



Research Article

PHYTOCHEMICAL EVALUATION AND IN-VITRO ANTI-BACTERIAL AND ANTIOXIDANT EFFECT OF EXTRACT OF SOLANUM XANTHOCARPUM AND ACHYRANTHES ASPERA

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ABSTRACT

Background: This study investigates the phytochemical composition, antioxidant activity, and antibacterial properties of extracts from *Solanum xanthocarpum* and *Achyranthes aspera*. *Achyranthes aspera* exhibited 65.864 % inhibition with an IC₅₀ value of 31.056 µg/mL, and *Solanum xanthocarpum* showed 55.385% inhibition with an IC₅₀ value of 51.920 µg/mL. **Methodology:** The total phenolic content (TPC) and total flavonoid content (TFC) were measured using the Folin-Ciocalteu assay and aluminum chloride colorimetric method, respectively. Antioxidant activities were evaluated using DPPH radical-scavenging and reducing power assays. The antibacterial activity against *Streptococcus mutans* was determined using the well diffusion method. **Result and Discussion:** The TPC of *Solanum xanthocarpum* and *Achyranthes aspera* extracts were 77.80 mg GAE/g and 98.40 mg GAE/g, respectively, while the TFC were 82.66 mg RE/g and 136.66 mg RE/g. In the DPPH assay, *Achyranthes aspera* exhibited 65.864 % inhibition with an IC₅₀ value of 31.056 µg/ml, and *Solanum xanthocarpum* showed 55.385% inhibition with an IC₅₀ value of 51.920 µg/mL. The reducing power assay indicated significant antioxidant potential, especially for *Achyranthes aspera*. The antibacterial activity against *Streptococcus mutans* MTCC 389 revealed that *Achyranthes aspera* exhibited a maximum inhibition zone of 23±1.732 mm. At the same time, *Solanum xanthocarpum* showed a maximum inhibition zone of 20±1 mm at a concentration of 2 mg/mL. **Conclusion:** The extracts of *Solanum xanthocarpum* and *Achyranthes aspera* demonstrate significant antioxidant and antibacterial activities, highlighting their potential as natural sources of antimicrobial and antioxidant agents. Further research is needed to isolate bioactive components, elucidate their mechanisms of action, and assess their potential cytotoxic effects on human cells.

INTRODUCTION

Natural compounds that mimic those of medicinal plants are important in the discovery and development of novel

medications [1]. Roughly 25% of pharmaceuticals include ingredients sourced from higher plants [2]. Pathogenic microorganisms are increasingly causing life-threatening

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infections all across the world, and these infections are becoming a major source of foreboding and mortality for people with weakened immune systems in affluent nations [3]. Controlling antimicrobial resistance through increased antibiotic use and decreased hospital cross-infection is critically important [4]; however, the creation of novel antibiotics is essential to preserving the efficacy of antibacterial therapy [5]. According to WHO estimates, 65-80% of people on Earth live in underdeveloped countries and primarily receive their primary medical care from plants [6,7].

Solanum xanthocarpum, also known as Yellow-berried Nightshade, is a spiny shrub from the Solanaceae family, prominently used in “Dasmul Asava” in Ayurvedic preparation [8]. This plant is renowned for its medicinal properties, especially for treating respiratory ailments such as asthma and bronchitis, thanks to its expectorant qualities that help clear mucus from the airways. Additionally, it is used to alleviate coughs and colds due to its anti-inflammatory effects on the respiratory tract. Beyond respiratory health, *Solanum xanthocarpum* is valued for its anti-inflammatory and analgesic properties, making it a remedy for pain in conditions such as arthritis and rheumatism [9,10]. It also helps manage digestive issues such as indigestion and flatulence, thanks to its carminative properties, which help expel gas from the intestines. Furthermore, it is used to treat intestinal worm infestations. The plant's antibacterial and antimicrobial properties make it effective against various infections, and it is applied topically to address skin conditions such as eczema, psoriasis, and fungal infections. The medicinal efficacy of *Solanum xanthocarpum* is attributed to its rich phytochemical content, including alkaloids, flavonoids, glycosides, saponins, and tannins, which contribute to its therapeutic effects [11].

Achyranthes aspera, commonly known as Devil's Horsewhip, is an herbaceous plant from the Amaranthaceae family with significant Anti-inflammatory activity. This plant is esteemed for its versatile medicinal properties and has been traditionally used to address various health issues. It is also known for promoting blood circulation and reducing blood stasis, thereby aiding the treatment of bruises and injuries. Additionally, *Achyranthes aspera* is used to support kidney health and improve urinary function, and is often used to treat conditions such as urinary tract infections and kidney stones [12]. Its diuretic properties help flush toxins from the body. The plant also plays

a role in reproductive health, where it is used to regulate menstrual cycles and alleviate menstrual pain. Furthermore, *Achyranthes aspera* is recognized for its wound-healing properties and is often applied externally to treat cuts, wounds, and skin infections. The therapeutic benefits of *Achyranthes aspera* are attributed to its rich phytochemical content, including alkaloids, saponins, and flavonoids, which contribute to its multifaceted healing properties in *Achyranthes aspera*. Therefore, this paper examines the antibacterial activity and antioxidant properties of extracts from the whole plants of *Achyranthes aspera* and *Solanum xanthocarpum*, which are traditionally used as dietary supplements for general health. This study set out to determine whether the selected plant extracts could inhibit the growth of harmful microbes.

Recently, there has been considerable interest in the antimicrobial and antioxidant properties of therapeutic vegetation [13]. Research on the medicinal benefits of plants' antioxidant qualities has been done [14]. It has been established that those phenolic chemicals, comprising vitamin E, ascorbic acid, phenolic acids, flavonoids, and other carotenoids, are principally in charge of the antioxidative capacity of these vegetative products [15]. Those naturally occurring antioxidant agents are extremely effective at preventing damage caused by oxidative radicals in the Free State [16]. Several clinical illnesses, for example, atherosclerosis, inflammation, cardiovascular disease [17], cancer, meditational damage, trauma, and autoimmune diseases, possess oxidative stress as a primary causative element in their development [18]. Many different molecules that scavenge free radicals are found in plants (fruits, vegetables, medicinal herbs), among these are vitamins, terpenoids, nitrogen compounds, phenolic compounds, and a few additional endogenous metabolites that are very active antioxidants [19–21].

