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Evaluation of phytochemical constituents, antibacterial, antifungal, anti-inflammatory and antioxidant analysis of *Solanum xanthocarpum* Schrad and Wendl.

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Abstract

Many organisms that are present in the oral cavity are not pathogenic. The pathogenicity of the microorganisms affects the host when the host is immunocompromised due to systemic disease or immunosuppressive medications. The pathogenicity usually manifests as an infection, which is treated using antimicrobial agents. There are many plants that can potentially act as antimicrobial agents. One of such plants is *Solanum xanthocarpum*. *S. xanthocarpum* is also known as Indian nightshade, from the Solanaceae family which known for its medicinal properties. Many tribes consider this plant a vegetable. In traditional medicine, this plant plays an important role in treating mankind. Since it has convincing medicinal benefits and is being used in traditional medicine, we chose to test the potentiality as anti-inflammatory agent, anti-mycotic, anti-oxidant, and the phytochemical assay of the S. *xanthocarpum*. The objective of this study was to assess anti-mycotic, anti-inflammatory, anti-oxidant, phytochemical and germicidal effects of the *S. Xanthocarpum.* The anti-fungal property was analyzed by using Kirby- Bauer on Sabourad Dextrose Agar (SDA) medium. Anti-inflammatory activity had been analyzed by inhibiting protein denaturation using 5 different concentrations of the plant extract. Phytochemical assay was done using 10 solutions. Anti-bacterial test was done by agar disc diffusion method using 4 different microorganisms at 3 different concentrations. The study proved that *S. xanthocarpum* can be used as a potential germicidal, anti-mycotic, anti-oxidant, anti-inflammatory agent.

Key words : *Solanum xanthocarpum*, Antioxidant, Antibacterial, Antifungal, Phytochemical.

Most oral fungal infections or oral mycosis are caused by opportunistic infections. In recent years the decrease in host resistance is mainly due to immunosuppressive drug therapies which lead to pathogenic diseases to spread locally in the oral cavity through colonization[2]. Candidiasis, Aspergillosis, Mucormycosis or Zygomycosis, Histoplasmosis, Blastomycosis, Cryptococcosis, Paracoccidio-idomycosis, and Geotrichosis are the familiar mycotic infections that occur in the oral cavity[19]. The diagnosis should be based on clinical presentation, followed by histopathological investigation of the lesional tissue[19]. The patient exhibits pain, erythema, ulcerations, white plaques, burning, and a foul taste in the mouth[19]. Treatment plan should involve clinical evaluation of oral findings, comprehending the type of the lesion, treating the underlying predisposing factors, and administering the necessary antimycotic agents[19]. In some cases of fungal infections, surgical debridement is indicated[19]. *S. xanthocarpum* is a plant with therapeutic properties that is also known as Indian nightshade or yellow berried nightshade. Kantakari, another name for *Solanum surattense*, is its common name. It is a member of the family Solanaceae. This plant is a woody perennial herb with a spiky, diffuse, bright green color.

The plant contains phytoconstituents that show potential medicinal properties[10]. It has been used for its health benefits for a long time. The reason behind these properties is mainly because of the bioactive compounds that are produced during secondary metabolism in plants. It has been reported to be safe for human consumption as it is non-toxic. Because of its anti-inflammatory qualities, the plant's edible fruits are used as traditional medicine by the people of Manipur, India[17]. The Irula tribes of Tamil Nadu, India's Hasanur Hills, consume the prepared, undeveloped fruits of *S. xanthocarpum* (Sx) as a vegetable[16]. The fruits and seeds of the *S. xanthocarpum* plant are consumed by the Kattunaikka, Paniya, and Kuruma tribes in Kerala's Wayanad district[14]. Fruits are used in conventional medicine in some parts of India. Phytoconstituents present in *S. xanthocarpum* are used as a potential fungicide and anti-inflammatory agent[7,20]. Since *S. xanthocarpum* has being used in traditional medicine the study aims at evaluating the phytochemical, anti-oxidant, anti-inflammatory, germicidal and anti-mycotic activity for pharmaceutical use.

Plant acquisition :

The leaves of *S. xanthocarpum* were collected from the herbal garden Anna Nagar, Chennai. The plant was identified as *S. xanthocarpum* by a qualified Botanist.

