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Research Article

Phytochemical Characterization and Antibacterial Activities of *Solanum xanthocarpum* Seed Extract

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Abstract

The main aim of the present work is to study the phytochemical, antioxidant, and antimicrobial studies of *Solanum xanthocarpum* of the Solanaceae family. The plant has immense medicinal value; particularly the root is used in wound healing, cough, and asthma by the native people. The seed extract showed significant antibacterial activity against Gram-positive and Gram-negative bacterial strains, such as *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (Gram-negative). The broth was analyzed for the secondary metabolites such as alkaloids, flavonoids, terpenes, sterols, tannins, and phenolic compounds were also studied and the results were provided. The results suggest that the seed extract from *S. Xanthocarpum* could be used as an antimicrobial agent for treating several bacterial diseases.

Keywords: *Solanum Xanthocarpum Seed Extract; Phytochemical Studies; Antibacterial; Antioxidant Activities*

Introduction

Medicinal plants contain numerous phytochemicals with immense therapeutic value and are considered natural and safe compared to synthetic drugs. They have many secondary metabolites, for instance, terpenes, flavonoids, alkaloids, and steroids. The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and the development of safe modern drugs. *Solanum xanthocarpum* is an annual herb, which grows as a wild plant in many parts of India. In vernacular it is also known as kantakari or Bharatiya. It is a rare medicinal plant used in traditional medicine. It belongs to the Solanaceae family. It is commonly known as yellow-berried nightshade, is a prickly plant, which grows wild in different regions of the Indo-Pakistan subcontinent. Fruits are berry, yellow, or with white-green strips, surrounded by enlarged calyx [1] Solanum *xanthocarpum* is a diffuse herb with a prickly stem. The root is almost cylindrical and tapering. Leaves are ovate-oblong, acute, pinnately lobed, sparsely stellate, and pubescent. Nodes and internodes are prominent in the stem. Flowers purple in color, few in axillary cymes. Seeds are smooth, compressed, and reniform; taste-bitter [2]. Local people use it as a folk medicine for the treatment of various ailments. In Kerala, the kattunaikka, paniya, and Kuruma tribes of the Wayanad district consume fruits and seeds as food [3]. Fruits are considered a valuable traditional use by traditional healers in the treatment of many common diseases in other parts of India. *Solanum xanthocarpum* is widely used to treat respiratory diseases in southern Indian traditional medicine. It is used in the treatment of bronchial asthma In Ayurveda [4]. Medicinal use of *Solanum xanthocarpum* is well documented. Phytoconstituents present in these are used as anti-inflammatory, anti-allergic agents, and as potential fungicides [5].

Medicinal properties

Solanum xanthocarpum is useful in bronchial asthma, hair fall, curing dropsy, leprosy, cough, chest pain, stopping vomiting, skin disease, and itching. This herb is also used in wound healing and cardiac disease. These plants have been used in veterinary treatment. The entire

plant decoction is used as an herbal drug to treat gonorrhoea and also used as pest control. Leaf paste is used as a pain-killer and roots act as an expectorant and diuretic, useful in the treatment of cataract fever, chest pain, and coughs. Its seeds are given as a remedy for asthma and cough. Fruits are effective in curing throat infections, rheumatism, indigestion, diabetes, and other inflammatory diseases. Fruit is an anthelmintic drug. Fruits stem and flowers are good medicine to get relief from the burning sensation in feet, as well as it has cardiotonic, cytotoxic, hypotensive, anti-anaphylactic, anti-tumor, and anti-spasmodic properties [6]. During the primary metabolic process, oxidative stress in human beings will be generated which requires antioxidants. This antioxidant property can be estimated in ethanol and DPPH system [7]. The plant's bioactive components are a source of making medicines [8]. The bioactive substances are known to behave as microbial inhibitors [9].

Aim of the Study

The present study aimed to investigate the phytochemical constituents from *xanthocarpum* seed extract and their biological applications like antioxidant, and anti-bacterial activities.

Materials and Methods

Collection of seeds

Healthy seeds of *Solanum xanthocarpum* were collected from Sirivaram village, Anantapur district, Andhra Pradesh, India. Seeds were collected and rinsed thrice with distilled water and the seeds were shade-dried at room temperature for a week. The shade-dried seeds were powered using the homogenizer stored in air-tight containers and used for further studies.