Antioxidants' scavenging of free radicals might slow the development of tissue fibrosis [22]. Antioxidants are believed to be possible prophylactics that reduce oxidative damage to the human body [23]. Fruits include a lot of antioxidants, which physically neutralize free radicals by providing them with electrons and turning them into harmless particles [24]. In addition to aging, free radicals, also known as reactive oxygen species (ROS), have been implicated in the etiology of several diseases, including cancer, cardiovascular disease, diabetes, hypertension, and neurological disorders [25,26]. The discovery

that many artificial antioxidant mechanisms have harmful or carcinogenic effects has prompted studies into the characteristics of logically formed ring antioxidants [14]. Antioxidants shield living things from damage caused by uncontrolled lipid peroxidation, protein degradation, reactive oxygen species, and DNA strand breakage [27,28]. Antioxidants can neutralize reactive free radicals, stop other molecules from oxidizing, and may consequently promote good health by preventing degenerative diseases [29,30].

Despite the documented traditional uses of *Solanum xanthocarpum* and *Achyranthes aspera*, limited comparative scientific evaluation has been conducted to assess their relative antioxidant and antibacterial potentials, particularly against oral pathogens. Both plants are reported to contain phenolic compounds, flavonoids, alkaloids, and saponins, which are known contributors to antimicrobial and free-radical-scavenging activities. However, variations in phytochemical composition may result in differential biological efficacy. Therefore, a comparative evaluation of these two plants is scientifically justified to determine whether their pharmacological effects are complementary, synergistic, or contrasting. The present study hypothesizes that differences in total phenolic and flavonoid content between *Achyranthes aspera* and *Solanum xanthocarpum* may lead to measurable variations in antioxidant capacity (DPPH and reducing power assays) and antibacterial activity against *Streptococcus mutans* MTCC 389. Establishing such comparative evidence may support their rational selection for natural oral healthcare formulations.

The rapid and ongoing rise in antimicrobial resistance (AMR) has renewed interest in alternative and complementary antimicrobial strategies, including plant-derived compounds that may act through multiple mechanisms, thereby reducing the risk of single-target resistance. Recent global surveillance and policy reviews highlight increasing resistance across common bacterial infections and underscore the urgent need for diverse discovery pipelines beyond conventional antibiotics. Plant secondary metabolites (phenolics, flavonoids, alkaloids, saponins, terpenoids) have shown promising broad-spectrum antibacterial activity in recent systematic reviews and experimental studies; these compounds can act as membrane disruptors, enzyme inhibitors, quorum-sensing modulators, or biofilm inhibitors — mechanisms that are particularly relevant when addressing multidrug-tolerant or biofilm-forming pathogens. The therapeutic potential and formulation/delivery challenges of

plant-derived antimicrobials have been summarized in recent comprehensive reviews (2023–2024), and justify systematic comparative screening of ethnomedicinal species for reproducible activity [31]. Plant secondary metabolites (phenolics, flavonoids, alkaloids, saponins, terpenoids) have shown promising broad-spectrum antibacterial activity in recent systematic reviews and experimental studies; these compounds can act as membrane disruptors, enzyme inhibitors, quorum-sensing modulators, or biofilm inhibitors — mechanisms that are particularly relevant when addressing multidrug-tolerant or biofilm-forming pathogens.

The therapeutic potential and formulation/delivery challenges of plant-derived antimicrobials have been summarized in recent comprehensive reviews (2023–2024), and justify systematic comparative screening of ethnomedicinal species for reproducible activity [32][33]. In the context of oral health, *Streptococcus mutans* remains a principal cariogenic species due to its acidogenicity, adhesion, and biofilm-forming traits; contemporary oral microbiome reviews emphasize both the persistence of *S. mutans* as a clinical target and the need for agents that reduce cariogenicity without broadly disrupting the oral microbiome. Given prior reports of antibacterial activity for both *Achyranthes aspera* and *Solanum xanthocarpum* against oral pathogens, a comparative evaluation — focusing on quantifiable phytochemical markers (Total Phenolic Content, Total Flavonoid Content) and functional assays (DPPH, reducing power, well diffusion against *S. mutans* MTCC 389) — is timely and scientifically warranted [34][35].

MATERIAL AND METHOD

Collection of Plant material

Fresh whole plants of *Achyranthes aspera* and *Solanum xanthocarpum* were collected from the Aravali forest region near Sohna, Gurugram, Haryana, and the surrounding GLA University campus. The plant materials were authenticated by NISCAIR (Accession Nos.: NISCAIR/RHMD/CONSULT/2019/3559-60-2 and NISCAIR/RHMD/CONSULT/2019/3559-60-1). The collected plant materials were washed, shade-dried, pulverized into a coarse powder, and extracted with appropriate solvents under laboratory conditions.

Preparation of Plant Extract by Maceration

The collected whole plants of *Achyranthes aspera* and *Solanum xanthocarpum* were thoroughly washed with distilled water to remove adhering soil and debris, then shade-dried at room

temperature (25–30°C) for approximately one month to ensure complete moisture removal. The dried plant materials were then coarsely powdered using a mechanical grinder. About 100 g of dried, powdered whole-plant material was subjected to cold maceration in a hydroalcoholic solvent system (water: ethanol, 50:50 v/v). The powdered material was immersed in 500 mL of solvent and left at room temperature for 72 hours with intermittent shaking to facilitate extraction.

After maceration, the mixture was filtered through muslin cloth, then through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure and dried to obtain a semi-solid extract. The dried extracts were stored in airtight containers at 4°C until further phytochemical and biological analysis. A 50:50 hydroalcoholic solvent was selected due to its balanced polarity, which allows efficient extraction of both polar and moderately non-polar phytoconstituents, including phenolic compounds and flavonoids responsible for antioxidant and antibacterial activity.

