



Phytochemical study of qualitative and antioxidant, antibacterial studies of solanum nigrum linn

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Abstract

In the solanaceae family, Solanum nigrum is known as Black Night Shade. Flavonoides, alkaloids, tannins, saponins, proteins, and phenols are among the biological compounds that have been studied qualitatively. Antioxidant activity was measured using the DPPH technique, while antibacterial activity was assessed under various conditions.

Keywords: phytochemicals, solanum nigrum l, anti oxidant activity, antibacterial activity

Introduction

Since ancient times, medicinal plants have been used in Homeopathy, Ayurvedic, Allopathy, and traditional medicine ^[1]. Medicinal plants play an important role in traditional and modern medicine ^[2]. Their use has been increased by numerous studies, and it reduces the use of synthetic drugs ^[3]. The use of synthetic drugs causes many side effects ^[4]. This technique of medicine meets the needs of nearly 75 percent of people living in rural areas near natural resources. Many chemical components of medicinal plants can be found in the leaves, stems, flowers, and other parts of the plants. The existence of many complex chemical compounds of varied formations that present as secondary Metabolites ^[6] determines the healing quality of herbal plants. Medicinal plants are quite valuable in terms of money. It manufactures the raw materials for native pharmaceuticals ^[7]. We must be aware of the chemical components found in plants since this knowledge aids us in detecting the most effective treatments for ailments. Alkaloids, tannin, flavonoids, phenolic content, and other bioavailable chemical ingredients in plants are responsible for biological and biochemical activity in the human anatomy ^[8]. Plant-based herbal species have anticancer and antioxidant properties. Plant components are environmentally beneficial. The medicinal plants offer a great deal of therapeutic value ^[9]. Solanum nigrum L., often known as Black Night Shade, is a member of the solanaceae family. It is an annual herbaceous plant with a green, smooth, semi-climbing stem that grows up to 10-60 cm long. The opposing leaves are slightly cogged and have a complete limb, oval and dimond form. River bunds, damp woods, waste land, old fields, and ditches, as well as roadside and wet cultivated land, are all popular places to find it. It is used medicinally to treat a variety of ailments, including pneumonia, hurting teeth, wing worms, discomfort, fever, inflammation, and stomach aches, as well as as a tonic, antioxidant, anti-inflammatory, and antipyretic ^[10].

Taxonomic Classification

Kingdom: Plantae

Division: Angiosperms

Class: Edicts

Sub Class: Asteroids

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: Solanum Nigrum

Plant Description

Materials and Methods

Plant Material Preparation

The leaves were taken from rural areas in Villupuram, Tamil Nadu, India, and carefully cleaned 2-3 times with running tap water before being air dried in the shade. The plant material was ground in the mixer after complete shade drying, and the powder was stored in little plastic bags with suitable labelling.

Plant Material Extraction

Preparation of aqueous extracts: First, 5 gm of ground leaves were weighed using an electronic scale, and 5 gm of plant material were crushed in 25 ml of sterile water, which was then heated to 50-60 c and filtered using

Whatman filter paper no.1. The filtrate was then centrifuged for 15 minutes at 250 rpm, collected in sterile bottles, and kept refrigerated at 5° C until use.

The Ethanolic Extract Is Made In The Following Way

Ethanolic extract was made according to Indian pharmacopoeia procedures (Anonymous, 1996). Batch extraction with 140ml ethanol and 60ml distilled water was performed individually and sequentially on the leaves powder material. Whatmann filter paper was used to filter these extracts. The extract was then stored in an airtight container ^[11].

Preliminary phytochemical Analysis

This was done using the procedures given in Qualitative phytochemicals analysis of the crude powder of *Solanum nigrum* L for tests of phytochemicals such as alkaloids, saponins, tannins, flavonoides, and protein, among others.

1. Test for Alkaloids

200 mg of plant material was collected, mixed with 10 mL Methanol, and filtered. After that, 2 ml filtrate was taken and 1 percent HCL was added with steam 1 ml filtrate and 6 drops Mayer's reagent/ Wagner's Dragendorff's were added. The presence of alkaloids is indicated by the presence of creamish/brown/red/orange precipitate.

