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Antifungal Activity, Phytochemical Analysis of *Solanum nigrum* (L.) - An Important Antiulcer Medicinal Plant

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Abstract

Solanum nigrum (L.) is commonly known as "Blacknight shade" and is belongs to solanacae family. The herb is antiseptic, anti dysenteric, antidiuretic and it has very important gastric ulcerogenic activities. Three solvent extracts from leaf,seed and roots of *Solanum nigrum* were assayed for antifungal activity against fungal strains such as *Penicillium notatum, Aspergillus niger, Fuserium oxisporium* and *Trichoderma viridae.* The zone of inhibitions was compared with the standard antibiotics. Phytochemical screening of the crude extracts reveals the presence of various secondary compounds such as alkaloids, flavonoids, steroids, tanins and phenols. The organic solvent extracts (ethanol, methanol and ethyl acetate) of seeds were exhibited strong antifungal activity against all the tested fungal strains compared to leaf and root extracts. Among all the extracts ethyl acetate seed extract showed high antifungal activity (8.0-16.0mm zone of inhibition) on all the tested fungal strains and relatively lowest MIC value in the range of (2.0-6.0μg/ml) were obtained with ethanol seed extracts.

Key Words: *Stevia rebaudiana*, Flower, Leaf extracts, Antibacterial activity, Phytochemical analysis

Introduction

The medicinal plants are plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc), extracts, infusions, decoctions and powders are used in the treatment of different diseases of humans, plants and animals [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]. The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and remerging infections disease. Thus it is anticipated that phytochemicals with adequate antimicrobial efficacy will be used for the treatment of various microbial infections. How ever 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 [3].

Medicinal plants synthesize a vast array of secondary metabolites that are important for human life. For medicinal purpose photochemical investigation of plants is an interesting area of research, leading to the isolation of several new compounds. Therefore, in recent years researches are increasing turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [4]. Some previous studies revealed a strong antimicrobial activity of tested medicinal plants on fungal and bacterial pathogens. Antimicrobial activity of the essential oil of *Cestrum diurnum* was studied [5]. *In vitro* antimicrobial activity of Bakuchoil against oral micro organism [6]. Anticancerous properties of *Withania somnifera* [7]. Antibacterial activity of flower extracts of *Cassia alata* [8] was studied.

The present investigation mainly aims at phytochemical screening for secondary compounds and antifungal 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 shade dried and powdered in 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 and ethyl acetate by keeping them in a shaker for 3 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 antifungal activity and phytochemical analysis.

Phytochemical analysis

Phytochemical analysis of all the evaporated solvent extras was conducted following the procedure of Indian Pharmacopoeia [10]. By this analysis, the presence of several phytochemicals listed in table-1 was tested. To test for alkaloids (200mg plant material in10ml methanol, filtered); a 2ml filtrate +1%HCL+steam, 1 ml filtrate+6drops 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.

Antifungal activity

The test fungal strains used for the study were *Penicillium notatum, Aspergillus niger, Fuserium oxisporium and Trichoderma viridae* were obtained from Department of Microbiology, Sri Venkateswara University, Tirupathi. All the fungal cultures were maintained on potato dextrose agar (PDA) slants at 4^0C.

Fungal inoculum preparation

Prior to experiment, fungi were cultured on PDA slants for 72 hrs until fully sporulated. Spores were collected by adding 10 ml. of medium (which contained 0.5% tween 80 and 0.5% Agar in sterile distilled water); [11] scarped with sterile loop and aseptically transferred into sterile test tubes. The final spore suspension concentration was adjusted to 2 x 10^6 spores/ml using hemocytometer.

