of herbal preparations, as well as in industrially-formulated drugs, is gaining momentum in many parts of the world. This is also true for Ethiopia. At the same time, more and more focus is also being given to the medicinal value of endemic Ethiopian medicinal plant species.

There is an encouraging legal framework to get permission for those medicinal plant products that can be safely used as herbal medicines in Ethiopia. At the national level, the recognition of traditional medicine in Ethiopia was accorded in 1942 (Proc. 27) where the legality of the practice is acknowledged as long as it does not have a negative impact on health. Articles in the Ethiopian Penal Code (512/1957) and the Civil Code (8/1967) provide guidelines for the practice of traditional medicine.

The Ethiopian government has policies and strategies with regard to medicinal plants that support the development and utilization of plant resources in a sustainable manner. The institutions that currently have the main mandates in this field are primarily the Institute of Biodiversity and Conservation (IBC) and the Ethiopian Health and Nutrition Research Institute (EHNRI).

The marketing opportunity of medicinal plant seedlings and herbal products is a new area that is developing. Local people are now much more aware of the importance of medicinal plants, and the need for conservation and protection measures to be taken for safeguarding these natural resources.

In light of the current case study, some recommendations can be made for sustaining such an innovative experience:

- Institutionalization and capacity-building of local people; for the transfer of indigenous knowledge, the conservation of medicinal plants as well as the proper utilization of the resources could help with regard to the utilization of plant biodiversity.
- Local indigenous groups could come together to protect their plant biodiversity and sustainably utilize the benefit.
- Local government and other non-government agencies can also assist in this endeavour.

- A community register of the uses of medicinal plants would be very useful, along with proper documentation for ensuring property rights and, eventually, obtaining patents.

Replicability

Many countries including Germany, Japan, Korea and the USA are major consumer countries of raw medicinal plant materials for their large pharmaceutical industries. In this regard our research results also have a potential market outside the country. In addition, in some African countries including Botswana, Kenya and Mozambique, the healthcare systems use traditional plants in a more formal trade in plant-derived allopathic drugs. Thus, it may be possible for Ethiopia to collaborate in similar endeavours and strengthen the contact and benefit from the results of medicinal plants such as *D. angustifolia* and *C. myricoides*.

Lesson Learned

Some of the major constraints were harvesting the plant material in time due to the growing season, acquisition of supplies including chemicals, and sufficient funds to run the research. Some chemicals are imported and we had to wait until they were delivered. The steps taken to overcome some of the obstacles included trying to obtain some materials from other sources. Communities use medicinal plants. However, this use is not always justified scientifically. What should be done is first of all to create a discussion forum at the village level about the need to scientifically prove the medicinal plants they use on a day-to-day basis. We must come to terms with this and then, when the innovative experience is fully justified, they can become the beneficiaries of their own knowledge, because the knowledge is theirs and not of the researcher.

Future Plans

The project is in its first phase of proving the efficacy of the plants against *Plasmodium* parasites in an animal model. In the next phase the following aspects need to be considered.

- Checking the extracts of both plants; *D. agnustifolia* and *C. myricoides* in human Plasmodium parasites (*P. falciparum*, *P. vivax*) in vitro models.
- Checking the toxicity of the active extracts/fractions on animal models.
- If these are found to be safe then proceeding to the preparation of herbal formulations.

- Further work on the chemistry of the active extracts followed by testing of their efficacy *in vivo* on mice models as well as *in vitro*.
- The final phase, if the plant extracts are found to be efficacious and safe, would be the start of using the plant products as leads to develop antimalarial drugs.

It is very important to identify the target groups and beneficiaries to ensure the success of any project. For this project, a stakeholder analysis was carried out during the inception phase and this helped in designing activities effectively. Research institutes, higher learning institutes, community leaders, religious leaders, members of local government bodies and the private sector will be involved in the project. They play a crucial role in shaping local people's opinions and the decision-making process and provide important technical and financial support.

Sustainable use of medicinal plants is becoming a serious concern in Ethiopia because of resource degradation in the lowlands and highlands alike. Ecosystem conservation will ensure *in situ* conservation of medicinal plants and enable sustainable harvesting methods for collecting medicinal plants from wild habitats.

If the project is sustained and comes to a positive outcome it will contribute to the healthcare system and the economic development of the country.

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