Preparation of seed extract

The air-dried seed powered (5g/100ml) was extracted by cold extraction method using distilled water. The extracts were filtered through Whatman filter paper and then concentrated and transferred to sterilized vials and stored at room temperature.

Phyto chemical analysis of seed extract of *Solanum xanthocarpum*

The phytochemical analysis of *Solanum xanthocarpum* seed broth extract was a test to determine phytochemical bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids and steroids [10].

Test for carbohydrates

- **Molisch's test:** For this, 2 ml of Seed extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube separately and 2 ml of concentrated acid was added carefully along the sides of the tubes. The formation of a violet ring at the junction may indicate the presence of carbohydrates.
- **Test for reducing sugars: Benedict's test:** Benedict's reagent was added to the test tube, and the 2 ml of seed extract mixture was boiled for 5 - 10 minutes in the water bath. Formation of green, yellow, or red color depending on the amount of reducing sugar present in each filtrate.
- **Test for saponins: Froth test:** Two grams of the powdered sample (seed) was mixed with 5 ml of distilled water and shaken vigorously for a few minutes. The formation of foam or froth indicates positive results for 15 minutes [10,11].
- **Test for proteins: Biuret test:** To the 2 ml seed extract few drops of 10% sodium hydroxide solution were added and heated in a test tube. Later a drop of 0.7% copper sulphate solution was added. The formation of purple violet color may indicate the presence of proteins.

- **Test for phenolic compounds and tannins:**

- **Iodine test:** The seed extract was added with diluted iodine solution, the appearance of a transient red color is an indication of the presence of phenolic compounds.
- **Tannin test:** A small amount of seed extract was shaken with water and warmed. Then 2 ml of 5% ferric chloride solution was added and incubated for some time and observed for the formation of brownish green or blue-black color which indicates the presence of tannins [10,11].
- **Test for alkaloids: Wagner's test:** 2 ml of seed extract was taken in a test tube and a few drops of Wagner's reagent were added. The formation of a reddish-brown precipitate is an indication of the presence of alkaloids [10,11].
- **Test for flavonoids: Shinoda test:** 2 ml of seed extract was taken in a test tube, 0.5% Mg powder was added and a few drops of concentrated HCl were added. change in color to orange, pink/red is an indication of a positive report [10,11].
- **Test for steroids: Liebermann Burchard test:** To the seed extract a few drops of acetic anhydride were added and it was boiled and cooled then few drops of concentrated sulphuric acid were added to the side of the tube. Positive results are indicated by forming a red top layer and a lower layer of sulphuric acid showing yellow and green colors [10,11].
- **Test for terpenoids:** Two milliliters of the seed extract were mixed with 5 ml of chloroform, and then 1.5 ml of conc.H_2SO_4 was added to the layer. Positive results are indicated by the formation of a reddish-brown color on the surface [10, 11].

- **Test for glycosides: Borntrager's test:** One milliliter of diluted H_2SO_4 was added to the seed extract and heated for 5 minutes then filtered; the supernatant was pipetted and cooled with an equal volume of dichloromethane. The formation of rose pink color indicates the presence of glycosides.

Antimicrobial activity of seed extract

The antibacterial activity of crude was carried out by the well diffusion method [11]. Nutrient agar medium plates were prepared, sterilized, and solidified. After the solidification of the media, both gram-positive *Staphylococcus aureus* and gram-negative *E. coli* bacterial cultures were swabbed on these plates. Different concentrations of seed crude extract were loaded in the wells of nutrient agar plates and incubated at 37°C for 24 hours. Streptomycin was used as a control. The formation of the zone of inhibition was measured after incubation by metric scale [12].

Antioxidant activity of seed extract

DPPH method (2, 2 di-phenyl-1-picrylhyrazyl)

DPPH radical scavenging activity or antioxidant activity was measured by the method of Bonomo., *et al*. [13] with slight modification. This method is based on measuring DPPH concentrations (2, 2-diphenyl1-picryl-hydrazyl). DPPH is a stable free radical that is red and turns yellow when scavenged. Antioxidant reacted with the stable free radical DPPH, pairing it off in the presence of a hydrogen donor and reducing it to DPPH, which lowers the DPPH's absorbance. The ability of DPPH to scavenge free radicals was demonstrated by this feature.

