ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL INVESTIGATIONS ON *NICOTIANA PLUMBAGINIFOLIA* VIV. (WILD TOBACCO)

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Nicotiana plumbaginifolia Viv (Solanaceae: Solanales) is a annual or perennial weedy herb that is also known as wild tobacco. Leaves of *N. plumbaginifolia* Viv. were collected air dried and powdered. Aqueous and methanol extracts were prepared and observed their antibacterial activity on five human pathogenic bacteria. Viz *Bacillus cereus, Bacillus fusiformis, Salmonella typhimurium Staphylococcus aureus and Pseudomonas aeruginosa* by paper disc diffusion method. The significant results were obtained by aqueous as well as methanolic extracts of leaves against all the tested bacteria. However, the aqueous extract showed strongest activity on *Bacillus fusiformis* and methanol leaf extract also showed strongest activity on *Bacillus fusiformis* .The leaves of *N.plumbaginifolia* were also evaluated for phytochemicals and were found to contain alkaloids, saponin, tannin, flavonoides, cardiac glycosides, phenolic compounds, steroids, terpenoides and carbohydrates.

Key words: Human pathogens, antibacterial activity, phytochemical and Nicotiana plumbaginifolia.

INTRODUCTION

There are over 2,75,000 species of flowering plants in the world today (Anonymous, 2000). Various plants and their parts have been used by man for the treatment of several diseases, particularly those caused by microorganisms. There is likelihood that all these plants used by the tribal people must have antimicrobial activities. A large number of antimicrobial agents already existing for various purposes have proved ineffective on target microorganisms (Babalola, 1988).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Shrivastava *et al.,* 1996). In the past few years, extensive studies on antimicrobial activity in solanaceae members have been carried out by various workers. *Cestrum diurnum* L. (Bhattacharjee *et al*., 2005), *Capsicum annum* (Cichewicz and Thrope, 1996), *Withania* spp. (Ramji *et al*., 2005) and *Physalis minima* (Shariff *et al*., 2006).

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Plants have been a valuable source of natural products for maintaining human health, especially in the past few decades, with more intensive studies for natural therapies. The use of plant compounds for the treatment of human ailments has gradually increased in India. Among the estimated plant diversity, only a small percentage of medicinal plants have been investigated phytochemically and the fraction submitted to biological or pharmacological screening is smaller. Thus, any phytochemicals investigation of a medicinal plant will reveal only a very narrow spectrum of its constituents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Gerhartz *et al*., 1985; Kroschwitz and Howe-Grant, 1992). These phytochemicals are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, and bactericidal and antimicrobial (Purohit and Mathur, 1999). Thus, it is an urgent need to screen a large number of medicinal plants and their isolated substances for new antimicrobial compounds represent an important potential source for new effective medicines.

Nicotiana plumbaginifolia Viv. (Solanaceae) commonly known a 'Wild Tobacco' is an annual or perennial weed herb with hairy stem, which is originated from Mexico and West Indies. It is found in damp situation by road side. It attains a height up to 60 cm with spreading radical and slender leaf branches. In India its has great medicinal properties because it is antispasmodic, diuretic, expectorant and it is widely used in the treatment of several human ailments like rheumatic, swelling in order to relieve the pain, dried leaves are used in the treatment of nausea and travel sickness.

In the light of above enumerated facts, the present investigations have been carried out on antibacterial activity in aqueous as well as methanol leaf extract of *Nicotiana plumbaginifolia* Viv, against five human pathogenic bacteria, namely *Bacillus cereus, Bacillus fusiformis, Salmonella typhimurium Staphylococcus aureus and Pseudomonas aeruginosa* and also screened for phytochemical evaluation of *N.plumbaginifolia* Viv.

MATERIALS AND METHODS

Collection of plant materials. The fresh leaves of *Nicotiana plumbaginifolia* Viv were collected from various places of Agra (U.P.), India. The leaves were washed under running tap water and shade dried for three weeks. The dried leaves were then homogenized by using a grinder to make fine powder and stored in air tight bottles.

Preparation of aqueous extract. The plant samples were air dried for 48 hours and ground into uniform powder using a grinder. 15 g of dried powder were taken in 250 ml distilled water in separate conical flasks ,air tight with cork and then kept

on a shaker for 8 hours. After it the extract was filtered by using a vacuum filtration system and stored at 4 ^{o}C degree in airtight containers.

Preparation of methanol extract. The collected leaves were washed twice in running tap water and once with sterile distilled water subsequently. The leaves were shade dried for three weeks and made to coarse powder. The powder of leaves was passed through whatman filter No. 40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxhlet apparatus with 85 % methanol solvent. The extract was dried using rotatory vacuum evaporator and they give molten extract and store at 4 ^{o}C until further use.

