

Studies on Phytochemical constituents, Functional Group Identification and Antimicrobial Activity of *Solanum nigrum* (Solanaceae)

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Abstract

Phytochemical constituents, functional group identification and antimicrobial activity of ethanolic extract of *Solanum nigrum* were investigated. The phytochemical screening of the crude extract revealed the presence of alkaloids, reducing sugars, tannis, flavonoids, Phlobatannis, and steriods. The extract were subjected for identification of functional groups using infra red spectrophotometer, presence of C=O, C-H, C=C and C-O were identified, these bonding structures are responsible for presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid and anhydrides. And the crude ethanolic extract of *Solanum nigrum* were assayed for in vitro antimicrobial activity against gram positive and gram negative bacteria and they were also subjected for antifungal activity and the zone of inhibition were compared with control drug Penicillin and Nystatin. *Staphylococcus aureus* as the most sensitive strains exhibited maximum zone of inhibition about 25.7mm among gram positive organism tested. *Escherichia coli* exhibits 30.1mm zone than that of standard penicillin positive control. *Trichophyton mentagrophytes* exhibits maximum zone of inhibition of about 26.1mm among the tested fungal species.

Key words

Solanum nigrum, *Staphylococcus aureus*, *Escherichia coli*, *Trichophyton mentagrophytes*, Infra red spectrophotometer.

Introduction

Natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be occurred to the patients and community (Patwardhan and Hopper, 1992). The antiseptic qualities of aromatic and medicinal plants and their extracts were recognized in the laboratory data back to the early (Dorman and Dean, 2000). As result of ecological and fitgeographical diversification, endemism of species is high (Tan, 1992; Dagic et al., 2002).

Ayurvedic remaining one of the most ancient and yet living traditions practiced widely in developing countries as well as developed countries and has a sound philosophical and experimental basis (Dahanukar and Thathe, 2000; Chopra and Doiphode, 2002).

In recent years investigation found several plants of the ethnomedicine posse's really interesting biological activities which could be of interest for all parts of the world (El- Fiky et al., 1995; Awadh Ali et al., 2001; Mothana and Lindequist, 2005; Mothana et al., 2006; Al-Fatimi et al., 2007).

Approximately 20% of the plants found in the world have submitted to pharmacological activities (Suffrendini et al., 2004). And the plants have organic chemicals (Evans et al., 1986). The plants posses chemotherapeutic, bacteriostatic and antimicrobial agents (Purohit and Mathur, 1999)

The source of plants is models for the synthesis of new drugs with better therapeutic, chemical (or) physical properties than the original compound (Baerheim svendsen and Scheffer, 1992). In recent year's plant material have been investigated as a source of medicinal agents (Krishnaraju et al., 2005). And the plants materials used for treatment bacterial infections (Balandrin et al., 1985). However world health organization also has recognized the importance of traditional medicine and has been active in crating strategies, guidelines and standards for botanical medicines (WHO, 2002).

Plant derived drugs remains important resource especially in developing countries, to combat serious disease. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness (Zhang, 2004). In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). And natural products play on important role in drug development programmes in the pharmaceutical industry (Baker et al., 1995).

There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999; Ignacimuthu et al., 1998).

The activities have been have been selected because of their great medicinal revelence with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin et al., 1999). Because natural products of higher plants may give a new source of antimicrobial agents. There may research group that are now engaged in medicinal plants research (Samy et al., 1998; Hamil et al., 2003; Motsei et al., 2003).

The development drug resistance in human pathogens against commonly used antibiotics has necessitate a search for new antimicrobial substance from other sources including plant screening of medicinal plants for

antimicrobial activities and photochemical is important for finding potential new compounds for therapeutic uses.

In present study focus phytochemical constituent, functional group identification and in vitro evaluation of antimicrobial activity of the separated fractions of ethanolic extracts from the aerial parts of *Solanum nigrum* are bioassay against pathogenic microorganism.

Materials and Methods

Collection of Plant materials and Identification

Plant material of *Solanum nigrum* was purchased from a local Ayurvedic medicinal shop "Chennai," and the material was identified and authenticated by the Chief Botanist, Tamil Nadu Aromatic Medicinal Plants Corporation Limited (TAMPCOL), Arignar Anna Siddha Medical College and Hospital Campus, Chennai, Tamil Nadu, India.

Ethanolic extraction

The plant materials were dried in shaded and powdered by mechanical grinder. The powder of *Solanum nigrum* plant material were initially defated with petroleum benzene (60 - 80°C) followed by 1000 ml of ethanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using whattman filter paper (No 1) and then concentrated in vaccum and dried at 45°C for ethanol elimination and the extract were kept in a sterile bottle under refrigeration condition of about 2-8°C.

