Vol. 14(9), pp. 445-450, September, 2020
DOI: 10.5897/JMPR2019.6900
Article Number: 2D4B74164339
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Larvicidal activity of *Juniperus procera* extract against anopheles mosquito in *in vitro*, North Western Ethiopia

Wondimu Ersino[1], Hirpasa Teressa[2]* and Tadele Alemayo[3]

[1]Department of Biology, College of Natural and Computational Sciences, Wachemo University, P. O. Box 667, Ethiopia.
[2]Department of Biology, College of Natural and Computational Sciences, Wolkite University, Ethiopia.
[3]Department of Animal Rangeland and Wildlife Science, College of Dryland Agriculture and Natural Resources, Mekelle University, Ethiopia.

Received 23 December, 2019; Accepted 22 June, 2020

Mosquitoes are the major health threat as they transmit plasmodium parasites that cause malaria. Since mosquitoes developed resistances towards insecticides, investigators are searching for alternative control measures. Bioactive plant chemicals have been identified to serve as a defense mechanism against insects' larvae. Therefore, the aim of the study was to evaluate larvicidal activities of *Juniperus procera* extracts in in vitro against 3rd to 4th instars larvae of Anopheles arabiensis. The leaves of the plant were collected from Fogera District and brought to Bahir Dar University where it was extracted with chloroform, petroleum ether, ethanol and acetone. The phytochemical screening was performed. A. arabiensis 3rd to 4th instar larvae were collected from Woreta town of Fogera District and bioassay was tested on 20 larvae at room temperature. Finally, the percentage mortality of larvae at 24, 48 and 72 h was calculated. The result showed that chloroform, petroleum ether, acetone and ethanol extracts of *J. procera* caused significant mortality of anopheles mosquito larvae (P=0.00). The mortality rate increased with the concentration of the extracts. Therefore, the study concluded that *J. procera* might be an alternative in mosquito control.

Key words: Anopheles arabiensis, *Juniperus procera*, Larvicidal activity, plant extract, solvent.

INTRODUCTION

Malaria is a disease caused by protozoan parasites of the genus *Plasmodium* and transmitted by female Anopheles mosquitoes (Eve et al., 2005). It is a tropical disease with high morbidity and mortality demanding a rapid and comprehensive effort to tackle (Bloland, 2001). According to World Health Organization, the global malaria death in 2017 was estimated to be 435,000. Children under 5 years are the most vulnerable group affected by malaria accounting for 61% of all malaria deaths worldwide. Furthermore, 93% of all malaria death has been reported from Africa alone (WHO, 2018a).

Being one of the tropical Sub-Saharan countries, malaria is a major public health problem in Ethiopia having two epidemiologically important parasitic species: Plasmodium falciparum and Plasmodium vivax which contribute to nearly 69 and 30% of all malaria cases respectively (WHO, 2018b). In Ethiopia, about three-fourth of the land below 2000 m is malarious and two-third of the population lives in areas at risk of malaria (Kassahun, 2004).

*Corresponding author. E-mail: hirpaifet100@gmail.com.

A study carried out in 2016 showed that there were an estimated 2,927,266 new malaria cases in Ethiopia with 4,782 deaths (Tadele et al., 2019). In addition, malaria is the most widespread disease being number one of the top ten diseases that causes high morbidity in the study site (Fogera District). It is highly prevalent in the rainy season mainly between May and July, and at the end of rainy season in October and December (Bake-Migongo et al., 2012).

Anopheles mosquito is the major public health threat as it is the only vector transmitting malaria which causes mortality, morbidity, economic loss and social disruption (Sakthivadivel and Daniel, 2008). Although adversely contaminating the environment, synthetic insecticides are available for controlling these mosquitoes. However, mosquitoes build up hereditary resistance to synthetic insecticides (Singh et al., 2007). The recent WHO report confirmed that malaria vectors developed resistance to the four commonly used insecticides: pyrethroids, organochlorines, carbamates and organophosphates across the world (WHO, 2018a). Since mosquitoes develop resistance towards chemical insecticides, researchers have been searching for alternative control mechanisms (Markouk et al., 2000).