2. Test for Saponins

5 ml distilled water was added to approximately 0.5 ml filtered water. The presence of saponins is indicated by the persistence of frothing.

3. Test for Tannins

200 mg plant material were taken and added 10 ml distilled water and then filtered. After that 2 ml filtered were taken and added 2 ml $FeCl_3$ Blue. Then black precipitate indicate the presence of Tannins & Phenols.

4. Test for Flavonoides

We took 200 mg of plant material and mixed it with 10 ml distilled water before filtering it. After that, 2 ml of filtered water was taken and 2 ml of $FeCl_3$ Blue was added. The presence of Tannins and Phenols is indicated by black precipitate.

5. Test for protein

Add a few drops of Millons reagent to 3-5 mL of plant extract or filtrate, stir thoroughly, and heat. After boiling, a white precipitate is generated, which turns brick red.

6. Phenol test

A few drops of a very dilute solution of neutral ferric chloride are added to a little amount of the material in water of alcohol. When phenol is present, a violet colour is generated.



Fig 1: Shows the Plant and Powder image of *Solanum Nigrum* Linn.

Result and Discussion

Phytochemical Screening

Basic phytochemical screening is using simple chemical tests to detect the presence of classes of compounds recognised to have medicinal potential in a plant extract, such as alkaloids, acetogenins, polyketides, isoprenoids,

and carbohydrates. The elements of a qualitative chemical can be used to determine the general composition of an unknown vegetable product. Steroids (Salkowski test), terpenoids, alkaloids (Wagner's Tests), flavonoids (Alkaline reagent test), tannins (Braymer's test), saponins (Forthing test), Coumains (NaOH test), carbohydrates (Molisch's Benedict's test and Fehling's test), proteins, and amino acids were all screened using standard protocols.

Table 1: Preliminary Phytochemical Analysis of Flowers and Leaves of *Solanum Nigrum* Linn

| S.No | Phytochemical Constituents | Aqueous Extract | Ethanol Extract |
|------|----------------------------|-----------------|-----------------|
| 1. | Alkaloids | + | + |
| 2. | Saponins | + | + |
| 3. | Tannins | – | + |
| 4. | Flavonoids | + | + |
| 5. | Proteins | + | – |
| 6. | Phenol | + | + |

+ = indicate presence of phytochemical, - = indicates absence of phytochemicals

The majority of current study is focused on the phytochemicals of the *Euphorbia Hirta* plant. Many phytochemicals were discovered in the *Solanum Nigrum* Linn leaf extract, which was extracted using the waterbath method. As a result, the study reveals that the plant extract has phytochemical properties.

***In Vitro* Antioxidant Activity**

DPPH, H₂O₂, and O₂⁻ radical scavenging assays were used to assess the extract *Solanum Nigrum* Linn's antioxidant activity *in vitro*. The IC₅₀ values were calculated and compared to ascorbic acid, a standard antioxidant (Table 2). The ABTS assay is a relatively new one that uses a more powerful, chemically created radical to screen complex antioxidant mixtures including plant extracts, drinks, and biological fluids. The solubility of ABTS^{•+} in both organic and aqueous environments, as well as its stability over a wide pH range, piqued researchers' interest in using it to estimate antioxidant activity [12].

When the DPPH-free radical combines with hydrogen donors, it forms a matching hydrazine. The DPPH radical is purple in appearance and turns yellow when it reacts with hydrogen donors. It's a discoloration test that involves adding the antioxidant to a DPPH solution in ethanol or methanol and measuring the decrease in absorbance at 490 nm. Free radical participation, particularly increased generation, appears to be a characteristic of most human diseases, including cardiovascular disease and cancer.

The addition of sodium hydroxide to air-saturated dimethyl sulfoxide produces superoxide radicals in the alkaline DMSO method (DMSO). At normal temperature, the produced superoxide remains stable in solution, reducing nitro blue tetrazolium to Formosan dye, which can be detected at 560 nm. The creation of a red dye formazan is inhibited by a superoxide scavenger capable of reacting [13]. Several oxidase enzymes produce hydrogen peroxide in the body. There is mounting evidence that hydrogen peroxide causes serious harm to biological systems, either directly or indirectly through its reduction product, the hydroxyl radical (OH•). The decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm when a scavenger is incubated with hydrogen peroxide in this method [14].

