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RESEARCH ARTICLE

Bioprospecting *Solanum nigrum* Linn. (Solanaceae) as a potential source of Anti-Microbial agents against selected Bacterial strains

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ABSTRACT

Solanum nigrum Linn. is used in Indian traditional and folklore medicines to cure various ailments. Crude extracts of different plant parts of *S. nigrum* were obtained using different solvents viz. petroleum ether, chloroform, acetone, ethanol and methanol. Phytochemical constituents in different solvent extracts were analyzed. In vitro antibacterial studies on the leaf extracts were carried out on selected bacterial strains *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia*, using disc diffusion assay. Results indicate that the leaf aqueous extract was more active against all the microbes tested however, with variations. This indicates that the composition of phytochemicals depend on the type of solvent system employed in extraction.

Keywords: Phytochemicals; *Solanum nigrum*; Bioprospecting; Antimicrobial agents; Medicinal plants.

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1. INTRODUCTION

The use of herbal drugs has been very minimal for reasons viz., less availability of herbs, high prices of medicinal plants and lack of technology for commercial cultivation. However, the indiscriminate use of antibiotics and dependence on synthetics has reached a point of saturation and people prefer to use natural pharmaceuticals with a hope to surmount antibiotic resistance developed by pathogenic microbes [1-5], Furthermores, some antibiotics have undesirable side effects which limit their applications, hence, ultimate goal of leading pharma giants is to hunt for novel therapeutic agents from plants that are effective with minimal side effects [6].

To ascertain the medicinal value of the phytochemicals pharmacological studies have been carried out by different groups world over. WHO depicts infectious diseases are the major cause of deaths worldwide. Infectious diseases alone account for more than 50 % of the deaths in tropical countries [7]. To combat these diseases in the last few decades, pharmacological industries have produced a number of antibiotics, but the resistance of microbes has also increased [8]. Further, it has been reported that bacterial strains have developed resistance to almost all the antibiotics that are available in the market. This has resulted multiple drug resistance in both human and plant pathogens due to indiscriminate use of synthetic drugs especially in the developing countries [9].

The efficacy of the plants in curing various ailments has been well established and a large volume of work has been done in this field by researchers in India. In this study, the most commonly used medicinal plant mentioned in the folkloric medicinal system to ameliorate the skin infections *S. nigrum* has been selected and phytochemical constituents were analyzed for their activity towards the selected microbial strains of clinical importance.

S. nigrum (Solanaceae) is an important medicinal plant. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates, protein, fat, crude fiber, and

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minerals. This herbal plant is used as medicine for asthma, vomiting of blood, reducing blood glucose level and bilious matter phlegmatic rheumatism and ulcer [10]. Further the leaves of *S. nigrum* has been reported to exhibit antibacterial, antihistaminic, antiallergic, antifungal, anticancer anti inflammatory, antimitotic, antioxidant and antitumor activity [11-15]. *S. nigrum* has been used as the important ingredient in Liv. 52, used for treating liver diseases [16].

2. MATERIALS AND METHODS

2.1. Collection of the plant material:

S. nigrum (Solanaceae) was collected from Periyakulam, Theni district, Tamilnadu, India. The Flora of Presidency of Madras [17] and The Flora of Tamil Nadu Carnatic [18] were used for identification of the plant. The plant was authenticated by Botanical Survey of India, Coimbatore. The plant samples were washed thoroughly in tap water and then with distilled water and kept for drying. Dried leaves were taken and powdered for extraction. Soxhlet extraction was used to isolate the active drug principle.

2.2. Test organisms:

Clinical strains of *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumonia* were obtained from Holy Redeemer Hospital, Theni, Tamil Nadu, India . All bacterial cultures were maintained in NA slants/ plates; stored at 4℃ and periodically sub-cultured.

2.3 Preliminary screening of the plant extracts:

Crude leaf extracts of *S. nigrum* were obtained using different solvents viz. petroleum ether, chloroform, acetone, ethanol and methanol. Preliminary qualitative phytochemical screening of the solvent extracts of plant parts for flavonoids, glycosides, phenols, reducing sugars, resins, saponins, steroid, sterols, tannins, terpenoids, and triterpenoids to detect the chemical constituents by following the standard protocols [19-22].

Test for Phenols:

The plant extract (50 mg) was dissolved in 5 ml of distilled water. To this a few drops of neutral 5% ferric chloride solution were added. Appearance of a dark green color indicates the presence of phenolic compounds in the extract.

Test for Sterols:

The insoluble residue was dissolved in chloroform and a few drops of acetic anhydride were added along with a few drops of conc. sulphuric acid from the sides of the test tube and was observed for the formation of blue to blood red color.

