Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 4(1): 218-225, February 2010

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Variations of anti-mosquito larvicidal constituents in the *Harrisonia abyssinica* species of Tanzania

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ABSTRACT

Harrisonia abyssinica (Simaroubaceae) is widely distributed and used in traditional medicine in Tanzania. Phytochemical studies of the plant report the presence of steroid and limonoid compounds while much of its biological studies were concentrated on its pharmacological activity on human pathogens. In the present study, eight extracts from plant materials collected from the Moist forest mosaic (Zone I) and the Coastal forest and thicket zone (Zone II) were tested against *Culex quinquefascintus* Say larvae. Detailed analysis of mosquito larvicidal activity of the eight extracts showed a dose dependent ($p>0.05$) trend with the dichloromethane and ethanol extracts of the root bark plant materials collected from Zone I having higher effectiveness. In 24 h, the dichloromethane and ethanol extracts of the root bark from Zone I achieved mortality of 90% and 100%, respectively, at 50 ppm. Likewise, at 5 ppm the two extracts were having 60% and 58% mortality, both been not significant different ($p>0.05$) but significantly different ($p<0.05$) to the rest of the extracts and the control. Follow-up isolation of the ethanol extract of the root bark from Zone I yielded two known limonoids, harrissonin (**1**) and pedonin (**2**) which were also present in the dichloromethane extract from the same Zone. Similarly, the toxicity of the ethanol extract of the root bark from Zone I (LC_{50} = 6.75 µg/ml) had high activity compared to other extracts. The variations of activity and chemical compounds in *Harrisonia abyssinica* suggest the importance of keeping pharmacopoeias of importance medicinal plants in our regions.
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Key words: *Harrisonia abbysinica*, Simaroubaceae, Limonoids, Harrissonin, Pedonin, Larvicides, Cytotoxic

INTRODUCTION

Mosquito borne diseases such as malaria, filariasis and yellow fever are of public and economic importance in developing countries (WHO, 1996). In Tanzania, *Culex quinquefascintus* Say is the most prevalent mosquito species being responsible for transmission of elephantiasis and lymphatic filariasis (Chavasse et al., 1995). Worldwide, it is estimated that, *Wuchereria bancrofti* infected about 80 million people in Asian, African and some South American countries and 750 million at risk (WHO, 1992, 1996). Although the use of insecticide treated bednets (ITN) is seen as a palatable solution for controlling these diseases, in urban areas, mosquitoes tend to bite outdoors making the nets slightly less effective as a control strategy. In the absence of promising vaccines, insecticide resistance, high cost and detrimental effects caused by synthetic chemical insecticides, plants

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continue to play a very important role in traditional medicine and in protection against mosquito vectors as well as drugs especially in African communities. *Harrisonia abyssinica* (Simaroubaceae) is used in traditional medicine to relief different abdominal pains (Johns et al., 1995; Chhabra et al., 1984, 1993), fever and malaria (Chhabra et al., 1993). Most of its reported scientific research was concentrated on its pharmacological activity on human pathogens (Sawhney et al., 1978; Balde et al., 1995; Tahir et al., 1999; Anani et al., 2000). But the fact that Simaroubaceae plants are rarely attacked by insects (Rugutt et al., 1996, 2001) and limonoids compounds which have been reported to cause growth regulating activity on insects are present in *Harrissonia abyssinica* (Rugutt et al., 2001; Kiprop et al., 2007; Rajab et al., 1997) warrant its inclusion as potential source of insecticides. In the present study, extracts from plant materials collected from two different ecological areas in Tanzania were tested against *Culex quinquefascintus* larvae.

MATERIALS AND METHODS

Collection of plant materials

Tanzania is divided into seven ecological zones (Figure 1). The two zones where the root and stem barks of *Harrisonia abyssinica* were collected in April 2007 are the Moist forest mosaic (Zone I) at Lamadi village in Magu district, Mwanza, and the Coastal forest and thicket zone (Zone II) at Changanyikeni village, 15 km west of Dar es Salaam city, Tanzania. Zone I is also called Lake Victoria phytochorion while zone II is called the Zanzibar-Inhambane regional Mosaic phytochorion. The voucher specimens HSO 5627 and HSO 5631 were deposited at the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Tanzania, after been verified by Mr H.O. Suleimani, a botanist at the Department of Botany, University of Dar es Salaam, Tanzania.