Test for Phytochemical analysis

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugars, Saponins, tannis, Carbonyls, Flavonoids, Phlobatannis and steriods (Adetuyi and Popoola 2001; Trease and Evans, 1989; Sofowora, 1982).

Test for Alkaloids

Weigh about 0.2 gm of plant extract in separate test tube and warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragencloffs reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Test for Cardiac glycoside

Keller-Killani Test

Weigh about 0.5 gm of plant extract in a separate test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo sulphate (VI) acid. And observe for brown ring formation at the interface (Finar, 1983).

Test for Terpenoids

Weigh about 0.5 g plant extract in separate test tubes with 2 ml of chloroform. And add concentrated Sulphuric acid carefully to form a layer. And observe for presence of reddish brown color interface was formed to show positive results for the presence of terpenoids.

Test for reducing sugars

Take a test tube and add 2 ml of crude plant extract and add 5 ml of Distill water and filtered. The filtrate was

boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. Observe for orange red precipitate indicates the presence of reducing sugars.

Test for Saponins

Weigh about 0.2 gm of plant extract in the test tube and add 5 ml of distill water and then heat to boil. Observe for frothing (appearance of creamy mass of small bubbles) shows the presence of Saponin

Test for Tannis

To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. And observe for dark green solutions indicates the presence of tannis

Test for Carbonyl

Take 2 ml of plant extract in separate test tubes and add few drops 2,4, di nitro phenyl hydrazine solution and shaken. And observe for the presence of yellow crystals immediately for the presence of an aldehyde.

Test for Flavonoids

Weigh about 0.2 gm plant extract in separate test tubes and dissolved diluted Sodium hydroxide and add diluted Hydrochloride. And observe for yellow solutions that turns colorless indicates the presence of flavonoids

Test for Phlobatanin

Weigh about 0.5 gm of plant extract in a test tubes and dissolved with distill water and filtered. The filtrate was boiled with 2% Hydrochloric acid solution. Observe for red precipitate shows the presence of Phlobatanin

Test for Steroids

To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid .Observe for the color change from violet to blue or green in samples indicating the presence of steriods

Identification of functional group

Instrument used : FTIR (Fourier Transformer Infra red Spectrophotometer)

Make : Shimadzu

Sample preparation for Infrared Spectrophotometer

A drop of ethanolic extract of *Solanum nigrum* was placed on the Sodium chloride cell to obtain the thin layer. Then the cell was placed in the FTIR compartment and scanned accordance with ASTM (American standard testing material) 1252-98 through IR region for about 25 scans and chromatogram was obtained.

Antibacterial activity

Bacterial strains and Growth conditions

The following cultures of gram positive organism were used *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus anthracis*,

Bacillus subtilis and gram negative organism of *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*, *Salmonella typhi*, *Salmonella paratyphi*, *Vibrio cholera* and *Pseudomonas aeruginosa*. The fungal cultures used were *Microsprum gypsum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Aspergillus niger*. The cultures are obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. The bacterial cultures were maintained in the nutrient agar slants and fungal cultures were maintained in Sabouroud dextrose agar slant at 2 - 8°C.

Preparation of plant extract, antibiotic and Inoculum preparation

The dried plant extract were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 5.0 mg/ml and antibiotic at 50µg/disc. Inoculum of bacterial cultures were prepared in nutrient broth medium and kept incubation at 35°C for 8 hours and fungal cultures at 25°C for 24 hours.

Procedure for performing the Disc Diffusion test (Bayer et al., 1986)

The required amount of Mueller-Hinton plates (Hi media) is prepared as per manufacturer instructions. A sterile cotton swab was dipped into the turbid culture suspension. The dried surface of Muller-Hinton agar plate was inoculated by streaking two more times rotating the plate approximately 60° each time. The lid may be left aside for 3-5 minutes and allow to dry for the excess surface moisture content.

The ready prepared sterile Himedia discs (0.7mm) were poured with different concentration of above prepared antibiotic and plant extract solutions, the discs were placed on the medium and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours for bacterial cultures and for fungal cultures the plates should be incubated at 25°C for 72 hours. The negative control was included without adding the cultures to know the sterile conditions. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc.

Results and Discussion

Ayurveda remains one of the most ancient and yet living traditions practiced widely in India. Medicinal herbs consist of wide variety of chemical compounds nearly 80% of the world population depends upon traditional system of health care. Survey had revealed that 50% of the top prescription drugs in the world are based on natural products. Hence this present study was conducted to study the in vitro antimicrobial activity of medicinal plant used by Indian peoples to show that the therapeutic properties of *Solanum nigrum* used in traditional medicine coincide with laboratory findings.

