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Research Article

Free radical scavenging and insecticidal activity of *Elaeagnus kologa* Schldl.

K.S. Vinayaka*[1], T.R. Prashith Kekuda [2], H.R. Nethravathi [3], N.B. Thippeswamy [3], S.J. Sudharshan[2]

[1] *Dept. of Studies and Research in Applied Botany, Jnanasahyadri, Shankaraghatta-577451, Karnataka, India*

[2] *Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India*

[3] *Dept. of Studies and Research in Microbiology, Jnanasahyadri, Shankaraghatta-577451, Karnataka. India*

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ABSTRACT

Free radicals contribute to more than one hundred disorders in humans. Mosquitoes are the most important single group of insects acting as vector for many tropical and subtropical diseases. The present study was conducted to screen various solvent extracts of *Elaeagnus kologa* Schldl. for Antioxidant activity by DPPH radical scavenging assay and insecticidal activity against second instar larvae of *Aedes aegypti*. The extracts have exhibited concentration dependent radical scavenging activity. Among the extracts, methanol extract exhibited high free radical scavenging activity. A concentration dependent larval mortality was observed in all the extracts. The larval mortality was recorded as 100% in the case of 15 and 20mg/ml of methanol, ethyl acetate and acetone extracts. The radical scavenging and insecticidal activity could be attributed to the presence of phytochemicals in the solvent extracts.

Keywords: **:** *Elaeagnus kologa* Schldl., Soxhlet extraction, DPPH assay, Insecticidal activity, *Aedes aegypti*

INTRODUCTION

Mosquitoes are the most important single group of insects acting as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and others [1]. The large-scale use of chemical pesticides in agriculture and public health leads to adverse effects such as development of pesticide resistance, frequent pest out breaks, emergence of new pests, pollution and health hazards. In order to search an environmentally safe alternative, scientists considered the pesticides of biological origin (biopesticides) in the place of synthetic insecticides. Throughout history, plant products have been successfully exploited as insecticides, insect repellents, and insect antifeedants [2]. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides [3].

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [4,5]. The synthetic antioxidants like BHA, BHT, gallic acid esters etc., have been suspected to cause or prompt negative health effects. Strong restrictions have been placed on their application [6,7]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits[8]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, the majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins[9]. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds [10]. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts [11]. Flavonoids are a group of polyphenolic compounds with known properties, which includes free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [12].

Elaeagnus kologa Schldl (Syn: *Elaeagnus latifolia* L.), belonging to the family Elaeagnaceae, is a scandent shrub with elliptic obovate leaves with prominent silvery and a few rusty scales below. Flowers are in axillary clusters and silvery in color. Fruits elliptic of oblong, red when ripe and endocarp ribbed, woolly thin. The plant is locally called by the name Hulige hannina gida and is used against cutaneous infections, dropsy, anasarca, venereal sores, deafness and dysentery [13]. In the present study, we have screened various solvent extracts of *E. kologa* for Antioxidant activity by DPPH radical scav-

***Corresponding author.**

K.S. Vinayaka

Dept. of Studies and Research in Applied Botany,
Jnanasahyadri, Shankaraghatta-577451,
Karnataka, India
Tel.: + 91-9986254721
E-mail: ks.vinayaka@rediffmail.com

enging assay and insecticidal activity against second instar larvae of *Aedes aegypti*.

MATERIALS AND METHODS

Collection and identification of plant

Elaeagnus latifolia was collected in the Sharavathi river basin of Central Western Ghats of Karnataka. The plant was authenticated in Dept. of Studies and Research in Applied Botany, Jnanasahyadri, Shankaraghatta and voucher specimen (KU/AB/KSV/237) were deposited in the department for future reference.

Extraction and Phytochemical constituents of solvent extracts

The leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material (200g) was extracted with solvents namely methanol, chloroform, petroleum ether, ethyl acetate and acetone by soxhlet extraction and exhaustively extracted for about 48 hours. The extracts were filtered through Whatman filter paper No. 1 and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator [14]. All the extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites [15].

Screening solvent extracts for Antioxidant activity

The antioxidant activity of solvent extracts and the standard (Ascorbic acid) was tested using DPPH free radical scavenging activity [16,17]. Different concentrations of solvent extracts and standard namely 0.125, 0.25, 0.5mg/ml and 1.0mg/ml were prepared in methanol. 0.002% of DPPH was prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution was mixed with 2ml of different concentrations of plant extract and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity in \%} = \frac{A - B}{A} \times 100$$

(A- Absorbance of DPPH; B- Absorbance of solution of DPPH and extract)

Screening of solvent extracts for Insecticidal activity

Insecticidal activity of solvent extracts was tested on Second instar larvae of *Aedes aegypti* mosquito. The larvae were collected from water stagnated area and identified in the Dept. of Entomology, UAS, Shivamogga, Karnataka, India. The larvae were maintained under suitable temperature and humidity. Different concentrations of solvent extracts (5, 10, 15 and 20mg/ml) were prepared in 10% DMSO and added to sterile labeled beakers containing about 100ml of water. Twenty larvae were placed in each of the beakers containing extracts. A control was kept containing 10%DMSO. After adding the larvae, the beakers were kept in the growth room maintained at room temperature. The larvicidal effect of extracts was determined by counting the number of dead larvae after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. Each test was repeated thrice; the percentage of larval mortality was determined [18].