Preparation of extracts

Stem and root barks of *Harrisonia abbysinica* were dried under shade for 14 days and then milled to obtain pulverised powders. About 250 g of each of the pulverised powder portions was soaked in 1L of dichloromethane for 48 h and then resoaked by using ethanol (95%). The crude extracts were obtained after evaporation of the solvent under reduced pressure at 40 ^{o}C on a rotary evaporator.

Bioassay-guided isolation and identification of active compounds

Following bioassay of crude extracts, the ethanol extract from Zone I which showed high activity was subjected to column chromatography over silica gel (Merck, 230-400 Mesh) eluting with pet ether/ethyl acetate. A known limonoid compound, pedonin (**2**) (Hassanali et al., 1987) was obtained from the second column chromatography fraction eluting with petroleum ether:ethyl acetate (1:4 v/v) while harrisonin (**1**) (Rajab et al., 1997) was obtained after a second chromatography on Sephadex® LH-20 (Pharmacia, 1:1 v/v MeOH/$CHCl_3$). 1D and 2D NMR spectral data were obtained on a Bruker Avance DPX 300 spectrometers, operating at 300 for ^{1}H NMR and 75 MHz for ^{13}C NMR (δ = 0; TMS internal standard); HRMS: TOF MS EI mass spectrometer operating at 70 eV.

Brine shrimp test

The brine shrimp lethality test (BST) was used to predict the presence of toxicity in the extracts. The experiment was set according to Meyer et al. (1982). Briefly, solutions of the extracts were made in DMSO, at concentrations of 8, 24, 40, 80 and 240 µg/mL except for the ethanol extract of the root bark from Zone I whose concentrations were 1, 2.5, 5, 10 and 20 µg/mL then incubated for 24 h in triplicate vials. Ten brine shrimp larvae in 5 ml of a mixture containing the extract, seawater and DMSO, for

