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Phytochemical screenings and evaluations of antibacterial and antioxidant activities of methanolic leaf extract of *Senna auriculata* (L). Roxb

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Abstract

The current study examineed the "Phytochemical contents, antimicrobial and antioxidant activities of the methanolic leaf extract of *S. auriculata*". Methanol was used to extract a leaf sample from a plant. The phytoconstituents and functional groups in the leaf extract had been investigated using Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier Transform Infrared (FTIR) analysis. Concurrently, the agar well-plate method was used to assess antimicrobial activity against ten bacterial strains. DPPH (1,1-diphenyl-2-picrylhydrazyl), reducing power activity, phosphomolybdenum activity, hydrogen peroxide activity, and nitric oxide inhibitory activity were employed to assess antioxidant activity. Carbohydrates, protein, tannins, saponins, and terpenoids were found in the phytochemical analysis. The GC-MS study of the leaf extract revealed the presence of 13 major bioactive substances with antimicrobial, free radical scavenging, and anticancer properties. The appearance of distinctive peak values with various functional groups, such as alcohol, alkanes, aromatic, esters, and amines, was indicated by the FTIR result. The leaf extract exhibited a potent antibacterial effect against all pathogens tested. The highest activity against *A. hydrophila* (15±0.6 mm) was found at a 100 µg/mL concentration. The antioxidant properties of leaf extract increased as concentration was increased. The leaf extract contains 108.13 mg GAE/g and 61.25 mg RE/g of phenolic and flavonoid content, respectively. Finally, the methanolic leaf extract of *S. auriculata* was found to have powerful antibacterial and antioxidant features and could be a valuable source of natural compounds for creating new medications. Although *S. auriculata* has been extensively studied for its antibacterial activity against pathogenic organisms, the findings showed that it is also effective against the fish *pathogen A. hydrophila*. More investigation is required to find and identify the biologically active compounds responsible for the plant's antibacterial and antioxidant activity.

Keywords**:** *activities; antibacterial; antioxidant; bioactive compounds; methanolic extract; phytochemical contents; Senna auriculata.*

1. Introduction

Many modern medicines are made directly from medicinal plants, which are a source of new drugs. Most of the drugs used in traditional medical systems are derived from natural sources or semi-synthetic derivatives of natural products. These organic plant products have immune-boosting, antibacterial, and anticancer properties (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017). The beneficial effect of medicinal plants has been used since olden times through a simple process that does not require the isolation of pure

compounds. The use of bioactive components for medicinal applications has gradually increased due to the side effects that some man-made drugs have and the emergence of drug resistance to treatments for infectious diseases currently in use. Phytochemicals are naturally occurring compounds found in medicinal plants, leaves, vegetables and roots that have defense mechanisms and protect humans from a wide range of diseases. Plant secondary metabolites have therapeutic properties, such as anticancer, anti-inflammatory, antibacterial and antioxidant activities (Altemimi et al., 2017). Antioxidants are biologically active compounds, such as phenols, flavonoids, ascorbic acid (Vitamin C) and carotenoids. A large class of bioactive substances known as phenolics and flavonoids have a variety of biochemical functions, with phenolics acting as antioxidants and anti-mutagens and having a significant impact on gene expression in addition to having antiviral, anticancer, anti-inflammatory, and antioxidant properties, flavonoids also have neuroprotective and cardioprotective effects (Ullah et al., 2020). Such natural antioxidants have become the focus of numerous research studies to locate a source of potentially safe, low-cost, and active antioxidants. Cardiovascular disease, stroke, cancer, metabolic disorders and Alzheimer's disease are a few serious illnesses that are prevented and treated with antioxidant-based medications. Since conventional antibiotics lose their potency over time, it has become more important in recent years to develop novel antimicrobials that are equally potent. Numerous studies have concentrated on evaluating pure secondary essential oils and microbial, and plant extracts as potential antibacterial agents (Alvarez-Martinez, Barrajon-Catalan, Herranz-Lopez, & Micol, 2021).

