SCREENING OF SOME MEDICNAL PLANTS OF ETHIOPIA FOR THEIR MOLLUSCICIDAL ACTIVITIES AND PHYTOCHEMICAL CONSTITUENTS

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Abstract

Schistosomiasis infection is on the rise in Ethiopia, affecting a substantial portion of the productive force. The transmission and life cycle of the schistosome parasite is effected between the molluscan intermediate host and the definitive host, i.e. man. Medicinal plants with molluscicidal properties have paramount importance for the local control of snails. This study was focused on the preliminary phytochemical and laboratory investigation of the molluscicidal properties as well as evaluation of the acute toxicity of the aqueous extracts of 19 different medicinal plants belonging to 23 families on mice. The effect of the aqueous extracts of 19 medicinal plants on experimental snails, *Biomphalaria pfeifferi*, *Bulinus sp*. and *Physa acuta* were evaluated. Portions of the same extracts were used for the identification of the major class of secondary metabolites. Determination of the LD_{50} of the extracts also carried out. Out of the tested plant extracts belonging to these plant families, four plants, *viz*., *Albizia gummifera, Balanites aegyptica, Hedera helix and Warbrugia ugandensis* exhibited promising molluscicidal activities against *Biomphalaria pfeifferi, Bulinus sp.* and *Physa acuta.* The chemical profile of the crude aqueous extracts showed the presence of some secondary metabolites *viz*., polyphenols, alkaloids, tannins, saponins and glycosides. Acute toxicity studies of the promising plant on mice showed medium lethal dose (LD_{50}) values ranging from 150 mg per Kg – 450 mg per Kg when the aqueous extracts were administered intraperitonealy. The crude extracts of the plants demonstrating stronger molluscicidal effect and safety on non-target organism stresses the need for extended laboratory and field evaluation, which could then be employed to play an important role in schistosomiasis control.

Keywords: Molluscicidal, Medicinal plants, *Biomphalaria pfeifferi, Bulinus sp., Physa acuta*.

1. Introduction

Vector-borne infectious diseases are among the major causes of public health problems in Ethiopia as elsewhere in developing countries. Among these, endemic schistosomiasis, affects over 200 million people in about 70 tropical countries (1). Intestinal schistosomiasis, caused by *Schiostosoma mansoni*, and the urinary form, caused by *S. haematobium* are endemic in Ethiopia with a wider distribution of the former than the latter (2, 3). With the construction of dams and expansion of irrigation based agriculture schemes and population movements, the incidence of schistosomiasis infection is on the rise in Ethiopia, affecting a substantial portion of the productive force, that in turn affects the economy (4 – 6).

Transmission and life cycle of the schistosome parasite is effected between the molluscan intermediate host and the definitive host, i.e. man. The involvement of the intermediate host in the development of the parasite apparently exuberates the complexity of the effort required in the control and prevention of the disease. This situation, therefore, necessitates further integration of all possible approaches including chemotherapy and snail control by the application of anti-mollusc synthetic or natural products. The synthetic molluscicides such as, Bayluscide^{R} (Niclosamide), have proved most effective and widely used for focal application (7).

How ever, the ever mounting cost of imported synthetic molluscicides coupled with increasing concern over the possibility of snail resistance to these compounds and their toxicity in non-target organisms have triggered the search for alternative plant-derived molluscicides (8-10). Therefore, to overcome such problems, a number of plants have been tested even though only a few were phytochemically examined (11).

The use of plants with molluscicidal properties on the other hand, is generally considered to be simple, inexpensive and appropriate technology for local control of snail vector. Thus far, several studies have been carried out to identify potential natural products against the snail intermediate hosts. In Ethiopia, studies have been limited to only a few plants despite the existence of a variety of traditionally claimed medicinal plants. Of special interest in this regard is the locally grown plant, *Phytolacca dodecandra* L'Heritz (Endod). Berries of *P. dodecandra* have been extensively studied and proved effective against molluscs in Ethiopia and elsewhere (12, 13). Though quiet effective, some limitations have been observed with the use of the berries. Its untoward effect on non-target organisms such as on fish and limited growth and cultivation area for the most potent variety are cited to hinder its wider applications. Further insight into different medicinal species is thus deemed necessary in increasing the options of finding better alternative plants with molluscicidal activities. Previous studies of some of the plants reported in this study revealed anti-bacterial, anti-fungal and larviciadal activities (14), and some of the plants also found to be distributed in schistosomiasis endemic area. In the present investigation 19 plants employed in the indigenous health care system were screened for their molluscicidal properties and for the class of compound(s) they accumulate.