Antifungal activity assay

The antifungal activity was determined by performing agar well diffusion method. In this method Potato dextrose agar plates after solidification was inoculated with test microorganisms by spreading the fungal suspensions of 1 µl (1 µl of adjusted spore suspension) and evenly spread with sterile bent rod in aseptic conditions. Agar plates were punched with sterile cork borer (5 mm in diameter) and it was filled with 100ml of plant extract of 500 µg/ml concentration. Antibiotics such as penicillin, cefotoxime were used as positive controls. All the culture plates were incubated at 37^0C for 72 hrs in a growth chamber. The antifungal activity was assayed by measuring the zone of inhibition for the respective plant extract and it was compared with standard antibiotic. Potato dextrose agar plates without adding cultures were used as negative controls.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by macro broth dilution method [12]. The reconstituted extract was serially diluted two-fold in 'PD' broth medium. Duplicate tubes of each dilution were inoculated with fungal suspensions of 1 µl (1 µl of adjusted spore suspension)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 of plant extracts

The preliminary qualitative phytochemical analysis of crude organic solvent extracts of leaves, seeds and roots of *Solanum nigrum* was carried out. The results, reveals the presence of various photochemicals such as alkaloids, flavonoids, phenols, steroids and tannins. Higher concentration of alkaloids was present in ethyl acetate seed extracts compared to root extracts. Higher concentration of flavonoids was present in methanol and ethyl acetate leaf and root extract compared to seed extracts. Higher concentrations of phenols were recorded in ethanol seed extracts when compared to root extracts. Ethyl acetate seed extracts shows higher concentrations of steroids. Ethanol root extracts shows a moderate activity. Lower concentrations of steroids were present in methanol leaf, seed and root extracts. Higher concentrations of tannins were present in ethyl acetate leaf extract. A moderate concentration of tannins was present in ethanol, leaf and seed extracts compared to root extracts.(Table-2)

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 so the preliminary phytochemical studies received pronounced importance, because the crude drugs posses varied composition of secondary metabolites [3].

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	+ +	+	++	++	+ + +
Ethyl acetate	+ + + +	+ +	+ + + +	+ + +	+ + +
SEED					
Ethanol	++++	+	++	++	+
Methanol	++	++	+	+++	++
Ethyl acetate	++++	++	++++	+++	+++
ROOT					
Ethanol	+++	++++	+	+	++
Methanol	+	++	+	+++	+
Ethyl acetate	+	++	+++	++++	++

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

Table: 2. Susceptibility of test fungal strains to leaf, seed and root extracts of *Solanum nigrum* and standard antibiotics.

Type of extract / antibiotic	Zone of inhibition or antifungal activity (in mm)			
	Aspergillus niger	*Penicillium notatum*	*Fuserium oxisporium*	*Trichoderma viridae*
LEAF				
Ethanol	-	8.0	12.0	-
Methanol	4.0	-	6.5	12.0
Ethyl acetate	12.0	6.0	-	5.5
SEED				
Ethanol	8.0	15.0	16.0	6.0
Methanol	6.5	5.5	5.0	14.5
Ethyl acetate	15.0	8.0	6.5	7.5
ROOT				
Ethanol	-	-	5.0	-
Methanol	3.0	-	-	-
Ethyl acetate	-	4.5	-	4.0
STANDARD ANTIBIOTICS				
Penicillin	8.0	10.0	12.0	8.5
Cefotoxime	14.8	14.0	16.0	12.0

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

Type of extract / antibiotic	MIC (in µg/ml)			
	Aspergillus niger	*Penicillium notatum*	*Fuserium oxisporium*	*Trichoderma viridae*
LEAF				
Ethanol	-	12.50	6.00	-
Methanol	28.0	-	12.50	6.00
Ethyl acetate	6.0	12.50	-	4.00
SEED				
Ethanol	6.00	2.00	2.00	6.00
Methanol	12.50	12.50	6.00	4.00
Ethyl acetate	4.00	8.50	6.00	12.50
ROOT				
Ethanol	-	-	45.0	-
Methanol	12.50	-	-	-
Ethyl acetate	-	28.0	-	45.0

Antifungal activity

The antifungal activity of *Solanum nigrum* plant extract was assayed by agar well diffusion method. The various solvent extracts of leaf, seed and root were showed high activity against the tested fungal strains and the activity was assayed by measuring the diameter of growth inhibition zone and its subsequent concentration was tabulated. Among all the solvent extracts used, ethanol seed extract shows high activity (6.0 -16.8 mm) against all the tested fungal strains. All the seed extracts (Ethanol, Methanol and Ethyl acetate) showed high activity (5.0 – 16.0 mm of zone inhibition) against all the tested fungal strains, compare to leaf extracts (Ehanol, Methanol and Ethyl acetate) which shows a moderate activity (4.0- 12.0mm). Ethanol seed extracts shows high activity against *Penicillum notatum*, *Fuserium oxisporium* (15.0 – 16.0 mm zone of inhibition). Ethyl acetate seed extracts exhibits strong activity against *Aspergillus niger* (15.0 mm of zone of inhibition). Ethanol and methanol root extracts shows less activity (5.0, 3.0 mm zone of inhibition) against *Fuserium* and *Aspergillus niger* respectively, which does not shows any