The ethanolic extract of *Solanum nigrum* were subjected for phytochemical analysis, the results shows that positive for tests of alkaloids, reducing sugars, tannis, flavonoids, phlobatanins, and sterioids but shows negative results for cardiac glycosides, terpenoids, saponins and carbonyl were discussed in the table -1

Table 1. Phytochemical constituents of *Solanum nigrum*.

S. no	Test parameters									
	Alkaloids	Cardiac glycosides	Terpenoids	Reducing sugars	Saponins	Tannis	Carbonyl	Flavonoids	Phlobatanin	Sterioids
1	+	-	-	+	-	+	-	+	+	+

Key + = Positive ; - = Negative

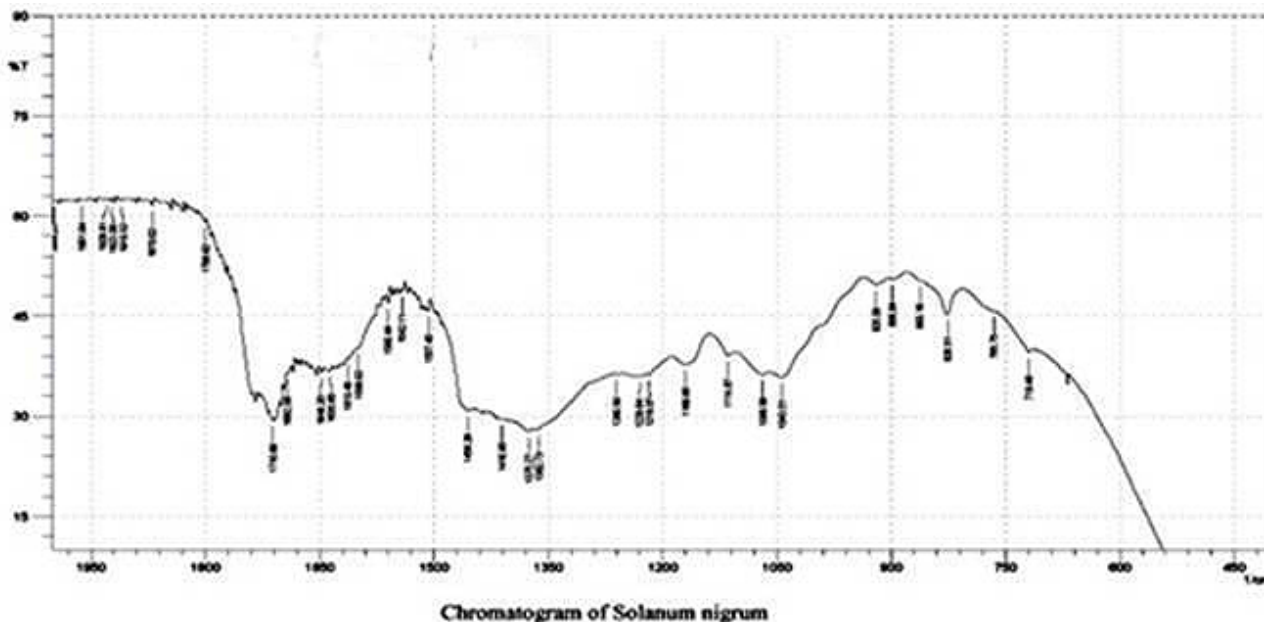
The Infra red spectroscopic (IR) analysis of ethanolic crude extract of *Solanum nigrum*, reveals that the presence of different functional group ranging from 4000 to 400 wave number, C-Hstr1, alkyl group, (2926.6cm⁻¹), C=O stretching for carbonyls (2162.1 cm⁻¹), C-O bending for alcohols, ethers, esters, carboxylic acid and anhydrides (1310.6 –1059.6cm⁻¹), C-H bending alkyl (1453.4 – 1376.2 cm⁻¹), C=C stretching (1660.0- 1667.0) and C-H bending for methyl group (864.1 – 668.4 cm⁻¹)

The spectroscopic analysis of the plant extract of *Solanum nigrum*, figure -1 shows peaks range from 2000 – 400 wave number were discussed in the table -2 the extract revealed the presence of C=O, C-H, C=C and C-O bond stretching. Generally O-H stretching will be present in all phenolic compounds and C-N is commonly found in all types of Alkaloids.

Table 2. IR Spectroscopic analysis of ethanolic extract of *Solanum nigrum*, which showing characteristic absorption peaks at IR range.

S. No	Component	Absorption peaks of <i>Solanum nigrum</i>
1	C-H	719.46, 765.75, 826.51, 863.16, 898.84
2	C=O	1710.89, 1799.62, 1870.02
3	C=C	1410.95, 1456.28, 1507.40, 1542.11, 1560.44, 1599.02, 1613.48, 1635.66, 1648.20, 1692.56
4	C-O	1043.51, 1068.58, 1114.87, 1169.85, 1218.07, 1229.64, 1260.50, 1362.73, 1375.27

Figure 1. IR Spectroscopic analysis of ethanolic extract of *Solanum nigrum*.