Microorganism and culture condition. Present investigations were carried out on five human pathogenic bacteria viz. *Bacillus cereus, Bacillus fusiformis, Salmonella typhimurium Staphylococcus aureus and Pseudomonas aeruginosa.* Bacteria cultured were maintained on Muller Hinton (MH) medium .The antibacterial activity was examined for aqueous and methanol leaf extract of *Nicotiana plumbaginifoila* Viv.

Antimicrobial Screening Screening of antibacterial activity was carried out by paper disc method (Gould and Bowie 1952). High media sterile disc was used for activity, saturated disc with the extract (0.04ml) and known quantity of standard reference antibiotic separately was air dried at room temperature. The molten Muller Hinton (hi media) was inoculated with 100 ml of the inoculums and poured into sterile Petri plates (borosil) .The disc with test compound was placed on the upper surface of sterilized Muller Hinton plate that had been inoculated with the test organism (using a sterile swab) and air dried to remove the surface moisture . The thickness of MH medium was kept equal in all Petri plates and the standard disc (tetracycline) was used in each plate as control. The plates were inoculated 24 hours at 37 degree C in incubator. After 24 hours growth of bacteria was measured for its zone of inhibition. The results were obtained by measuring the zone diameter. The experiment was conducted in replicates of 3 and the mean value is presented. The results were compared with the control tetracycline.

Phytochemical Screening Methods. Chemical constituents were checked in aqueous and methanol leaf extracts by standard chemical testing. The tests are as follows:

(a) Test for tannins and phenolic compounds. (ferric chloride test): Few drops of ferric chloride are added to 0.5 ml of test solution in a test tube. Appearance of blue – green color confirms the presence of tannins and phenolic compounds in the sample.

(b) Test for flavonoids: Shinoda test (magnesium hydro chloride reduction test) – To test the solution (0.5-1 ml), few reagent of magnesium ribbon were added and concentrated hydrochloric acid were added drop wise. Crimson red color appears after few minutes it confirms the presence of flavonoids in sample

(c) Test for saponins: (frothing test): About 0.5 ml extract were added in 5ml distilled water. Frothing persistence meant saponins were present in the sample.

(d) Test for proteins: (Ninhydrin test): About 0.5-1 ml of sample was taken in a test tube and it is boiled with 0.2 present solution of ninhydrin. If violet color appears, aromatic proteins are present in the test sample.

(e) Test for steroids and terpenoids: (Salkowski test). About 0.5-1 ml of test solution was treated with chloroform in a test tube. A few drops of concentrated sulphuric acid were added ,shaken well and then wait for sometime appearance of red color at the lower layer which indicates the presence of steroids and formation of yellow layer indicates the presence of terpenoids.

(f) Test for alkaloids (Mayer s test): 0.5-1 ml of sample was taken in a test tube. A few drops of Mayer's reagent were added; it was shaken well and allows standing for some time. Cream color precipitate indicates the presence of alkaloids in the sample.

(g) Test for carbohydrate: (Benedict's test) took 2 ml of Benedict reagent in a test tube. 5 drops of 0.5 percent test solution were added slowly. Reducing sugars gives a red precipitate.

(h) Test for cardiac glycosides (Keller-Killani test): 5 ml of each extract were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. It is treated with conc. H_2SO_4. A greenish color confirms the presence of cardiac glycosides.

RESULTS AND DISCUSSION

The results on antibacterial activity of aqueous as well as methanol leaf extract of *Nicotiana plumbaginifolia* Viv. against five human pathogenic bacteria, namely *Bacillus cereus, Bacillus fusiformis, Salmonella typhimurium Staphylococcus aureus and Pseudomonas aeruginosa* are represented in Tables 1 and 2. The present study demonstrates that aqueous as well as methanol leaf extract exhibit potential antibacterial activity against all the tested bacteria. The highest antibacterial activity of aqueous leaf extract was recorded in a higher concentration (600 ppm) on *Bacillus fusiformis* with a maximum (16.00 mm) zone of inhibition and least activity was observed a lower concentration (200 ppm) on *Bacillus cereus* with (12.00 mm) minimum zone of inhibition. However, methanol leaf extract showed the highest antibacterial activity in a higher concentration (600 ppm) on *Bacillus fusiformis* with (19.50 mm) maximum zone of inhibition and the lowest activity was recorded in a lower concentration (200 ppm) on *Salmonella typhimurium* with (13.00 mm) minimum zone of inhibition.

The extracts of higher plants can be a very good source of antibiotics against various fungal and bacterial pathogens. Plant based antibacterial compounds have enormous therapeutic potential as they can serve the purpose without any side

effects that are often associated with synthetic antimicrobials (Fridous *et al.,* 1990). A large number of species of family *Solanaceae* which grow mainly in the tropical and temperate region are rich in phytochemicals of medicinal values. Some of these plants have great antibacterial activity against human pathogenic bacteria (Maiti *et al.*, 2002).