Extract preparation :

The plant was identified and the leaves were shade dried in the laboratory for a week of time. The dried leaves were blended using mortar and pestle to increase the surface area for extraction. The powdered samples were kept in airtight containers for further study.

Materials :

Soxhlet apparatus, Heating mantle, Rotary Evaporator, Weighing Balance, Solvent.

Procedure :

Plant material is placed in a thimble-

holder in a standard Soxhlet system (Figure 1). The thimble-holder solution is aspirated by a syphon when the liquid reaches the overflow level, and in the following step, a boiling flask, referred to as a distillation flask, is filled with the solvent (ethanol) and attached to an isomantle's Soxhlet extractor and condenser. The crushed plant material is filled in the thimble positioned in the soxhelt extractor. The isomantle heats the solvent, and travels through the device to the condenser after the evaporation. The thimble into which the condensate flows acts as reservoir. The solvent level reaches the syphon and the cycle restarts by the flow of it back into the flask. Ten hours should be allotted to the process. After the procedure was complete, ethanol (a solvent) was used to extract 25g of the material. To lower the pressure at 50°C a rotary evaporator was used to condense the extracts. To yield a stock of 100 mg/ml the samples were reconstituted in their corresponding solvents after condensation which was then refrigerated for additional research[9]. The extracts were used for further analysis.

Anti-inflammatory assay :

Inhibition of protein denaturation :

Protein denaturation is a process in which proteins lose their ability to retain their structural integrity when exposed to external stimuli as strong acids or bases, concentrated inorganic salts, organic solvents, or heat treatment. It has been noted that denaturation of proteins reduces their biological effectiveness. Since denatured proteins are thought to be one of the mediators of inflammation, substances that stop the precipitation of denatured protein clumps and protein condensation are beneficial[9,20].

Procedure :

500µl of 1% BSA was added to 100µl of test. For ten minutes, the mixture was incubated at 37°C for twenty minutes. At 51°C, the contents must be heated in a water bath for 20 minutes. The blank value is compared to the absorbance at 660 after cooling to room temperature. Acetyl salicylic acid serves as the positive control while water served as the product control.

Calculation: %Inhibition = (Control O.D - Sample O.D)/Control O.D*100} (Table-1).

Table–1. Anti-inflammatory activity of Sample

S. no	Concen-tration (µg/ml)	Sample (O.D)	% Inhi-bition
1	20	0.544	22.94
2	40	0.477	32.43
3	60	0.410	41.92
4	80	0.352	50.14
5	100	0.285	59.63

Control Optical Dentistry (O.D): 0.706

The graph (Figure 2) suggests, from the analysis of the inhibition of the O.D. values at 660 nm by various concentrations that a test for the anti-inflammatory activity of the sample was assessed. Inhibition of inflammation, from the data in the table, is directly proportional to concentration since it increases from 22.94% inhibition at 20µg/mL to 59.63% inhibition at 100µg/mL. The sample's capacity to efficiently suppress anti-inflammatory processes is indicated by decrease in O.D values. The maximum inhibition of 59.63% at 1000µg/mL indicates significant anti-inflammatory property.

The graph illustrated the evaluation of the sample's anti-inflammatory potential across several concentrations (20 to 100 µg/mL), with the percentage inhibition of inflammation determined. The results showed concentration-response type increase in anti-inflammatory activity. The graph is also linear in nature, that the increasing concentration of sample shows a perfect correlation to increased anti-inflammatory activity. It is well evident from this that it has shown concentration-responsive action towards inhibiting it. At its highest concentration, which was 100 µg/mL, the activity showed great potential as an anti-inflammatory drug. These results vindicate its potency as such.

Antifungal assay :

The disc diffusion method is the best way to test for antifungal susceptibility because it is low-tech, low-cost, and requires minimal equipment. The study was done by taking one sample and 3 microorganisms *Candida albicans, Trichoderma viridae, Aspergillus niger*. The standard was Amphotericin B (20µl/disc).

Preparation of inoculum :

On Sabourad Dextrose agar siant, stock cultures were kept at 4°C. Stock cultures were transferred into test tubes filled with Sabourad Dextrose broth and cultured for 48 hours at room temperature to create active cultures for the tests. The experiment used agar disc diffusion method.