Traditionally, plant products have been used by the people for many years in insect control. Secondary metabolites present in plants serve as defense mechanism against insect attacks and their derivatives provide alternative source in the control of mosquito (Akhtar et al., 2010). Furthermore, plants possess eco-friendly bioactive chemicals that are alternative to control mosquitoes (Raghavendra et al., 2009). Plant based pesticides are less toxic, easily biodegradable and delay the development of resistance because of its new structure (Kamaraj and Rahuma, 2010). Recent researches have proved effectiveness of plant derived compounds including bioflavonoid, saponin, steroids, alkaloids, essential oils and tannins has potential mosquito larvicidies (Vatandoost and Hanafi-Bojd, 2008; Akhtar et al., 2010).

J. procera has traditional importance for malaria treatment in most rural area of Ethiopia (Desta, 1995). Although researchers have tried to test the plant's larvicidal activity and its medicinal value in malaria treatment (Kaliyaperumal et al., 2014; Ismat et al., 2018), they have not studied it in *in vitro*, using different solvents like chloroform, petroleum ether, acetone and ethanol extracts. Therefore, the aim of the study was to evaluate larvicidal activities of *J. procera* extracts in *in vitro* against 3rd – 4th instars larvae of *A. arabiensis*.

MATERIALS AND METHODS

Experimental site

This study was conducted in Fogera District, North western Ethiopia. Fogera is one of the Districts in the South Gondar Zone of Amhara Region and located at 11°58' latitude and 37°41' longitude (FWARDO, 2014). Woreta town is the capital of the District and found at 625 km to the northwest of the capital, Addis Ababa and 60 km to the north of the Regional capital, Bahir Dar. The District has a total area of 117,414 ha, where 44% is cultivated land, 24% is pasture land, 20% is covered by water bodies and the rest are others. The total population of the District is 251,714 from which 220,421 people are known to live in the rural area. The District has 20°C mean annual temperature and 1216.3 mm mean annual rainfall. The area has wider suitable land for agricultural activities and the altitude ranges from 1774 up to 2410 masl (IPMS, 2005).

Experimental design of the research

Experimental design of the research work was arranged in 4x3 factorial randomized completely block design (RCBD) with three replications. 1000 ml distilled water was used for both experimental and control groups.

Plant collection

J. procera leaves were collected from Fogera District and brought to Bahir Dar University, Chemistry laboratory. The plant was morphologically identified using standard keys as stated in Sue et al. (2000). The leaves were washed thoroughly by tap water to remove dusts and unwanted materials, and air dried in dark at room temperature. Finally, the dried leaves were made powder using electrical grind mill and then stored at room temperature (Ncube et al., 2008).

Preparation of plant extracts

Four solvents chloroform, petroleum ether, ethanol and acetone were used for the extraction with polarity gradient method (Ncube et al., 2008). The four aforementioned solvents were used for extraction to see the effect of their polarity differences on the extracts since ethanol and acetone are soluble in water, but chloroform is slightly soluble and petroleum ether is insoluble. Then, 200 g of *J. procera* leaves' powder was soaked in 1000 ml petroleum ether in Erlenmeyer flasks. The soaked crude extract was placed on orbital shaker at 120 rpm for 48 h. The mixture was filtered by cotton and passed through Buchner funnel with Whatman filter paper. Before extracting the residue with other solvent, remains of the first solvent was removed using rotary evaporator. Then the macerated residue was extracted consecutively by the same amount of ethanol, acetone and chloroform using polarity gradient as done for the petroleum. All plant extracts by the aforementioned solvents were filtrated using Buchner funnel with Whatman filter paper. From the above extracts, 200 mg/L, 400 and 600 mg/L solutions were prepared by dissolving the extract in distilled water and introduced into separate labeled bowels. Then, the solutions were stored in a refrigerator at 4°C until larvicidal bioassay test according to WHO standard (Das et al., 2010). Furthermore, phytochemical screening was carried out for each solvent extract as per the standardized method (Audu et al., 2007; Das et al., 2010; Obasi et al., 2010).

