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**Research Article**

# SCREENING OF *SOLANUM NIGRUM* FOR ITS PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY AGAINST RESPIRATORY TRACT PATHOGENS

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## ABSTRACT

Medicinal plants are extensively used to cure various infectious diseases in human beings, hence S. *nigrum*, was investigated for its activity against the isolated pathogens from sputum samples. The study was designed to screen and characterize the bacteria isolated from the respiratory tract infected patients. Aqueous, ethanol and diethyl ether extract of *S. nigrum* was prepared; four different concentrations of each extract was taken to determine the antibacterial activity against the isolated bacteria. The ethanolic extract of *S. nigrum* showed highest antimicrobial activity in comparison to aqueous and diethyl ether extracts. Phytochemical analysis of the plant showed the presence alkaloids, terpenoids, flavonoids, saponins, steroids and phenols. Thus *S. nigrum* has antimicrobial activity and can be used clinically to find novel antibacterial compounds for respiratory tract pathogens.

**Keywords**: Respiratory tract infections, Antibacterial activity, Phytochemical activity, *S. nigrum*, Secondary metabolites.

## INTRODUCTION

Respiratory tract infections (RTIs), which involve the upper or lower respiratory tract, frequently occurs after birth (Sethi *et al*., 2013). RTIs, such as sore throat, earaches, laryngitis, common cold, sinusitis, and mastoiditis, are the most frequently-occurred infections of all human diseases and have been frequently documented (Sazawal and Black, 2003; Roncevic *et al*., 2002). RTIs are amongst the most wide spread and serious infections, accounting for over 50 million deaths globally each year. Each year approximately seven million peoples die as direct consequences of acute and chronic respiratory infection. Bronchitis and pneumonia are the most common infection. Respiratory pathogens like *Klebsiella pneumoniae*, *Pseudomonas aeroginosa* and *Staphylococcus aureus* are some of the causative agents responsible for bronchitis and pneumonia (Ponni *et al*., 2000).

Most respiratory tract infections are caused by viral and bacterial pathogens responsible for higher morbidity and mortality (Qadri, 1993). The leading causes of non-communicable disease deaths in 2008 due to respiratory diseases were 3.9% and 4.2 million deaths were reported due to asthma and Chronic Obstructive Pulmonary Disease globally (WHO, 2010). Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combating diseases. Currently, people of Asia especially India are utilizing plants as part of their routine health management (Samy *et al*., 2008). Medicinal properties of plants are hinged on the presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and essential oils amongst

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others (Karou *et al*., 2006). The primary benefits of using plant derived medicines are that they are relatively cheaper than synthetic drugs, offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine. As a result, many potent drugs have been purified from plants, including emetin, quinine, artemisin and introduced to modern medical practice.

*Solanum nigrum* commonly known as "Black night shade" belongs to solanacae family. It is called as Manathakkali in Tamil. It shows medicinal properties like an-timicrobial, anti-oxidant, cytotoxic properties, antiulcerogenic, and hepatoprotective activity. It is an African pediatric plant utilized for several ailments that are responsible for infant mortality especially feverish convulsions, eye diseases, hydrophobia and chronic skin ailments. It is a potential herbal alternative that acts as an anti-cancer agent (Jain *et al*., 2011). *Solanum nigrum* belongs to the family *Solanaceae.* and is commonly known as Makoi or black nightshade. *S. nigrum* has been extensively used in traditional treatment for various ailments such as pain, inflammation and fever. The plant also possesses antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic properties (Ayesha *et al.*, 2010). The berries have been used in the treatment of stomach ulcers in folk medicine (Singh *et al*., 2011).

Therefore in the current investigation bacteria responsible for respiratory tract infections were isolated from sputum samples of RTI patients and later on characterized. *Solanum nigrum* leaves were subjected to extraction with solvents like ethanol, petroleum ether and water. The antimicrobial effects of the prepared extracts were evaluated against isolated respiratory tract pathogens.

