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Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Cassia didymobotrya* (Fresenius) Irwin & Barneby against immature stages of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae)

Raja Nagappan

Department of Biology, Faculty of Natural and Computational Sciences, Post Box 196, University of Gondar, Gondar, Ethiopia

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ABSTRACT

Objective: To evaluate aqueous and ethanol extract of *Cassia didymobotrya* leaves against immature stages of *Culex quinquefasciatus*. **Methods:** The mortality rate of immature mosquitoes was tested in wide and narrow range concentration of the plant extract based on WHO standard protocol. The wide range concentration tested in the present study was 10 000, 1 000, 100, 10 and 1 mg/L and narrow range concentration was 50, 100, 150, 200 and 250 mg/L. **Results:** 2nd instar larvae exposed to 100 mg/L and above concentration of ethanol extract showed 100% mortality. Remaining stages such as 3rd, 4th and pupa, 100% mortality was observed at 1 000 mg/L and above concentration after 24 h exposure period. In aqueous extract all the stages 100% mortality was recorded at 1 000 mg/L and above concentration. In narrow range concentration 2nd instar larvae 100% mortality was observed at 150 mg/L and above concentration of ethanol extract. The remaining stages 100% mortality was recorded at 250 mg/L. In aqueous extract all the tested immature stages 100% mortality was observed at 250 mg/L concentration after 24 h exposure period. The results clearly indicate that the rate of mortality was based dose of the plant extract and stage of the mosquitoes. **Conclusions:** From this study it is confirmed and concluded that *Cassia didymobotrya* is having active principle which is responsible for controlling *Culex quinquefasciatus*. The isolation of bioactive molecules and development of simple formulation technique is important for large scale implementation.

1. Introduction

Cassia didymobotrya (*C. didymobotrya*) (syn *Senna didymobotrya*) belonging to the family Fabaceae (Leguminosae) is a widely used medicinal plant in East Africa. It is native plant to tropical Africa found from Congo east to Ethiopia and south to Namibia, Zimbabwe and Mozambigue[1]. In tropical Asia and America these plants were originally introduced as a fodder, green manure and cover crop. However, it is now mainly cultivated as an ornamental plant. These evergreen East African native plants tolerate in full sun even with very little water and blooming with beautiful attractive yellow flowers. In Ethiopia, it is found in dry and moist Kolla and Weyna Dega agroclimatic zones of Arsi, Sidamo, Wolego, Shewa and in the western part of Welo at 1 400-2 400 meter above sea level[2].

C. didymobotrya is a potential medicinal plant and the medicinal values are explored well in many parts of the world by traditional practitioners. In Kenya, traditionally Kipsigis communities were using these plants to control malaria as well as diarrhea. In addition, use to treat skin conditions of humans and livestock infections as well[3]. In Congo, Rwanda, Brundi, Kenya, Uganda, Tanzania, root decoction of these plants was used for the treatment of malaria, other fevers, jaundice and intestinal worm. In addition, root or leaf mixed with water or decoction of fresh parts was used to treat abscess of the skeletal muscle and venereal diseases[4]. The plant is also useful for the treatment of fungal, bacterial infections, hypertension, haemorrhoides, sickle cell anemia, a range of women's diseases such as inflammation of fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion[1, 4]. The antibacterial activities of hexane extract against *Microsporum gypsum* (*M. gypsum*),

*Corresponding author: Raja Nagappan, Department of Biology, Faculty of Natural and Computational Sciences, Post Box 196, University of Gondar, Gondar, Ethiopia.
E-mail: nagappanraja@yahoo.com
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dichloromethane extract against *Trichophyton mentagrophyte* and *M. gypsum* was reported[5]. According to Joji Reddy *et al*[6] presence of phenolic compounds, flavonoids and carotenoids in the ethyl acetate extract of leaves are responsible for pronounced antibacterial activities which are comparable with standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin.