Since the 15th century, *Senna auriculata* (L.) Roxb., also known as *Cassia auriculata* L., is one of the medicinal herbs that have been traditionally utilized in Ayurveda, Siddha and Unani (Nille, Mishra, Chaudhary, & Reddy, 2021). Polyphenols, anthraquinones, saponins, tannins and steroids are abundant in cassia species. The plant components are also used as a medication for ulcers, leprosy and liver illnesses because they have anti-diabetic, antipyretic, anti-hyperglycemic activity, wound-healing, antioxidantidant, anti-inflammatory, hepatoprotective and antibacterial characteristics (Vedavathy, & Rao, 1991). *S. auriculata* is frequently used in Ayurvedic medicine as an astringent, tonic and treatment for liver, skin, diabetes, conjunctivitis, ulcers and other illnesses. Seeds are used to treat diarrhea, hyperglycemia and ophthalmology conditions. Impotence can be effectively treated with bark decoction. The fruit is used to heal skin infections and dyspepsia. The root is used to treat diabetes, urinary problems and fever. Diabetes treatment uses floral and flower bud extracts. When *S. auriculata* is consumed, the beta-pancreatic cell's insulin synthesis becomes active. It eradicates several diabetic symptoms like frequent urination and excessive thirst by slowing down the metabolism of starch into glucose (Kainsa, Kumar, & Rani, 2012). It is widely used to prevent and cure kidney stones, painful urination, and urinary distension. It aids in menstrual cycle regulation and reduces excessive endometrium. For ladies who have irregular periods, Senna flower tea is beneficial. Additionally, Senna decreases blood cholesterol levels while assisting with fat loss and maintaining a healthy body weight (Nambirajan et al., 2018).

One of the most prevalent freshwater infections worldwide is *Aeromonas hydrophila*. It can transmit a number of zoonotic illnesses to humans and fish. It causes gastroenteritis in people. Economic losses have been incurred by both micro and macro scale fish farmers as a result of the illnesses that are affecting aquaculture. According to reports, many of the plants are vulnerable to fish infections. *Thymus vulgaris* ethanolic leaf extract showed improved efficacy against *A. hydrophila* isolated from *Cyprinus carpio* (Al-laham et al., 2014). The Kirby-Bauer disc-diffusion technique was employed by Agnieszka et al., (2021) to investigate the antibacterial effect of 41 ficus species against *Aeromonas* strains. Most extracts greatly reduced the growth of *A. sobria, A. salmonicida* and *A. hydrophila* bacteria compared to oxytetracycline and gentamicin. The essential oils from *Melaleuca alternifolia, Thymus vulgaris* and *Eugenia caryophyllus* can prevent the development of *Salmonicida, A. sobria*, and *A. hydrophila* (Kot et al., 2019).

2. Objectives

The aims of the present study are,

1) To identify the bioactive compounds and functional groups present in the methanolic leaf extract of *S. auriculata* by phytochemical screening, FTIR and GC-MS analysis.

2) To test the total phenolic and total flavonoid content as well as the DPPH activity, reducing power activity, nitric oxide scavenging activity, phosphomolybdenum activity and hydrogen peroxide activity to determine the antioxidant activity of leaf extract.

3) To assess the antibacterial effectiveness against gram-positive and gram-negative microorganisms.

3. Materials and methods

3.1 Collection and extraction of *S. auriculata*

Young and healthy leaves of *S. auriculata* were gathered from the herbal garden of Thiruvalluvar University, Vellore. An expert in botany from the Department of Botany at Ayya Nadar Janaki Ammal College in Sivakasi verified the plant materials identification. The leaves were carefully inspected, and any fungus-damaged, infected, or old leaves were removed. 2-3 times of thorough washing with distilled water were applied to the plant material. The cleaned leaves were blended into a fine powder using a sterile electronic blender and airtight storage for 15 days of shade drying. Figure 1 shows a thorough description of the research studies approach. In order to make the extract, 5 grams of dry powder sample was soaked in 50 mL of methanol. It was allowed to macerate for 24 hours at room temperature in a shaker. After that, Whatman no. 1 filter paper was used to filter the extract. The substance was eliminated by evaporating the methanol at room temperature in a fume hood. The yield of leaf extract in methanol was 16%. Extracts from the process were saved for subsequent examination.