2. Materials and Methods

2.1 Plant material

2.1.1 Collection and identification:

The plants or parts thereof used in this study were collected between February and May, 2005 from several sites of Ethiopia in the wild at altitudinal range of 900 – 3900 m. They were identified by a taxonomist using standard Flora, and voucher specimens were deposited in the Herbarium of the Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa.

2.1.2 Extract preparation and phytochemical screening:

Air dried and powdered plant material (250 - 500 g) was macerated in water for 2 hours in a shaker. The filtrate was lyophilized to give (as a percentage of powdered plant material) 3 – 6 % amorphous powder. The extracts were kept in tightly stoppered bottle in a desicator at room temperature until required for molluscicidal testing. Portion of the same extract that was used for molluscicidal activity was used for the identification of the major class of secondary metabolites by employing the methodology out lined by Marini-Bettolo, *et al*., 1981 and Harborne, 1984 (15, 16).

2.2 Laboratory assessment of molluscicidal effect

2.2.1 Experimental snail collection

The experimental snails, *Biomphalaria pfeifferi*, intermediate host of *S. mansoni*, *Bulinus sp*. and *Physa acuta* were collected in Wonji, about 110 Km southeast of Addis Ababa, the capital city from water courses of an irrigation scheme that has not been treated with molluscicide. The snails were identified in accordance with standard identification keys (17, 18), and were left to acclimatize to laboratory conditions in aquaria at a temperature of 25 - 28 ^{O}C for 4 - 5 days before testing.

2.2.2 Molluscicidal testing

The WHO working guideline and the standard test procedures were followed for the evaluation of the plant molluscicides (19, 20). A similar snail size of about 6 – 8 mm in diameter as in the case of *B. pfeifferi* and shell height of the *Bulinus sp*. and *P. acuta* were used for the experiment. From a stock solution of 100 ppm, dilutions of 1, 5, 10, 20, 40, 60, 80 and 100 ppm were prepared for each crude plant extract in one liter. Each concentration contained five snails in four replicates in a container of 300 to 500 ml capacity, amounting to twenty experimental snails per concentration and ten more snails in a separate de-chlorinated water that served as control. In total, 160 experimental and 10 control snails were used to test each plant extract. The snails were then exposed for 24 hours at room temperature in the extract solution. They were, then, washed four to five times in de-chlorinated water and kept in extract free water for another 24 hours recovery period. Mortality was determined by the absence of movement, failure of the flesh portion to withdraw into the shell upon mechanical stimuli (probe) and at times when bleeding was observed.

2.3 Determination of LD_{50} on mice

The study was conducted in Swiss albino mice (25 – 30 g body weight). The animals were divided into four groups each containing 6 mice of both sexes (3 males, 3 females). The extract was administered by intragastric and intraperitoneal route starting from smaller to higher doses in 1ml of vehicle. Records of mortality and manifestation of toxicity were made during 24 hrs. Based on the mortality rate, probit values, the LD_{50} was determined as described by McLeod (1976) (21).

2.4. Statistical analysis:

Mortality rates were compared between experimental and control group for different plant extracts using X^2-square test. Mortality rates also compared across all the 19 species for those plant extracts which showed significant effect using same statistical procedure. A P- value < 0.05 was considered as statistically significant. Lethal doses for 50% and 90 % (LD_{50} and $LD_{90)}$ were computed for those plant extracts which demonstrated significant effect.

3. Results

Locally grown 19 different medicinal plants belonging to 23 families were collected and the extracts of their various parts were tested for molluscicidal activities. The assay of the investigated plant species were carried out using different concentrations of the aqueous extracts. The mortality rate on the experimental snails in comparison with the control group is shown in Table 1 and 2.

The highest value that killed 90 % of the snail population (LD_{90}) ranged from 24 ppm to 70 ppm for the same plant species, respectively. *B. pfeifferi* was found to be the most susceptible as demonstrated by the highest mortality rate by all the four species mentioned above (Table 2). The molluscicidal activity of the aqueous extracts of the various plant parts considered in this study appears to be due to the presence of alkaloids, tannins, polyphenols, saponins and terpenes (Table 1).

Table 1 Molluscicidal effects of traditionally used medicinal plants aqueous extracts.

