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Investigation of *in vitro* Babeosis Treatment Using *Swartzia madagascarensis* Root and Pod Extracts

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ABSTRACT
The root bark and pods of Swartzia madagascarensis were collected from Chikomba District, Mashonaland East Province, Zimbabwe in July 2011. They were taken to the Government Herbarium, Ministry of Agriculture, Zimbabwe, for identification. The samples were processed in the Department of Chemistry at the University of Zimbabwe and Bioassays were carried out in the Central Veterinary Laboratories of the Ministry of Agriculture, Harare. Both the ethyl acetate and the methanol extracts were effective against Babesia bigemina results of their fortification of chloroquine showed that they might have a synergistic relationship with chloroquine. Results revealed that the extracts could be developed into antibabesial agents.
Key words: *Antibabesia, antimalarial, Babesia bigemina, berenil, chloroquine, fansidar, Swartzia madagascarensis.*

INTRODUCTION

Sustainable management of traditional medicinal plants is important because of their value as a potential source of new drugs and due to peoples' reliance on traditional medicinal products for health. The vast majority (70-80%) of people in Africa consult traditional medical practitioners for healthcare [1]. People of Africa and other parts of the world still use plant extracts widely in the treatment of malaria and other ailments [2].

Although the vast majority of the people of Africa do so for economic reasons, there are people who use traditional medicines because they believe them to be useful, in some cases more useful than western processed medicines. Moreover, there are some diseases which cannot be cured using Western medicines. There are many instances where a patient visited a clinic or hospital, got treated and could not recover and was discharged because nothing else could be done for the patient. Some patients have been to hospitals for cancer treatment and were told to go back and prepare for amputation. The patient got cured using traditional medicines and need for amputation disappeared [3]. Hence traditional medicines should not be discarded and ignored. Instead, they should be researched on and developed to make them more efficacious and more user-friendly. It is for that reason that we have embarked on studies of the type being reported here.

INFORMATION ON THE PLANT BEING DISCUSSED

Swartzia madagascarensis (Leguminosae). Common names: Msekeseke (Swahili); Snake bean (English); Mucherechese (Shona, Zimbabwe).

Swartzia madagascarensis is widely distributed across wooded habitats in Africa, often on sandy soil. It is usually small, with thick, rough grey bark with longitudinal cracks and a light, rounded crown. The tree is bare for several months of the year, but the cylindrical chocolate-brown seed pods remain and make it quite distinctive. When damaged the tree exudes a dark-coloured gum and a pea-like scent. The leaves have an odd number of greyish coloured leaflets with yellow hairs on the underside.

The tree bears fragrant pale, pea-like flowers followed by the pods that release 10-15 seeds when the sticky yellow flesh around them rots on the ground. The tree produces a dark, fine-grained timber suitable for turnery and carving. The timber is termite-resistant and is also valued for its medicinal properties as well. The pods are nitrogen-rich food for cattle. They are also used in the preparation of pesticides in Zambia and are also widely used for fish poison, besides being effective against snails [4].

Swartzia madagascarensis is one of the most important leguminous trees with phytochemicals used for medicinal and other purposes. Leaves and pods are used to cure scarbies and fungal cutaneous infections; root bark is used to cure toothaches; extracts from flowers are used as mosquitocides and the pods have been reported to have molluscidal activity. *Swartzia madagascarensis* is also extensively used in West Africa to treat malaria. In vitro screening of methanol and aqueous methanol extracts revealed that the extracts were active (5 g/ml). The varied use of the plant and its relative ease of cultivation have demonstrated the need to optimize the growth and establishment of sustainable availability of harvestable products of this plant and studies to optimize its seed germination have been carried out [5, 6].

Phytochemical investigation of the dried fruits of *Swartzia madagascarensis* afforded five triterpenoid saponins, shown to be glucuronides of oleanolic acid and of gypsogenin by chemical and spectrophotometrical means. Of these, 3-O-[O-α-L-rhamnopyranosyl-(1→3)-(ß-D-glucopyranosyluronic acid)oleanolic acid, was responsible for the high molluscidal activity of *Swartzia madagascarensis* fruits against the schistomiasis-transmitting snails, *Biomphalaria glabrata* and *Bulinus globasus* [7].

In Zimbabwe, Swartzia madagascarensis is used for cure of fungal diseases, malaria, fevers in domestic animals and humans. Some of the fevers may be due to malaria in humans or due to babesiosis in domestic animals. The parasites that cause malaria and those that cause babesiosis are very difficult to distinguish between them, even with the very sophisticated modern equipment. Many patients have been treated for malaria when their problem was babesiosis. Drugs which were developed for malaria treatment are being used to treat babesiosis, but in different combinations [8, 9]. This is possible because of the similarity between the two diseases. In the present study, we are investigating the efficacy of a plant we have known to cure fevers and drugs which are known to cure malaria and babesisosis. Hence we are comparing the efficacies or plant extracts with the efficacy of fansidar, chloroquine and berenil. The first two are malaria curing agents and berenil is an antibabesial agent.