The in vitro antimicrobial activity of ethanolic extract of *Solanum nigrum* was assessed qualitatively and quantitatively by the presence or absence of inhibition zones, Zone diameter values, the ethanolic extract of

Solanum nigrum showed great in vitro antimicrobial activities against 16 types of bacteria and 4 fungus species tested. In this study the antimicrobial activities of ethanolic extract were compared with standard antibiotics such as Penicillin and Nystatin used as positive control.

Results from the antimicrobial disc diffusion assay were furnished in table-3, the data indicates that gram positive *Staphylococcus aureus* was the most sensitive strain tested to the ethanolic extract of *Solanum nigrum* with greatest inhibition zone of about 25.7mm. *Staphylococcus epidermidis*, *Bacillus anthracis* and *Bacillus subtilis* were also found more sensitive with zone of inhibition 22.3mm, 20.1mm and 22.1mm. *Streptococcus pyogenes* show less activity when compared with control standard standard penicillin with zone of inhibition about 10.2mm.

The ethanolic extract of *Solanum nigrum* also shows excellent activity against *Escherichia coli*, tested gram negative organism with 30.1mm zone of inhibition to that of standard penicillin positive control.

Klebsiella pneumoniae, *Shigella flexneri*, *Salmonella typhi* and *Pseudomonas aeruginosa* exhibited maximum significant zones to the tested ethanolic crude extract of *Solanum nigrum* with inhibited zones of about 28.1mm, 26.0mm, 18.2mm, and 21.5mm. *Shigella boydii*, *Salmonella paratyphi* and *Vibro cholera* exhibited better significant zones of about 10.0mm, 09.1mm and 12.5mm.

The ethanolic extract of *Solanum nigrum* also exhibited the highest antifungal activity against *Microsporium gypsum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Aspergillus niger* with the inhibition zone of 24.2mm, 26.1mm, 22.1mm and 18.7mm when compared with control standard Nystatin

Table 3. Antimicrobial activity expressed as minimum inhibitory concentration (MIC) of ethanolic extract of *Solanum nigrum* by disc diffusion method.

S. No	Microorganism	Ethanolic extract Inhibition zone(mm)	Antimicrobial agent Inhibition zone(mm)
1	Gram positive organism	<i>Aegle marmelos</i>	Penicillin
	<i>Streptococcus pyogenes</i>	10.2	16.2
	<i>Streptococcus agalactiae</i>	14.9	18.4
	<i>Streptococcus faecalis</i>	20.1	24.1
	<i>Staphylococcus aureus</i>	25.7	22.1
	<i>Staphylococcus epidermidis</i>	22.3	17.2
	<i>Bacillus anthracis</i>	20.1	16.1
	<i>Bacillus subtilis</i>	22.1	15.0
2	Gram Negative organism		
	<i>Escherichia coli</i>	30.1	12.5
	<i>Klebsiella pneumoniae</i>	28.1	22.1
	<i>Shigella flexneri</i>	26.0	16.4
	<i>Shigella sonnei</i>	14.0	18.3

	<i>Shigella boydii</i>	10.0	17.1
	<i>Salmonella typhi</i>	18.2	14.2
	<i>Salmonella paratyphi</i>	09.9	10.5
	<i>Vibro cholera</i>	12.5	16.1
	<i>Pseudomonas aeruginosa</i>	21.5	16.1
3	Fungal species		Nystatin
	<i>Microsporium gypsum</i>	24.2	16
	<i>Trichophyton mentagrophytes</i>	26.1	20
	<i>Epidermatophyton flocosum</i>	22.1	18
	<i>Aspergillus niger</i>	18.7	17

Note

Values in the means of triplicate

Ethanol extract concentration 5.0 mg/ml

Penicillin 50µg/disc

Nystatin 50µg/disc

Conclusion

This research work states that the presence of presence of alkaloids, reducing sugars, tannis, flavonoids, Phlobatannis, and steriods are responsible for the antimicrobial activity, the ethanolic extract of *Solanum nigrum* have of C=O, C-H, C=C and C-O bond stretching compounds exhibits maximum zone of inhibition against the tested organism when compared with control drug penicillin and Nystatin .Hence present study suggests that pathogenic microorganism become resistant to the existing drugs. And this study shows that the plant is promising development of phytomedicine for antimicrobial properties. In this endeavor, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs. The drugs derived from herbs may have the possibility of using in medicine because of its antibacterial activity.

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