Agar diffusion method :

Antifungal activity was assessed by using the disc diffusion method on Sabourad Dextrose agar (SDA) medium (Table-2) (Figure 3). The petriplate was filled with Sabourad Dextrose Agar (SDA) medium. After the medium hardened, the inoculums were applied to the solid plates using a sterile swab saturated with the fungal solution. As a positive control, amphotericin-B was used. Sterile discs containing 20µl of samples and a positive control were inserted, and the discs were then put into SDA plates. For twenty-four hours, the plates were incubated at 28°C. The antifungal activity was calculated using the zone of inhibition's diameter (Table 2).

Table–2. Anti-fungal activity of sample

Organisms	Zone of inhibition (mm)			
	Sample (µg/ml)			Standard
	1000	750	500	
Candida albicans	28	26	23	32
Trichoderma viride	25	16	11	32
Aspergillus niger	29	18	18	32

Inference:

The table indicated the zone of inhibition increases as the concentration of the sample increased suggesting dose-dependent antifungal activity across different fungal organisms. The standard consistently showed 32mm across all the concentrations tested. The plant extract exhibited maximum zone of inhibition of *Aspergillus niger* was at 29mm at 1000µg/mL.

This showed the plant extract has significant antifungal activity which increases with concentration. Although the extract's activity did not surpass the standard drug, the increasing inhibition zone at higher concentrations highlights its potential as a natural antifungal agent.

Figure 1. Soxhlet apparatus used for extraction of *Solanum xanthocarpum* leaves

Antioxidant assay :

DPPH Assay :

Because the spare electron delocalizes across the molecule, DPPH (1,1-diphenyl-2-picrylhydrazyl) is a persistent free radical. This implies that, in contrast to the vast majority of other free radicals, the molecules do not dimerize. The deep violet color, which arises due to an absorption band in an ethanol solution centered at about 520 nm, is also a consequence of delocalization. The reduced form is the description given by Blois in 1958 when the solution of DPPH mixes with the chemical capable of donating the hydrogen atom, leading to a loss of its violet color although a pale yellowish tinge from the picryl group is retained. This results in the following basic reaction: Z• + AH = ZH + A•. In this reaction, ZH is referred to as the reduced form and A• is the free radical formed here. Here, Z• and AH have been referred to as the donor molecule and the DPPH radical, respectively. The final stoichiometry will, thus depend on the subsequent reactions that it produces, as these will specify the number of DPPH molecules reduced (decolored) by the one reductant molecule.

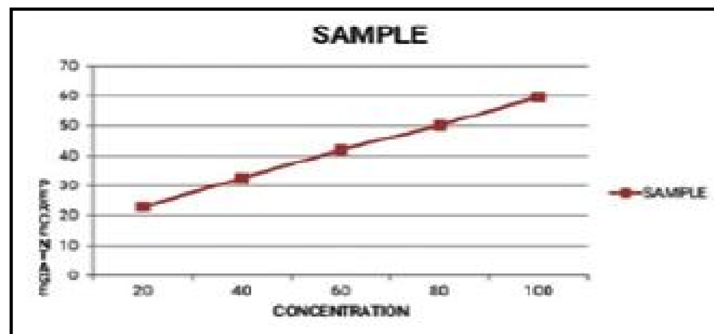

Figure 2. Anti-inflammatory activity of Solanum xanthocarpum extract showing inhibition of protein denaturation at concentrations of 20–100 µg/mL.

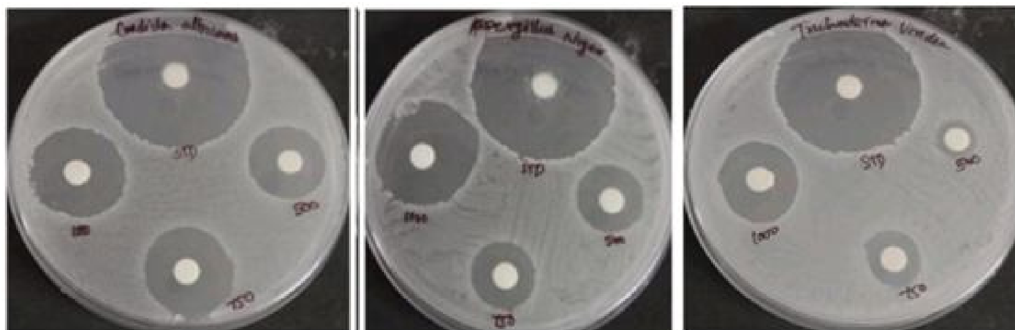

Figure 3. Antifungal activity of *Solanum xanthocarpum* extract against a) *Candida albicans*, b) *Aspergillus niger* c) *Trichoderma viride* as determined by the agar disc