RESEARCH DESIGN AND METHODOLOGY

Swartzia madagascarensis root bark, fruit pods and a branch were obtained from Manyene village, Chikomba District in July 2011, following interviews with local people. The plant parts were taken to the National Botanical Gardens (National Herbarium), Harare, for identification. The root bark and pods were dried in the shade for two weeks, chopped to small pieces with a small axe, ground to powders using a motorized laboratory grinding mill in the Chemistry Department at the University of Zimbabwe, and the powders accurately weighed into 50g samples. The powders were exhaustively extracted with ethyl acetate (50 g powder, 500ml EtoAc, 24h x 3), decanted, filtered and the EtoAc recovered using a rotary evaporator at 40^{o}C. The extracts were combined, re-dissolved in MeOH, homogenized, the MeOH removed at the rotary evaporator, yielding 1.0050g pod extract as a yellow gum, and 1.203g root extract as an oxblood coloured gum. The extracts were stored in the fridge until used. The residues were then exhaustively extracted with MeOH (500 ml, 24h x 3), decanted, filtered and the MeOH recovered using a rotator evaporator at 50^{o}C. The extracts were combined, re-dissolved in MeOH, homogenized, the MeOH removed at the rotary evaporator, yielding 2.0157g of a yellowish red gum of the pod extract and 1.9256g of an oxblood coloured gum of the root extract. The extracts were stored in the fridge until used.

All sensitivity tests were carried out at the Central Veterinary Laboratories using blood from cattle infected with 1993 Babesia bigemina Rusape Field Strain. Paratisized blood was drawn intravernously from the neck of cattle using a 500ml syringe, and the blood immediately stored in 10 x 100ml heparinised sample tubes and kept in a water bath incubator maintained at 37^{o}C. The cattle annul temperature was regularly monitored using a clinical thermometer and inoculated with berenil and imizol if their condition deteriorated.

Determination of Sensitivities of the extracts against *Babesia bigemina*

Babesia bigemina and *Plasmodium falciparum* are intraerythrocytic and structurally similar. Antimalarials have been used to treat babesial diseases with a degree of success. The sensitivity tests were based on microscopic examination of parasites as well as the condition of the red blood cells after exposure to a drug. Random sampling was used to estimate the parasite population. The

average of individual estimates was used to evaluate the drug's potency at different concentration levels, whilst excluding bacteria, rejecting slides that were contaminated with bacteria.

Measurement of Packed Cell Volume

Packed Cell Volume (PCV) is the ratio of live red blood cells to the total volume of blood sample. The PCV was determined by centrifuging heparinized blood in a capillary tube at 1000RPM for 5 minutes, separating the blood into 2 layers, and measuring the length of each layer. PCV determines whether the drugs used killed parasites only, or both the parasites and blood cells. Agents that kill both parasites and blood cells lead to anaemia, among other complications.

Microscope Slide Preparations

The materials include Giemsa stain, 100% methanol, bibulous paper and a microscope withx100 oil immersion lens, 10x10 grid eyepiece and microscope immersion oil. The slides were fixed in 100% methanol for about 3 minutes and rinsed in tap water. Fresh solution of 10% Giemsa stain in distilled water was added and the slides were left to dry for about 30 minutes, and the slides rinsed in tap water and dried thoroughly using bibulous paper. A light microscope with a 100x100 magnification was used to observe the parasites, red blood cells and white blood cells.

Parasites estimation

The slides were viewed under oil immersion with a 100x objective. Parasitemia was estimated by counting the number of infected cells. A 10x10 grid square in the eye piece facilitated counting. An even-blood-smear yields about 100 red blood cells per 10x10 grid. A one infected blood cell in a 10x10 grid would be 0.1 parasitemia. An average-of-10 fields are counted and the average taken to obtain a representative estimate [10].

Preparation of Standard Solutions of berenil, fansidar and chloroquine

Exactly 0.0200g of each of the 3 powders and each of the EtoAc and MeOH extracts was weighed using an electronic balance and dissolved in 20ml distilled water, giving 0.0010g/ml of each drug, which was then halved and the volume made up with distilled water, giving 0.0005g of each. This was further halved and made up to give 0.00025g/ml for each of berenil, fansidar, chloroquine, EtoAc and MeOH extracts of the root and pod.