Table 1

Antibacterial activity of aqueous leaf extract of *Nicotiana plumbaginifolia* Viv. against five human pathogens as tested by disc diffusion assay

Species of microorganisms	Zone of Inhibition (mm)				
	600 ppm	400 ppm	200 ppm	I	II
Bacillus cereus	14.50	15.00	12.00	13.00	0
Bacillus fusiformis	16.00	13.00	14.00	15.00	0
Salmonella typhimurium	15.00	13.75	15.50	14.00	0
Staphylococcus aureus	15.50	12.50	15.00	15.75	0
Pseudomonas aeruginosa	14.00	13.50	14.50	12.50	0

I – antibiotic tetracycline (1 mg/ml)
II – Control (Methanol)

Table 2

Antibacterial acivity of methanol leaf extract of *Nicotiana plumbaginifolia* Viv. against five human pathogens as tested by disc diffusion assay

Species of microorganisms	Zone of Inhibition (mm)				
	600 ppm	400 ppm	200 ppm	I	II
Bacillus cereus	14.50	17.00	49.00	17.75	0

Table 2

(continued)

Bacillus fusiformis	14.20	14.70	19.50	12.50	0
Salmonella typhimurium	13.00	13.50	15.20	15.00	0
Staphylococcus aureus	16.00	15.00	19.20	15.75	0
Pseudomonas aeruginosa	17.00	14.00	19.00	17.50	0

I – antibiotic tetracycline (1 mg/ml)
II – Control (Methanol)

Table 3

Phytochemical screening in aqueous and methanol leaf extract of *Nicotiana plumbaginifolia* Viv.

Phytochemical constituents	Aqueous leaf extract	Methanol leaf extract
Carbohydrates	+	+
Proteins	+	+
Cardiac glycosides	+	+
Tannins	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Saponins	–	–
Steroids	–	–
Terpenoides	+	+
Alkaloids	+	+

(+): Presence
(–): Absence

Besides the antibacterial activity some member of solanaceae like *Capsicum annum* and *Capsicum fruitescences* also inhibit the growth of fungi *Alternaria*

solani and *Saprolegnia parasitica* (Khallil, 2001). Sanches *et al.,* (1997) and Silva *et al*., (1999) reported that the aqueous and ethanolic extract of *Physalis angulata* inhibited the growth of *Staphylococcus aureus* and *Escherichia coli.*

Suffredini *et al.*, (2004) also tested aqueous extracts of Solanaceae members from native of Amazon rain forest and Atlantic forest for antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* following broth micro dilution method and they showed some degree of inhibition of bacterial growth at concentrations of 100 µ/ml.

Nair *et al.*, (2005) also reported that aqueous and ethanolic extracts from some plants used in allopathic medicine are potential sources of antiviral, antibacterial, antitumoral agents.

The phytochemical investigations in *Nicotiana plumbaginifolia* Viv had been studied in aqueous as well as methanol leaf extracts. It is clear from Table 3 that aqueous and methanol leaf extract of *N. plumbaginifolia* Viv were rich in phytochemicals, namely carbohydrates, cardiac glycosides tannins, phenolic compounds, flavonoids, terpenoides and alkaloids; however, these extracts did not showed the presence of saponins and steroids.

Similar results have been reported by Asirvatham and Rangasamydhanabalan, (2008). They showed preliminary phytochemical screening of leaf extracts of *Solanum trilobatum* Linn. According to them, leaf extract revealed the presence of sugar, proteins, alkaloids, terpenoides, saponins, tannins, cardiac glycosides, terpenoides and lipids.

Jawahar *et al.*, (2004) also investigated calcium, iron, phosphorus, carbohydrates, protein, fat and fiber from the leaves of *Solanum trilobatum* (*Solanaceae*).

Phytochemicalds compounds from plant material is depend on the type of solvent used in the extraction method. The solubility of the active constituents in solution (methanol extract) showed some degree of antibacterial activity (Romero *et al.*, 2005). Trial of solvent of various polarities will explore the effect of solvent composition on extract efficacy. It was reviewed by several workers (Chandan *et al*., 2003; Jaime, 2006; Sassi *et al.,* 2008; Gosh *et al*., 2008).

CONCLUSIONS

In conclusion, the present study revealed the significant antibacterial activity of aqueous as well as methanol leaf extract of *N. plumbaginifolia* Viv. against all the tested bacteria. The antibacterial activity can be attributed to the metabolites which is responsible for activity. It is, therefore, suggested that these extracts can be used in the treatment of an infectious disease caused by those bacteria against which the extracts showed significant activity. Thus the pant can be used as an antibacterial agent and may serve as leads for the pharmaceuticals industries in developing countries like India.

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