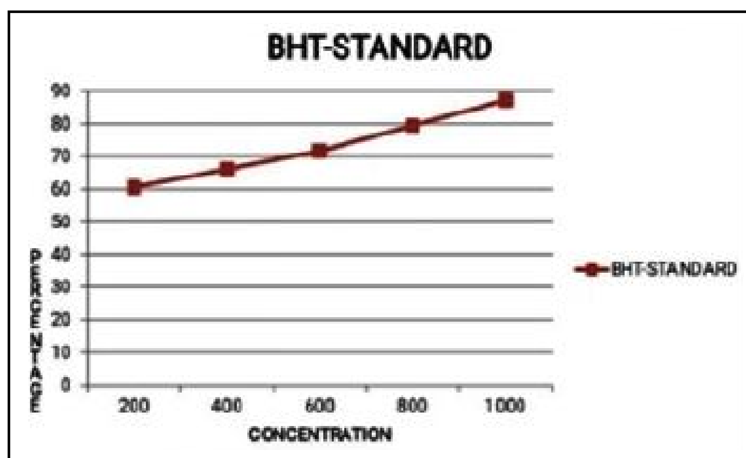

Figure 4. Antioxidant activity of *Solanum xanthocarpum* extract measured by DPPH radical scavenging assay, indicating concentration-dependent activity (200–1000 µg/mL).

Chemicals used: 1,1 – diphenyl -2- picrylhydrazyl (DPPH), Dimethylsilphoxide (DMSO), 1.6mg/ml in Methanol was used as BHT (standard). Samples of desired concentration from 1 mg /ml to max of 5 mg / ml (in /DMSO).

Procedure :

Aliquot In each test tube, 3.7 ml of absolute methanol was supplied, while the blank received 3.8 ml. Put 100 µl of BHT in the standard tube and 100 µl of the corresponding sample in each of the test tubes. All of the test tubes, including the blank, received 200 µl of the DPPH reagent. All test tubes should be incubated for 30 minutes at room temperature in a dark environment. At 517 nm, the absorbance of every sample was measured (Table-3) (Figure 4).

Calculation: % Antioxidant Activity = (Absorbance at Blank). - (Absorbance at test) * 100 (Absorbance at blank).

Figure 5. Phytochemical profile of *Solanum xanthocarpum* extract demonstrating the presence of tannins, flavonoids, alkaloids, proteins, steroids, phenols, and carbohydrates.