Coll. No.	Species	Vernacular name	Parts used	Class of compounds	Mortality Rate (95%CI)		Traditional use
					Experimental	Control	
GL-2024	*Gardenia lutea* Fres. (*Rubiaceae*)	Gambilo	Sb	C, D, E	0.6 (0.0-1.5)	1.7 (0.0-5.8)	Ant, Syp, Swt
BA-2130	*Bersama abyssinica* Fresen. (*Melianthaceae*)	Azamir	Rb	B, C, E	4.0 (1.4-6.7)	3.8 (0.0-8.0)	Syp, Asc, Dys
AG-2006	*Albizia gummifera* (JF.Gmel.) C.A.Sm. (*Leguminaseae*)	Ambabesa, Sesa	Sd	A, C, E, F, G	52.5(32.6-72.4)8***	2.5 (0.0-5.6)	Scb
HH-2038	*Hedera helix* L. (*Araliaceae*)	Ivy	Fr	C, E, F	57.3 (38.8-75.7)***	6.3 (1.5-11.0)	
"	" "	"	Lv	C, E, F,G	53.1 (32.8-73.4)***	7.1 (4.2-10.0)	
CM-1194	*Croton macrostachyus* Hochst. (*Euphorbiacieae*)	Bisana	Fr	A, C, E, G	1.0 (0.0-2.0)	1.9 (0.0-4.0)	Lep, Ved, Skd, Mal An, Gon, Rgw, Syp, Ltb
AC-2070	*Acacia nilotica* (L) Wild. Ex Del. (*Leguminoseae*)	Wangegea	Sd	C, D, E	2.8 (0.4-5.2)	1.9 (0.0-4.8)	Dia, Cgh

Table 1 Cont.

Coll. No.	Species	Vernacular name	Parts used	Class of compounds	Mortality Rate (95%CI)		Traditional use
					Experimental	Control	
CH-2022	*Clematis hirsuta* Perr. & Guill. (*Ranunculaceae*)	Azo-hareg	Ar	C, F	0.6 (0.0-1.5)	1.9 (0.0-4.8)	Wod, Gon
SL-2028	*Securidaca longepedunculata* Fres. *(Polygalaceae)*	Etsemenahe	Sd	C, F, G	13.1 (0.1-26.1)	10.6 (1.0-20.2)	Gpn, Lep. Syp, Ltb, Cgh
CA-2012	*Calpurnia aura* (Ait.) Benth. (Leguminosae)	Degita, Cheka	Sd	A, C, E, G	1.9 (0.5-3.2)	3.1 (0.0-6.3)	Dys, Hyp
BA-2054	*Balanites aegyptica* (L.) Del. *(Balanitaceae)*	Bedana	Fr	C, E, F, G	78.8 (61.2-96.3)***	13.8(4.6-22.9)	Wod
EA-2004	*Entada abyssinica (Leguminosae)*	Ambilta	Sd	A, C, E, F, G	1.2 (0.0-2.8)	1.9 (0.0-4.0)	Hyp, Sch
JC-2037	*Jatropha curcas* L. *(Euphorbinacea)*	Ayderke	Sd	C, D, E, H	1.6 (0.0-3.2)	0.6(0.0-2.0)	Hyp, Men, Gon,
MF-2049	*Milletia feruginea (Leguminosae)*	Birbera	Sd	C, D, E, G	12.5 (6.4-8.6)	6.3 (1.1-11.4)	
Pl-2032	*Plumbago zeylanica* L. *(Plumbugacea)*	Mertese	Rt	C, D, E	10.6 (4.2-17.0)	4.6(0.9-8.3)	Lep, Gon, Syp, Ltb

Table 1 Cont.

Coll. No.	Species	Vernacular name	Parts used	Class of compounds	Mortality Rate (95%CI)		Traditional use
					Experimental	Control	
PS-2061	*Polygonum senegalense* Meisn. *(Polygonaceae)*	Doke arem	Ar	C, D, E, F, G	4.0 (2.1-5.9)	2.0 (0.0-5.1)	Tap
RC-2069	*Ruta chalepensis* L. *(Rutaceae)*	Tena adam	Ar	C, D, E	4.19 (1.0-7.2)	2.7 (0.0-6.0)	Wpc
TB-2103	*Tephrosia vogale (Leguminosae)*		Sd	C, D, E, G	0.0 (0.0-0.0)	0. 0(0.0-0.0)	
WU-2045	*Warburgia ugandensis* Sprague *(Cannaleaceae)*	Muka biftu	Lv	C, D, E	51.7 (27.3-76.0)	5.7 (1.3-10.1)	Cgh
BP-2058	*Bidens pilosa* L. (ompositae)	Yseytane merfe	Ar	C, D, E	1.5 (0.0-3.2)	0.6 (0.0-2.0)	Sta, Hed