RESULTS

Table-1: Phytochemical constituents in the methanol extract of *E. kogola*

Phytochemical group	Methanol extract
Tannins	+
Terpenoid	-
Alkaloid	-
Steroid	-
Saponins	+
Flavonoids	+

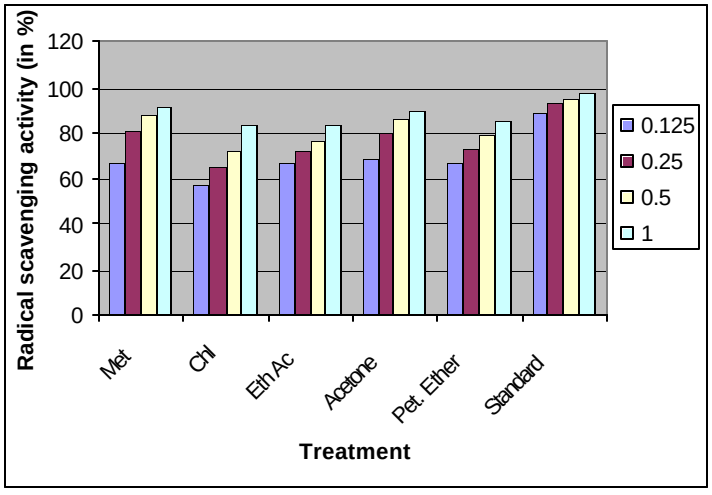
'+' Detected; '-' Not detected

The result of preliminary phytochemical analysis of the methanol extract of *E. kogola* is presented in Table-1. The methanol extract showed the presence of tannins, saponins and flavonoids.

Table-2: Antioxidant activity of solvent extracts of *Elaegnus latifolia*

Solvent extract	Radical scavenging activity (%) of different concentrations of solvent extracts (mg/ml)			
	0.125	0.250	0.500	1.000
Methanol	67.26	80.50	87.40	91.63
Chloroform	57.64	64.60	71.56	82.84
Ethyl acetate	66.30	71.83	76.14	83.43
Acetone	68.60	80.01	85.83	89.59
Petroleum ether	66.04	72.18	79.01	84.98
Standard (Ascorbic acid)	88.69	92.52	95.12	97.33

Fig-1: Radical scavenging activity of solvent extracts and standard by DPPH assay

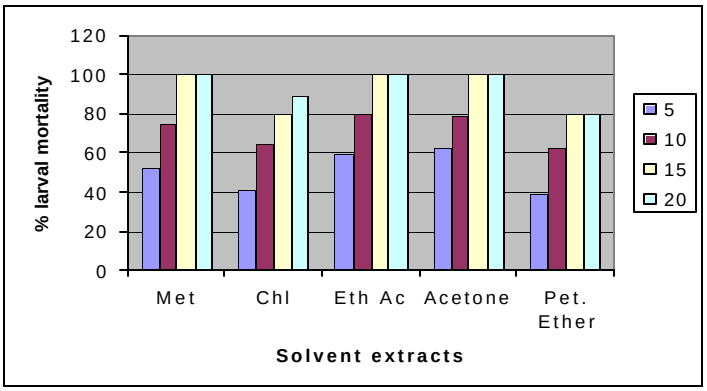


The result of antioxidant activity of solvent extracts is shown in Table-2 and Fig-1. The crude solvent extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extracts have exhibited concentration dependent radical scavenging activity i.e., higher the concentration, more scavenging potential. Among the extracts, methanol extract exhibited high free radical scavenging activity followed by acetone, ethyl acetate, chloroform and petroleum ether extracts. The scavenging activity of standard (ascorbic acid) was greater than that of solvent extracts.

Table-3: Percent larval mortality by different concentrations of solvent extracts

Solvent extract	Larval mortality (in %) by different concentrations of solvent extracts(mg/ml)			
	5	10	15	20
Methanol	53	75	100	100
Chloroform	41	65	80	89
Ethyl acetate	59	80	100	100
Acetone	63	79	100	100
Petroleum ether	39	63	80	80

Fig-2: Percentage mortality of larvae in different concentration of solvent extracts



The percent mortality of the second instar larvae of *A. aegypti* in different concentrations of various solvent extracts shown in Table 2. It is evident from the results that ethyl acetate and methanol extracts were the most effective among all the tested extracts. Concentration dependent mortality was observed in all the extracts. The larval mortality was recorded as 100% in the case of 15 and 20mg/ml of methanol, ethyl acetate and acetone extracts. Among extracts tested, least activity was observed in case of petroleum ether extract which is followed by chloroform extract. All the extracts, at concentration of 5mg/ml, exhibited over 50% larval mortality except chloroform and petroleum ether extracts (Table-3; Fig-2).

DISCUSSION

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [19,5]. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators [20]. The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants [21,22]. DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule [23]. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses color stoichometrically depending on the number of electrons taken up [24]. In this study, the scavenging activity was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Killing larvae of mosquitoes is a successful way of minimizing mosquito densities in breeding grounds before they reach adult stage. It largely depends on the use of synthetic chemical insecticides. But their repeated use has caused environmental problems and widespread development of resistance. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment. It is observed that the carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins are having mosquito larvicidal activity [18]. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever [25]. The larvicidal activity of solvent extracts of *E. latifolia* could be mainly due to the presence of various phytoconstituents.

CONCLUSION

Plant extracts have been used in the control of mosquito borne diseases as the chemical agents have caused some ill effects and also the mosquitoes developed resistance against them. The results of the present study are in justification of this and the extracts of plants selected in this study could be used in control of arboviral infections transmitted by *Aedes aegypti*. In recent years much attention has been devoted to natural antioxidant and their association with health benefits. The antioxidant activity of methanolic extracts could be chiefly due to the presence of various phytoconstituents such as flavonoids, phenolics etc., that play important roles. Thus, the plant could be exploited as the natural antioxidants that could protect the body against damage caused by free radicals.

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