Determination of Solubility

The solubility of the dried extracts in various solvents (water, ethanol, methanol, ethyl acetate, and petroleum ether) was determined by adding an excess of extract to 10 mL of each solvent in a sealed vial, then shaking at 25°C for 24 hours. The solution was then filtered, and the solvent was evaporated to dryness. The residue was weighed, and solubility was expressed as mg/mL of solvent.

Quantitative assessment of phytochemistry

Total Phenolic Content by Spectrophotometric Analysis

The total phenolic content of the sample was determined using the Folin-Ciocalteu Assay. The distilled water was used to dilute the extracts of *Achyranthes aspera* and *Solanum xanthocarpum* (including 0.2 milliliters of the canned solution) to a volume of 7 milliliters. Additionally, 2.5 milliliters of Folin-Ciocalteu reagent and 2 milliliters of 7.5% sodium carbonate were combined. For the final two hours at room temperature, a spectrophotometer was used to measure the absorbance at 760 nm. Calibration curves using Paradig solutions of Gallic Acid Equivalent (GAE) mg/g were produced. Gallic acid agglomerations of 20, 40, 60, 80, & 100 µg/mL were made. Polyphenols and other reducing agents can be detected by the Folin-Ciocalteu reagent. In response, they turn blue. Using spectrophotometry, the blue color was identified [36].

Quantification of total flavonoid content by spectrophotometric analysis

The concentration of flavonoids was determined using the aluminum chloride method [37]. 0.5 milliliter *Solanum xanthocarpum* and 2 milliliter distilled water were combined with the extract solution of *Achyranthes aspera*. After that, Sodium nitrite (5%) was introduced in 0.15 ml quantities and mixed. Subsequently, two milliliters of 4% sodium hydroxide were added, and 0.15 milliliters of 10% aluminum chloride were left to stand for up to six minutes. The mixture was then diluted to 5 milliliters by aggressively blending distilled water. Using a UV spectrophotometer, the mixture's absorbance was measured at 510 nm. Standard solutions of Rutin Equivalent (RE), mg/g, were used to create calibration curves. We prepared Rutin concentrations of 20, 40, 60, 80, and 100 µg/mL. Rutin-equivalent milligrams, weighted by the grams of dry extract, were calculated from the total flavonoid concentration using the calibration curve [38].

In vitro Antioxidant Performance

The activity of DPPH Radical Scavenging

McCune and Johns's modified approach was used to assess the ability of extracts from different plant species to scavenge free radicals. The methanol was used to dissolve the extracts. To bring the volume of the DPPH solution in methanol to 1 mL, different extract volumes (20–100 µL) were added. After adding 2 milliliters of DPPH reagent (0.1 mg/mL) to this, the mixture was thoroughly shaken and left at room temperature for 30 minutes. The absorbance of the resulting solution at 517 nm was measured. The radical-scavenging activity is measured by determining the amount of antioxidants required to reduce the initial DPPH absorbance by 50% (IC50) [39]. The following formula was used to calculate the sample's or standard's fraction of antioxidant activity:

$$\% \text{ Inhibition} = \frac{\text{Ab of control} - \text{Ab of sample}}{\text{Ab of control}} \times 100$$

Reducing power assay

The standard solution was prepared by dissolving 3 mg of ascorbic acid in distilled water, and extracts were prepared by combining 1 mg of dried extract with methanol to obtain sample concentrations.

Procedure for reducing power

In this procedure, 1.0 ml of deionized water, 2.5 ml of pH 6.6 phosphate buffer, and 2.5 ml of 1 percent potassium ferricyanide

are combined with extract aliquots ranging from 20 to 100 µg/ml. Ascorbic acid was added as a reference concentration. The amalgamation was incubated for 20 minutes at 50 °C in a water bath once it had cooled. The mixture was combined with aliquots of 2.5 ml (10 percent) trichloroacetic acid and centrifuged for ten minutes at 3000 rpm. Five milliliters of freshly made ferric chloride (0.1%) solution, and two milliliters of distilled water were combined with the upper layer of the solution. To measure the absorbance at 700 nm, the Systronic twin-beam UV-2201 UV spectrometer was used. The extract was not added when the blank was prepared.

Antibacterial Activity (Well Diffusion Assay)

Well diffusion assay

Sample dilutions were prepared at concentrations of 0.5, 1, 1.5, 2, and 1 mg/mL, with volume makeup using distilled water. Nutrient agar media was prepared by dissolving 28g in distilled water, sterilizing it in an autoclave, and solidifying it. The shaker was used to maintain the suspension of microorganisms, which was calibrated to 10⁸ CFU/mL. Then 100 µl of inocula of bacterial strains (*S. mutans* MTCC 389) was dispersed across the NAM (nutrient Agar-formatted media). Inoculum was applied to the entire sterile agar plate using a sterile spreader to cover the surface and inoculate it. Using a sterile cork borer, four 6-mm wells were drilled into the inoculation material.

A negative control (solvent blank) was included in the well diffusion assay to confirm that the observed antibacterial activity was due to the plant extracts and not the solvent used for sample preparation. Distilled water, used as the extraction/dilution solvent, was introduced into one well (6 mm diameter) under identical experimental conditions. The plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured in millimeters. The sample concentrations (0.5, 1, 1.5, 2, and 1 mg/mL) varied across wells. After incubation for 18 to 24 hours at 37 °C, it was left at room temperature for half an hour to disperse. Following incubation, the development of a clear area surrounding the well—a sign of the tested drugs' antibacterial efficacy —was monitored on the plates. We measured and observed the millimeter-based zone of inhibition (ZOI). Using a scale on the reverse side of the Petri plate, zones were measured to the nearest millimeter. A black, non-reflective background was held a few inches above the Petri plate. The zones of full inhibition's diameters, as determined by the unassisted judgment, were measured, encompassing the well's distance [40].

RESULTS AND DISCUSSION

Solubility establishment of *Solanum xanthocarpum*, *Achyranthes aspera* Extract

The result of the solubility value of Powder of *Achyranthes aspera* and *Solanum xanthocarpum* was observed. The solubility in water, ethyl acetate, methanol, and petroleum ether is shown in Table 1.

Table 1: Solubility Determination of *Solanum xanthocarpum* and *Achyranthes aspera* Extract.

