SCREENING OF SOME ETHIOPIAN MEDICINAL PLANTS FOR MOSQUITO LARVICIDAL EFFECTS AND PHYTOCHEMICAL CONSTITUENTS

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Abstract

Vector borne diseases are among the major causes of illness and death in many developing countries affecting substantial portion of the productive force. Medicinal plants with larvicidal properties have paramount importance for the local control of mosquito. This study was therefore focused on the phytochemical screening and laboratory investigation of the larvicidal properties of the aqueous extracts of 33 medicinal plants belonging to 27 families. The effects of aqueous extracts of 33 plants on laboratory reared *Aedes agypti, Aedes africanus* and *Culex quinquefasciatus* were evaluated using the standard WHO protocol. Portions of the same extracts were used for the identification of the major classes of secondary metabolites. Determination of the LD_{50} of the most active plants extracts was also carried out on mice. Out of the tested 33 plant extracts, five plants, *viz*., *Albizia gummifera* (seeds), *Balanites aegyptica* (fruits), *Hedera helix* (leaves and fruits), *Millettia ferruginea* (seeds) and *Warburgia ugandensis* (leaves) exhibited promising larvicidal activities against *Aedes agypti*, *Aedes africanus*, and *Culex quinquefasciatus,* respectively. Acute toxicity studies of these plants on mice showed medium lethal dose (LD_{50}) values ranges from 150 mg per Kg to 450 mg per Kg when the aqueous extracts were administered intraperitonealy. Phytochemical investigation of the aqueous extracts used for the test revealed the presence of saponins, polyphenols, alkaloids and glycosides as major classes of compounds in most of the plants. The crude extracts of these plants demonstrating stronger larvicidal 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 the control of the larvae of the vectors at their breeding site.

Key words: Larvicidal, Medicinal plants, *Aedes agypti*, *Aedes africanus*, and *Culex quinquefasciatus,*

Introduction

Vector control offers a viable alternative to reduce the spread of vector born diseases. Larvicides offer a better option since it is aimed at killing the larvae or act as a growth inhibitor, before the vector develops into an adult that can transmit all vector borne diseases such as malaria, filariasis, dangue fever, yellow fever, etc. The use of plant derived compounds for mosquito control is appealing. Unlike synthetic insecticides or pesticides, they are biodegradable, economically cheap and environmentally sound alternative control measures. Plants used in traditional medicine or recorded in ethno-pharmacological literature provide a source of information for such investigation. The experimental findings supporting the above information could facilitate or play an important role in vector control programs involving community participation especially at the village level. (1)

There are some reports of medicinal plants with insecticidal activity including ovicidal, anti feedant, larvicidal, repellence from plants belonging to Annonaceae, Papilionaceae, Meliaceae, Mimosaceae and Lamiaceae (2). A number of unsaturated N-(2-methyl propyl) amides with larvicidal activities have been reported from plants in Compositae, Piperaceae and Rutaceae (3).

Plant extracts are also found to be advantageous for field use in mosquito control program as indicated in some studies (3-6). In Ethiopia there are quite a large number of plant species that are traditionally used as insect repellents or insecticides (7). Thus exploring plant species that adopt to a variety of geographical and environmental conditions is believed a worthwhile effort. In the present investigation 33 medicinal plants employed in the indigenous health care system were screened for their larvicidal properties and the class of compound(s) they accumulate.

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 - 2006 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 Department of the Ethiopian Health and Nutrition Research Institute, Addis Ababa.

2.1.2 Extract preparation and phytochemical screening

Air dried and powdered plant material (50 - 250 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 larvicidal testing. Portion of the same extract that was used for larvicidal activity was used for the identification of the major class of secondary metabolites by employing the methodology out lined by (8, 9).

2.2 Larval susceptibility tests

Laboratory reared early fourth instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes africanus* were used through out the experiment. The larval suceptibility tests were carried out according to the standard WHO procedure (10). Test solutions of different concentration were prepared and larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Aedes africanus* mosquitoes were then placed in each test solution to observe the larvicidal property as per the following procedure.

a). Groups of twenty larvae (for each mosquito species) were placed in 250 ml glass beakers containing 200 ml of the extract solution. Same number of larval at the same growth stage were kept in distilled water as a control group.

b). The larvae in each solution were then left for 24 hours, after which they were transferred into distilled water containing larval food for another 24 hours to check for any sign of recovery.

c). Finally mortality was recorded as a function of failure of the larval to swim to the surface or their inabilities as submerge to the bottom in response to mechanical probing. Each treatment was replicated four times.

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 six 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 and probit values, the LD_{50} was determined as described by McLeod, (1976) (11).