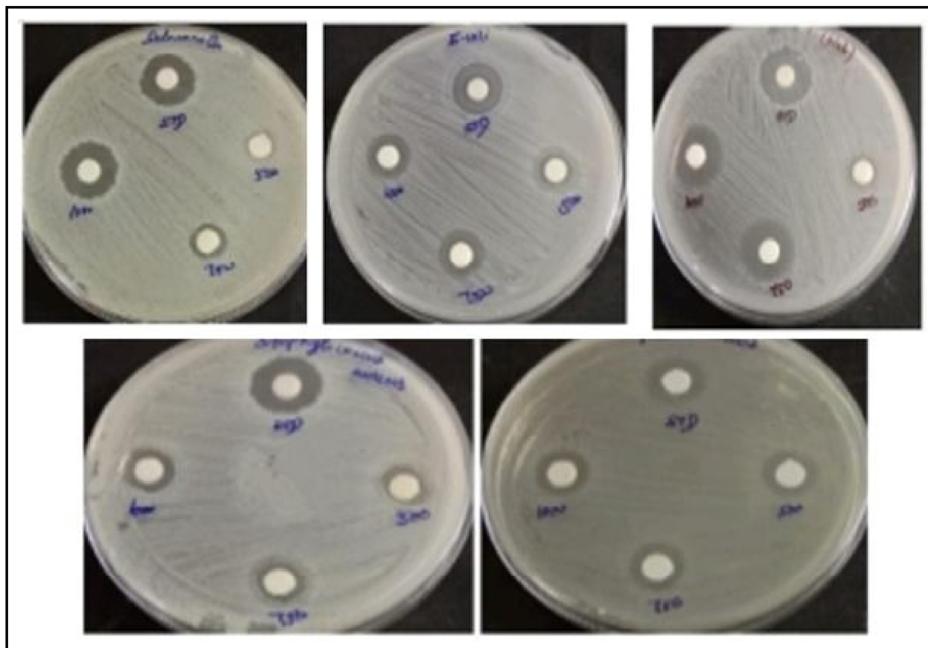

Figure 6. Antibacterial activity of *Solanum xanthocarpum* extract against, a) *Salmonella typhi*, b) *Escherichia coli,* c) *Klebsiella pneumoniae* d) *Staphylococcus aureus* and e) *Pseudomonas aeruginosa* using the agar disc diffusion method.

Table–3 Anti-oxidant property of the sample (DPPH Std-BHT)

Sl. no.	Concentration (µg/ml)	Sample O.D	DPPH Activity %
1	200	0.100	60.31
2	400	0.086	65.87
3	600	0.072	71.42
4	800	0.053	78.96
5	1000	0.033	86.90

Control Optical Dentistry (O.D): 0.252

Table–4. Anti-oxidant property of the sample (Sample 1)

Sl. no.	Concentration (µg/ml)	Sample O.D	DPPH Activity %
1	200	0.550	9.53
2	400	0.442	27.30
3	600	0.345	43.25
4	800	0.228	62.50
5	1000	0.147	75.82

Control Optical Dentistry (O.D): 0.608

Inference :

Table-4 demonstrated that the plant extract exhibits antioxidant activity as measured by DPPH scavenging ability and this activity increased with higher concentrations. BHT (Butylated Hydroxy Toulene) a commonly used synthetic antioxidant, also demonstrated concentration- dependent antioxidant activity. The decreased optical density (O.D) of the DPPH solution decreased with the addition of the plant extract or BHT. This indicated that the antioxidant compounds neutralized the DPPH free radicals This decrease in O.D. was compatible with the increased DPPH activity as both the concentration of the plant extract and the concentration of BHT were increased. With the increasing sample concentration, the DPPH activity (%) increased and was dose-dependent.

Phytochemical Analysis :

The various chemical components found in plants, such as alkaloids, flavonoids, terpenoids, steroids, tannins, glycosides, etc., produce chemicals known as phytochemicals. When it comes to detecting all of the biochemical components that plants create, qualitative phytochemical screening is essential[11,15].

Tannins test : A millilitre of the sample was taken and a few drops of 0.1% ferric chloride were added and the sample was analyzed for brownish green or blue-black coloration.

Saponins test : Two millilitres of water were added to one millilitre of the sample. For 15 minutes, the suspension was shaken in a graded cylinder. Saponins are indicated by an outer layer of foam.

Flavonoids test : After taking one millilitre of the sample, strong hydrochloric acid was mixed in to see a white colour, and NaOH was added to produce a yellow tint.

Alkaloids test : A couple of drops of the Drangandoff the reagent were applied onto a single millilitre of the sample. The test is considered positive if there is a significant yellow precipitate.

Protein test : One millilitre of sample was obtained, and a few drops of Millon's reagent was added. A white precipitate indicates the presence of Protein.

Steroids test : One millilitre of sample was obtained. Two drops of ten percent

concentrated sulphuric acid was added, and the brown hue was seen.

Anthraquinones test : A millilitre of sample was obtained, aqueous ammonia was added, and the color was observed to alter. Pink, crimson, or violet hue in the aqueous layer indicates the presence of anthraquinones.

Phenols test : One millilitre of sample was obtained, and three millilitres of ten percent concentration of lead acetate was added. A substantial white precipitate on the surface suggests the presence of phenolic chemicals.

Trepenoids test : To the extract, 2 ml of chloroform was added followed by 3 ml of concentrated sulphuric acid, and this made a total volume of 0.5 ml. An appearance of red-brown color at the interface serves as proof of the presence of terpenoids.

