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Research Article

INSECTICIDAL, ANTIBACTERIAL, AND ANTIRADICAL ACTIVITY OF *NICOTIANA PLUMBAGINIFOLIA* VIV. (SOLANACEAE)

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ABSTRACT

Objectives: *Nicotiana plumbaginifolia* Viv. belongs to the family Solanaceae. The present study was carried out to determine insecticidal, antibacterial, and antiradical activity of aerial parts of *N. plumbaginifolia*.

Methods: Extraction was carried out by maceration process using methanol. Insecticidal activity was assessed, in terms of larvicidal effect, against II and IV instar larvae of *Aedes aegypti*. Antibacterial activity was evaluated against a panel of 7 bacteria by agar well diffusion assay. Antiradical activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) free radical scavenging assays.

Results: The extract was found to cause dose-dependent mortality of larvae of *A. aegypti*. The lethal concentration 50 value of extract for II and IV instar larvae was found to be 0.41 and 0.99 mg/ml, respectively. Extract was effective in inhibiting all bacteria. Gram-positive bacteria displayed marked susceptibility when compared to Gram-negative bacteria. *Bacillus cereus* and *Escherichia coli* were susceptible to highest and least extent, respectively. Extract scavenged both DPPH radicals and ABTS radicals dose dependently. Extract scavenged ABTS radicals more efficiently (inhibitory concentration [IC_{50}] value 13.51 µg/ml) when compared to DPPH radicals (IC_{50} value 17.43 µg/ml).

Conclusions: The plant *N. plumbaginifolia* appears to be a promising resource for developing agents with insecticidal, antibacterial, and antiradical activity. The observed bioactivities could be attributed to the presence of phytochemicals which are to be isolated, characterized, and subjected for bioactivity determinations. The plant can be used to prevent arboviral diseases, infectious diseases, and oxidative damage.

Keywords: *Nicotiana plumbaginifolia*, Maceration, Larvicidal, *Aedes aegypti*, Agar well diffusion, Free radical, 1,1-Diphenyl-2-picrylhydrazyl, 2,2-Azinobis 3-ethylbenzothiazoline 6-sulfonate.

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INTRODUCTION

Mosquito-borne diseases are prevalent in many countries across the world and are known to affect millions of people every year. Diseases such as malaria, dengue, yellow fever, and Japanese encephalitis that are transmitted by mosquitoes are known to cause massive mortality and morbidity in human population. Hence, it is very important to prevent and control mosquito-borne diseases. Synthetic insecticides such as organochlorine and organophosphate compounds are extensively used for controlling mosquitoes. However, their extensive use is associated with certain negative health and environmental impacts such as residual content in environment, adverse effects on human health, and other non-target populations. Besides, they are costly, non-biodegradable, and may not be effective as insects readily develop resistance against them. This situation triggered immense interest in scientific community to look for alternative strategies for mosquito control and the use of botanicals as pesticides is shown to be one of the promising strategies [1-8].

Many microorganisms are known to cause diseases in humans and result in considerable morbidity and mortality. Discovery of antibiotics is considered as one of the most important milestones in the field of medicine. Therapy using antibiotics has saved plenty of people from death since discovery. However, the use of antibiotics suffers from several drawbacks among which the development of resistance in pathogenic bacteria (due to overuse and abuse of antibiotics) seems to be most important one. Besides, high cost and possible side effects of antibiotics limit their use. This triggered much interest in scientific community to search alternative strategies for disease therapy. Natural products offer a promising alternative for treatment of infectious diseases. Plants, plant-based formulations, and purified compounds from plants have shown inhibitory activity against a variety of pathogenic bacteria including drug-resistant strains [9-17].

Molecular oxygen is very essential to generate energy and is relatively harmless. However, during aerobic metabolism and exposure to conditions such as drugs, radiations, and pollution, some reactive species are produced from oxygen. These species are termed as reactive oxygen species and include free radicals such as superoxide radicals and hydroxyl radicals and non-radical species such as hydrogen peroxide. Free radicals are chemical species having an unpaired electron in an atomic orbital and are known to be unstable and highly reactive. They are highly reactive and are known to damage proteins, lipids, and nucleic acids. Free radicals are known to cause oxidative damage which is implicated in cancer, aging, cardiovascular diseases, and neurodegenerative disorders. Cells have an antioxidant defense system which includes enzymatic and non-enzymatic system. A balance should always exist between free radical generation and antioxidant defense to have normal health. Interest in natural antioxidants is triggered due to suspected negative effects of synthetic antioxidants. Botanicals are proven to be promising resources of agents with antioxidant potential [12,16,18-21].