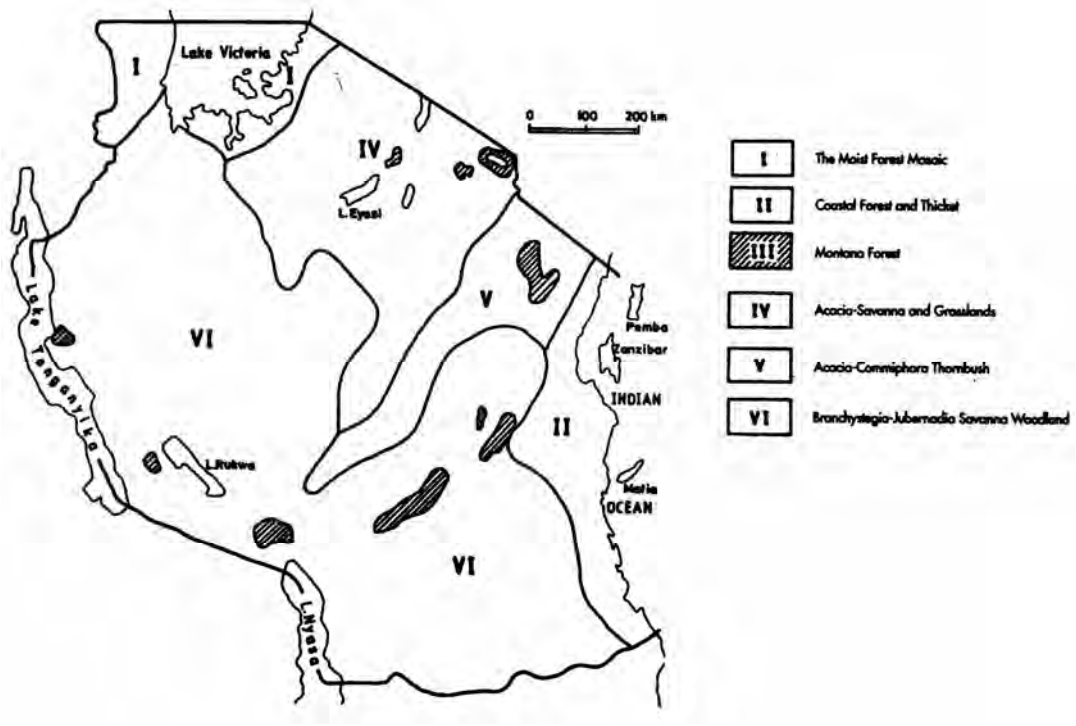

Figure 1: The map of Tanzania showing the ecological zones where the two samples of *Harrisonia abyssinica* were collected.

treatment experiment and seawater and DMSO only for control experiment. The average number of survived larvae in each triplicate was recorded after 24 h and the mean percentage mortality obtained was used in calculation of LC_{50} using SAS program (SAS, 2000).

Larvicidal test

Larvicidal experiment was done according to WHO protocol of 1996 (WHO, 1996). Thus, different concentrations of 50, 25, 10 and 5 ppm were prepared from the stock solution of each plant extract to make up 250 ml of the mixture of extract, water and DMSO. Twenty late third instar mosquito larvae were then incubated in triplicate beakers having different concentrations and mortality was observed after 24 h. A control experiment contained the mixture of water and DMSO only. All tests were carried out under controlled temperature (26 ± 2 ^{o}C) and relative humidity of 75-85% using the laboratory reared *C. quinquefascintus* larvae. The average number of dead larvae was recorded after 24 h and the mean percentage mortality obtained was used in analysis of variance. Student-Newman–Keuls test (SNK) of the SAS package was used to compare the means (SAS, 2000).

RESULTS

Soaking of the plant materials from the root and stem barks of *Harrissonia abyssinica* from Zone I and Zone II resulted into different extract yields (Table 1). In general, the yields were higher for the ethanol extracts especially those from the roots (Table 1). Toxicity studies against brine shrimp larvae for the ethanol extracts from the root barks collected

from Zone II and dichloromethane extract of the stem bark materials collected from Zone I showed moderate activity (LC_{50} values ≥ 30 µg/mL) which were significant different ($p > 0.05$) to the rest of the extracts and the control (Table 1) with the cytotoxicity of the ethanol extract of the root bark from Zone I (LC_{50} = 6.75 µg/ml) been high.

Figure 2 shows the proportion of mosquito larvicidal effectiveness of each extract. It was observed that the dichloromethane and ethanol extract of the

Table 1: Percentage yield and brine shrimp lethality results of the eight extracts from *Harrisonia abyssinica.*

Plant part	Solvent used	Zone I			Zone II		
		Yield (%)	LC_{50} (µg/ml)	95% CI (µg/ml)	Yield (%)	LC_{50} (µg/ml)	95% CI (µg/ml)
Stem bark	Dichloromethane	0.60	60.62	49.79, 72.85	0.65	67.85	55.94, 82.67
	Ethanol	3.25	74.50	58.73, 93.87	3.18	74.11	62.08, 88.71
Root bark	Dichloromethane	1.76	30.42	18.98, 41.79	1.11	150.35	128.76, 180.67
	Ethanol	5.58	6.75	5.69, 7.94	7.01	30.87	21.83, 40.14

LC_{50} values are significant at 95% confidence level, lower and upper confidence limits which coincides are not significantly different.