Solvent	Result	
	<i>S. xanthocarpum</i>	<i>A. aspera</i>
Water	Soluble	Soluble
Ethyl Acetate	Sparingly soluble	Sparingly soluble
Methanol	Sparingly soluble	Sparingly soluble
Petroleum Ether	insoluble	insoluble
Ethanol	Sparingly soluble	Sparingly soluble

Qualitative Phytochemical Examination of Various Extracts

The Results of the phytochemical tests of *S. xanthocarpum* and *A. aspera* using different methods are shown in Table 2.

Quantitative Phytochemical Analysis of *Solanum xanthocarpum* and *Achyranthes aspera* extract

TPC Estimation

The Total Phenolic Content (TPC) of the plant extract was determined using the Folin-Ciocalteu method, with gallic acid serving as the standard. A standard curve was constructed by measuring the absorbance of gallic acid at various concentrations. The absorbance values were 0.112 at 760 nm for 20 µg/mL, 0.234 for 40 µg/mL, 0.359 for 60 µg/mL, 0.468 for 80 µg/mL, and 0.574 for 100 µg/mL. These values were plotted to create a calibration curve, which was used to quantify the phenolic content in the plant extract. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract, enabling comparative analysis of phenolic content relative to the standard. Standard curve data for gallic acid are shown in Table 3 and Figure 1. The total phenolic content (TPC) of the extracts of *Solanum xanthocarpum* and *Achyranthes aspera* was determined using a standard curve of gallic acid. The absorbance of both extracts was measured, resulting in a mean absorbance of 0.391 ± 0.002 for both plants. The TPC for *Solanum xanthocarpum* was found to be 77.80 mg GAE/g extract, whereas *Achyranthes aspera* exhibited a higher TPC of 98.40 mg GAE/g extract. These values highlight the presence of phenolic compounds in the extracts, which are known for their significant antioxidant properties (Table 4).

Table 2: Phytochemical evaluation of *Solanum xanthocarpum* and *Achyranthes aspera* extract

S. No.	Experiment	Result	
		<i>S. xanthocarpum</i>	<i>A. aspera</i>
Carbohydrates			
1.	Molisch's Test	AB	P
2.	Fehling's Test	P	P
3.	Benedict's Test	P	P
Alkaloids			
1.	Mayer's Test	AB	AB
2.	Hager's Test	P	P
3.	Wagner's Test	P	P
Terpenoids			
1.	Salkowski Test	P	P
2.	Liebermann-Burchard's Test	AB	AB
Test for Flavonoids			
1.	Lead Acetate Test	P	P
2.	Alkaline Reagent Test	P	P
Tannins and Phenolic Compounds			
1.	FeCl ₃ Test	P	P
2.	Lead Acetate Test	P	P
3.	Gelatine Test	P	P
Saponins			
1.	Foam Test	P	P
Protein and Amino Acids			
1.	Ninhydrin Test	P	AB
2.	Biuret's Test	P	P
Glycosides			
1.	Legal's Test	P	P
2.	Keller Killani Test	AB	P
3.	Bontrager Test	P	P

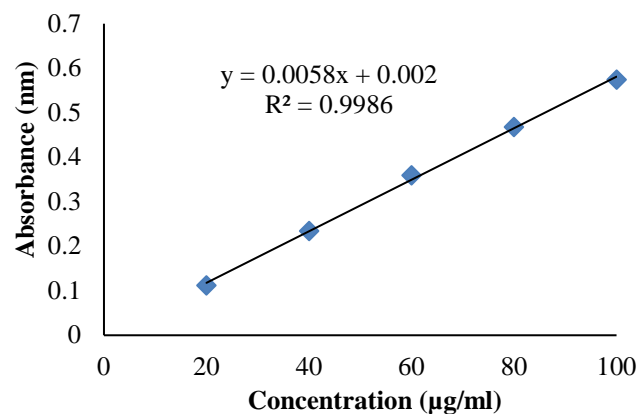
Where AB means Absent and P means Present

Table 3: Standard curve data for Gallic Acid

Conc. in $\mu\text{g per mL}$	Absorbance (at 760 nm)
20	0.112
40	0.234
60	0.359
80	0.468
100	0.574

Table 4: Total Phenolic Content in *Solanum xanthocarpum* and *Achyranthes aspera* extract.

Extracts	Absorbance (Mean \pm SD)	TPC (mg GAE/g extract)
<i>S. xanthocarpum</i>	0.391 \pm 0.002	77.80
<i>A. aspera</i>	0.391 \pm 0.002	98.40

**Figure 1: Graph of TPC estimation.****TFC Estimation**

The Total Flavonoid Content (TFC) of the plant extract was quantified using the aluminum chloride colorimetric method, with rutin as the standard. A standard curve was established by measuring the absorbance of rutin at various concentrations. The absorbance values recorded were 0.088 for 20 $\mu\text{g/mL}$, 0.163 for 40 $\mu\text{g/mL}$, 0.229 for 60 $\mu\text{g/mL}$, 0.308 for 80 $\mu\text{g/mL}$, and 0.404 for 100 $\mu\text{g/mL}$. These absorbance values were plotted to create a calibration curve, which was subsequently used to determine the flavonoid content in the plant extract. The TFC was expressed as milligrams of rutin equivalents (RE) per gram of dry extract, providing a standardized measure of flavonoid concentration (Table 5). The total flavonoid content (TFC) of the extracts of *S. xanthocarpum* and *A. aspera*, expressed in mg per gram equivalent to rutin, was analyzed. The absorbance of *S. xanthocarpum* extract was recorded at 0.253 ± 0.002 , corresponding to a TFC of 82.66 mg RE/g extract. In contrast, the *A. aspera* extract showed a higher absorbance of 0.415 ± 0.004 , resulting in a TFC of 136.66 mg RE/g extract. These results indicate a significant presence of flavonoids in both extracts, with *A. aspera* showing higher flavonoid content than *S. xanthocarpum* (Table 6).