Plant Part: Ar = Aerial part, Rt = Root, Fr = Fruit, Sd = Seed, Sb = Stem bark, Lv = Leaves, Wp = Whole plant, St = Stem, Rb=Root bark.
Class of Compounds identified: A = Alkaloids, B = Cardiac glycosides, C = Polyphenols, D = Tannins, E = unsaturated sterol/or triterpens, F = Saponins, G = Glycosides/ and or Carbohydrates.
Traditional Uses: Amb=Amebiasis, Ant=Antihelmintic, Anx = Anthrax, Ane = Anti-emetic, Anf = Anti-fungal, Asc = Ascariasis, Cgh = Cough, Dia = diarrhoea, Dys = Dysentery, Eyd = Eye disease, Gon = Gonorrhoea, Hed = Headache, Hok = Hookworm, Les = Leshimaniasis, Lep = Leprosy, Mal = Malaria, Mas = Mastitis, Mls = Measles, Pne = Pneumonia, Poa = Poison antidote, Rab = Rabies, Rgw = Ringworm, Sch = Schistosomiasis, Skd = Skin diseases, Sot = Sore throat, Sta = Stomach-ache, Swl = Swelling, Syp = syphilis, Tap = Tapeworm, Ltb = Lung Tb, Ton = tonsillitis, Toa = Toothache, Try = Trypanosomiasis, Ved = Venereal disease, Vm = Vermifuge, Wpc = Whooping-cough, Wod = Wound dressing, Hyp = Hypertension, Men= Menorrhegia. *** $p < 0.001$.

Of all the tested 19 medicinal plants, extracts from four species, *viz*., *Albizia gummifera* (JF.Gmel.) C.A.Sm (seeds), *Balanites aegyptica* (L.) Del. (fruits), *Hedera helix* L. (leaves and fruits) and *Warburgia ugandensis* Sprague (leaves) showed significantly high mortality rate ($P < 0.01$) on the two species of experimental snails *viz*., *B. pfeifferi* and *Bulinus sp*. while *A. gummifera* and *H. helix* are also found to be potent on *P. acuta* (Table 2). The relationship between the concentration of the extracts of the four species and percent mortality is also presented in Figures 1- 4.

Fig 1. Mortality (%) of the three snail species after being exposed to the aqueous extract of *Albizia gummifera (seeds)*

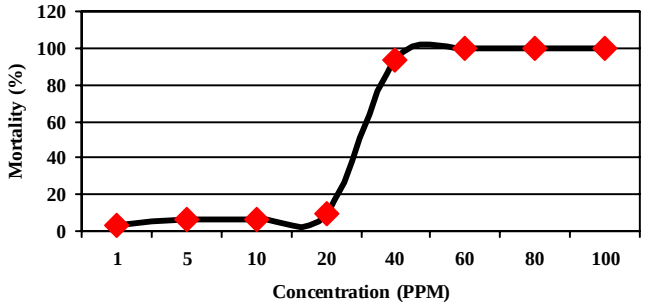
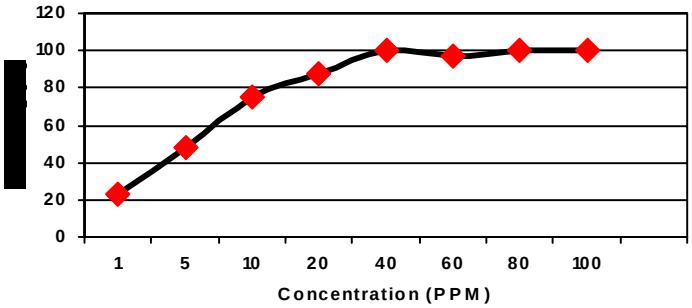
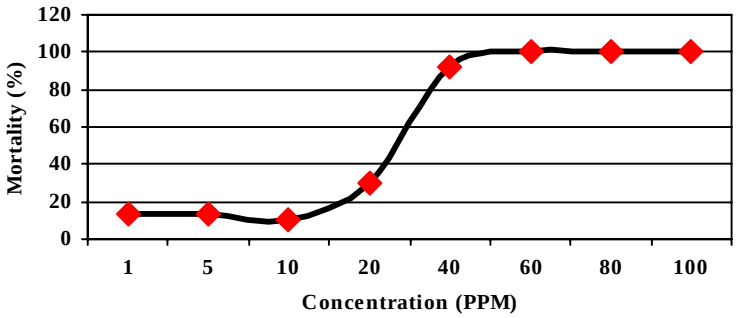