Different concentrations of seed extracts were taken in test tubes and made up to suitable volute (ml 1000 µl) with methanol. Ascorbic acid was used as standard, and DPPH as control. Later 1 ml of 0.1 mM DPPH solution was taken in test tubes. The test tubes were shaken and incubated for 30 minutes in a dark room. The absorbance was recorded at 517 nm against a blank of DPPH and ascorbic acid used as reference using a UV-spectrophotometer [14-17].

% of inhibition = (A control- A sample/A control) × 100.

A sample = Absorbance of the sample.

A control= Absorbance of control.

Hydrogen peroxide scavenging activity

Different concentrations of crude were added to the phosphate buffer (0.1M) and 0.6 ml hydrogen peroxide solution (43 Mm H_2O_2 in PBS). The mixture was kept for incubation for 10 minutes and then absorbance of hydrogen peroxide was determined against the blank solution containing phosphate buffer without hydrogen peroxide [18,19] and values were read at 230 nm in UV-Spectrophotometer. Ascorbic acid was used as a standard.

H_2O_2 Scavenging effect % = A°- A1÷A°×100.

A°= Absorbance of the control reaction.

A1 = Absorbance of the sample.

Results and Discussion

Collection of seed sample

Healthy seeds of *Solanum xanthocarpum* were collected from Sirivaram village, Anantapur district, Andhra Pradesh, India. Collected seeds were rinsed with distilled water and then shade-dried at room temperature for ten days.

Figure 1: *Seeds of Solanum xanthocarpum.*

Preparation of seed extract

The air-dried seed powered (5g/100ml) was extracted by cold extraction method using distilled water. The extracts were filtered through Whatman No. 1 filter paper and stored in cold for further analytical studies.

Figure 2: *Seed extract (filtrate).*

Phytochemical analysis of seed extract

The phytochemical analysis of seed extract shown in table 1 revealed the presence of carbohydrates, tannins, phenols, alkaloids and steroids and the absence of proteins, anthraquinones, saponins and flavonoids. Similar reports were made in the phytochemical analysis of root extracts of *Solanum xanthocarpum* [20].

Tests	Observation and Interpretation	Result
Test for carbohydrates		
Molisch's test	Violet coloration at junctions of two layers was observed indicating the presence of carbohydrates	+ve
Benedict's test	Brick red coloration was not observed indicating the absence of reducing sugars	-ve
Test for proteins		
Biuret's test	Purplish violet coloration was not observed indicating the absence of proteins	-ve
Test for Anthraquinone glycosides		
Borntrager's test	Change of Rose pink to red coloration was not observed indicating the absence of anthraquinone glycosides	-ve
Test for saponins		
Froth test	Foam not formed indicating absence of saponins	-ve
Test for tannins and phenolic compounds		
Iodine test	Transient Red Coloration was observed indicating the presence of phenolic compounds and tannins	+ve
Ferric chloride test	Green colour was formed, indicates the presence of phenolic compounds	+ve
Test for alkaloids		
Wagner's test	Reddish brown precipitate was observed, indicating presence of alkaloids	+ve

Test for flavonoids		
Shinoda's test	Pink Coloration was not observed indicating absence of flavonoids	_ve
Test for steroids		
Liebermann-Burchard test	Brown rings at the junctions of two layers and upper layer turning green was observed indicating presence of steroids	+ve

Table 1: *Phytochemical analysis of seed extract of Solanum xanthocarpum.*

Antibacterial activity

The results of a study on the antibacterial activity of plant leaf extract against bacterial strains are shown in table 2. Both Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria were effectively eradicated by the seed extract of *Solanum xanthocarpum*. The zone of inhibition for gram-positive bacteria was 0.9 cm at the lowest concentration of seed extract, and it was 1.5 cm at the highest concentration. The zone of inhibition for gram-negative bacteria was 1.2 cm at the lowest concentration and it was 1.4 cm at the highest concentration. The zone of inhibition also increased as seed extract concentration did. Similar reports were seen in *Solanum xanthocarpum* methanol extract exhibited antibacterial efficacy against a wide range of human pathogen-causing infections [21].