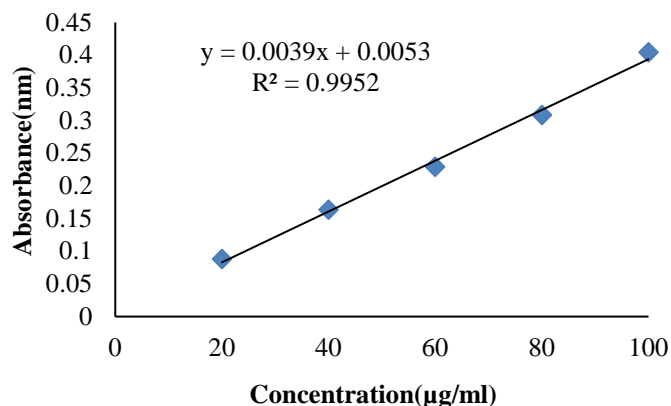
**Figure 2: Standard curve of Rutin**

Table 5: Standard curve data for Rutin.

Conc. in $\mu\text{g per mL}$	Absorbance (at 510 nm)
20	0.088
40	0.163
60	0.229
80	0.308
100	0.404

Table 6: TFC in *Solanum xanthocarpum* and *Achyranthes aspera* extract.

TFC content in mg RE/g extract to Rutin		
Extracts	Absorbance (Mean \pm SD)	TFC (mg RE/g extract)
<i>S. xanthocarpum</i>	0.253 \pm 0.002	82.66
<i>A. aspera</i>	0.415 \pm 0.004	136.66

ANTI-OXIDANT ACTIVITY

DPPH Assay

The antioxidant activity of the plant extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, with ascorbic acid as the standard. Various concentrations of ascorbic acid (20, 40, 60, 80, and 100 $\mu\text{g/mL}$) were tested, and the corresponding absorbance values were measured at 517 nm. The absorbance values for ascorbic acid were 0.4295 at 20 $\mu\text{g/mL}$, 0.3295 at 40 $\mu\text{g/mL}$, 0.2539 at 60 $\mu\text{g/mL}$, 0.165 at 80 $\mu\text{g/mL}$, and 0.098 at 100 $\mu\text{g/mL}$, with a control absorbance of 0.958. The percentage inhibition of DPPH radicals increased with higher concentrations of ascorbic acid, showing 50.97% inhibition at 20 $\mu\text{g/mL}$, 61.41% at 40 $\mu\text{g/mL}$, 69.30% at 60 $\mu\text{g/mL}$, 78.58% at 80 $\mu\text{g/mL}$, and 85.57% at 100 $\mu\text{g/mL}$. These results demonstrated a dose-dependent increase in radical scavenging activity, indicating the strong antioxidant potential of ascorbic acid. The plant extract's DPPH radical-scavenging activity was then compared with this standard to determine its antioxidant efficacy (Table 7).

The % inhibition of *Achyranthes aspera* and *Solanum xanthocarpum* was measured at varying concentrations (20, 40, 60, 80, and 100 $\mu\text{g/mL}$). At 20 $\mu\text{g/mL}$, *Achyranthes aspera* showed a % inhibition of 46.822, while *Solanum xanthocarpum* exhibited a % inhibition of 46.000. As the concentration increased to 40 $\mu\text{g/mL}$, *Achyranthes aspera* had a % inhibition of 51.229, compared to 48.104 for *Solanum xanthocarpum*. At 60 $\mu\text{g/mL}$, the % inhibition for *Achyranthes aspera* was 59.406, whereas for *Solanum xanthocarpum* it was 51.302. Further increasing the concentration to 80 $\mu\text{g/mL}$, *Achyranthes aspera* showed a % inhibition of 62.406, while *Solanum xanthocarpum* showed 54.406. At the highest concentration of 100 $\mu\text{g/mL}$,

Achyranthes aspera exhibited a % inhibition of 65.864, and *Solanum xanthocarpum* had a % inhibition of 55.385. The IC₅₀ values were determined to be 31.056 $\mu\text{g/mL}$ for *Achyranthes aspera* and 51.920 $\mu\text{g/mL}$ for *Solanum xanthocarpum*, indicating a higher inhibitory potential for *Achyranthes aspera* compared to *Solanum xanthocarpum*. The IC₅₀ value represents the concentration required to inhibit 50% of DPPH free radicals, and lower IC₅₀ values indicate higher antioxidant potency.

In the present study, *Achyranthes aspera* exhibited a markedly lower IC₅₀ value (31.056 $\mu\text{g/mL}$) than *Solanum xanthocarpum* (51.920 $\mu\text{g/mL}$), indicating significantly greater free radical-scavenging efficiency. This enhanced antioxidant potential may be attributed to the higher total phenolic (98.40 mg GAE/g) and total flavonoid content (136.66 mg RE/g) observed in *Achyranthes aspera*, as phenolic and flavonoid compounds are well-established hydrogen donors capable of neutralizing reactive oxygen species. The findings suggest that *A. aspera* possesses superior antioxidant potency, making it a more promising candidate for natural antioxidant formulations. (Table 8).

Table 7: Scavenging of DPPH radicals by Ascorbic acid.

Conc. in $\mu\text{g/mL}$	Abs (at 517 nm)	% Inhibition
20	0.4295	50.96701
40	0.3295	61.40543
60	0.2539	69.29687
80	0.165	78.57662
100	0.098	85.57035
Control	0.958	
IC ₅₀		15.661