The sensitivity and haemolytic properties of each of the standards and each of the extracts at the 3 concentrations above were assessed using the level of parasitemia and PCV as the measurable parameters. The initial test to verify the general sensitivities of the extracts consisted of mixing 1.0ml of parasitized blood with 1.0ml of each of the herbal extracts at the 3 concentrations (Table). The experiment was repeated with lower volumes of drug and/or herbal concentrations (Table). Reducing the volume of herbal extract to 125 L being added to 1.0ml of parasitized blood maintained at 37^{0}C using a float water bath. All the parasitized blood test samples were stored and used in heparinized sample tubes. Unused blood for the day was stored in a fridge at 4^{0}C. Heparinized blood is blood that contains heparin to prolong the life span of blood cells to about 3 days.

RESULT AND DISCUSSION

Parasitemia indicated that the root and pod ethyl acetate extracts were equally effective against *Babesia bigemina* and closely resembled fansidar (Table 1). The methanol extracts of the pod and root were not as effective as the ethyl acetate extracts and much less effective than fansidar. PCV figures and the appearance of red blood cells indicated that the root ethyl acetate extract was good , causing no haemolysis, but the pod extract caused haemolysis. One sample of the methanol extract of the pod wiped out all parasites, gave an outstanding PCV figure of 18, but no red blood cells could be seen after the addition. This was possibly an outlying result which must be ignored. Berenil behaved similarly to water. Parasites could be distinctly seen and the drug did not appear to have any effect on the parasites.

Parasitemia (Table 2) indicated that the methanol extracts of both the pod and the root were not as effective as the ethyl acetate extracts. However, PCV indicated that the pod methanol extract was more friendly to the red blood cells compared to the root methanol extract which caused haemolysis.

Fortification of chloroquine with pod and root extracts gave very good parasitemia results for the fortified chloroquine indicating that the extracts enhanced the efficacy of chloroquine, probably as a result of synergism between chloroquine and the extracts (Table 3). Chloroquine on its own gave

a parasitemia of 0.14. Fortification lowered the parasitemia to 0.08. Chloroquine caused haemolysis as indicated by PCV and the appearance of red blood cells. Fortification removed haemolysis and led to normal looking red blood cells. Such synergism or potentiation has been observed in connection with fortification of fansider with plant extracts [11, 12], and in connection with herbal malaria treatment in general [13].

Table 1. Sensitivity ofEtoAc Extracts on *Babesia bigemina*

Sample	Conc.	parasitemia	PCV out 25	Sensitivity	RBC cond
S. madagascarensis root	0.0010g/ml	0.20	14	sensitive	intact
	0.0005g/ml	0.13	14	v. sensitive	intact
	0.00025g/ml	0.10	14	v. sensitive	intact
S. madagascarensis pod	0.0010g/ml	0.13	13	v. sensitive	Fairly intact
	0.0005g/ml	0.14	14	sensitive	intact
	0.00025g/ml	0.13	10	v. sensitive	haemolysis
Fansidar	0.0010g/ml	0.17	15	sensitive	intact
	0.0005g/ml	0.10	15	v. sensitive	intact
	0.00025g/ml	0.10	7	v. sensitive	haemolysis
Berenil	0.00025g/ml	3	15	No effect	intact
Water		3	15	No effect	intact
Blood		4	18	No effect	intact

Table 2. Sensitivity of MeOH Extracts on *Babesia bigemina*

Sample	Conc.	parasitemia	PCV out 25	Sensitivity	RBC cond
S. madagascarensis root	0.0010g/ml	0.13	7	sensitive	Some intact
	0.0005g/ml	0.60	8	v. sensitive	haemolysis
	0.00025g/ml	0.20	14	sensitive	intact
S. madagascarensis pod	0.0010g/ml	0.25	14	sensitive	intact
	0.0005g/ml	0.00	18	v. sensitive	No cells
	0.00025g/ml	0.25	14	sensitive	intact

Table 3. Results of fortification of chloroquine with extract

Sample	Sample	parasitemia	Sensitivity	PCV	RBC cond.
5 L S. madag. root	120 L Chloroquine	0.080	v. sensitive	14	Intact
10 L S. mada. root	115 L Chloroquine	0.080	v. sensitive	14	Intact
15 L S. mada. root	110 L Chloroquine	0.080	v. sensitive	14	Intact
5 L S. mada pod	120 L Chloroquine	0.080	v. sensitive	14	Intact
10 L S. mada pod	115 L Chloroquine	0.081	v. sensitive	14	Intact
15 L S. mada pod	110 L Chloroquine	0.080	v. sensitive	14	intact
	125 L Chloroquine	0.14	sensitive	9	haemolysis

Key:
S. mada = Swartzia madagascarensis; RBC cond = condition of red blood cells

Thus a case for more in depth studies towards development Swartzia madagascarensis root and pod as antibabesial drugs as well as fortifying agents to chloroquine in the enhancement of chloroquine as an antibabesial agent has been made.

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