Nicotiana plumbaginifolia Viv. (Solanaceae) is an erect, shallow-rooted, pubescent, annual, or perennial herb and is 1-3 m tall. It is commonly called Tex-Mex tobacco and wild tobacco. It grows in damp places near water. It is originated in West Indies and Mexico [22-25]. The plant

is used traditionally as an insecticide and to treat various ailments such as toothache, nausea, wounds, pain, itching, syphilis, and piles [8,23,26-30]. *N. plumbaginifolia* is shown to exhibit bioactivities such as antioxidant [24], antimicrobial [24,31], insecticidal [8], analgesic [23], neuropharmacological [23], and hepatoprotective [32] activities. The present study was carried out to investigate insecticidal, antibacterial, and free radical scavenging activity of aerial parts of *N. plumbaginifolia*.

METHODS

Collection and extraction of *N. plumbaginifolia*

The plant was collected near Matturu, Shivamogga, Karnataka, during January 2017. The plant was identified by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences. The plant material (aerial parts) was washed to remove extraneous matter, dried under shade and powdered. Extraction of plant material was carried out by maceration process using methanol. In a stoppered container, the powdered material (10 g) was left in methanol (100 ml) for 48 hrs with occasional stirrings. The content was filtered and the filtrate was evaporated at room temperature, and crude extract was obtained [17,33].

Insecticidal activity of *N. plumbaginifolia*

We determined insecticidal activity of extract (in terms of larvicidal potential) against II and IV instar larvae of *Aedes aegypti*. In brief, 20 larvae (II and IV instar) were transferred into flasks containing 50 ml of water with different concentrations of extract 0.0-2.0 mg/ml. The flasks were incubated for 24 hrs. Later, the number of dead larvae in each of the flasks was counted, and the mortality rate was determined using the following formula:

Mortality of larvae (%) = (Number of dead larvae/total number of larvae) ×100. Lethal concentration 50 (LC_{50}) value was calculated using linear regression analysis. LC_{50} value indicates the concentration of extract which is required to cause 50% mortality [4,5].

Antibacterial activity of *N. plumbaginifolia*

Agar well diffusion method was carried out to investigate antibacterial potential of extract against four Gram-positive bacteria, namely, *Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493, *Bacillus subtilis* NCIM 2063, and *Bacillus cereus* NCIM 2016 and three Gram-negative bacteria, namely, *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200, and *Salmonella typhimurium* NCIM 2501. 24 hrs old nutrient broth (HiMedia, Mumbai) cultures of test bacteria were swab inoculated on sterile nutrient agar (HiMedia, Mumbai) plates aseptically followed by punching wells of 8mm diameter using sterile cork borer. 100 µl of extract (20 mg/ml of dimethyl sulfoxide [DMSO]), chloramphenicol (reference antibiotic; 1 mg/ml of sterile water), and DMSO were transferred into respective wells in the inoculated plates. The plates were incubated for 24 hrs at 37°C, and the zones of inhibition were measured using a ruler [17,33].

Free radical scavenging activity of *N. plumbaginifolia*

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Various concentrations of extract and ascorbic acid (6.25-200 µg/ml) were prepared in 1 ml of methanol in clean and dry tubes. To each of the tube, 3 ml of DPPH radical solution was added and the tubes were incubated in the dark for 30 minutes. The absorbance was measured spectrophotometrically at 520 nm. Methanol replacing extract served as control. Radical scavenging potential of extract was determined using the following formula:

DPPH radical scavenging potential (%) = (Ac-At/Ac) ×100, where Ac and At denote the absorbance of DPPH control and absorbance of DPPH in the presence of extract/ascorbic acid, respectively. The inhibitory concentration (IC_{50}) value was calculated using linear regression analysis, and the value obtained indicates the concentration of extract/ascorbic acid required to scavenge 50% of free radicals [17,33].

2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical scavenging activity

ABTS radicals were generated by mixing ABTS stock (7 mM) with potassium persulfate (2.45 mM) and leaving the reaction mixture to stand for 16 hrs in dark. 3 ml of ABTS radical solution was transferred into each of the tubes containing various concentrations (6.25-200 µg/ml) of extract and ascorbic acid. The tubes were incubated for 30 minutes in the dark, and the absorbance of content of each tube was measured spectrophotometrically at 730 nm. Methanol replacing extract served as control. ABTS radical scavenging potential of extract was determined using the formula:

Scavenging potential (%) = (Ac-At/Ac) ×100, where Ac and At denote the absorbance of ABTS control and absorbance of ABTS in the presence of extract/ascorbic acid, respectively. The IC_{50} value was calculated using linear regression analysis, and the value obtained indicates the concentration of extract/ascorbic acid required to scavenge 50% of free radicals [17,33].