Many species of the plants belongs to the genus Cassia possess potential larvicidial, ovicidal, repellant activities against wide species of immature and adult vector mosquitoes[7-10]. Ojewole *et al*[11] evaluated leaves and stem barks of *C. didymobotrya* against the malarial vector *Anopheles fluviatilis* and reported pronounced lethal effect to early stages. The aqueous extract of stem bark at 1%, 0.1% or 0.01% W/V solution showed potential larvicidal activity. In addition, insecticidal properties are also reported but comparatively lesser than nicotine[12]. Therefore, *C. didymobotrya* is unquestionably potential medicinal plant but there is no much work on their bioactivities against mosquitoes in Gondar area, Amhara region, Ethiopia. The feather likes leaves bearing shrubs or small trees are extensively growing in the study area. Therefore, an attempt was made to evaluate aqueous and ethanol extract of *C. didymobotrya* leaves against filarial vector, *Culex quinquefasciatus* (*Cx. quinquefasciatus*)[13]. It is a vector of lymphatic filariasis, widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation[14].

2. Materials and methods

Larvicidal and pupicidal experiment was conducted in the botany laboratory, Faculty of Natural and Computational Sciences, University of Gondar, Ethiopia from February 2010 to May 2010.

2.1. Plant materials collection and processing

Fully matured dark green leaves of *C. didymobotrya* were collected in and around the vicinity of Tewodros campus, University of Gondar in the month of February 2010. Plant species was identified by verifying the colour pictures followed by description and identification characters[2]. The plant leaves were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade in the botany laboratory for 20 d. The dried leaves were powdered by using electric blender. Finally, fine powder was collected from the powdered leaves by sieving through the kitchen strainer and used for extraction.

2.2. Extraction procedure

Twenty gram of powdered plant material was kept in 200 mL conical flask and added 100 mL of solvent such as water and ethanol individually. The mouth of the conical flask was covered with aluminum foil and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent. Then, extract was filtered by using muslin cloth followed by Whatman no 1 filter paper and finally filtered by using vacuum and pressure pump (AP-9925 Auto Science). The solvent from the extract was removed by using rotary vacuum evaporator RE52 with the water bath temperature of 50℃. Finally, the residues were collected and used for the experiment.

2.3. Test concentrations preparation

Stock solution of 10 000 mg/L concentration was prepared by adding 1 g of plant residue with 5 mL of acetone and make up to 100 mL by adding tap water. From the stock solution 0.1% of soap powder was added for emulsification purpose. From the stock solution 1 000, 100, 10 and 1 mg/L concentration was prepared for wide range of tolerance test. After that narrow range concentration such as 50, 100, 150, 200 and 250 mg/L was prepared and tested against immature stages of *Cx. quinquefasciatus*.

2.4. Maintenance of immature mosquitoes

The immature stages of *Cx. quinquefasciatus* were collected from the stagnant water with rich organic pollution in and around Tewodros campus and also nearby places around University of Gondar. Mosquito larval collection was done from the breeding site by using large kitchen strainer and transferred to large plastic container and transported to the laboratory. In the laboratory the larvae was kept in enamel try with tap water and provided powdered dog biscuit along with yeast powder (3:1 ratio) as a feed. After acclimatization of the mosquito larvae in the laboratory conditions, subsequent experiments were conducted.

2.5. Larvicidal activity

Larvicidal activity of aqueous and ethanol extract of *C. didymobotrya* was evaluated by using WHO method[15]. Twenty five 2nd, 3rd, 4th and pupal stage was released in to 250 mL of glass beaker individually. In each beaker, concentration of aqueous and ethanol extract was maintained at 10 000, 1 000, 100, 10 and 1 mg/L concentration with the final water volume of 200 mL for wide range tolerance test. In control experiment except plant materials remaining all added as mentioned in the concentration preparation. The larval mortality rates were recorded at 24 h exposure period[16]. The dead larvae in five replicates were counted individually and converted in to percentage of mortality. Dead larvae were identified when they failed to move when

the water was disturbed. Based on immature mortality in wide range test narrow range concentration such as 50, 100, 150, 200 and 250 mg/L was prepared and tested like wide range of concentration. Both the experiment was replicated five times and the percentage mortality was calculated. The corrected percentage of mortality was calculated by using Abbott's formula[17].

Corrected % mortality = (% mortality in test − % mortality in control)/(100−% mortality in control) × 100.