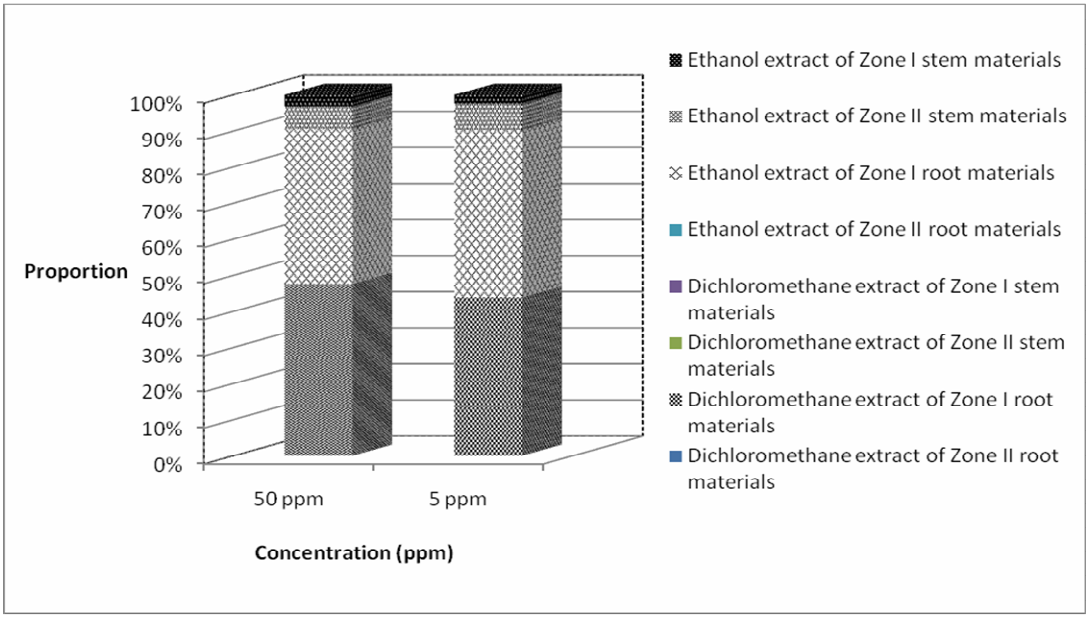

Figure 2: Proportional of mosquito larvicidal effectiveness of the eight extract from the stem and root bark of *Harrissonia abyssinica* from Zone I and Zone II at 5 and 50 ppm.

root bark plant materials collected from Zone I had almost same effectiveness and pre-dominated other extracts (Figure 2). Detailed analysis of mosquito larvicidal results of the eight extracts showed a dose dependent ($p>0.05$) trend. In 24 h, the dichloromethane and ethanol extract of the root bark from Zone I achieved mortality of 90% and 100%, respectively, at 50 ppm. Likewise at 5 ppm the two extracts were having 60% and 58% mortality, both been not significant different ($p>0.05$) but significant different ($p<0.05$) to the rest of the extract and the control (Figure 3). None of the stem bark extracts from either Zone I or Zone II was effective as larvicidal agent (Figure 3). Due to this observation, about 2 mg of each extract in 10 mL of methanol was made and the resulting solution used for spotting in a thin layer chromatography (TLC). The resulting pattern of compounds eluted with petroleum ether and ethyl acetate (9:1 v/v) indicated clearly that, extracts from the root bark of the plant materials collected from Zone I contained two more compound which were lacking in the rest of the extracts (Figure 4). Follow-up isolation of these compounds resulted into two known limonoids, harrissonin (**1**) and pedonin (**2**) (Figure 5) whose NMR data were in agreement with previous reports (Hassanali et al., 1987; Rugutt et al., 2001; Kiprop et al., 2007; Rajab et al., 1997).