2.4. Statistical analysis

Data were entered and analyzed using SPSS version 10 and STATA software version 8. P- value of less than 0.05 was considered statistically significant. Mortality rates of larvae and activity expression (log probit analysis) as effective concentration (LC_{50} and LC_{95}) were compared across all the plant species investigated. Lethal dose for 50% (LD_{50}) was computed in mice for those plant extracts which demonstrated significant larvicidal effect.

Results

A total of 33 locally grown different medicinal plants belonging to 27 families were collected and the extracts of their various parts were tested for larvicidal effects. The assay of the investigated plant species were carried out using different concentrations of the aqueous extracts on three different larval species (Table 1). Of the 33 tested medicinal plants, at least extracts of nine plants showed significant larvicidal effects (≥ 90%) at the cut off concentration of 200 ppm against *Aedes africanus* and *Aedes aegypti*. Eight plant extracts showed ≥ 70% lethality effect at the cut off concentration of 1200 ppm for *Culex quinquefasciatus* (Table 1).

The relationship between the effective concentrations (LC_{50} and LC_{95}) of the extracts of the five most potent plant species and their respective lethal dose values (LD_{50}) in mice is

presented in Table 2. The plants that showed potent larvicidal activity against *Aedes aegypti*, *Aedes africanus*, and *Culex quinquefasciatus* were *Albizia gummifera* (JF.Gmel.) C.A.Sm (seeds), *Balanites aegyptica* (L.) Del. (fruits), *Hedera helix* L. (leaves and fruits) and *Warburgia ugandensis* Sprague (leaves) and *Millettia ferruginea* (Hocst.) Beker (seeds). *H. helix* and *W. ugandensis* showed significantly higher mortality rate ($P < 0.01$) on all the larvae species. The dose that killed 50 % of the larvae population (LC_{50}) for the most promising plants ranged from 28.6 ppm for *H. helix* (fruits) to 92.6 ppm for *M. ferruginea* (seeds) against *Ae. africanus*; 23.6 ppm for *A. gummifera* (seeds) to 146.7 ppm for *H. helix* (fruits) against *Ae. aegypti*; 36.1 ppm for *M. ferruginea* (seeds) to 137.0 for *H. helix* (fruits) against *C. quinquefasciatus* (Table 2).

Table 1 Larvicidal effects of some traditionally used plants of aqueous extracts

Coll. No.	Species	Ver. Name	Parts used	Class of compounds	Activity expressed as effective concentration						Trad use
					LC_{50} (95% CI)			LC_{95} (95% CI)			
					Aedes africanus	*Aedes aegyptia*	*Culex quniqufasciatus*	*Aedes africanus*	*Aedes aegyptia*	*Culex quniqufasciatus*	
AG-2006	*Albizia gummifera* (JF.Gmel.) C.A.Sm. (*Leguminaseae*)	Ambabesa, Sesa	Sd	A, C, E, F, G	80.8 (60.5-106.60	23.6 (20.6-27.4)	69.9 (-3.84-143.3)	159.5 (127.6-227.3)	40.9 (35.2-51.1)	240.3 (158.2-773.3)	Scb
SL-2028	*Securidaca longepedunculata* Fres. *(Polygalaceae)*	Etsemenahe	Sd	C, F, G	103.1 (93.7-113.3)	105.1 (86.4-127.9)	275.9 (227.8-396.7)	167.3 (152.4-188.0)	190.6 (160.4-245.4)	441.9 (344.4-703.7)	Gpn, Lep. Syp, Ltb, Cgh
HH-2038	*Hedera helix* L. (*Araliaceae*)	Ivy	Fr	C, E, F	42.5 (37.5-49.1)	33.1 (24.0-46.0)	135.7 (101.4-192.6)	72.9 (63.2-89.5)	58.1 (45.4-96.8)	255.1 (196.6-426.1)	
HH-2038	*Hedera helix* L. (*Araliaceae*)	Ivy	Lv	C, E, F,G	28.6 (19.9-40.4)	146.7 (25.5-174.7)	137.0 (98.8-208.0)	49.2 (38.3-84.0)	236.4 (201.2-307.7)	263.5 (197.5-486.4)	
EA-2004	*Entada abyssinica* *(Leguminosae)*	Ambilta	Sd	A, C, E, F, G	108.5 (87.6-133.9)	64.2 (34.1-105.6)	166.8 (130.6-244.2)	185.2 (154.5-247.0)	140.4 (101.0-278.7)	307.8 (234.8-529.3)	Hyp, Sch

Table 1 Cont.