2.6. Pupicidal activity

Freshly emerged pupa was used for pupicidal activity. The concentration of plant extract and methods were followed as that of larvicidal activity. For each concentration 25 numbers of freshly emerged pupae were released individually and the percentage of mortality was recorded after 24 h exposure period. The experiment was replicated five times and percentage of mortality was calculated. The corrected percentage pupal mortality was calculated based on Abbott's formula as mentioned in larvicidal activity.

2.7. Statistical analysis

The experimental data was subjected to statistical analysis to derive mean and standard deviation. The significant difference in concentration of the plant extract and exposure period was confirmed by one way analysis of variance (ANOVA). Further individual mean significant difference was calculated by using post hoc Least Significant Difference test by using SPSS software version 16. The mean percentage larval mortality was subjected to probit analysis for LC_{50} and LC_{90}, 95% upper and lower confidence (LCL−UCL) limits and *Chi*−square (X^2) significance by using EPA computer probit analysis software program version 1.5[18].

3. Results

Aqueous and ethanol extracts of *C. didymobotrya* was tested in wide and narrow range of concentrations against immature stages of *Cx. quinquefasciatus*. According to WHO[13] experimental protocol wide range of tolerance test is important to fix the concentration for narrow range test. In wide range test the mean percentage mortality of immature mosquitoes exposed to ethanol extract after 24 h exposure period was presented in Table 1. Results revealed that 2nd instar larvae exposed to 100mg/L and above concentrations showed 100% mortality. Remaining stages such as 3rd, 4th and pupa, 100% mortality was observed at 1 000 mg/L concentration and above. The immature mosquitoes exposed to aqueous extracts was also presented in Table 1. In aqueous extract all the stages 100% mortality was recorded at 1000mg/L and above concentration. Based on this result narrow range of concentration was prepared and tested.

Table 1
Mean percentage mortality of immature *Cx. quinquefasciatus* exposed to wide range concentration of ethanol extract of *C. didymobotrya* after 24 h.

Concentration tested in mg/L	Ethanol extract				Aqueous extract			
	2nd instar	3rd instar	4th instar	Pupa	2nd instar	3rd instar	4th instar	Pupa
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
10	17.60±4.56	12.00±2.82	11.20±3.34	10.40±2.19	5.60±2.19	3.20±1.78	2.40±2.19	2.40±2.19
100	100.00±0.00	74.40±4.56	69.60±6.06	64.80±3.34	73.60±8.29	68.80±6.57	63.20±6.57	65.60±4.56
1 000	100.00±0.00	100.0±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
10 000	100.00±0.00	100.0±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Values are mean percentage±standard deviation of five replications.

Table 2
Mean percentage mortality of immature *Cx. quinquefasciatus* exposed to narrow range concentration of *C. didymobotrya* ethanol and aqueous extract after 24 h.

Concentration tested in mg/L	Ethanol extract				Aqueous extract			
	2nd instar	3rd instar	4th instar	Pupa	2nd instar	3rd instar	4th instar	Pupa
50	44.00±6.32^a	32.00±6.32^a	28.00±2.82^a	27.20±1.78^a	41.20±7.69^a	28.80±3.34^a	26.40±2.19^a	26.40±2.19^a
100	97.60±3.57^b	72.00±5.93^b	67.20±3.34^b	64.00±2.82^b	72.00±8.94^b	69.60±6.06^b	64.80±3.34^b	63.20±1.78^b
150	100.00±0.00^c	80.00±5.21^c	72.00±4.0^c	68.80±3.34^b	82.00±4.00^c	74.40±4.56^c	69.00±4.56^b	67.20±3.34^b
200	100.00±0.00^c	94.40±4.56^d	86.40±4.56^d	84.0±6.32^c	94.40±4.56^d	88.00±4.89^d	84.80±5.93^c	82.40±5.36^c
250	100.00±0.00^c	100.00±0.00^e	100.00±0.00^e	100.00±0.00^d	100.00±0.00^e	100.0±0.00^e	100.00±0.00^d	100.00±0.00^d
F−value	272.39	146.29	328.37	296.28	69.42	196.5	264.17	388.73
LC_{50} (LCL−UCL) (mg/L/L)	52.43 (48.36−56.26)	70.27 (61.67−78.18)	78.84 (37.39−110.01)	82.47 (35.36−117.76)	62.66 (53.26−71.09)	75.71 (37.13−104.6)	82.65 (36.62−117.4)	84.93 (31.69−124.55)
LC_{90} (LCL−UCL) (mg/L/L)	78.17 (71.08−90.55)	167.24 (149.4−192.7)	205.02 (143.0−572.4)	217.97 (147.8−789.2)	164.94 (145.8−193.0)	193.97 (137.5−480.8)	213.42 (145.8−724.2)	227.48 (150.1−1152.2)
Chi−square X^2	0.043	7.571	13.315	15.249	6.766	12.439	15.363	17.328