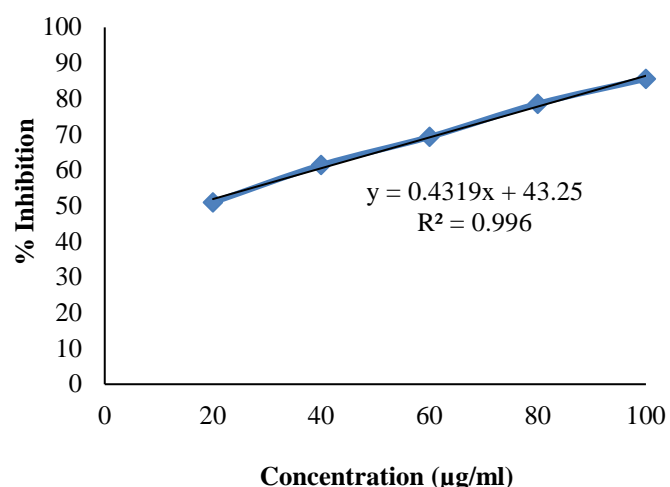
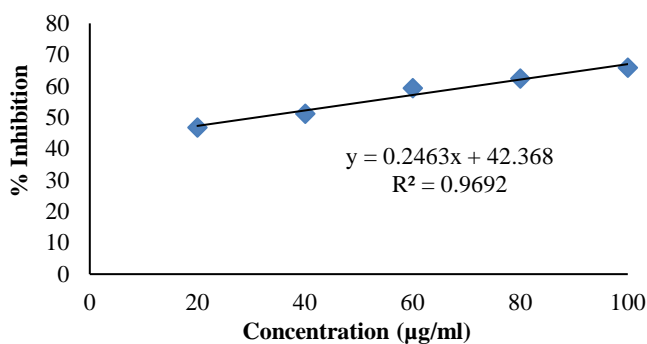
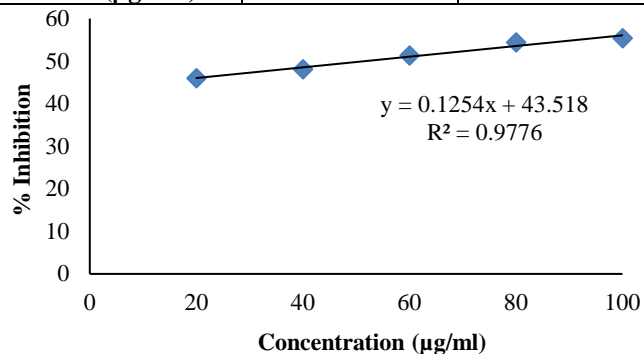
**Figure 3: Graph of Percentage Inhibition vs. concentration of Ascorbic acid**

Table 8: Scavenging of DPPH radicals activity for *Achyranthes aspera* and *Solanum xanthocarpum* extract.

Conc. (µg/mL)	% Inhibition of <i>A. aspera</i>	% Inhibition <i>S. xanthocarpum</i>
20	46.822	46.000
40	51.229	48.104
60	59.406	51.302
80	62.406	54.406
100	65.864	55.385
IC50 (µg/mL)	31.056	51.920

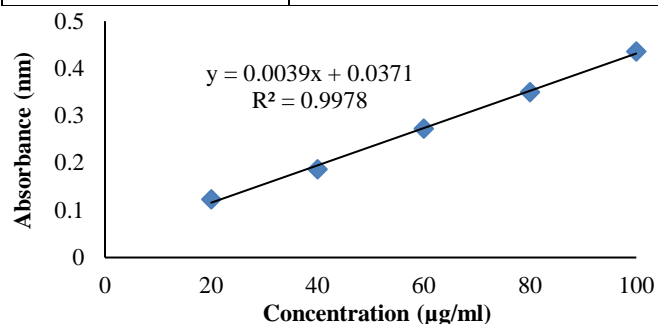
**Figure 4: Graphs representing the Percentage Inhibition vs. concentration of *S. xanthocarpum* and *A. aspera* extract.****Reducing power scavenging activity**

The reducing-power scavenging activity of the plant extract was assessed and compared with that of ascorbic acid, the standard. Different concentrations of ascorbic acid (20, 40, 60, 80, and 100 µg/mL) were prepared, and their absorbance values were measured at 700 nm. The absorbance values for ascorbic acid were 0.123 at 20 µg/mL, 0.187 at 40 µg/mL, 0.273 at 60 µg/mL, 0.35 at 80 µg/mL, and 0.436 at 100 µg/mL. These values showed a concentration-dependent increase in absorbance, indicating greater reducing power at higher ascorbic acid concentrations. The plant extract's reducing power was then evaluated and compared to these standard values to determine its antioxidant capacity. The results suggest that the plant extract may possess significant reducing power, contributing to its overall antioxidant activity (Table 9). The absorbance analysis of *Solanum xanthocarpum* and *Achyranthes aspera* at various

concentrations shows a consistent increase with concentration. At a concentration of 20 µg/mL, *Solanum xanthocarpum* exhibits an absorbance of 0.1961, while *Achyranthes aspera* shows a slightly higher absorbance of 0.2215. As the concentration increases to 40 µg/mL, the absorbance values also increase to 0.2504 for *Solanum xanthocarpum* and 0.3309 for *Achyranthes aspera*. This trend continues, with *Solanum xanthocarpum* reaching an absorbance of 0.3398 and *Achyranthes aspera* at 0.4383 at 60 µg/ml. At 80 µg/ml, the absorbance for *Solanum xanthocarpum* is 0.3738, and for *Achyranthes aspera* is 0.4998. Finally, at the highest tested concentration of 100 µg/mL, *Solanum xanthocarpum* has an absorbance of 0.4985, and *Achyranthes aspera* reaches 0.5707. These results indicate that both plant extracts exhibit a proportional increase in absorbance with increasing concentration, with *Achyranthes aspera* consistently showing higher absorbance values than *Solanum xanthocarpum* across all tested concentrations (Table 10).

Table 9: Scavenging reducing power activity of Ascorbic acid

Conc. in µg per mL	Absorbance (at 700 nm)
20	0.123
40	0.187
60	0.273
80	0.35
100	0.436

**Figure 5: Representation of Absorbance vs. concentration of Ascorbic acid****Table 10: Reducing power scavenging activity for *Solanum xanthocarpum* and *Achyranthes aspera* extract.**

Conc. (µg/mL)	Absorbance (at 700 nm) <i>S. xanthocarpum</i>	Absorbance (at 700 nm) <i>A. aspera</i>
20	0.1961	0.2215
40	0.2504	0.3309
60	0.3398	0.4383
80	0.3738	0.4998
100	0.4985	0.5707

