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In Vitro Antibacterial Activity and Phytochemical Analysis of *Solanum nigrum* (Linn.) - An Important Antiulcer Medicinal Plant

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Abstract

Solanum nigrum (L) is an important herbaceous medicinal plant belongs to Solanacae family. The herb is antiseptic, antidysentric, antidiueretic and is recommended in ayurveda for the management of gastric ulcers. Six solvent extracts from leaf, seed and roots of *Solanum nigrum* were assayed for in *vitro* antibacterial activity against pathogenic bacteria such as *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, klebsiella pneumonia E.coli, Proteous vulgaris, Pseudomonas putrida,* and the zone of inhibition were compared with different standard antibiotics. Phytochemical screening of the crude extracts revealed the presence of secondary compounds such as alkaloids, flavonoids, steroids, tainns, and phenols. The organic solvent extracts (ethanol, methanol, ethyl acetate, diethyl ether, chloroform and hexane) of seeds were exhibited strong antibacterial activity against different pathogenic bacteria compared to leaf and root solvent extracts. The ethyl acetate seed extracts of *Solanum nigrum* exhibited strong activity against *Pseudomonas, Proteous vulgaris, Klebsiella* (20.5 – 21.0mm of zone of inhibation). Among different types of extracts tested ethyl acetate seed extract showed lowest MIC values (1.50-4.50 μg/m) against all the bacterial isolates tested. A lowest MIC value was recorded against *pseudomonas putrida*, *Proteus vulgaris, Klebsiella pneumonia*.

Key Words: *Solanum nigrum* (L.), six solvent extracts, antibacterial activity, phytochemical analysis

Introduction

Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for discovering new drugs. Extracts of many plants are highly efficient against parasitic as well as microbial infections. It is estimated that around 70,000 plant species from lichens to tall trees, have been used at one time to other for medicinal purposes [1] .The use of different parts of several medicinal parts to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely, ayurvedic, siddha and unani have been in existence for several centuries. This system of medicine caters the needs of nearly 70% of the population residing in villages [2]. Plant derived drugs remains important resource especially in developing countries, to combat serious disease.

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial in infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [3]. The natural products play an important role in drug development programmes in the pharmaceutical industry [4]. However World Health Organization (WHO) also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines [5]. Therefore, in recent years researches are increasing turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [6].

In recent years, secondary plant metabolites have been investigated as a source of medicinal agents [7]. The photochemicals with adequate antibacterial activity will be used for the treatment of bacterial infections [8].Hence the present study mainly aims at phytochemical screening for secondary compounds and antbacterial activity of *Solanum nigrum*.

Materials and Methods

Collection of plant material and identification

Solanum nigrum plant material was collected from rural villages of Tirupathi, A.P, India. Botanical identification of plant material was done based on the data present in previous literature and placed in herbarium and it was properly documented.

Preparation of plant extracts

The plant materials (leaf, seed and root) were dried in shade and powdered by mechanical grinder. The leaves, seeds and roots were powdered and extracted following the published procedure with slight modifications [9]. The powdered material was isolated in ethanol, methanol,

chloroform, hexane, ethyl acetate and diethyl ether by keeping them in a shaker for three days. The extracts were reduced to 10% of its original volume and filtered. The filtered organic solvents were concentrated in vacuum using a rotatory evaporator, while aqueous extracts were subjected to antimicrobial activity and phytochemical analysis.

Phytochemical analysis

Phytochemical analysis of all the evaporated solvent extracts was conducted following the procedure of Indian Pharmacopoeia [10]. By this analysis, the presence of several phytochemicals listed in table 2 was tested. To test for alkaloids (200mg plant material in10ml methanol, filtered); a 2ml filtrate +1%HCL+steam, 1 ml filtrate+6 drops of Mayor's reagents/Wagner's regent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate respectively indicated the presence of respective alkaloids. For tannins (200mg plant material in 10 ml distilled water, filtered); a 2ml filtrate+2ml Fecl3, blue-black precipitate indicated the presence of tannins. For flavonoids (200 mg plant material in 10ml ethanol, filtered); a 2ml filtrate+conc.HCl+magnesium ribbon pink-tomato red colour indicated the presence of flavonoids. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered) a 2 ml filtrate+2ml acetic anhydride +conc.H_2SO_4. Blue-green ring indicated the presence of steroids. For phenols, 1ml of each solvent extracts dissolved in alcohol or water was separately treated with a few ml of neutral ferric chloride solution. The change in colour indicated the presence of phenols.