Carbohydrates test : Sample (0.5 ml) was taken followed by the addition of 0.5 ml of Benedict's reagent to this reaction mixture, while it was being mixed up well. After which it was kept for 2 minutes in the water-bath. Precipitated color indicates presence of Sugar.

Phytochemical assay :

Table–5. The Phytochemical studies of the sample

Test	Result
Tannins	Positive
Saponins	Negative
Flavonoids	Positive
Alkaloids	Positive
Proteins	Positive
Steroids	Positive
Anthraquinones	Negative
Phenols	Positive
Carbohydrates	Positive

Inference :

The sample's phytochemical screening identified a number of bioactive substances (Table-5) (Figure 5). Notably, tannins, flavonoids, alkaloids, proteins, steroids, phenols and carbohydrates were detected indicating a rich composition of secondary metabolites. These compounds are widely recognized for their significant biological activities. The absence of saponins and anthraquinones suggests the sample lacks certain metabolic pathways or precursor compounds necessary for their synthesis. The presence of tannins and flavonoids suggests potential antioxidant properties. Alkaloids and phenols are commonly associated with antimicrobial and anti-inflammatory activities. Steroids and carbohydrates further indicate the potential for metabolic and structural contributions. Proteins in the sample could enhance its nutritional or functional value. Overall, the phytochemical profile demonstrates the sample's potential for therapeutic application.

Antibacterial assay :

One sample was taken for antibacterial assay and 5 organisms were selected (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae)* Ampicillin (20µl/disc) was taken as standard.

Preparation of inoculum :

The stock culture was maintained at 4°C on a nutrient agar slant. A loop full of culture was added from the stock cultures into test tubes holding the nutrient broth in order to produce active cultures for the tests. The tubes

were then cultured for 24 hours at 37°C. The agar disc diffusion method was used to carry out the assay.

Agar disc diffusion method :

The disc diffusion method was used to do an initial assessment of the antibacterial effect. The bactericidal qualities of the extracts were assessed using the disc diffusion method on Muller Hinton agar (MHA) medium. The Petri plate is filled with Muller Hinton Agar (MHA). A sterile brush wet with the bacterial solution was used to disperse the inoculums across the plates once the medium had set. 20 µl of material (concentration: 1000 µg, 750 µg, and 500 µg) was added after the disc had been placed on MHA plates. For twenty-four hours, the plates were incubated at 37°C. After that, the antibacterial activity was calculated using the diameter of the zone of inhibition (Table-6), (Figure 6).

Table-6. Antibacterial activity of the sample

Organisms	Zone of inhibition (mm)			
	Sample (µg/ml)			Standard
	1000	750	500	
Staphylococcus aureus	13	11	11	18
Escherichia coli	14	14	10	17
Salmonella typhi	19	14	-	19
Pseudomonas aeruginosa	13	13	13	15
Klebsiella pneumoniae	18	18	8	18

Inference :

Using the agar disc diffusion method, the sample's antibacterial activity was assessed against five bacterial strains: *Salmonella typhi,* Escherichia coli, Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The results were measured by the zone of inhibition. At the highest concentration (1000µg/mL) the sample exhibited the most significant activity against Salmonella typhi followed by Escherichia coli and Staphylococcus aureus. Moderate activity was observed against Pseudomonas aeruginosa while *Klebsiella pneumoniae* demonstrated the least sensitivity. As the sample concentration decreased, the zone oh inhibition consistently reduced indicating a concentration-dependent antibacterial effect. Compared to the standard antibiotic, the sample showed comparable activity against certain strains (Escherichia coli and Staphylococcus aureus), though its overall potency was lower. These findings suggest that the sample contains bioactive compounds with antibacterial properties.

Most oral infections are due to the immune function dysfunction such as HIV, AIDS or as a result of local or systemic medical treatment[2]. Candida species are the most commonly cause oral fungal infection [2]. Most oral fungal infections are diagnosed based on the clinical manifestations[19]. Oral infections can be treated by treating the underlying