Values are mean percentage±standard deviation of five replications. F−values are based on one way ANOVA for individual instars. For 2nd instar the value of 200 and 250 mg/L concentrations of *C. didymobotrya* ethanol extract are not included for *Chi*−square analysis. Within the column similar alphabets are not statistically significant by LSD (P>0.05).

In narrow range test mean percentage mortality of immature mosquitoes exposed to ethanol extract after 24 h exposure period was presented in Table 2. Result revealed that in 2nd instar larvae 100% mortality was observed at 150 mg/L and above concentrations. Therefore, results of 50, 100, 150 mg/L concentrations were subjected to calculate LC_{50}, LC_{90} and values. The LC_{50}, LC_{90} and values was 52.42 mg/L, 78.17 mg/L and 0.043, respectively. The results of X^2 values were not statistically significant ($P>0.05$) because the calculated value was lower than table value (3.841). However, in general one way ANOVA followed by LSD showed significant difference (F=273.39; $P<0.05$). The range of 95% confidence limits (LCL-UCL) of LC_{50} and LC_{90} values was 48.36-56.26 mg/L and 71.08-90.55 mg/L, respectively. In 3rd, 4th and pupal stages all those values are greater than 2nd instar stage. The LC_{50} value of 70.27 mg/L and LC_{90} value of 167.24 mg/L was calculated for 3rd instar stage. The value of 3rd stage was not significantly different ($P>0.05$) because the calculated value (7.571) was lower than table value (7.815). However, one way ANOVA followed by LSD showed statistically significant results (F=7.571; F=146.29; $P<0.05$). The LC_{50}, LC_{90} and X^2 value of 4th instar stage was 78.84 mg/L, 205.02 mg/L and 13.315, respectively. In 4th instar stage X^2, one way ANOVA and LSD results showed significant difference at 5% level (F=328.37; $P<0.05$). In pupal stage LC_{50}, LC_{90} and value was 82.46 mg/L, 217.96 mg/L and 15.249, respectively. The comparison of mean percentage mortality of pupal stage, X^2, one way ANOVA and LSD results showed significant difference at 5% level (F=296.28; $P<0.05$).

The result of immature stage mosquitoes exposed to aqueous extract was presented in Table 2. Result revealed that in 2nd instar larvae 100% mortality was observed at 250 mg/L concentration. The LC_{50}, LC_{90} and X^2 values was 62.66 mg/L, 164.93 mg/L and 6.766, respectively. The results of X^2 value was not statistically significant ($P>0.05$) because calculated value was lower than table value (7.815). However, in general one way ANOVA followed by LSD showed significant difference (F=69.415; $P<0.05$). The range of 95% confidence limits (LCL-UCL) of LC_{50} and LC_{90} values was 53.26-71.09 mg/L and 145.80-193.03 mg/L, respectively. In 3rd, 4th and pupal stages all those values are greater than 2nd instar stage. The LC_{50} value of 75.70 mg/L and LC_{90} value of 193.97 mg/L was recorded for 3rd instar stage. The value showed statistically significant difference at 5% level (X^2=12.439; $P<0.05$). In addition, one way ANOVA followed by LSD was also confirmed statistically significant results (F=196.50; P<0.05). The LC_{50}, LC_{90} and value of 4th instar stage was 82.65 mg/L, 213.42 mg/L and 15.363 mg/L, respectively. The statistical comparison of mean percentage mortality of 4th instar stage, X^2, one way ANOVA and LSD results showed significant difference at 5% level (X^2=15.363; F=264.17; $P<0.05$). In pupal stage LC_{50}, LC_{90} and X^2 value was 84.93 mg/L, 227.48 mg/L and 17.328, respectively. The comparison of mean percentage mortality of pupal stage, X^2, one way ANOVA and LSD results showed significant difference at 5% level. (X^2=17.328; F=388.73; $P<0.05$).

