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Phytochemical, antioxidant and antibacterial activity of Solanum Trilobatum

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Abstract
The leaves of Solanum trilobatum were employed in phytochemical research. The biological compounds alkaloids, tannins, saponins, terpenoid, flavanoids, proteins, and phenols have all been examined qualitatively. Antioxidant activity was determined using the DPPH method, and antibacterial activity was determined using the disc diffusion method with test microorganisms such as Bacillus Subtilis and Escherichia coli at varied concentrations of the extracts (50, 100, 150 g/ml).

Keywords: Solanum Trilobatum, phytochemical study, antioxidant, antibacterial

Introduction
Solanum trilobatum L. (Solanaceae) has long been known as nightshade, a plant that has been used to treat a variety of ailments by people from all walks of life. Earlier research on Solanum trilobatum concentrated on a variety of pharmacological activities, with a few focusing on antioxidant and hepatoprotective properties. The current study aimed to assess the antioxidant capability and hepatoprotective effects of S. trilobatum L. on TAA intoxication in Wistar albino rats [1]. The rats were divided into four groups, each with six animals. Solanum trilobatum is a medicinal plant that is widely available in different parts of the world and is used in Indian medicine. It is bioactive and contains drugs such as Sobatum, Sobasodine, tomatidine, disogenin, and solanine, which can be found in various parts of the plant, including the leaf, stem, root, flowers, and berries. It's antibacterial, anti-inflammatory, antioxidant, cytotoxic, anti-diabetic, and immodulatory properties have all been investigated [2] It's also used to treat tumours, cancer, cells, and other diseases, as well as to boost immunity. Natural nutrional and non-nutrional components found in a variety of edible plant species play a role in pharmacological action [3] Because of higher cultural acceptability, compatibility with the human body, and less side effects, herbal medicine provides the foundation for around 75 percent to 80 percent of the world's population, primarily targeting primary health care in underdeveloped countries. Plants should also be evaluated for their efficacy against human disease and for the creation of safe modern medications, according to the World Health Organization [WHO].
Medicinal plants are a diverse set of commercially significant plants that offer the primary raw material for domestic medications. They are employed as nutraceuticals, food supplements, folt medications, and for drug production in the modern medicinal industry [4]. Solanum trilobatum leaves and stems are said to have antimicrobial, anti-inflammatory, and anti-ulcerogenic effects [5].
The extracts from the leaves are used to boost male fertility and treat snake poisoning [6]. Because the plants are powerful against human disease and its causes, they are being exploited to generate safe modern medications [7].
The roots, berries, and blooms of this plant, known as 'Alarka' in Telugu and 'Tuduvalai' in Tamil, are used to alleviate cough [8].
Solanum is the chemical in this plant that suppresses tumour growth. Plant-derived substances have recently piqued interest due to their wide range of applications. Therapeutic plants are a local heritage with worldwide significance, and the globe is endowed with a vast array of medicinal plants [9]. Common sugars, amino acids, proteins, and chlorophyll are the primary ingredients. Alkaloids, terpenoids, flavonoids, tannins, and phenolic chemicals are secondary constituents [10]. I could study the phytochemical and biological activity based on the report.

Taxonomic classification
Kingdom: Plantae
Division: Tracheophyta
Class: Dicotyledons
Order: Solanales
Family: Solanaceae
Genus: Solanum
Species: Solanum trilobatum

Plant Description

Fig 1: Solanum Trilobatum plant and powder image.

Materials and Methods

1. Plant material preparation

Generally fresh leaves were collected from the rural village of villupuram washed 2-3 times with running tap water in order to remove the impurities from the leave materials. The leave materials was dried in air under shade after it dried the plant materials was grind with the help of mixer, The powder was kept in small plastic containers or zip lock cover with proper labelling.

2. Plant material extraction

Preparation of aqueous extracts in the first grind leaf materials, take 5gm of leaf powder and weighed by using an electronic balance then 5gm of plant material were crushed in 25ml of sterile water then heated at 50-60^{o}C then it was filtered by using whattman filter paper, The filtrate was centrifuged at 2500 rpm for 15min and filtrate was collected in sterile bottle and stored in the refrigerator at 5^{o}C now the filtrate was ready for test analysis

Phytochemical Screening (Preliminary)

All extracts were screened for phytochemicals according to established protocols.