Coll. No.	Species	Ver. Name	Parts used	Class of compounds	Activity expresses as effective concentration						Trad use
					LC_{50} (95% CI)			LC_{95} (95% CI)			
					Aedes africanus	***Aedes aegyptia***	***Culex quniqufasciatus***	***Aedes africanus***	***Aedes aegyptia***	***Culex quniqufasciatus***	
BA-2054	*Balanites aegyptica* (L.) Del. *(Balanitaceae)*	Bedana	Fr	C, E, F, G	86.7 (63.2-118.1)	66.9 (38.6-106.6)	111.9 (68.9-177.7)	154.4 (121.8-236.1)	139.6 (102.0-263.9)	219.6 (161.5-438.1)	Wod
BA-2054	*Balanites aegyptica* (L.) Del. *(Balanitaceae)*	Bedana	StBr	C, F, G	84.8 (46.0-140.2)	208.0 (190.8-240.7)	231.5 (202.1-288.2)	182.0 (130.9-373.8)	294.8 (256.4-384.2)	368.0 (305.4-501.4)	Wod
EK-2946	*Echinops kebericho*	Kibercho	Rt	E, H	99.4 (81.6-120.3)	128.5 (117.1-141.2)	275.8 (227.8-396.7)	168.7 (142.7-271.1)	215.8 (195.9-244.0)	441.9 (344.9-703.7)	Mal
MF-2049	*Millettia ferruginea* (Hochst,) Baker *(Leguminosae)*	Birbera	Sd	C, D, E, G	92.6 (64.0-131.6)	89.5 (65.8-120.0)	36.1 (-121.2-102.5)	169.3 130.7-277.2)	172.4 (136.8-253.5)	140.4 (85.2-1064.1)	
AC-2070	*Acacia nilotica* (L) Wild. Ex Del. (*Leguminoseae*)	Wangegea	Sd	C, D, E	108.5 87.6-133.9)	76.6 (41.4-132.6)	190.0 (171.5-217.2)	185.2 (154.5-247.0)	145.2 (103.9-327.2)	281.6 (247.2-340.4)	Dia, Cgh

Table 1 Cont.

Coll. No.	Species	Ver. name	Parts used	Class of compounds	Activity expresses as effective concentration						Trad use
					LC_{50} (95% CI)			LC_{95} (95% CI)			
					Aedes africanus	*Aedes aegyptia*	*Culex quniqufasciatus*	*Aedes africanus*	*Aedes aegyptia*	*Culex quniqufasciatus*	
WU-2045	*Warburgia ugandensis* Sprague *(Cannaleaceae)*	Muka biftu	Lv	C, D, E	76.7 (53.5-107.6)	73.6 (51.0-104)	58.8 (41.0-85.3)	148.6 (115.1-232.9)	150.2 (116.1-234.1)	113.2 (86.4-187.4)	Cgh
TB-2103	*Tephrosia vogale (Leguminosae)*	Yetota atere	Sd	C, D, E, G	102.3 (71.4-143.7)	146.7 (125.5-174.7)	231.5 (202.1-288.2)	209.1 (161.4-332.9)	236.4 (201.1-307.6)	368.0 (305.4-501.4)	Mal
PD-2071	*Phytolacca dodecandra* L'Heritz (Type 44)	Endod	Sd	E, F	223.6 (207.6-1059.5)	237.3	140.0 (110.0-156,6)	269.8 (232.8-2537.3)	287.2	258.4 (205.0-391.5)	Mol
AI-2072	*Azadirachta indica* Juss. (*Maliaceae*)	Neem	Lv	D, E, F	178.2 (169.4-186.8)	249.4 (216.2-360.4)	140.0 (110.0-186.6)	215.3 (203.7-235.5)	356.7 (287.0-613.0)	258.4 (205.0-391.5)	Mal

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, H=Dterepenoid. **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., Mol=Moluscicdal

The value that killed 95 % of the larvae population (LC_{95}) ranged from 49.2 ppm for *H. helix* (fruits) to 169.3 ppm for *M. ferruginea* (seeds) against *A. africanus*; 40.9 ppm for *A. gummifera* (seeds) to 236.4 ppm for *H. helix* (fruits) against *A. agypti*; 113.2 ppm for *W. ugandensis* (leaves) to 263.5 ppm for *H. helix* (fruits) against *C. quinquefasciatus* (Table 2).

In the present investigation, two different plants parts were used for *H. helix* (fruits and leaves) and *B. aegyptica* (fruits and stem bark) to screen and select the most active plant part for future consideration. The larvicidal effect of the fruits and stem bark of *B. aegyptica* were not significantly different against *Ae. africanus* ($p=0.737$) and *C. quinqiefasitatus* ($p=0.09$) but there is significance against *Ae. aegypti* ($p<0.03$). The larvicidal effect of the fruits and leaves of *H. helix* were not significantly different against *C. quinquifasitaus* ($p=0.88$). There is high significance against *Ae. aegypti* for the fruits ($p<0.008$) and against *Ae. africanus* for the leaves ($p<0.003$).