The inclusion of phytochemicals such as alkaloids, carbohydrates, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids in the *Solanum Nigrum* Linn extract resulted in a higher antioxidant activity. By chemical technique, the *Solanum Nigrum* Linn extract has the least antioxidant activity. All of the *Solanum Nigrum* Linn show strong antioxidant activity when compared to typical antioxidants such as ascorbic acid, based on the above antioxidant findings. All of the evaluated methods had the same antioxidant activity order. Because it includes a high amount of phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, the *Solanum Nigrum* Linn has remarkable antioxidant action when compared to conventional ascorbic acid.

The inhibition-based *in vitro* approaches are used. The inhibition of free radical action is evaluated after samples are added to a free radical producing system, and this inhibition is connected to the sample's antioxidant activity. The generated radical, the reproducibility of the creation procedure, and the endpoint employed for the determination all differ significantly. Despite the fact that *in vitro* methods provide a valuable indication of antioxidant activity, data derived from *in vitro* methods are challenging to adapt to biological systems and do not always predict similar *in vivo* antioxidant activity. It's important to remember that all of the methodologies established have advantages and disadvantages, and that a single measurement of antioxidant capacity is rarely enough. It's possible that a variety of approaches will be required.

The *Solanum Nigrum* Linn and standard solutions had concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 g mL⁻¹. To avoid extract agglomeration, the dilute solution of *Solanum Nigrum* Linn were sonicated for 30 minutes at room temperature in a sonicator bath. The absorbance was compared to the equivalent blank solutions using spectrophotometry. Using the following formula, the % inhibition was calculated:

$$\text{Radical scavenging activity \%} = \frac{OD \text{ control} - OD \text{ sample}}{OD \text{ control}} \times 100$$

Invitro Antioxidant Activity

1. Assay for DPPH

The experiment was done in a 96-well micro titer plate. In each well of the micro titer plate, 10 l of each sample or standard solution was added separately to 200 l of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) solution. The absorbance of each solution was measured at 490 nm after 30 minutes of incubation at 37 °C.

The DPPH radical scavenging assay method was used to assess the antioxidant activity of the extract Solanum Nigrum Linn. The anti-oxidant activity of Solanum Nigrum Linn equal doses of 100 nM measured at 230 nm is shown in Table 2.

2. Assay Hydroxyl Radical Scavenging

Various amounts of samples or standard (0.5 mL) were added to a reaction mixture including ferric chloride (0.5 mL, 0.1 mM); EDTA (0.5 mL, 0.1 mM); ascorbic acid (0.5 mL, 0.1 mM); hydrogen peroxide (0.5 mL, 2 mM); and p-nitrosodimethyl aniline (p-NDA; 0.5 mL, 0.01 mM) By combining 0.5 mL sample with 2.5 mL phosphate buffer, a sample blank was created. These solutions' absorbance was measured at 440nm.

H₂O₂ radical scavenging assay technique was used to study the antioxidant activity of extract Solanum Nigrum Linn. The anti-oxidant activity of Solanum Nigrum Linn equal doses of 100 nM measured at 230 nm is shown in Table 2.

3. Assay for Superoxide Radical Scavenging (Alkaline DMSO Method)

0.1 mL of nitro blue tetrazolium (NBT; 1 mg mL⁻¹) was added to a reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) and 0.3 mL of the sample in freshly distilled DMSO at varied concentrations. At 560nm, the absorbance was measured.