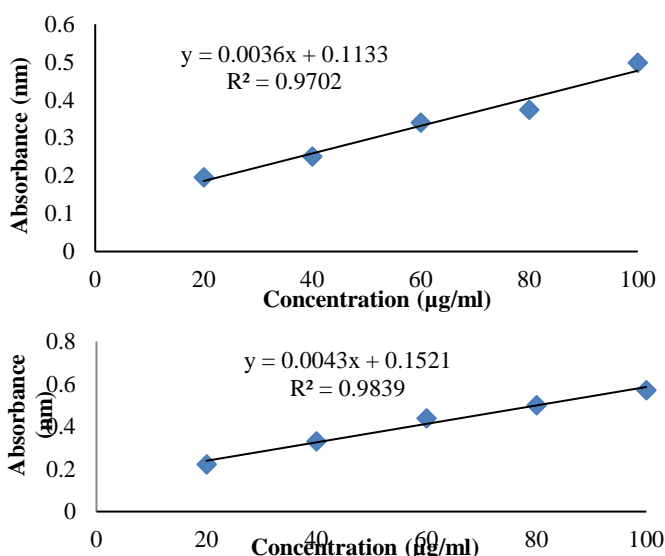


Figure 6: Graph representing the absorbance vs. concentration of *S. xanthocarpum* and *A. aspera* extract.

Antimicrobial Activity (Well Diffusion Assay)

The antibacterial activity of *Achyranthes aspera* against *S. mutans* was assessed using the well diffusion assay, with concentrations ranging from 0.5 mg/mL to 2 mg/mL. The results demonstrated a concentration-dependent increase in antibacterial action. At the lowest concentration of 0.5 mg/mL,

the mean inhibition zone was 9.333 ± 0.577 mm.

Increasing the concentration to 1 mg/mL resulted in a mean inhibition zone of 12 ± 1 mm. Further increases to 1.5 mg/mL and 2.0 mg/mL yielded mean inhibition zones of 15.666 ± 0.577 mm and 23 ± 1.732 mm, respectively. These results indicate that *Achyranthes aspera* exhibits significant antibacterial activity against *S. mutans*, with greater inhibition observed at higher concentrations (Table 11). The antibacterial action of *Solanum xanthocarpum* against *Streptococcus mutans* was evaluated at various concentrations, revealing a dose-dependent increase in the inhibition zone diameter. At a concentration of 0.5 mg/mL, the inhibition zones measured 7 mm, 8 mm, and 7 mm across three plates, with a mean value of 7.33 ± 0.577 mm. Increasing the concentration to 1 mg/mL resulted in inhibition zones of 10 mm, 11 mm, and 9 mm, yielding a mean of 10 ± 1 mm. At 1.5 mg/mL, the inhibition zones were 14 mm, 15 mm, and 15 mm, with a mean value of 14.666 ± 0.577 mm. The highest concentration tested, 2 mg/mL, produced inhibition zones of 19 mm, 21 mm, and 20 mm, resulting in a mean of 20 ± 1 mm. These findings demonstrate that *Solanum xanthocarpum* exhibits significant antibacterial activity against *S. mutans*, with the extent of inhibition increasing with higher concentrations (Table 12).

Table 11: Antibacterial action of *A. aspera* against *S. mutans*.

Conc. in mg per mL	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean ± SD
0.5	9	9	10	9.333±0.577
1	13	12	11	12±1
1.5	15	16	16	15.666±0.577
2	22	25	22	23±1.732



Plate -1



Plate -2

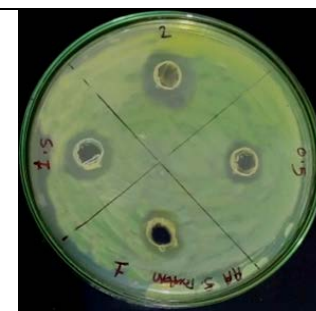


Plate -3

Table 12: Antibacterial action of *Solanum xanthocarpum* against *S. mutans*.

Conc. in mg per mL	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean ± SD
0.5	7	8	7	7.33±0.577
1	10	11	9	10±1
1.5	14	15	15	14.666±0.577
2	19	21	20	20±1

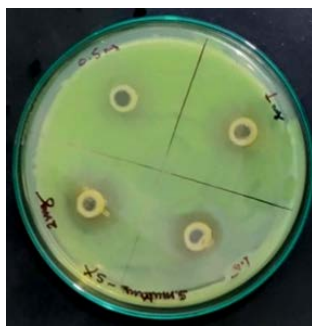


Plate -1

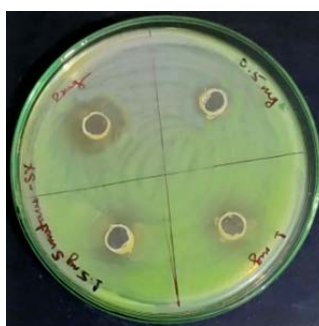


Plate -2



Plate -3

Results of the antibacterial action of Standard against *S. mutans*

The antimicrobial activity of the standard agent chlorhexidine against *Streptococcus mutans* was assessed by measuring the zone of inhibition. At a concentration of 10 µg/mL, chlorhexidine produced a substantial inhibition zone of 34 mm. This result indicates that chlorhexidine is highly effective in inhibiting the growth of *S. mutans*, demonstrating its potent antimicrobial properties.

Table 13: Antimicrobial activity of Standard (Chlorohexidine) against *S. mutans*

Microorganism	Zone of inhibition (mm)
<i>S. mutans</i> (10 µg/mL)	34 mm

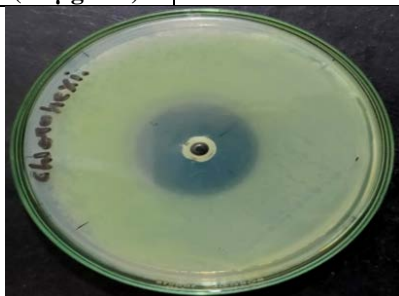


Figure 7: Antimicrobial activity of standard (Chlorohexidine) against *S. mutans*.