## MATERIALS AND METHODS

### Collection and authentication of Plant Material

The plant material *S. nigrum* (Figure 1) was collected from in and around Chennai, Tamilnadu, India. The plant was authenticated by Dr. D. Aravind, Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai, Tami Nadu, India.

**Figure 1. *Solanum nigrum.***

### Preparation of Extracts

Fresh leaves of *S. nigrum* was washed thoroughly in tap water and with distilled water and air dried in the shade at room temperature for five days. Shade dried leaves were powdered.The plant powders (100 g) were successively extracted using ethanol and petroleum ether in soxhlet apparatus and crude extraction was done with water. The extracts were dried in vacuum desiccator and were stored in a sterile container for further use.

### Collection of Samples

The sputum samples were collected from patient's aseptically in well-labelled sterile, wide mouthed glass bottles with screw cap from Karpaga Vinayaga Institute of Medicinal Science and Government Hospital. Samples were then taken to the laboratory immediately for analysis (Kolawale *et al*., 2009). On the labels were marked the name, age, sex of the patients and the time of sample collection.

### Isolation and identification of pathogens in sputum samples

The collected samples were processed as per the standard procedure. For isolation and characterization of bacterial flora, the samples were inoculated into Blood agar (BA) media and incubated at 37°C for 24 hours (Cheesborough, 2006). Characteristic colonies from the plates

were isolated and then sub cultured to obtain pure culture. All the bacteria were isolated and identified using morphological and biochemical tests adopting standard procedures (Cowan and Steel, 1974). Stock culture was maintained in both Agar slant and 20% sterile buffered glycerol.

**Phytochemical Screening**

The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolic, alkaloids, tannins, flavonoids, saponin and steroids under qualitative analysis; the screening was carried for all the extracts of the plant (Harborne, 1998).

**Antibacterial activity of various extracts:**

Muller-Hinton Agar (MHA) plates were seeded with 24 hours old culture of the isolates. The organic fractions were dissolved in dimethyl sulfoxide (DMSO). Wells were bored using sterilized syringe of pore size 8 mm. Various concentrations of the extracts (250µl, 500µl, 750µl and 1000µl) were added into the sterile 8mm diameter well. Incubation was made at $37^0$C for 24 hrs. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The experiment was repeated four times and the average values were calculated for antibacterial activity (Sridhar *et al*., 2011).

## RESULTS AND DISCUSSION

**Phytochemical activity**

The phytochemicals present in *Solanum nigrum* were alkaloids, flavonoids, steroids, tannins and phlobatannins (Table 1), this is in accordance with an earlier investigation which showed the presence of various phytochemicals such as alkaloids, flavonoids, phenols, steroids and tannins in the crude extract of *S. nigrum* (Perez *et al*., 1990). These compounds are known to be biologically active because they protect the plants against infection. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available regarding anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties of plants (Cushnie and Lamb, 2005).

**Table 1. Phytochemical Constituents of *Solanum nigrum***

| Sl. No. | Phytochemical | Ethanolic extract | Aqueous Extract | Petroleum Ether extract |
|---|---|---|---|---|
| 1 | Alkaloids | + | + | + |
| 2 | Terpnoides | - | - | - |
| 3 | Saponins | - | - | - |
| 4 | Tannins | + | + | + |
| 5 | Flavonoids | + | + | + |
| 6 | Phlobatannins | + | - | - |
| 7 | Steroid | + | + | + |
| 8 | Phenols | - | - | - |

Tannins are known to possess general antimicrobial and antioxidant activities (Mohanta *et al*., 2011). Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents (De-Lucca *et al*., 2005). Other compounds like saponins also have anti-fungal properties (Mandal *et al*., 2005). Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory and weight loss, etc. It is also known to have anti-fungal properties (Manjunatha, 2006). Saponins have been implicated as bioactive antibacterial agents of plants (Arts and Hollman, 2005; Scalbert *et al.,* 2005). Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti- microbial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammtory (Abubakar, 2009).