RESULTS AND DISCUSSION

Insecticidal activity of *N. plumbaginifolia*

Prevention of mosquito-borne diseases involves various strategies. One of the important methods of prevention is the killing of mosquito larvae in stagnant water. In the present study, we evaluated insecticidal potential of *N. plumbaginifolia* against larvae of *A. aegypti* and the result is shown in Fig. 1. The extract showed dose-dependent mortality of larvae. The extract caused >50% of mortality of II and IV instar larvae at concentration 0.5 mg/ml and higher and 1 mg/ml and higher, respectively. Extract was found to be more effective against II instar larvae when compared to IV instar larvae. The LC_{50} value of extract for II and IV instar larvae was found to be 0.41 mg/ml and 0.99 mg/ml, respectively. In an earlier study, Singh *et al*. [8] evaluated larvicidal potential of leaf extract of *N. plumbaginifolia* against *Anopheles stephensi* and found dose-dependent larvicidal activity. It is clear from the result of present study that extract is more lethal to initial stages of larval development. Similar result was observed in the study of Singh *et al*. [8] in which the leaf extract of *N. plumbaginifolia* exhibited marked larvicidal effect against initial stages of larval development.

Antibacterial activity of *N. plumbaginifolia*

Higher plants are considered to be promising resources of agents with activity against pathogenic microbes including drug-resistant bacteria [17,34,35]. In the present study, we evaluated antibacterial potential of *N. plumbaginifolia* by agar well diffusion assay which is one of the most widely used *in vitro* assays to determine antibacterial potential of plant extracts. In this assay, the presence of an inhibition zone around the well is taken as positive result while the absence of inhibition zone is negative for antibacterial activity [17,36-38]. The extract of *N. plumbaginifolia* was found to exhibit inhibitory activity against all test bacteria with zone of inhibition ranging from 1.0 cm to 1.8 cm. The extract was more effective against Gram-positive bacteria when compared to Gram-negative bacteria. *B. cereus* and *E. coli* were inhibited by extract to highest and least extent, respectively. *S. aureus* and *S. epidermidis* were inhibited to similar extent (zone of inhibition 1.4 cm). Inhibitory activity of extract against *P. aeruginosa* and *S. typhimurium* was similar (zone of inhibition 1.2 cm). Reference antibiotic caused marked antibacterial activity when compared to extract while DMSO did not show inhibition of test bacteria (Table 1). In an earlier study, Singh *et al*. [22] showed antibacterial potential of aqueous extract obtained from leaves of *N. plumbaginifolia* against Gram-positive and Gram-negative bacteria. Recently, Ajaib *et al*. [24] observed potent antibacterial activity in leaf, stem, root, and fruit of *N. plumbaginifolia*.

DPPH radical scavenging activity of *N. plumbaginifolia*

The assay involving scavenging of DPPH radicals was developed by Blois. It is one of the most widely used *in vitro* assays for determining free radical scavenging nature of various kinds of samples including

Table 1: Antibacterial activity of *Nicotiana plumbaginifolia*

Test bacteria	Zone of inhibition in cm		
	Extract	Antibiotic	DMSO
Bacillus cereus	1.80±0.10	3.80±0.00	0.00±0.00
Bacillus subtilis	1.60±0.00	3.66±0.05	0.00±0.00
Staphylococcus aureus	1.40±0.10	3.40±0.10	0.00±0.00
Staphylococcus epidermidis	1.40±0.00	3.10±0.00	0.00±0.00
Pseudomonas aeruginosa	1.20±0.00	2.96±0.05	0.00±0.00
Escherichia coli	1.00±0.00	2.50±0.00	0.00±0.00
Salmonella typhimurium	1.23±0.05	2.80±0.00	0.00±0.00

DMSO: Dimethyl sulfoxide

plant extracts. The assay is simple and cheaper and uses stable, organic, nitrogen-centered free radical which need not be generated as in case of ABTS assay. Substances having the potential of donating hydrogen will convert the purple-colored DPPH radical into a yellow-colored non-radical form DPPHH which can be monitored at 515-520 nm spectrophotometrically [16,17,21,33,39-42]. In the present study, we evaluated radical scavenging potential of extract of *N. plumbaginifolia* by DPPH assay, and the result is shown in Fig. 2. The extract exhibited concentration-dependent scavenging of DPPH radicals with an IC_{50} value of 17.43 µg/ml. A scavenging activity of >50% was observed at extract concentration of 25 µg/ml and higher. When compared to extract, ascorbic acid scavenged DPPH radicals more efficiently with an IC_{50} value of 3.06 µg/ml. The study of Ajaib *et al*. [24] showed concentration-dependent scavenging of DPPH radicals by different parts of *N. plumbaginifolia*. In the present study, the radical scavenging potential by *N. plumbaginifolia* was lesser than that of ascorbic acid; however, it is clear that the extract possesses hydrogen-donating property, and hence, it can act as a free radical scavenger.