Bacterial strains and growth conditions

The following cultures of gram +ve bacteria such as *Bacillus subtilis, Bacillus megaterium, Proteus vulgaris, Staphylococcus aureus,* and gram –ve bacteria such as *E.coli, Pseudomonas putrida, Klebsiella pneumonia* were used for screening antibacterial activity. All the cultures were obtained from Microbiology department, S.V.University, Tirupathi. The bacterial cultures were maintained in nutrient agar slants at 2 – 8^0c.

Inoculum preparation

The bacterial strains were inoculated on nutrient broth (0.5 % peptone, 0.5% NaCl, 0.15% yeast extract, P^H 7.4) and incubated at 37^0c for over night. The bacterial cells were harvested by centrifuging at 5000g for 15 minutes. The pellet formed was washed twice with PBS (phosphate buffer saline, 10mM sodium chloride, P^H 7.4) and the cells were counted by heamocytometer. The bacterial cells were diluted to approximately 10^5 cfu /ml before use [11].

Determination of antibacterial activity

The antibacterial activity of leaf, seed and root extracts was determined by using agar well diffusion method with slight modification of published procedure [12]. Nutrient agar slants after solidification was inoculated with the test microorganisms, by spreading the bacterial inoculums under aseptic conditions. Wells of 5mm diameter were punched in the agar medium with sterile cork borer and filled with 100 ml of plant extract. The antibiotics such as penicillin, kanamycin, tetracycline and cefotoxine at 100 µg/ml concentration were used in the test system as positive controls. The plates were incubated at 37^0 c for 24 hrs. The negative control was added with out adding the cultures to know the sterile conditions. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective plant extract and antibiotic.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by macro broth dilution method [13]. The reconstituted extract was serially diluted two-fold in nutrient broth medium. Duplicate tubes of each dilution were inoculated with 5 x 10^5 cells (cfu) of the test bacterial strain and cultures incubated at 37^0C for 18 hours. Two-fold serial dilutions of penicillin were included in each experiment as controls. MIC was taken as the highest dilution (least concentration) of controls. MIC was taken as the highest dilution (least concentration) of extract showing no detectable growth in the macro-broth assay.

Results and Discussion

Phytochemical analysis (Table-1)

The preliminary phytochemical analysis of crude organic extracts of two month old callus tissue, tissue cultured and field grown plants of *Solanum nigrum* was carried out. The results were represented in table-1 which reveals the presence of various phytochemicals such as alkaloids, flavonoids, phenols, steroids and tainns. Higher concentration of alkaloids was present in ethyl acetate seed extracts compared to root extracts. Higher concentrations of flavonoids were present in methanol and ethyl acetate leaf and root extracts compared to seed extracts. Higher concentrations of phenols were recorded in the diethyl ether leaf and seed extracts compared to seed extracts. Higher concentrations of phenols were recorded in the diethyl ether leaf and seed extracts, ethanol seed extracts when compared to root extracts. Ethyl acetate seed extracts, diethyl ether root extracts showed higher concentrations of steroids. Diethyl ether leaf, seed, chloroform, seed and root extracts, ethanol root extracts shows a moderate activity. Lower concentrations of steroids were present in hexane methanol leaf, seed and root extracts. Higher concentrations of tainns were present in ethyl acetate leaf extract. A moderate concentration of tainns was present in ethanol, chloroform leaf extract and diethyl seed extracts compared to root extracts.