The bacteria were isolated and identified using morphological and biochemical tests following standard procedures. Out of the 50 Sputum samples, 23 bacterial isolates were recovered and the biochemical tests revealed that, these isolates belong to 5 species (Table 4). The bacteria isolated from the samples were *Klebsiella pneumoniae*, *Streptococcus pyrogens*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from the sputum samples. In an earlier study following bacteria were isolated from the sputum sample of RTI patients *S. aureus, E. coli, P. aeruginosa, S. pneumoniae* and *K. pneumonia* (Siddiqui *et al*., 2009); this is in accordance with the present investigation except *S. pneumoniae*.

**Table 2.** Biochemical characterization of isolated bacteria from RTI patients.

| S. No. | Characteristics | *E. coli* | *K. pneumoniae* | *S. aureus* | *P. Aeruginosa* | *S. pyrogens* |
|---|---|---|---|---|---|---|
| 1 | Shape | Rods | Rods | Cocci | Rods | Cocci |
| 2 | Gram Test | - | - | + | - | + |
| 3 | Coagulase | - | - | + | - | + |
| 4 | Catalase | + | + | + | - | + |
| 5 | Citrate | - | + | + | + | + |
| 6 | Oxidase | - | - | - | + | - |
| 7 | Indole | + | - | - | - | - |
| 8 | MR | + | - | - | - | - |
| 9 | VP | - | + | + | - | + |
| 10 | Motility | Motile | Motile | Non- Motile | Motile | Non- Motile |

**Antibacterial activity**

The results of the antibacterial activities (Table 3) showed that the plant extracts exhibit remarkable activity against the test organisms isolated (*E. coli, K. pneumonia, P. aeruginosa, S. aureus,* and *S. pyrogens*) with zone of inhibition ranging from 5 to 25 mm. From the table it is evident that for the four concentrations taken for each extract; the highest concentration 100 mg/ml showed the maximum zone of inhibition for the five bacterial species. Ethanolic extract of *S. nigrum shows* maximum zone of inhibition for P. *aeruginosa* followed by the *S. pyrogens.* Aqueous extract of *S. nigrum shows* maximum zone of inhibition for P. *S. aureus* followed by *P. aeruginosa and S. pyrogens.* Petroleum ether extract of *S. nigrum shows* maximum zone of inhibition for *S. pyrogens* followed by *S. aureus*. Among the three extracts, ethanolic extract exhibited maximum antibacterial activity. Based on earlier reports, among the great variety of secondary compounds found in *S. nigrum* 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 (Singh *et al*., 2011).

**Table 3.** Antibacterial activity of leaf extracts of *Solanum nigrum* (Zone of diameter of three replicates).

| | | Zone of inhibition (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. No. | Microorganism | Ethanolic Extract | | | | Aqueous Extract | | | | Petroleum ether extract | | | | Streptomycin (10 mg/ml) |
| | | A | B | C | D | A | B | C | D | A | B | C | D | |
| 1 | *E. coli* | 12 | 11 | 10 | 9 | 11 | 9 | 8 | 7 | 10 | 9 | 7 | 5 | 21 |
| 2 | *K. pneumonia* | 13 | 10 | 11 | 7 | 11 | 9 | 10 | 7 | 12 | 9 | 10 | 6 | 15 |
| 3 | *S. aureus* | 15 | 13 | 8 | 6 | 16 | 13 | 10 | 6 | 15 | 10 | 9 | 7 | 16 |
| 4 | *P. aeruginosa* | 17 | 15 | 13 | 5 | 15 | 14 | 11 | 9 | 12 | 13 | 11 | 9 | 17 |
| 5 | *S. pyrogens* | 16 | 12 | 11 | 9 | 15 | 13 | 10 | 7 | 16 | 13 | 10 | 7 | 20 |

A, B, C, D indicates 100, 75, 50 and 25 mg/ml concentrations.

## CONCLUSION

This study shows that *S. nigrum* possess antimicrobial activity against bacteria associated with respiratory tract infectious. The plant can be used as a source of oral drug against respiratory tract infections; however, further studies are required to isolate the active principle from the crude extract for proper drug development.

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