Fig 2. Mortality (%) of the three snail species after being exposed to the aqueous extract of *Balanites aegptica* (fruits)



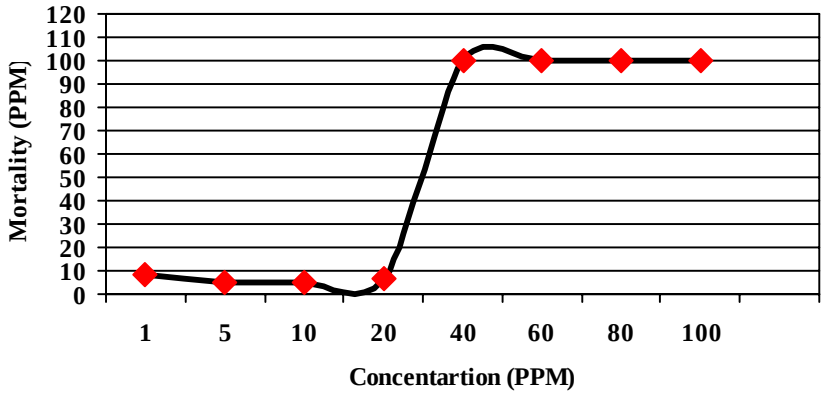
The killing effect of *B. aegyptica* and *W. ugandensis* were significantly higher on *B. pfeifferi* than on the other two snail species ($P<0.05$, $P<0.01$), respectively. The lowest dose that killed 50 % of the snail population (LD_{50}) for the most promising plants ranges from 5.5 ppm for *B. aegyptica* to 30 ppm for *W. ugandensis* (Figure 2 and 4).

Fig 3. 1 Mortality (%) of the three snail species after being exposed to the aqueous extract of *Hedera helix* (seeds)



The most potent extracts were also evaluated for the possibility of toxic effects in mice so as to assess the potability of the water treated with such extracts intended for controlling snails. Thus, the Medium Lethal Dose (LD_{50}) value determined on mice ranges from 150 mg / Kg for *H. helix* (seeds) to 450 mg / Kg for *B. aegyptica* (fruits), when the extracts were administered intraperitonealy. In oral administration of the aqueous extracts of all the promising plants, up to 2.5 g / Kg in mice did not produce either toxicity nor lethality (Table 2).

Fig 3.2. Mortality (%) of the three snail species after being exposed to the aqueous extract of *Hedera helix* (leaves)



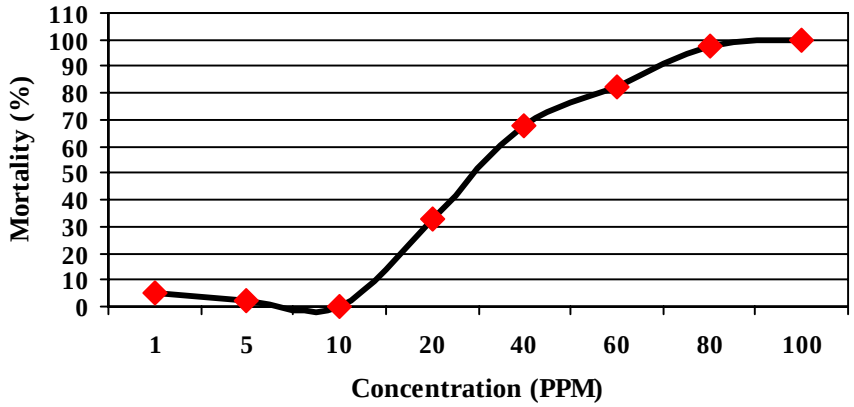
4. Discussion

Snail control using molluscicides is a major component in the control of schistosomiasis. Thus, investigation into plants as potential molluscicides is considered as viable and preferred alternative in the control of the vector snails at the community level. This preference is usually based on merits which focus on the crude extract of a regenerating part or parts of a plant showing an activity at concentrations lower than 100 ppm with low or no toxicity to non-target organisms. Moreover, the plant should be locally available or easily cultivable at local level (22).