The superoxide scavenging assay method was used to assess the antioxidant activity of Solanum Nigrum Linn once more. The superoxide scavenging activity of Solanum Nigrum Linn at a concentration of 100 nM was measured at 560 nm in this work. Table. 2

Table 2: Antioxidant scavenging percentages against DPPH, Hydrogen peroxide, and Superoxide radicals in Solanum Nigrum Linn compared to normal

| Compound | Free Radical Scavenging Activity (%) | | |
|---------------------|--------------------------------------|-------------------------------|-----------------------------|
| | DPPH | H ₂ O ₂ | O ₂ ⁻ |
| Solanum Nigrum Linn | 65.3 | 68.2 | 70.2 |
| Ascorbic Acid | 67.2 | 70.0 | 73.2 |

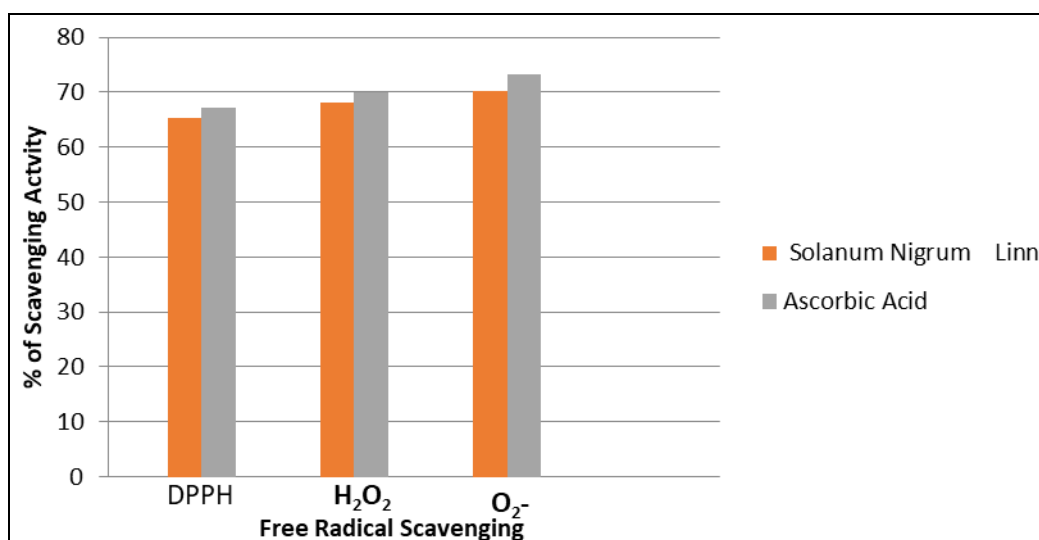


Fig 2: *In vitro* antioxidant activity various free radical assay method compared with Standard Ascorbic Acid.

DPPH, H₂O₂, and superoxide radical scavenging assays were used to study the antioxidant activity of the extract Solanum Nigrum Linn. Figure 4 compares Solanum Nigrum Linn's anti-oxidant activity to that of normal ascorbic acid. Superoxide radical scavenging has stronger antioxidant activity than hydrogen peroxide radical scavenging, according to free radical scavenging.

In comparison to other free radical scavenging methods, DPPH free radical scavenging activity reveals superior antioxidant activity. It has 58 % activity compared to 68 % for normal ascorbic acid.

H₂O₂ free radical scavenging activity is superior to other free radical scavenging methods in terms of antioxidant activity. It has a 62 % activity compared to 70 % for normal ascorbic acid.

In comparison to other free radical scavenging methods, superoxide free radical scavenging activity demonstrates the best antioxidant activity. It shows a 63 % increase in activity when compared to standard ascorbic acid 73%.

Antibacterial Activity

Infectious diseases caused by bacterial and fungal organisms pose a severe hazard to public health around the world. Antibiotics are a common therapeutic option for bacterial and fungal illnesses. The emergence of antimicrobial resistance and toxicity concerns, on the other hand, have reduced the usage of antibacterial agents. Antibiotics' safety and efficacy limits complement biological research on the antibacterial role of plants, which have similar toxicity and efficacy. The antibacterial properties of *Solanum Nigrum* Linn ethanolic extract against pathogenic bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli* were investigated. The antibacterial and antifungal potential of ethanolic extracts was measured in terms of bacterial and fungal growth inhibition zones. The antibacterial outcome has sparked interest in the development of alternative antimicrobial medications for the treatment of infectious disorders. without any negative side effects The consequences of such colonisation in vital human organs as the lungs, urinary tract, and kidneys might be lethal. This bacterium is found on and in medical equipment, especially catheters, because it thrives on damp surfaces, causing cross-infections in hospitals and clinics. It can also breakdown hydrocarbons, therefore it's been used to break down tar balls and oil spills. By disc diffusion method, *Solanum Nigrum* Linn leaf extract has strong activity against *Pseudomonas aeruginosa* with a zone of inhibition of 18 mm and *E. coli* with a zone of inhibition of 21 mm.