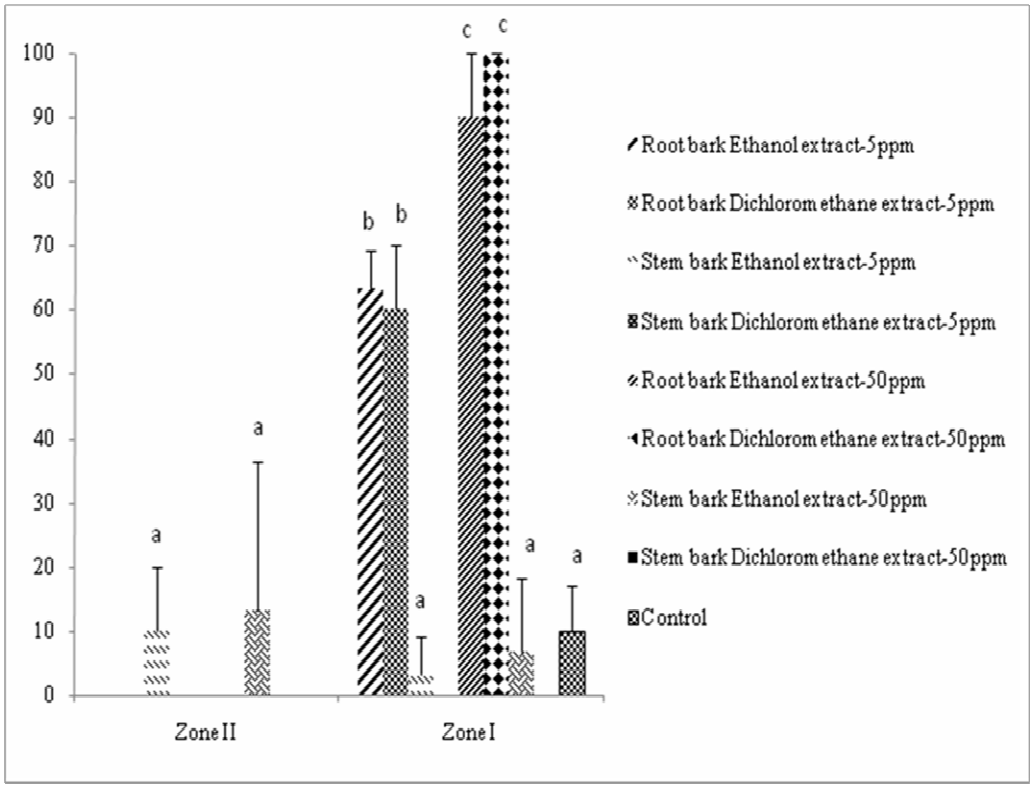

Figure 3: Mean percentage mortality (±SE) of the eight extract from the stem and root bark of *Harrissonia abyssinica* from Zone I and Zone II at 5 and 50 ppm. Columns with the same letters are not significantly different at $p < 0.05$.

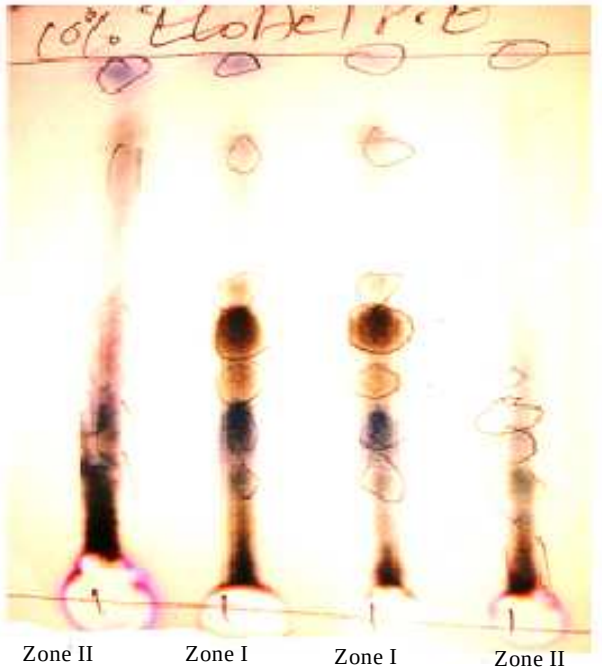

Figure 4: Thin Layer Chromatograph fingerprint of the compounds from the root barks of *Harrisonia abyssiniaca* from Zone I and Zone II.