factors, administering ant-fungal medications and sometimes by incision and drainage[19]. Traditional medicine derives its medication from plants, which are pharmaceutical in nature[3]. In Chattisgarh, *S. xanthocarpum* is considered a traditional healer since it is used to cure a variety of common diseases[10]. *S. xanthocarpum* has been a valuable plant due to its therapeutic properties[13,18]. As a result, we assessed and emphasized the antifungal, antibacterial, anti-inflammatory, and phyto-chemical properties of *S. xanthocarpum*. According to research by Mahalakshmi *et al*.,[6] *S. xanthocarpum* extracts are just as effective as commercially available chlorhexidine[6]. Protein denaturation serves as a critical marker of inflammation because it is a precursor to immune system activation and inflammatory mediator release. Assays measuring the ability of compounds to inhibit protein denaturation provide a valuable tool for screening potential anti-inflammatory agents, including plant extracts. In the study Bovine Serum Albumin (SBA) was used to assess that *S. xanthocarpum* extracts have anti-inflammatory advantages. The outcomes demonstrated the potential of *S. xanthocarpum* extracts as anti-inflammatory agents.

Free radicals and reactive oxygen species (ROS), which can cause oxidative stress, should be neutralized by antioxidants. Bioactive compounds like phenolics, flavonoids, alkaloids, tannins, saponins and glycosides plays pivotal role in anti-oxidant activity of *S. xanthocarpum*. Kumar S. *et al.* investigated and reported that the phytoconstituents found in *S. xanthocarpum* have cytotoxic and antioxidant properties[4]. Poongothai *et al.* researched and demonstrated that extracts from the leaves of *S. xanthocarpum* had antioxidant properties[12]. Kumar *et al.* established via their investigation that root[5] and fruits extracts exhibited considerable free radical scavenging activity in the DPPH radical scavenging experiment[18]. Our findings indicated that *S. xanthocarpum* may directly scavenge free radicals or inhibit their production. Mahalakshmi *et al*.[6] and Arumuganainar *et al*.[1] investigated and reported that the antibacterial activity of *S. xanthocarpum* was significant. This study reported high levels of phenolics and flavonoids in *S. xanthocarpum* which correlate with its antioxidant properties. One of the most used techniques for assessing the antioxidant capacity of plant extracts or any other material is the DPPH assay. The DPPH assay relies on antioxidants' capacity to neutralise the DPPH radical. With a nitrogen centre, the DPPH radical is a stable free radical. DPPH assay was also done using 1,1 – diphenyl -2- picrylhydrazyl (DPPH), Dimethylsilphoxide (DMSO) chemicals and methanol as standard. When the DPPH test was performed in the study the plant extract showed a strong ability to scavenge radicals from *S. xanthocarpum*.

Since pathogenic fungi are becoming more resistant, plant-based antifungal medicines have drawn a lot of interest as safe and effective substitutes for synthetic antifungal medications[17]. These characteristics are mostly ascribed to bioactive substances found in plants, which use a variety of ways to target fungal cells. Because of the bioactive substances they contain, such as alkaloids, flavonoids, terpenoids, and saponins, plants have antifungal properties. Plant extracts exhibit antifungal activity by interfering with the structural integrity and

functionality of fungal cells. By disrupting the structural integrity and functionality of fungal cells, plant extracts demonstrate antifungal action. To assess the antifungal activity in vivo or in vitro techniques can be used. According to Singh *et al*. *S. xanthocarpum* has the potential to be employed as an antifungal medication[17]. In this study, we investigated the antifungal activities of *S. xanthocarpum* using the agar disc diffusion technique. According to the results, three microorganisms — *Candida albicans, Trichoderma viridae*, and *Aspergillus niger* are strongly inhibited by the plant extract. The active components of the *S. xanthocarpum* extract may be used in antifungal therapy.

Plant extracts are useful in the fight against bacterial infections, including those caused by drug-resistant strains, because they show potential antibacterial qualities. Plant extracts have shown efficacy against various gram-positive and gram-negative bacteria[8]. In the present investigation, we evaluated *S. xanthocarpum* antibacterial properties using the agar disc diffusion technique which is a qualitative analysis. Antibacterial activity is indicated by the zone of inhibition. The findings demonstrated that *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were all inhibited by the *S. xanthocarpum* extract.

Investigating the phytochemical, antioxidant, antifungal, antibacterial, and anti-inflammatory qualities of *S. xanthocarpum* —a medicinal plant with a long history of use was the aim of this study. The findings of this research shed light on the potential benefits of *S. xanthocarpum* as anti-bacterial, anti-fungal, anti-oxidant, anti-inflammation with phyto-chemical properties.

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