Test for Alkaloids

For the production of an orange colour precipitate, a few drops of Dragondraff's reagents were added to 5ml of the extract.

Test for Tannins

The extracts were dissolved in water and then placed in a water bath at 300^{0}C for 1 hour before being treated with ferric chloride sodium and monitored for the production of a dark green colour.

Test for Sapanions

A little amount of the extract was mixed with a few drops of distilled water and forcefully shaken until a persistent foam formed.

Test for Terpenoids

A few drops of chloroform and con.H_2SO_4 were carefully added to 5ml of the extract to form a layer on the walls of the test tube, and the presence of reddish brown colour was noted.

Wagner's test

To 5ml of the extract few drops of wagner's reagent was added for the formation of reddish brown colored precipitate.

Test for flavanoids

Magnesium ribbons were dipped in 3ml of the extract and then dried. HCl was put over them and absorbed for the production of magenta (brick red) colour, demonstrating the presence of flavanoids.

Test for proteins
For the development of a violet or purple tint, a few drops of 10% Sodium Chloride and 1% Copper Sulfate were added to 3ml of the extract. It darkens when alkali is added.

Test for phenols
Ferric chloride test
Add 2ml of distilled water to 1ml of plant extract, then a few drops of 10% $FeCl_3$ to generate a green colour, which indicates the presence of phenols.

In Vitro Antioxidant Activity
To properly analyse in vitro antioxidant activity of a specific molecule or antioxidant capacity of a biological fluid, a variety of approaches may be required. Standard methods were used to investigate the antioxidant potentials of the extract Solanum trilobatum. The concentrations of the Solanum trilobatum Extract and standard solutions were 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 g mL1, respectively. To avoid extract Solanum trilobatum agglomeration, the dilute solution of extract Solanum trilobatum was sonicated for 30 minutes at room temperature using a sonicator bath. The absorbance was compared to the equivalent blank solutions using spectrophotometry. Using the following formula, the % inhibition was calculated:

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

DPPH Assay
The experiment was done in a 96-well microtiter plate. In each well of the microtiter 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.

Hydroxyl Radical Scavenging Assay (p-NDA Method)
Various amounts of samplesor 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); andp-nitrosodimethyl aniline (p-NDA; 0.5 mL, 0.01 mM 0.5 mL sample and 2.5 mL phosphate buffer were combined to make a sample blank. These solutions' absorbance was measured at 440 nm.

Superoxide Radical Scavenging Assay (Alkaline DMSO Method)
To the 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 various concentrations, 0.1 mL of nitro blue tetrazolium (NBT;1 mg mL−1) was added to make a final volume of 1.4 mL.The absorbance was measured at 560 nm.

Hydrogen Peroxide Radical Scavenging Assay
In phosphate-buffered saline, a solution of hydrogen peroxide (20 mM) was produced (pH 7.4). 1 mL of the samples and standard were added to 2 mL of hydrogen peroxide solution in PBS at various concentrations. The absorbance was measured at 230 nm after 10 minutes.

Antibacterial activity
Preparation of nutrient agar medium
Peptone - 0.150mg
NaCl - 0.150mg
Beef extract - 0.45mg
Yeast extract - 0.045mg
Agar agar - 0.450mg
Water - 30ml

The aforesaid materials were dissolved in 30mL of distilled water and autoclaved for 15 minutes at 121°C. The medium's pH was kept constant at 7.2. Pour into sterile Petri plates after thoroughly mixing.

Antibacterial activity of Solanum trilobatum
The disc diffusion method was used to test the antibacterial activity of an ethanolic extract of Solanum trilobatum. Different concentrations of the extracts (50, 100, 150g/ml) were generated by reconstituting with water. The test microorganisms, such as Pseudomonas and E.coli, were seeded into the appropriate medium using a spread plate method of 10l (106 cells/ml) and bacteria cultures developed in nutritional broth for 24 hours. After the extracts had solidified, sterile filter paper discs (6mm in diameter) soaked with them were placed on test organism plates. The typical antibiotic was gentamycin (20 g/ml). The anti assay plates were incubated for 24 hours at 37 degrees Celsius. The inhibitory zones' sizes were determined in millimetres.