Test for Flavonoids:

One ml of the extract, a few drops of dilute sodium hydroxide was added. Formation of intense yellow color in the plant extract, which become colorless on addition of a few drops of diluted HCl indicates the presence of flavonoids.

Test for Resins:

5ml of distilled water was added to the extract and observed for turbidity.

Test for Steroids:

One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Tannins:

About 0.5gm of the each extract was taken in a boiling tube and boiled with 20ml distilled water and then filtered. To the filtrate Five ml of the extract and a few drops of 1 % lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

Test for Glycosides:

The extract was hydrolyzed with HCI for few hours on a water bath. To the hydrolysate, 1 ml of pyridine was added and a few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides.

Test for Terpenoids:

To 0.5 gm of the extract 2ml of chloroform and conc. H_2So_4 (3ml) were carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Triterpenoids:

Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H2S04, Formation of reddish violet color indicates the presence of triterpenoids.

Test for reducing sugar:

A few drops of Fehling's solution A and B were added in equal volume to the dilute extracts, heated for 30 minutes and observed for the formation of brick red color precipitate.

Test for Saponins:

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

2.4. ANTIMICROBIAL ACTIVITY TEST

Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer *et al* [23] and Ncube *et al* [24]. Plant extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the NA slant stock

was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm, Hi-media, Mumbai, India) impregnated with the plant extracts (1.0 mg/disc and 5.0 mg/disc) were placed on the cultured plates and incubated for 24 h at 37°C. The solvent loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

3. RESULTS

Phytochemicals present in the methanol, ethanol, acetone, chloroform and petroleum ether extracts of *S. nigrum* are listed in Table 1. Observed data indicates that flavonoids and glycosides are present in all the solvent and tannins are not present in this plant.

Phytochemicals	Solvent system used for extraction of the phytochemicals				
	Methanol	Ethanol	Acetone	Chloroform	Petroleum Ether
Flavonoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Phenols	-	-	+	+	+
Reducing sugars	-	-	-	+	-
Resins	-	-	+	-	-
Saponins	+	+	-	-	-
Steroid	-	-	+	+	-
Sterols	+	+	-	-	-
Tannins	-	-	-	-	-
Terpenoids	+	+	+	+	-
Triterpenoids	+	+	+	+	-

Table 1 Phytochemicals of *S. nigrum* in different solvent extracts

(+) - Present; (-) – Absent

The anti-microbial activity of the plant extracts against selected bacterial strains is tabulated in Table 2. Antimicrobial activity exhibited by the extracts was compared to the commercially available broad spectrum antibiotics chloramphenicol. The zones of inhibition exhibited by the test organisms are represented in Table 2. Methanolic extracts exhibited significant antibacterial activity against all the strains tested.

Solvent	*S. aureus*	*B. subtilis*	*S. typhi*	*K. pneumonia*
Methanol	+	++	+	+
Ethanol	+	++	+	+
Acetone	+	++	-	+
Chloroform	-	+	+	+
Petroleum Ether	-	-	-	-
Chloramphenicol	++	++	++	++

Table 2 Antimicrobial activities of leaf extracts of *S. nigrum*

(-) No zone of inhibition; (+) Zone of inhibition between <10 mm; (++) Zone of inhibition between 11-20 mm; (+++) Zone of inhibition >20mm.

4. DISCUSSION

S. nigrum leaves extract and fractions exhibited mild antibacterial activity. It has been reported that *S. nigrum* seed extract also exhibited selective antifungal activity, with strong zone of inhibition against *P. nicotianae* [25]. Previously it has been reported that the most susceptible bacterial strain was *B. subtilis* and *S. aureus* to extract and fractions of *S. nigrum* leaves [13], however, similar results were obtained for *S. typhi* and *K. pneumonia* also. This indicates that phytochemicals in *S. nigrum* leaf extracts are active against both Gram positive and Gram Negative bacterial strains tested.

It has been reported that comparing the hot extracts of leaf completely lost its antimicrobial activity whereas the cold extract had significant antimicrobial activity, indicating that, the temperature may be responsible for the loss of the drug principle's activity. Thus, it is likely that the compounds may be heat labile.

Polar solvent extracts of *S. nigrum* are exhibiting significant antimicrobial activity whereas there is no report of activity in the non-polar solvent extracts. Hence, polar or the aqueous extracts may be the best solvent system in extracting drug principle from *S. nigrum*. The gram negative pathogens *S. typhi, K. pneumonia* is controlled by *S. nigrum* ethanol cold extract. The responsible phytochemicals could be phenols, sterols, tannins and terpenoids.

S. nigrum leaf extracts exhibited maximum antibacterial activity against the selected microbial strains, Hence the tested solvent extracts must be analysed further by purification and characterization of the active compounds that may serve as leads for the development of novel pharmaceuticals.

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Conflict of Interest: None Declared