Table-1: Phytochemical analysis of secondary metabolites such as alkaloids, flavonoids, phenols and tannins in different plant parts of *Solanum nigrum*

Type of extract	Alkaloids	Flavonoids	Phenols	Steroids	Tannins
LEAF					
Ethanol	+	+ + + +	-	-	+ +
Methanol	+ +	-	+	-	+ + +
Chloroform	-	+ + +	+	+	+
Hexane	+ + +	+ + + +	+ +	+ +	+ + + +
Ethyl acetate	+ + + +	+ +	+ + + +	+ + +	+ + +
Diethyl ether	+ + + +	+ + +	+ + + +	+ +	-
SEED					
Ethanol	++++	+	+	-	-
Methanol	++	++	+	+++	++
Chloroform	+	++++	+	+	+
Hexane	+++	++++	+++	++++	-
Ethyl acetate	++++	++	++++	+++	+++
Diethyl ether	+++	++	-	+++	+
ROOT					
Ethanol	+++	++++	+	+	++
Methanol	+	++	+	+++	+
Chloroform	-	-	-	+	+
Hexane	++	+++	+++	+++	++
Ethyl acetate	+	++	+++	++++	++
Diethyl ether	+++	++	++	-	+++

+ Less; + + Moderate; + + + High; + + + + Very high

Table-2: Susceptibility of test bacterial strains to leaf, seed and root extracts of *Solanum nigrum* (L.) and standard antibiotics.

Type of extract / antibiotic	Zone of inhibition or antibacterial activity (in mm)						
	Bacillus subtilis	*Escheritia coli*	*Bacillus megaterium*	*Pseudomonas putrida*	*Proteus vulgaris*	*Klebsiella pneumonia*	*Staphylococcus aureus*
LEAF							
Ethanol	13.0	12.0	15.0	16.0	13.5	10.5	7.0
Methanol	-	-	15.0	10.3	13.0	10.0	8.0
Ethyl acetate	15.2	16.0	15.5	15.0	14.5	16.0	14.0
Chloroform	-	-	-	-	15.0	10.0	-
Hexane	-	-	-	7.0	-	7.5	-
Diethyl ether	13.0	-	16.0	14.0	14.0	18.0	15.0
SEED							
Ethanol	17.5	15.0	18.1	19.5	18.0	13.0	12.0
Methanol	10.5	8.0	20.0	10.0	10.0	7.8	15.0
Ethyl acetate	14.0	17.5	12.0	20.5	21.0	21.0	18.0
Chloroform	10.0	13.0	16.0	17.0	10.5	10.5	13.0
Hexane	8.5	8.1	10.5	8.0	16.0	8.0	10.5
Diethyl ether	14.1	10.0	18.0	16.0	12.0	15.0	17.5
ROOT							
Ethanol	-	11.0	-	11.0	10.5	6.0	10.0
Methanol	-	-	6.0	-	-	-	-
Ethyl acetate	12.0	-	8.0	6.0	-	7.0	10.5
Chloroform	-	7.5	-	-	6.0	-	-
Hexane	-	-	-	8.0	-	-	6.0
Diethyl ether	-	5.0	-	-	-	4.5	-
STANDARD ANTIBIOTICS							
Cefotoxime	10.5	14.0	12.0	11.0	20.0	10.0	12.0
Penicillin	11.5	12.5	8.0	5.0	12.0	7.0	5.5
Kanamycin	15.0	12.0	14.0	14.0	14.5	11.5	10.5

Antibacterial activity (Table-2)

The antibacterial activity of *Solanum nigrum* plant extracts was assayed by agar well diffusion method. The various solvent extracts of leaf, seed and roots were used and were showed high range of activity against all the tested organisms comparing with standard antibiotics. The zone of inhibition, and its subsequent concentrations was tabulated and represented in table–2 and fig-1 respectively. Among all the solvent extracts used ethyl acetate seed extract shows very high activity (14-21.0mm zone of inhibition) against all the tested organisms. All the seed extracts (ethanol, methanol, chloroform, hexane, ethyl acetate, diethyl ether) were also shows high activity (8.0-21mm of inhibition) when compared to leaf extracts (ethanol, methanol, chloroform, hexane, ethyl acetate, diethyl ether) which showed a moderate activity (6.0-17.0 mm of zone of inhibition). Among the root solvent extracts, methanol, chloroform, hexane does not shows any activity against the tested organism. Ethanol root extracts shows a moderate to less activity (6- 11mm of zone of inhibition) against *E.coli,*