Measurement of zone of inhibition

The antibacterial potential of test compounds was measured in millimetres by the mean diameter of the inhibitory zone around the disc. A millimetre scale was used to assess the zones of inhibition of the tested microorganisms by the extracts.

Result and Discussion

Table 1: Qualitative analysis of solanum trilobatum leaves

S. No	Test	Observation
1	Alkaloids	+
2	Tannins	+
3	Saponins	+
4	Terpenoids	+
5	flavanoids	+
6	proteins	+
7	Phenol	+

(+) Presence (-) Absence

Fig 2: Qualitative analysis of Solanum trilobatum

Antioxidant Activity

Antioxidants' redox capabilities are crucial for absorbing and neutralising free radicals, quenching singlet and triplet oxygen, and degrading peroxides. The antioxidants are oxidised as a result of this process. This necessitates the regular replenishment of antioxidants. The DPPH test was used to assess the antioxidant capabilities of S. trilobatum. The antioxidant activity, as measured by the percentage of inhibition, was 40% when the aqueous extract was consumed in varied concentrations ranging from 10 to 60 gmL.

Antibacterial Activity

The antibacterial activity of an ethanolic extract of Solanum trilobatum against the pathogenic microorganisms Bacillus subtilis and Escherichia coli was investigated. 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. By disc diffusion method, leaf extract of Solanum trilobatum shows high activity against Bacillus subtilis with a zone of inhibition of 12 mm and E. coli with a zone of inhibition of 14 mm, indicating that solanum trilobatum has better antibacterial activity at al;l concentrations and that the zone of inhibition increases as concentration increases.

Table 2: Zone of inhibition of the extract Solanum trilobatum

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	Bacillus Subtilis	7	9	11	12	15
2	Escherichia coli	7	10	12	14	16
3	Control (DMSO)	NI	NI	NI	NI	NI

NI: No Inhibition

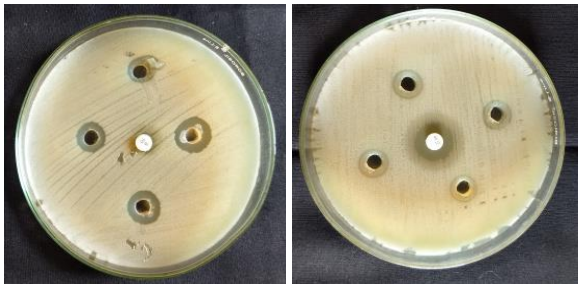

Fig 3: Antibacterial activity of the Solanum trilobatum against Bacillus Subtilis and Escherichia coli

Antioxidant Activity

In DPPH, H_2O_2, O_2-, NO, and OH radical scavenging assays were used to investigate the extract Solanum trilobatum's antioxidant activity in vitro. (See 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 [11] 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 hallmark of most human diseases, including cardiovascular disorders and cancer. The creation of a red dye formazan is inhibited by a superoxide scavenger capable of reacting [12] 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 presence of phytochemicals such as alkaloids, sugars, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids contribute to the extract Solanum trilobatum's greater antioxidant activity. With 67 percent antioxidant activity, the extract Solanum trilobatum chemical technique has the lowest antioxidant activity. In comparison to typical antioxidants like ascorbic acid, all of the extracts from Solanum trilobatum demonstrate high antioxidant activity. All of the evaluated methods had the same antioxidant activity order. Because it includes a considerable quantity of phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, the extract Solanum trilobatum has excellent antioxidant action [13, 14]

Table 3: shows the percentage of DPPH radical, Hydrogen peroxide radical, Superoxide radical, Nitric oxide radical and Hydroxyl radical scavenging activity in extract Solanum trilobatum compared with standard

Compound	Free radical Scavenging activity (%)				
	DPPH	H_2O_2	O_2-	NO	OH
Extract Solanum trilobatum	67.1	63.2	61.2	65.7	64.5
Ascorbic Acid	70.7	76.0	71.9	70.6	74.9