DISCUSSION

The current investigation showed that the extracts of *Solanum xanthocarpum* and *Achyranthes aspera* contain phytochemical constituents such as carbohydrates, flavonoids, glycosides, proteins, amino acids, terpenoids, steroids, tannins, phenolics, saponins, and alkaloids (Table 2), as determined through phytochemical analysis. The total phenolic content is calculated and reported as mg/g dry weight of the sample and as mg gallic acid equivalents. TPC values for *Solanum xanthocarpum* and *Achyranthes aspera* extracts were 77.80 and 98.40 mg GAE/g extract, respectively (Table 4). The total flavonoid content of the

extracts was expressed as Rutin mg RE/g of the test extract dry mass. The total flavonoid content for *Solanum xanthocarpum* and *Achyranthes aspera* extract, respectively, was 82.66 and 136.66 mg RE/g extract (Table 6). Antioxidants are essential for preventing disease because they reduce the oxidative damage caused by Reactive Oxygen Species to biological components. The antioxidant capacity of plant extracts was assessed in vitro using methods such as the DPPH and Reducing Power Assays. This study evaluated the in vitro antioxidant activity of extracts from *Solanum xanthocarpum* and *Achyranthes aspera*.

Results showed percent inhibitions of 55.385% and 65.864%, respectively, with IC₅₀ values of 51.920 and 31.056 µg/ml (Table 8). As a reference substance, ascorbic acid showed a % inhibition of 85.570%, reaping the IC₅₀ assessment of 15.661 µg/mL. The compound's ability to reduce may serve as a notable indicator of its potential antioxidant activity. It is possible for compounds with reducing capacity to function as both primary and secondary antioxidants by reducing oxidized intermediates in lipid peroxidation, indicating that they are electron donors. *Achyranthes aspera* aqueous extract demonstrated a potent lowering ability in comparison to *Solanum xanthocarpum* aqueous extract (Table 10).

The antibacterial activity of extracts from *Solanum xanthocarpum* and *Achyranthes aspera* was evaluated in vitro against *S. mutans* using the agar well diffusion method. The *Achyranthes aspera* extract was discovered to be more active against *S. mutans*, followed by *Solanum xanthocarpum* extract (23±1.732 mm and 20±1mm at 2mg/ml). *Achyranthes aspera* extract displayed the optimal inhibition zone of 23±1.732mm in diameter at a concentration of 2mg/ml against *S. mutans*. On the other hand, *Solanum xanthocarpum* extract Ped showed optimal inhibition zones of 20±1mm in diameter at a concentration of 2mg/ml. A critical comparison

between the plant extracts and the standard antimicrobial agent chlorhexidine reveals a substantial difference in potency. Chlorhexidine produced a 34 mm zone of inhibition at a concentration of only 10 µg/mL. In contrast, the maximum inhibition zones for *Achyranthes aspera* and *Solanum xanthocarpum* (23 ± 1.732 mm and 20 ± 1 mm, respectively) were achieved at a much higher concentration of 2 mg/mL (2000 µg/mL). This indicates that chlorhexidine is considerably more potent on a per-microgram basis. The markedly higher concentrations required for the plant extracts to achieve moderate inhibition may be attributed to their crude nature, which contains a complex mixture of bioactive and inactive constituents. Unlike chlorhexidine, which is a purified, highly concentrated synthetic antimicrobial agent with a well-defined mechanism of action, crude plant extracts often exhibit lower apparent potency due to limited diffusion in agar, variability in phytochemical concentrations, and the presence of inactive components.

Despite their comparatively lower potency, the plant extracts demonstrated significant antibacterial activity, suggesting the presence of biologically active compounds that may serve as lead molecules. Moreover, plant-derived antimicrobials may offer advantages such as reduced side effects, lower risk of resistance development, and additional antioxidant benefits. Further fractionation, purification, and characterization of the active constituents may enhance antibacterial efficacy and provide a more accurate comparison with standard agents. Alkaloids are known to interfere with DNA replication and disrupt membrane integrity. Saponins increase membrane permeability through interaction with sterols. Flavonoids inhibit nucleic acid synthesis and scavenge reactive oxygen species via hydrogen atom donation. Phenolic compounds alter membrane permeability and denature microbial proteins. The primary factors determining the size difference of the inhibition zone are the phyto-constituents (found in the extracts) and their ability to diffuse into the agar medium, as well as the development and metabolic processes of the bacteria within the medium and the antimicrobial activity of the substances that have diffused. Since Gram-negative bacteria contain higher quantities of lipids in their cell walls than Gram-positive bacteria, the inhibition zone diameter can also be related to the polarity of the compounds in the tested extracts and the composition of the test organisms. *Streptococcus mutans* was selected as the test organism because it is a primary etiological agent responsible for dental caries and

oral biofilm formation. Its ability to produce extracellular polysaccharides and tolerate acidic environments makes it a major contributor to tooth decay. Since both *Solanum xanthocarpum* and *Achyranthes aspera* have been traditionally used for oral and dental health applications, evaluating their activity against *S. mutans* provides relevant insight into their potential therapeutic application in oral care formulations.

CONCLUSION

The present study demonstrates that extracts of *Solanum xanthocarpum* and *Achyranthes aspera* possess significant antioxidant and antibacterial activities, which may be attributed to their rich content of phenolics, flavonoids, alkaloids, and saponins. Although the crude extracts exhibited measurable inhibitory activity against *Streptococcus mutans*, their potency was considerably lower than that of chlorhexidine, highlighting the need for further refinement. Future research should focus on bioassay-guided fractionation to isolate and identify the specific bioactive constituents responsible for the observed antimicrobial and antioxidant effects. Subsequent purification and structural elucidation using advanced analytical techniques such as HPLC, LC-MS, and NMR spectroscopy will be essential to characterize the active molecules. Additionally, mechanistic studies investigating membrane integrity disruption, enzyme inhibition, and modulation of oxidative stress at the molecular level are warranted. Further evaluation against a broader panel of Gram-positive and Gram-negative reference strains is recommended to determine the spectrum of antimicrobial activity. Toxicological assessment and cytotoxicity studies on human cell lines should also be conducted to establish safety profiles. Ultimately, isolation of potent compounds may facilitate the development of novel plant-derived antimicrobial agents or adjunct formulations for oral healthcare applications.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Kanchan Mathur contributed to the conception and design of the study, data collection, experimental work, and manuscript drafting. Reena Gupta contributed to data analysis and interpretation and critically revised the manuscript for important intellectual content. Both authors read and approved the final version of the manuscript.

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