Table 3: Zone of inhibition of the synthesized *Solanum Nigrum* Linn

| S. No | Positive and negative Pathogen | Zone of inhibition (diameter in mm) | | | | Standard (Gentamicin) |
|-------|--------------------------------|-------------------------------------|----------|----------|-----------|-----------------------|
| | | 25 µg/mL | 50 µg/mL | 75 µg/mL | 100 µg/mL | |
| 1 | <i>Staphylococcus aureus</i> | 8 | 12 | 15 | 17 | 10 |
| 2 | <i>Pseudomonas aeruginosa</i> | 9 | 10 | 12 | 16 | 12 |
| 3 | Control (DMSO) | NI | NI | NI | NI | NI |

NI: No Inhibition

The antibacterial properties of *Solanum Nigrum* Linn ethanolic extract were investigated against the pathogenic microorganisms *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial activity of ethanolic extracts was measured in terms of bacterial growth inhibition zone. The antibacterial outcome has sparked interest in developing new antimicrobial medications that are free of adverse effects for the treatment of infectious disorders. The consequences of such colonisation in vital human organs as the lungs, urinary tract, and kidneys might be lethal. This bacterium is found on and in medical equipment, especially catheters, because it thrives on damp surfaces, causing cross-infections in hospitals and clinics. It can also degrade hydrocarbons and has been used to oil spills produce tar balls and oil. By disc diffusion technique, *Solanum Nigrum* Linn leaf extract has high activity against *Staphylococcus aureus* with a zone of inhibition of 17 mm and *Pseudomonas aeruginosa* with a zone of inhibition of 16 mm.

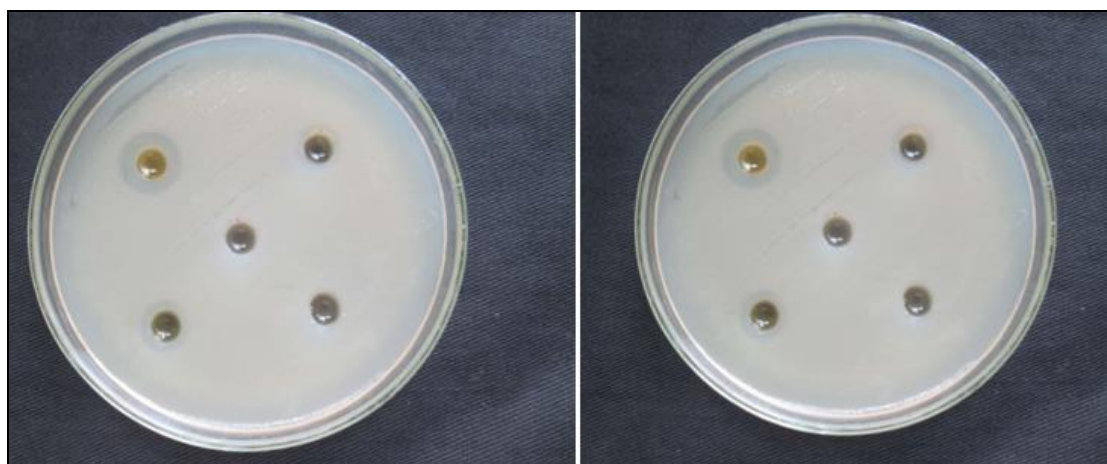


Fig 5: *Solanum Nigrum* Linn's Antibacterial Activity

Conculsion

The antioxidant activity of *Solanum Nigrum* extract was investigated using the DPPH, H₂O₂, and O₂- free radical assay methods. *In vitro* antioxidant studies using various methodologies reveal that *Solanum Nigrum* Linn has significant antioxidant activity when compared to typical medicines. Because it contains various phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, *Solanum Nigrum* Linn displays exceptional good activities in all biological research.

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