ABTS radical scavenging activity of *N. plumbaginifolia*

The assay involving scavenging of ABTS radicals is another popular *in vitro* antiradical assays. Unlike DPPH assay, it requires the generation of ABTS radicals which can be done by reacting ABTS stock with an oxidizing agent such as potassium permanganate or potassium persulfate. Substances with the potential to donate electron will convert blue-green colored ABTS radical solution into a colorless neutral form. The ABTS scavenging activity is widely used to evaluate radical scavenging potential of various plants [17,33,41-47]. In the present study, we determined the antiradical activity of *N. plumbaginifolia* by ABTS assay, and the result is shown in Fig. 3. The extract was found to scavenge ABTS radicals in a dose-dependent manner with an IC_{50} value of 13.51 µg/ml. The scavenging potential of ascorbic acid (IC_{50} value 2.48 µg/ml) was higher than that of extract. Although the extract of *N. plumbaginifolia* exhibited lower scavenging potential when compared to ascorbic acid, it is clear from the result of this study that the extract possesses electron-donating potential, and hence, it can act as a free radical scavenger.

CONCLUSIONS

Plants have been widely used as a remedy against various ailments/disorders. In the present study, we observed antibacterial, insecticidal, and antiradical potential of *N. plumbaginifolia*. In a suitable form, the plant can be exploited for treating infectious diseases, oxidative damage caused by free radicals and for controlling insect vectors which transmit viral infections such as dengue and chickungunya. The observed bioactivites could be related to the phytochemicals present in the plant which are to be isolated, characterized, and tested for their potential as antibacterial, free radical scavenging, and insecticidal agents.

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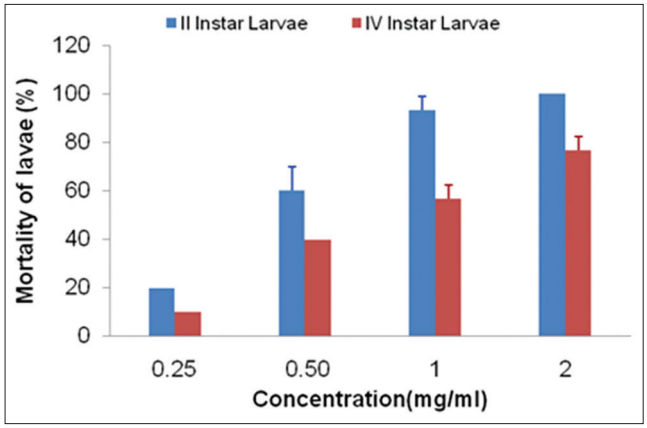

Fig. 1: Mortality of larvae (%) at different concentrations of extract

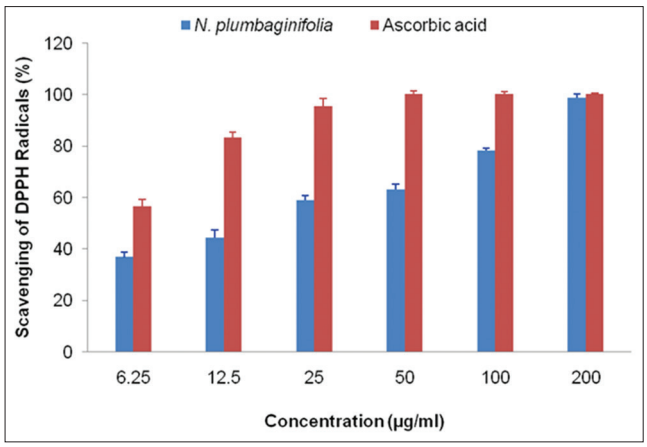

Fig. 2: Scavenging of 1,1-diphenyl-2-picrylhydrazyl radicals by *Nicotiana plumbaginifolia*

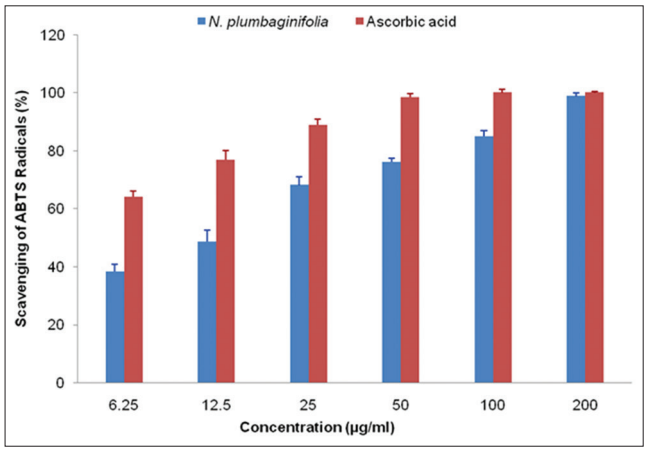

Fig. 3: Scavenging of 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate radicals by *Nicotiana plumbaginifolia*

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