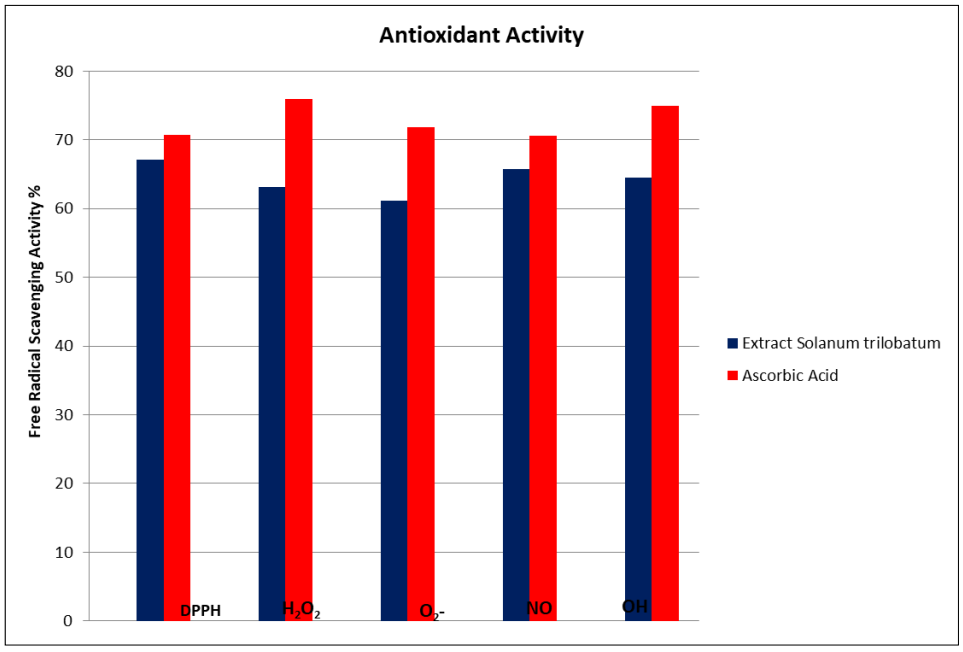

Fig 4: In vitro antioxidant activity various free radical assay method compared with Standard ascorbic acid

H_2O_2 radical scavenging assay

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. 35 H_2O_2 radical scavenging assay method was used to reexamine the antioxidant activity of extract Solanum trilobatum. Table 3 compares the anti-oxidant activity of extract Solanum trilobatum equal concentrations of 100 nM measured at 230nm to standard ascorbic acid with a 63 percent increase.

Superoxide radical scavenging assay

The antioxidant activity of the Extract Solanum trilobatum was again evaluated by superoxide scavenging assay method. In the present study, superoxide scavenging activity Extract Solanum trilobatum at equal concentration of 100 nM was determined at 560 nm with 61% with compared to standard ascorbic acid. Table 3.

Nitric Oxide radical scavenging assay

When a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm. The antioxidant activity of extract Solanum trilobatum was reexamined using the 35 H_2O_2 radical scavenging assay technique. Table 3 shows that extract Solanum trilobatum identical concentrations of 100 nM measured at 230nm has a 63 percent higher anti-oxidant activity than normal ascorbic acid.

Hydroxyl radical scavenging activity

In the current investigation, the hydroxyl radical scavenging method was also used to test antioxidant behaviour, and it showed 65 percent free radical scavenging when compared to ascorbic acid.

Conclusion

This study was completely worked on the plant S. trilobatum to check the activity in terms of Antimicrobial, Antioxidant and Antidiabetic. The physio-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. It is important to assess the quality of the plant material to suggest it as drug for application. So, plant extracts containing alkaloids, flavanoids, phenolic, proteins, amino acid, sterols, fixed oil, carotenoid, carbohydrate, terpenoids, tannin, cardiac glycosides, saponin quinones and anthroquinones were all analysed qualitatively and the quantitative tests includes alkaloids, flavanoids, tannins, terpenoids, saponins, proteins, cardiac glycosides and amino acid.

The antioxidant activity of Solanum trilobatum extract was investigated using the DPPH, H_2O_2, and O^{2-} 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 and it show better antibacterial activity with tested pathogen against Bacillus Subtilis and Escherichia coli.

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