activity against other fungal strains tested. Ethyl acetate root extracts also shows less activity against *Penicillium notatum, Trichoderma viridae* (4.0 – 4.5mm of zone of inhibition) and it does not affect other fugal strains tested. The obtained results were compared with standard antibiotics such as penicillin, cefotoxine. Among all the extracts, ethanol seed extract shows high activity (6.0- 16.0 mm of zone of inhibition) against all the tested fungal strains which is more sensitive compares to standard antibiotics such penicillin, cefotoxime (8.0 – 15.0 mm of zone inhibition). (Table-2 and Figure-1)

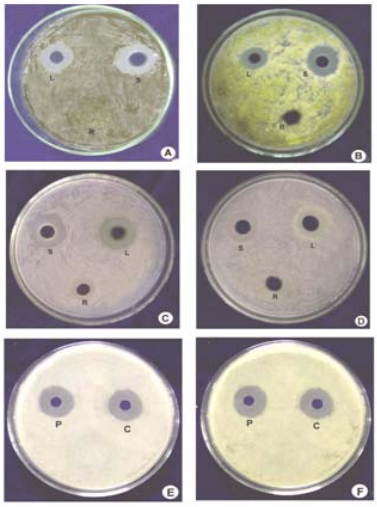

Figure: 1: Antifungal activity of leaf (L), seed (S) and root (R) solvent extracts of field grown *Solanum nigrum* plants

A) Plate tested with ethyl acetate leaf, seed and root extracts against *Aspergillus niger.*
B) Plate tested with ethanol leaf, seed and root extracts against *Penicillium notatum*
C) Plate tested with ethanol leaf, seed and root extracts against *Fuserium oxisporium*
D) Plate tested with methanol leaf and seed extracts against *Trichoderma viridae.*
E-F) Standard antibiotics such as pencillin (p) and cefotoxime (c) used as a positive control against *Penicillium notatum, Trichoderma viridae.*

The fungi are eukaryotic achlorophyllous and heterotrophic in nature and comprise about 1.5 million species of which only 74,000 species are described. And more than 300 species are potentially cause allergy systems in man [13]. Many species of fungi cause serious diseases of useful plants. Which includes wheat, rice, maize, barley, oat, cruciferous plants, potatoes, tomatoes and other fruit plants. So, fungi are regarded as the chief causative agents of plant pathology [14]. Similarly man and other mammalians, fishes, amphibians and reptiles are also susceptible to fungal infections [15]. *Penicillium* and *Aspergillus* species are the common spoilage organism in bakery products. Antifungal activity of crude extracts have been due to the presence of liphophihic compound that may bind with in or internal to the cytoplasmic membrane [16] are quinines [17] or thionine, which effect growth of filamentous fungi mainly by causing membrane permeabilization [18].

Minimum inhibitory concentrations (MIC) of the crude extracts of Solanum nigrum against test fungal strains

The lowest concentration (highest dilution) of the extract that produced no visible fungal growth (no turbidity when compared with the control tubes) was regarded as MIC. Among the different type of extracts tested, Ethanol seed extracts showed lowest MIC values in the range of (2.0-6.0 μg/ml). A lowest MIC values (2.0 μg/ml) were recorded against *penicillium notatum* and *Fuserium oxisporium*. In leaf extracts the MIC values were recorded in the range of (6.0-28.0 μg/ml), where as in root extracts very high MIC values were recorded in the range of (12.50-45.0 μg/ml) against the tested fungal strains. (Table-3)

Conclusion

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. *Solanum nigrum* crude extracts posses a broad spectrum of activity against a panel of fungal pathogens responsible for common microbial infections. Hence these promissory extracts open the possibility of finding new clinically effective antimicrobial compounds.

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