Collection and identification of mosquito larvae

Larvae (3rd – 4th instar) of *A. arabiensis* were collected from the stream water near town of Woreta. The collected mosquito larvae were brought to Woreta Agriculture College where morphological identification and larvacidal bioassay was carried out (Theodore et al., 2005). Finally, the larvae were transferred to a molder with tap water and kept in small room.

Table 1. Experimental design of the study.

Candidate plant	Type of solvent	Experimental group					Control group
		Concentration of extract (mg/L)/1000 ml			No. of larvae	No. of replicates	No. of larvae in 1000 ml water with replicates
J. procera	Chloroform	200	400	600	20	3	20 × 3
	Petroleum ether	200	400	600	20	3	
	Ethanol	200	400	600	20	3	
	Acetone	200	400	600	20	3	

Table 2. Phytochemicals screened from *J. procera* extracted by chloroform, petroleum ether, ethanol, and acetone solvents.

Detection of secondary metabolites	Test for secondary metabolites of *J. procera* extracts
Saponins	+
Phenols	+
Alkaloids	+
Carbohydrates	+
Proteins	+
Glycosides	+
Phytosterols	+
Tannins	+

+: shows the presence of secondary metabolites in the extracts.

Larvicidal bioassay

The existing standard was used to evaluate larvicidal efficacy of *J. procera* extracts against anopheles mosquito larvae (WHO, 2005). The bioassay was performed at relative humidity of 70-85%, room temperature, and pH 7.0 of distilled water. Twenty 3rd- 4th instar anopheles larvae were taken by pipette and added to each flask containing 1000 mL distilled water. Flasks with mosquito larvae were treated with 200, 400, and 600 mg/L of all solvent extracts of *J. procera*. Each treatment for experimental group was conducted in three replicates as designed above (Table 1). For control group, twenty 3rd-4th instar larvae were also transferred to 1000 ml distilled water. The larvae of both experimental and control groups were fed an equal amount of sucrose every 24 h. Finally, the effect of the plant extracts on larvae was tested by counting the number of dead larvae. Larvae counting were conducted at 24, 48 and 72 h of treatment and the percentage mortality was calculated. The computed mortality of larvae was adjusted using Abbott's formula (Abbott, 1925) as shown below:

$$\text{Percentage of mortality} = \frac{\text{number of dead larvae}}{\text{number of larvae introduced}} \times 100$$

Data analysis

Data was analyzed using SPSS version 16. Difference in concentration of plant extracts, type of solvents, and time of exposures were used as independent variables and mortality of larvae was used as dependent variable. One way ANOVA was used to determine the significant difference of the mortality of *A. arabiensis* larvae between the control and the experimental group and value of 0.05 was taken as significance level.

RESULTS

In the current study, *J. procera* extracts were tested to have significant secondary metabolites (Table 2). Similar secondary metabolites were screened from *J. procera* regardless of using different solvents in extraction. Accordingly, from *J. procera* extracted by chloroform, petroleum ether, ethanol, and acetone solvents, phytochemicals including alkaloids, proteins, carbohydrates, glycosides, phytosterols, saponins, tannins and phenols were identified.