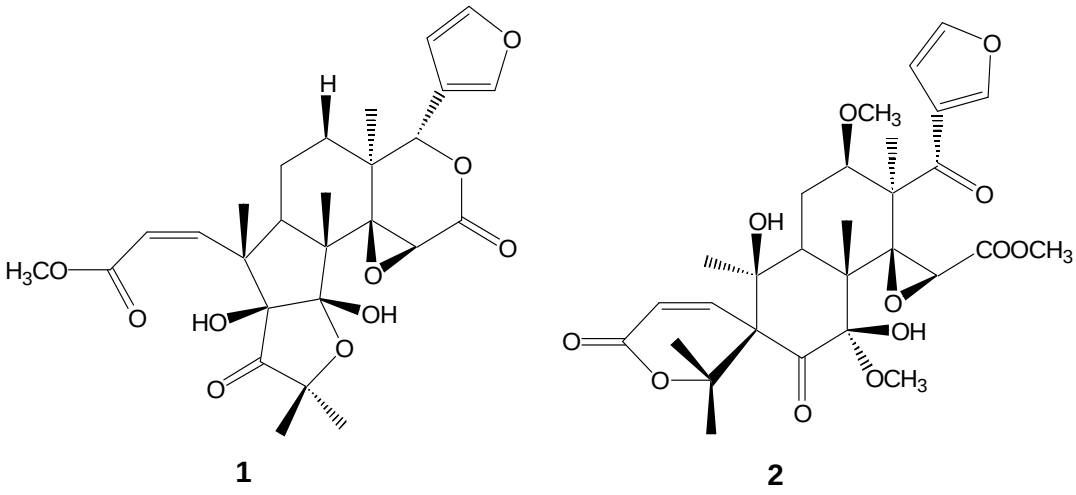

Figure 5: Chemical structures of harrisonin (1) and pedonin (2) from the root barks of *Harrisonia abyssinica* from Zone I.

DISCUSSION

Chemical and biological studies of the extracts from *Harrissonia abyssinica* indicates variations due to the place of collection and plant part studied. This phenomenon may be attributed to ecological variations between the two places of collection, and also to the distribution of compounds within the plant species. Previous reports showed that steroids predominate in the stem barks (Balde et al. 2000) while the root barks are concentrated with limonoids (Rugutt et al., 2001; Kiprop et al., 2007; Rajab et al., 1997). Harrissonin (**1**) and pedonin (**2**) have been established to exhibit antifeedant activity against *Spodoptera* (Hassanali et al., 1986) and larvicidal activity against *Aedes aegypti* (Kiprop et al., 2006). Compounds **1** and **2** are the medium polar limonoids, thus they appeared in the dichloromethane and methanol extracts (Figure 4). Previously, Meliaceae limonoids in extracts of medium polarity fractions were associated with synergistic effects of mosquito larvicidal activity against *An. gambiae s.s* (Ndung'u et al., 2004). The cytotoxicity activity of *Harrissonia abyssinica* extracts agree with other reports which show moderate anti-cancer activity (Masele and Nshimo, 1995; Anani et al., 2000; Kamuhabwa et al., 2000). Similarly, limonoids isolated from *Melia azedarach*, *Melia toosendan* and *Azadirachta indica* exhibited cytotoxic and anticancer activity (Roy and Saraf, 2006) but were free of any toxic effects in animal models (Roy and Saraf, 2006; Jacob et al., 2000). This suggests more research be done to guide the exploitation process of bioactive components in *Harrissonia abyssinica* growing in different ecological areas in order to have viable usefulness of the plant in mosquito control and medicinal applications.

Conclusions

Proper exploitation of *Harrisonia abyssinica* can lead into useful phytomedicine to be used in traditional medicine and mosquito control. In this regard, the presence of the two compounds in *Harrisonia abyssinica* extracts from Zone I warrant its use in crude form as well as pure compounds in the control of immature mosquitoes. Furthermore, difference in plant constituents from the two zones, suggest the important of keeping pharmacopoeias of important medicinal plants from our regions.

ACKNOWLEDGEMENTS

This study was supported by SIDA-SAREC research funds from the Directorate of Research and Publication, Muhimbili University of Health and Allied Sciences. The authors would like to thank Mr. H.O. Suleimani for identification and authentification of the plant materials.

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