Pseudomonas, Proteous vulgaris, Klebsiella, and Staphylococcus aureus. Ethyl acetate root extracts also shows activity (7.0- 12.0mm of zone of inhibition) against *Bacillus subtilis, Bacillus megaterium, Pseudomonas, Klebsiella, and Staphylococcus aureus.* Diethyl ether root extracts showed very less activity (5.0mm of zone of inhibition) against *E.coli and Klebsiella pneumonia* and it does not have any effect on other organisms. The obtained results of the crude extracts were compared with the standard antibiotics such as penicillin, kanamycin, cefotoxime and tetracycline. All the tested organisms are highly sensitive to the ethyl acetate seed extract (14.0-21.0mm) than the standard antibiotics such as kanamycin, penicillin, tetracycline, and cefotoxime which showed more or less activity (8.0-20.0mm) on the tested organisms compared with ethyl acetate seed extract (14.0-21.0mm).

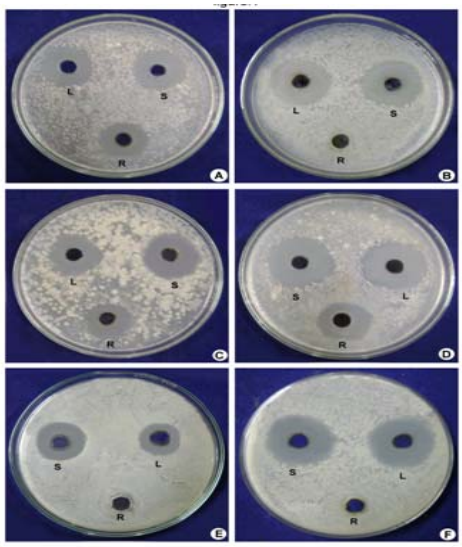

Figure – 1: A) Highest antibacterial activity exhibited by ethyl acetate leaf, seed and root extrcts against *Bacillus subtilis*
B) Highest antibacterial activity showed by ethyl acetate leaf and seed extracts against *E. coli.*
C) Hghest antibacterial ctivity showed by methanol leaf, seed and root extracts against *Bacillus megaterium.*
D) Highest antibacterial activity exhibited by ethanol leaf, seed and root extracts against *preudomonas*
E) Highest antibacterial activity showed by chloroform leaf, seed extractsagainst *Proteous vulgaris*
F) Highest antibacterial activity showed by diethys ether leaf and seed extracts against S*taphylococcus aureus.*

Minimum inhibitory concentrations (MIC) of the crude extracts of Solanum nigrum against test bacterial strains. (Table - 3)

MIC was determined by macro broth dilution method [13]. The lowest concentration (highest dilution) of the extract showing no detectable bacterial growth in the macro broth assay when compared with the control tubes was considered as minimum inhibitory concentration (MIC). Among different types of extracts tested; ethyl acetate seed extract showed lowest MIC values (1.50-4.50 μg/ml) against all the bacterial isolates tested. A lowest MIC value was recorded against *pseudomonas putrida*, *Proteus vulgaris, Klebsiella pneumonia*. Ethanol seed extracts showed the MIC values in the range of (4.50-8.50 μg/ml), where as diethyl ether seed extracts showed MIC values in the range of (2.50-8.0 μg/ml). Among the leaf extracts ethyl acetate leaf extracts showed MIC values in the range of (4.50-32.50μg/ml), Diethyl ether leaf extracts showed MIC values in the range of (2.50-7.0 μg/ml). In root extracts ethanol and ethyl acetate root extracts showed MIC values in the range of (7.0-65 μg/ml). From the data obtained it is evident that lowest MIC values were recorded in seed extracts followed by leaf extracts, where as in root extracts highest MIC values (65.0 μg/ml) were recorded.