In the present work *J. procera* extracts were also evaluated to kill Anopheles mosquito larvae. Accordingly, chloroform, ethanol, acetone, and petroleum ether extracts of *J. procera* applied at a concentration of 200 mg/L, 400 mg/L and 600 mg/L showed significant mean *A. arabiensis* 3rd- 4th instars larvae mortality at different time exposure (P=0.00). However, no larval mortality was recorded for the control group within the given time intervals (Table 3). Variations in toxicity of plant extracts against the larvae varied with the solvent used in extraction. Ethanol extracts of *J. procera* was effective against Anopheles mosquito larvae in which it showed linear increasing of mortality rate for *A. arabiensis* larvae than others. Although mortality rate increased with concentrations, it decreased with increased time exposure. The most effective time exposure for mortality of larvae was within 24 h. For all solvents used, maximum mean mortality was recorded for the highest

Table 3. The effect of different solvents extracts of *J. procera* against 20 *A. arabiensis* larvae in *in vitro* with different concentrations and time exposures.

Solvent	Concentration vs. distilled water with time exposure in h for *J. procera* extract				Time (h)	P-value
	Mean ± SD					
	Experimental group			Control group		
	200 mg/L	400 mg/L	600 mg/L	1000 mL		
Petroleum ether	8.66 ±1.11	10.33 ±1.11	12.66 ±1.11	20 ± 0.00	24	0.00
	4.00 ± 0.66	6.33 ±0.88	5.33 ± 0.44	20 ± 0.00	48	
	4.66 ±1.11	3.33±0.44	2.33 ± 1.77	20 ± 0.00	72	
Chloroform	5.00 ± 1.33	12.66 ±1.52	13.33 ± 2.44	20 ± 0.00	24	0.00
	7.66 ±1.11	6.66 ±1.55	5.66 ±1.77	20 ± 0.00	48	
	4.33 ±1.56	0.66 ±0.88	2.00 ±1.33	20 ± 0.00	72	
Acetone	10.66 ± 0.44	12.66 ±1.11	16.00 ±1.33	20 ± 0.00	24	0.00
	6.66 ± 0.45	5.66 ±1.11	3.66 ±0.89	20 ± 0.00	48	
	2.66 ± 0.44	2.00 ±0.67	0.33 ±0.44	20 ± 0.00	72	
Ethanol	6.66 ± 1.78	7.33 ±1.11	15.67 ± 0.4	20 ± 0.00	24	0.00
	2.67 ±0.44	8.33 ±1.56	7.66 ±1.77	20 ± 0.00	48	
	1.67 ±0.44	4.33 ±0.44	5.67 ±1.11	20 ± 0.00	72	

concentration (600 mg/L).

DISCUSSION

In the current study, *J. procera* extracted with chloroform, petroleum ether, ethanol, and acetone solvents had similar phytochemicals including alkaloids, proteins, carbohydrates, glycosides, phytosterols, saponins, tannins and phenols. In agreement with the present finding, other researchers also identified that *J. procera* leaves extracts are a source of phytochemicals such as tannins, phenols, saponins, alkaloids and flavonoids in which alkaloids and phenolics have been reported to be insecticides (Shaalan et al., 2005; Ismat et al., 2018). Moreover, studies identified that plants offer great promise as source of phytochemicals with potential as insecticides that can play crucial role in the control of mosquitoes (Ebong, 2008; Pathak et al., 2018). Therefore, screening secondary metabolites from *J. procera* might have incredible contribution in order to develop new alternative drugs in malaria control.

In the present study *J. procera* extracted with chloroform, ethanol, acetone, and petroleum ether applied at a concentration of 200, 400 and 600 mg/L killed A. *arabiensis* larvae. Kaliyaperumal et al. (2014) also evaluated larvicidal activity of *J. procera* against *A. arabiensis* and suggested that the plant can be the potential larvicidal agent. In agreement with our study, researchers have shown that plant derived compounds have larvicidal activities and can be efficient in insect control (Vatandoost and Hanafi-Bojd, 2008; Akhtar, 2010; Pathak et al., 2018). Thus, *J. procera* which has also been identified by the current work to contain significant plant derived compounds such as alkaloids, glycosides, saponins, phytosterols, phenols, and tannins can be an alternative in mosquito control. Such plant derived compounds are more important than other alternatives since they are less toxic and easily biodegradable in the environment (Kamaraj and Rahuman, 2010).