Ae. africanus was found to be the most susceptible as demonstrated by the lowest dose required and highest mortality rate of the larval species by all the five species mentioned above (Table 2). The larvicidal 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). The most potent plant 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 mosquito larvae. Thus, the Medium Lethal Dose (LD_{50}) value determined on mice ranges from 150 mg per Kg for *H. helix* (seeds) to 450mg per 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 2.5g per Kg in mice did not produce either toxicity nor lethality (Table 2).

Table 2 Comparison of the larvicdal efficiency (LC_{50} and LC_{95}) values of active medicinal plants by mosquito larvae species and LD_{50} value of their aqueous extracts in mice

Coll. No	Species	LC_{50} and LC_{95} values						LD_{50} value in mice	
		LC_{50} (95% CI)			LC_{95} (95% CI)				
		Aedes africanus	*Aedes aegyptia*	*Culex quniqufasciatus*	*Aedes africanus*	*Aedes aegyptia*	*Culex quniqufasciatus*	Oral route (g / kg body weight)	Intaperitoneal route (mg / kg body weight)
AG - 2006	*Albizia gummifera* (seeds)	80.8 (60.5-106.60	23.6 (20.6-27.4)	69.9 (-3.84-143.3)	159.5 (127.6-227.3)	40.9 (35.2-51.1)	240.3 (158.2-773.3)	2.50	250
BA-2054	*Balanites aegyptica* (fruits)	86.7 (63.2-118.1)	66.9 (38.6-106.6)	111.9 (68.9-177.7)	154.4 (121.8-236.1)	139.6 (102.0-263.9)	219.6 (161.5-438.1)	15.0	450
HH-2038	*Hedera helix* (leaves)	42.5 (37.5-49.1)	33.1* (24.0-46.0)	135.7 (101.4-192.6)	72.9 (63.2-89.5)	58.1 ** (45.4-96.8)	255.1 (196.6-426.1)	2.50	200
HH-2038	*Hedera helix* (fruits)	28.6* (19.9-40.4)	146.7 (25.5-174.7)	137.0 (98.8-208.0)	49.2 ** (38.3-84.0)	236.4 (201.2-307.7)	263.5 (197.5-486.4)	2.00	150
MF-2049	*Milletia ferruginea* (Seeds)	92.6 (64.0-131.6)	89.5 (65.8-120.0)	36.1 (-121.2-102.5)	169.3 130.7-277.2)	172.4 (136.8-253.5)	140.4 (85.2-1064.1)	2.30	220
WV-2045	*Warburgia ugandensis* (leaves)	76.7 (53.5-107.6)	73.6 (51.0-104)	58.8 (41.0-85.3)	148.6 (115.1-232.9)	150.2 (116.1-234.1)	113.2 (86.4-187.4)	2.50	300

** $p < 0.003, 0.008$; *$p < 0.05$

Discussion

Mosquito larvae control using larvicidal agents is a major component in the control of vector borne diseases. Thus, investigation into plants as potential larvicides is considered as viable and preferred alternative in the control of the mosquito species at the community level. Moreover, the plant should be locally available or easily cultivable at local level (12).

In the present screening study, the aqueous extracts of five plant species *viz*., *A. gummifera*, *H. helix*, *W. ugandensis*, *M. ferruginea* and *B. aegyptica*, appear promising as they exhibited larvicidal activities on 50 % and 95 % larvae population of the tested mosquito species at concentrations ranging from 23.6 to 263.5 ppm**.** Most of the species screened during this study for their larvicidal 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 five promising species, therefore, may account for their stronger potency compared to the remaining species investigated. The present observation with regards to the larvicidal action of the extract from neem plant in our test on *C. quinquifasitaus* did not correspond with that of the previous report (13). Although some methodological differences could exist, there seems to be a difference between the reported lethal dose estimation and that of our observation. In the present study, the LC_{50} and LC_{95} values of extracts from neem plant on *C. quinquifasitaus* was ranging 140.0 to 258.4ppm. This difference in results may probably be attributes to variation in localities or to the different parts employed ***i.e.*** leaves but not kernel were used in our study.

From the results of the present investigation, the larvicidal activities observed for the extracts of *A. gummifera* (seeds), *H. helix* (fruits) and *M. ferruginea* (seeds) are of special interest. The LC_{50} and LC_{95} for these plant extract range from 23.6-40.9, 28.6-49.2 and 36.1-140.4ppm, indicating their high potential to serve as a larvicidal agent against *A. agypti*, *Ae. africanus a*nd *C. quinquifasciatus*, respectively.

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

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

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