In the present preliminary study, at least five aqueous extracts from four different plant species *viz*., *A. gummifera*, *H. helix*, *W. ugandensis*, and *B. aegyptica*, appear promising as they exhibited molluscicidal activities at concentrations ranging from 5.5 to 30 ppm. WHO, 1993 (23) recommends that plant extracts showing LD_{50} values less than 40 ppm may be employed directly against mollusk populations, whilst active extracts may very well provide sources of new lead compounds with molluscicidal activities. Most of the species screened during this study for their molluscicidal activity more or less accumulate similar classes of compounds. Probably higher concentration of some of the constituents, particularly the polyphenols, triterpenes and saponins in the four promising species, therefore, may account for their stronger potency compared to the remaining species investigated.

The present observation with regards to the molluscicidal action of the extract from *H. helix* on our test snails and on *B. glabrata* observed previously was likely due to its triterpenoid saponins cited by (10).

Fig 4. Mortality (%) of the three snail species after being exposed to the aqueous extract of *Warburgia ugandensis* (leaves)



The added advantage of this plant is that the extracts with identical molluscicidal activity could be obtained not only from the fruits but also from its regenerating parts like the leaves. Some of the plants that were investigated in the present study were also reported to exhibit triterpeniod saponins (24). High molluscicidal activity of the extract from the bark of the East African medicinal tree, *W. ugandensis* on *Biomphalaria* snails at a very low concentration of 2 ppm was attributed to sesquiterpenes as reviewed by Hostettsmann (1984) (10). Although some methodological differences could exist, there seems to be an apparent disagreement between the reported lethal dose estimation and that of our observation. In the present study, the LD_{50} and LD_{90} values of extracts from *W. ugandensis* on *Biomphalaria* snails were 30 ppm and 70 ppm, respectively.

From the results of the present investigation, the molluscicidal activities observed for the extract of *B. aegyptica* is of special interest. The LD_{50} and LD_{90} for this plant extract were 5.5 ppm and 24 ppm, respectively, indicating high potential of this plant to serve as a molluscicide. Its killing effect on snails and cercariae including for its medicinal use in the Sudan was also reported previously (25). In this study molluscicidal activities were not observed for *A. nilotica*, which otherwise was proved effective elsewhere against *Bulinus truncatus* at 50 ppm and against *B. pfeifferi* at 75 ppm (26-28). This difference in results may be due to the different parts employed. i.e. seeds but not fruit / pods were used in our study.

The much higher Medium Lethal Dose (LD_{50}) values of the plant species with potent molluscicidal activity determined in mice showed that the plants are safe as far as the potability of the water is concerned. The results of this preliminary laboratory investigation of the indigenous plants showed promising molluscicidal activity on some of them from their regenerating parts at reasonably low concentrations.

The findings stress the need for extended laboratory and field evaluation to determine the optimum conditions of application in the control of snails with out endangering non-target aquatic organisms such as fish, toad, etc.

Table 2. Comparison of mortality rate (in experiment group) by snail species and LD_{50} value of the aqueous extracts in mice

Coll. No	Species	Mortality rate				LD_{50} value in mice	
		B. pfeiffter	*Bulinus Sp.*	*P. acuta*	Average value	Oral route (g / kg body weight)	Intaperitoneal route (mg / kg body weight)
AG -2006	*Albizia gummifera* (seeds)	50.0	51.9	55.6	52.5	2.50	250
BA-2054	*Balanites aegyptica* (fruits)	88.8*	68.8	Not done	78.8	15.0	450
HH-2038	*Hedera helix* (leaves)	65.6	47.5	58.8	57.3	2.50	200
HH-2038	*Hedera helix* (fruits)	56.2	50.6	52.5	53.1	2.00	150
WV-2045	*Warburgia ugandensis* (leaves)	66.4**	38.8	Not done	52.6	2.50	300

**p <0.01 *p <0.05

Acknowledgements

The authors are grateful to the staff of the Wonji Hospital and the traditional medical practitioners in the various regions for their assistance during the collection of specimens in the field work**.** This investigation was financially supported by the Ethiopian Health and Nutrition Research Institute (EHNRI).

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