The extraction yield and biological activity of the plant extracts can be affected by solvents used in extraction due to differences of solubility in different solvents (Ajanal et al., 2012; Barchan et al., 2014). As also reported by Ghosh et al. (2012), since insecticidal effects of plant extracts can vary due to the polarity of solvents used during extraction, ethanol extract of *J. procera* showed linear increase in mortality rate for *A. arabiensis* larvae than others. In agreement with the current work other study also identified the highest phenolic contents from *L. aromatica* using ethanol (Quy et al., 2014). The current work also agrees with the report of Sukumar et al. (1991), which indicated variations in toxicity of plant extracts against the larvae that varied with the solvent used in extraction. Although mortality rate increased with concentrations, it decreased with increased time exposure for unknown reason to the current study. The most effective time of exposure and concentration for maximum mean mortality of larvae was within 24 h and 600 mg/L respectively. In agreement to our work, another study done on larvicidal activity of *J. procera* showed that larval mortality rate is time and dose-dependent

(Kaliyaperumal et al., 2014). Ali et al. (2014) also identified that the total mortality is positively correlated with increasing concentrations.

Conclusion

Phytochemical screening of chloroform, petroleum ether, ethanol, and acetone extracts of *J. procera* showed positive result for alkaloids, carbohydrates, glycosides, phenols, flavonoids, proteins and tannins. *J. procera* extracted with the above solvents showed significant mean mortality of *A. arabiensis* larvae within 24, 48 and 72 h at different concentration (200, 400 and 600 mg/L).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank Debre Tabor University for financing this study. Bahir Dar University, Department of Chemistry is also appreciated for the laboratory materials and chemicals, as well as Woreta Agriculture College for providing the laboratory room.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265-266.

Ajanal M, Gundkalle M, Nayak S (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Science of Life 31(4):198-201.

Akhtar P, Ali M, Sharma PM, Farooqi H, Khan NH (2010). Phytochemical investigation of fruits of *Corylus colurna* Linn. Journal of Phytology 2(3):89-100.

Ali A, Tabanca N, Kurkcuoglu M, Duran A, Eugene K, Blythe A, Khan I, Husnu K, Baser C (2014). Chemical Composition, Larvicidal, and Biting Deterrent Activity of Essential Oils of Two Subspecies of *Tanacetum argenteum* (Asterales: Asteraceae) and Individual Constituents against *Aedes aegypti*. Journal of Medical Entomology 51(4):824-830.

Audu SA, Mohammed I, Kaita HA (2007). Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Science Journal 4(4):75-79.

Barchan A, Bakkali M, Arakrak A, Pagan R, Laglaoui A (2014). The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts. International Journal of Current Microbiology and Applied Sciences 3(11):399-412.

Bake-Migongo C, Catacutan D, Namirembe S (2012). Assessment of the headwaters of the Blue Nile in Ethiopia. ICRAF Working Paper No 149, Nairobi: World Agroforestry Centre.

Bloland PB (2001). Drug resistance in malaria. Geneva: World Health Organization. (WHO/CDS/CSR/DRS/4-25.

Das K, Tiwari RKS, Shrivastava DK (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 4(2):104-111.

Desta B (1995). Ethiopian traditional herbal drugs. Part III: Antifertility activity of 70 Medicinal Plants. Journal of Ethno Pharmacology 44:199-209.

Ebong P, Atangwho I, Eyong E, Egbung G (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* and *Vernonia amygdalina*. Journal of Biochemical and Biotechnology 4:239-244.

Eve W, Suprotik B, Kara H (2005). Is malaria a disease of poverty? Tropical Medicine and International Health 10(10):1047-1059.