4. Discussion

Exploring bioactive medicinal plants in vector and insect pest management program is one of the eco-friendly approaches because they are easily biodegradable in nature. Naturally plants are rich store houses for potential bioactive compounds which are gaining appreciation in recent times among the scientific communities. Any plant species showing promising bioactivity in aqueous extract is a valuable source for the wealth of resource poor communities. According to Berenbaum[19] crude extracts of the plants may have mixtures of active compounds which act synergistically and their overall bioactivity was also greater than individual compounds[20]. In the present study aqueous and ethanol extracts of *C. didymobotrya* was showed promising bioactive potential against immature stages of *Cx. quinquefasciatus*.

Bioactivity of *C. didymobotrya* extract was significantly varied based the solvents used for extraction, concentration of the extract and stage of the immature mosquitoes tested. In this present study IInd instar larva was highly susceptible than late stages. The percentage of mortality was also significantly greater in higher concentration. Irrespective of solvent used for extraction 100% mortality was recorded at 150 mg/L concentration of ethanol extract in 2nd instar larva and the remaining stages 100% mortality was recorded at 250 mg/L concentration. In general, ethanol extract was showed promising result in all the immature mosquitoes. However, aqueous extract was also showed significant result but the concentration of the extract was little greater. When compare to cost effect aqueous extract was far better than ethanol extract. Present findings are in agreement with the report of Ojewole *et al*[11] reported that pronounced larvicidal activity in early stages of *Anopheles fluviatilis* and the larvicidal activity was dependent on concentration and incubation time. In their studies 100% mortality of the larvae was observed at 1% solution of root bark, stem bark and leaf extracts. However, present study 100% mortality was recorded at 250 mg/L concentration of leaves extract. The variation may be due to presence of active principle varied in geographical distribution of plant species and another possible reason *Anopheles fluviatilis* may be highly resistant than *Cx. quinquefasciatus*[21]. Obviously it is true in our study area chemical pesticide utilization in mosquito control program was very meager. Therefore, present study *Cx. quinquefasiatus* may be highly sensitive to exposed plant extracts that may be one of the reasons for higher mortality in less concentration.

In the present study LC_{50} and LC_{90} values of *C. didymobotrya* showed great variation in 2nd instar larvae in ethanol and water extract compared to other immatures. The LC_{50} value of 2nd instar larvae exposed to ethanol extract was 52.42 mg/L and aqueous extract it was 62.66 mg/

L. This variation was attributed with dissolving nature of the active ingredients in respective solvent or the polarity of the solvent was also another important factor to determine dissolving nature of active principles. Many earlier reports confirmed the variation in the percentage of larval mortality in different group of mosquito species. Rajkumar and Jebanesan[8] reported ethanol extract of *C. obtusifolia* was showed LC_{50} value of 52.2 mg/L against 3rd late instar larvae of Anopheles stephensi. Govindarajan[7] reported LC_{50} value of methanol, benzene and acetone extract of Cassia fistula was 8.45 mg/L, 18.27 mg/L and 23.95 mg/L, respectively against *Aedes aegypti*. However, similar solvent extracts of the same plant LC_{50} value of Culex tritaeniorhynchus and Anopheles subpictus was 45.57 mg/L, 48.55 mg/L, 52.17 mg/L and 33.76 mg/L, 36.43 mg/L, 39.01 mg/L, respectively after 24 h exposure period. These earlier findings and current reports clearly indicate the important of solvent used for extraction. In conclusion, present study clearly revealed that leaf extracts of *C. didymobotrya* possessing bioactive compound to control immature stages of *Cx. quinquefasciatus*. However, hypothesis such does the plant extract are effective in natural mosquitoes breeding places need to be addressed. Further isolation of bioactive compound in a cost effective way and to develop simple formulation techniques is very much useful in large scale implementation of these plant extracts in mosquito control program.

Conflict of interest statement

We declare that we have no conflict of interest.

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