Table-3: Minimum inhibitory concentrations (MIC) of the crude extract of *Solanum nigrum* against the test bacterial strains.

Type of extract	MIC (μg/ml)						
	Bacillus subtilis	*Escheritia coli*	*Bacillus megaterium*	*Pseudomonas putrida*	*Proteus vulgaris*	*Klebsiella pneumonia*	*Staphylococcus aureus*
LEAF							
Ethanol	14.50	32.50	7.00	4.50	32.50	32.50	4.50
Methanol	-	-	4.50	14.50	32.50	4.00	7.00
Ethyl acetate	8.00	7.00	14.50	8.00	8.50	7.00	8.00
Chloroform	-	-	-	14.50	32.50	65.0	-
Hexane	-	-	-	7.00	-	14.50	-
Diethyl ether	4.50	-	4.50	4.50	7.00	2.50	4.00

SEED							
Ethanol	7.00	8.50	4.50	4.50	7.00	4.50	4.50
Methanol	14.50	14.50	7.00	14.50	7.00	14.50	32.50
Ethyl acetate	4.50	4.50	2.50	1.50	1.50	1.50	2.50
Chloroform	32.50	32.50	14.50	8.00	32.50	32.50	7.00
Hexane	32.50	14.50	32.50	32.50	14.50	32.50	8.00
Diethyl ether	4.50	7.00	2.50	4.50	7.00	8.00	2.50
ROOT							
Ethanol	-	-	-	7.00	14.50	32.50	65.0
Methanol	-	-	32.50	32.50	-	-	-
Ethyl acetate	8.00	-	14.50	-	-	14.50	65.0
Chloroform	-	32.50	-	-	65.0	-	-
Hexane	-	-	-	65.0	-	-	65.0
Diethyl ether	-	7.00	-	-	-	14.50	-

Discussion

The most of the phytochemicals classified as secondary metabolites are produce mainly by the shoot part of the plant, often their function in the plant is unknown, but certain phytochemicals have structural, functional and general defense against plant pathogens. Considering the high economical and pharmacological importance of secondary plant metabolites industries are deeply interested in utilizing plant tissue culture technique for large scale production of these substances [14].

In the present investigation on over all, seed extracts shows high inhibitory activity against all the tested bacterial strains followed by leaf, than root extracts. Among the bacterial strains *Bacillus subtilis,* gram-ve bacteria contaminates wounds*, Staphylococcus aureus* is one of the causative bacterium in community acquired pneumonia and blood species. *Kelebsiella* species causes urinary and respiratory track infections, opportunistic infectious, nosocomial pathogens and pneumonia [15, 16]. Antibacterial properties of leaf and seed extracts against the tested bacterial strains suggest that the respective crude extracts can be effectively used for wound healing, septicemia and other common infections diseases. Similar results were reported in *Adhatoda vasica* as a wound healing agent [1, 17] *Suregada angustifolia* against gram-ve *Klelebsiella* species [18] .The present study reveals that the active principles present in the aerial parts of plant are very active against all the tested bacterial strains compare to roots. Similar results were also reported in antimicrobial action of natural products from the *Speranhtus indicus* [19]. Based on earlier reports, among the great variety of secondary compounds found in plants, phenolics and terpenoids represent the main antimicrobial agents. Aromatic compounds such as phenols, phenolic acids, alkaloids and lectins and its derivative e.g., flavonoids have been identified as antimicrobial agents [20]. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects .Continued further exploration of plant derived antimicrobials is needed today.

Conclusion

In conclusion *Solanum nigrum* crude extracts posses a broad spectrum of activity against a pannel of bacterial strains responsible for common bacterial infections. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. However, the present study of *in vitro* antibacterial evaluation of *Solanum nigrum* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

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