Fogera Woreda Agricultural and Rural Development Office (FWARDO) (2014). Annual progress report for the year 2014.

Ghosh A, Chowdhury N, Chandra G (2012). Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research 135:581-598.

IPMS (2005). Fogera Pilot Learning, Woreda Diagnosis and Program Design, Ethiopia.

Ismat H, Mohamed H, Suleiman A (2018). Effect of Acid Extract of Leaves of *Juniperus procera* on Corrosion Inhibition of Carbon Steel in HCl Solutions. International Journal of Electrochemical Science 13:3910-3922.

Kaliyaperumal K, Askual G, Samuel F (2014). Larvicidal efficacy of Ethiopian ethnomedicinal plant *Juniperus procera* essential oil against Afrotropical malaria vector *Anopheles arabiensis* (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 4(1):99-106.

Kamaraj C, Rahuman AA (2010). Larvacidal and adulticide potential of medicinal plant extracts from south India against vectors. Asian Pacific Journal of Tropical Medicine 3:948-953.

Kassahun N (2004). Ethiopia Roll Back Malaria Consultative Mission: Essential Actions to Support the Attainment of the Abuja Targets. Ethiopia RBM Country Consultative Mission Final Report.

Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek, H, Jana M (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. Journal of Ethno Pharmacology 73:293-297.

Ncube NS, Afolayan MG, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African Journal of Biotechnology 7(12):1797-1806.

Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM (2010). Comparative photochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. African Journal of Pure and Applied Chemistry 4(9):206-212.

Pathak M, Kumar R, Kumar MS, Maurya O, Bharati KA (2018). A Review on Traditional Larvicidal Plants. Mintage Journal of Pharmaceutical and Medical Sciences 7(3):1-9.

Quy DD, Artik EA, Phuong LT, Lien HH, Felycia E, Suryadi I, Yi-HsuJu (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic.* Journal of Food and Drug Analysis 22(3):296-302.

Raghavendra K, Singh SP, Subbarao SK, Dash AP (2009). Laboratory studies on mosquito larvicidal efficacy of aqueous and hexane extracts of dried fruit of *Solanum nigrum* Linn. Indian Journal of Medical Research 130(1):74-77.

Sakthivadivel M, Daniel T (2008). Evaluation of certain insecticidal plants for the control of vector mosquitos' viz. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Applied Entomology and Zoology 43(1):57-63.

Shaalan EAS, Canyonb D, Younesc MWF, Abdel-Wahaba H, Mansoura AH (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International 3:1149-1166.

Singh RK, Dhiman RC, Mittal PK (2007). Studies on mosquito larvicidal properties of *Eucalyptus citriodora* Hook (family- Myrtaceae). Journal of Communicable Diseases 39:233-236.

Sue E, Mesfin T, Sebsibe D, Inga H (2000). Flora of Ethiopia and Eritrea. The National Herbarium 2(1):78-598.

Sukumar K, Perich MJ, Boobar LR (1991). Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association 7(2):210-237.

Tadele G, Shumbej T Misgun Sh (2019). Burden of malaria in Ethiopia, 2000-2016: findings from the Global Health Estimates. Tropical Diseases, Travel Medicine and Vaccines 5:11.

Theodore G, Andreadis TG, Thomas MC, Shepard JJ (2005). Identification guide to the mosquitoes of Connecticut: The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, Bulletin No. 966.

Vatandoost H, Hanafi-Bojd AA (2008). Laboratory evaluation of 3

repellents against *Anopheles stephensi* in the Islamic Republic of Iran. East Mediterranean Health Journal 14(2):260-267.
World Health Organization (2018a). World Malaria Report 2018. Geneva. Licence: CC BY-NC-SA 3.0 IGO.
World Health Organization (2018b). Global report on insecticide resistance in malaria vectors: 2010-2016. Geneva.
World Health Organization (2005). Malaria Control Today. Roll Back Malaria Department, World Health Organization, pp. 81-91.