Conc of seed extract (in µl)	**Zone of inhibition (in cm)**	
	S. aureus	***E. coli***
50	0.9	1.2
75	1.1	1.1
100	1.5	1.4
Streptomycin	0.9	0.9

Table 2: *Antibacterial activity of seed extract of Solanum xanthocarpum.*

Antioxidant activity

DPPH (2, 2 di-phenyl-1-picrylhyrazyl) method

The antioxidant activity of S. *xanthocarpum* seed extract was studied by DPPH free radical scavenging assay. This method is dependent on reducing DPPH radical to the ion-radical form DPPH-H in the presence of a hydrogen-donating antioxidant. The radical scavenging activity (RSA) values of Ascorbic acid and crude extract values are represented in figure 3. Similar reports were made in *Solanum xanthocarpum* root extract with DPPH assay [20]. DPPH scavenging activity percentage (%) at 0.5 ml is 5, 1.0 ml is 3.46, 1.5 ml is 28.125 and 2.0 ml is 23.4. Indicated that the percentage of inhibition is more at 1.5 ml.

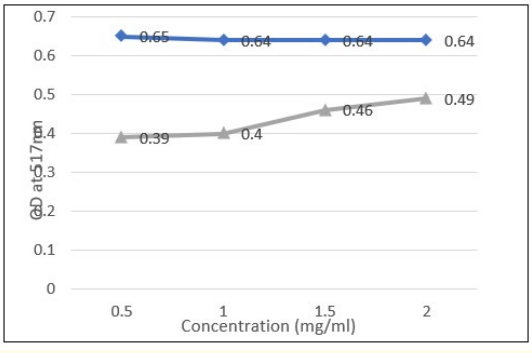

Figure 3: *DPPH activity of control and seed extract of Solanum xanthocarpum.*

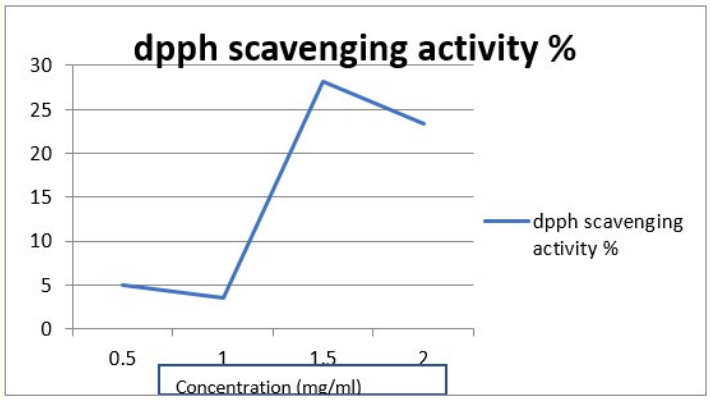

Figure 4: *DPPH scavenging activity.*

Hydrogen peroxide scavenging activity

The radical scavenging activity exhibits very good results shown in figure 5. The primary indication of good results is color change by adding crude. The hydrogen peroxidase activity increased as the concentration of the sample increased. Similar reports were made in *Solanum surattense* leaf extract [22]. H_2O_2 scavenging activity % at 0.5 ml is 18.6, at 1 ml is 21.2, at 1.5 ml is 21.7 and at 2 ml is 11.6.

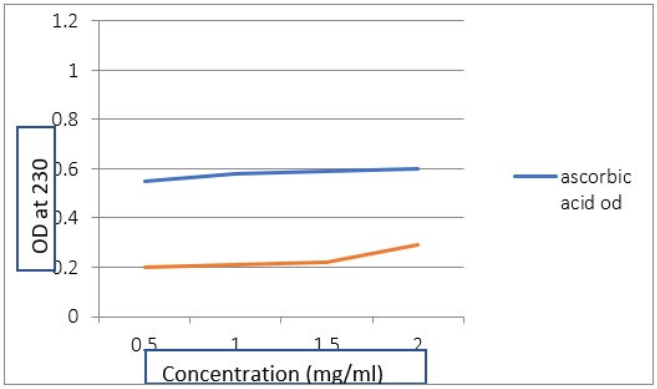

Figure 5: *H_2O_2 activity seed extract of Solanum xanthocarpum.*

Conclusion

According to the study findings, *Solanum xanthocarpum* seed extract contains a variety of phytochemicals, which are biologically active molecules that can serve and function as an inhibitor of microbial development. The antimicrobial and antioxidant properties of crude extracts are an indication of the phytomedical properties of plants and they could be used as antimicrobial and anticancer agents for modern medicine.

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