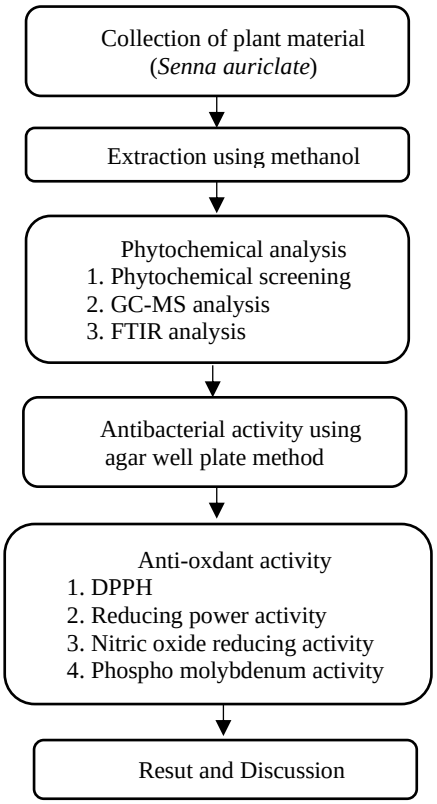

Figure 1 Research Conceptual Design

3.2 Phytochemical screening

To identify the presence of different phytoconstituents using the standard screening method, the methanolic leaf extract of *S. auriculata* was submitted to preliminary phytochemical screening (Harborne, 1998).

3.3 Antibacterial activity of the plant extract

The antibacterial activity of methanolic leaf extract of *S. auriculata* was determined by the agar well diffusion method. *Aeromonas hydrophila* (MTCC 1739), *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 10619), *Vibrio cholera* (MTCC 3906), *Proteus vulgaris* (MTCC 1771), *Klebsiella pneumoniae* (MTCC 3384), *Salmonella typhimurium* (ATCC 23564), *Corynebacterium glutamicum* (ATCC 21831), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 25619) are the bacterial strains used for this study. The bacterial strains were sub-cultured overnight at 37°C in Mueller-Hilton broth (HiMedia) medium. Aseptically prepared Mueller-Hilton agar medium was added to the sterile petri plates. Additionally, wells were created using a cork borer after the bacterial pathogens were swabbed into the agar plates. Methanol was employed as a control, and various extract concentrations (25, 50, 75, and 100 µg/mL) were poured into the corresponding wells. 48 hours were spent incubating the plates at 37°C. The zone of inhibition was measured in millimetres (mm) after the required incubation time (Win, & Min, 2018).

3.4 Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, reducing power test, nitric oxide scavenging assessment, phosphomolybdenum activity and hydrogen peroxide activity were used to analyze the antioxidant properties of the methanolic leaf extract of *S. auriculata*.

3.4.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

Antioxidant activity of methanolic leaf extracts was explored against 2, 2-diphenyl-1-picrylhydrazyl radical by ultraviolet spectrophotometer. The activity was carried out using Prieto, Pineda, and Aguilar (1999) methodology. In methanol, 0.1 mM solution of DPPH was prepared. 1 mL of the prepared solution was mixed with 1 mL of extract at various concentrations (100-1000 µg/mL). The combination was kept at room temperature for 30 min. At 517 nm, optical density was measured. Ascorbic acid was used as a reference. Higher activity is indicated by lower absorbance values. The percent antioxidant activity was calculated by the following formula:

$$\% \text{ inhibition} = \left(\frac{A_C - A_E}{A_C}\right) \times 100 \qquad (1)$$

Where, A_C is the optical density of the standard, and A_E is the optical density of the tested sample.

3.4.2 Reducing power assay

The method proposed by Srinivasan et al. (2011) was used to determine the results of the reducing power assay. Different extract strengths (100–1000 µg/mL) were combined with 1 mL of distilled water, 2.5 mL of potassium ferricyanide (1% w/v), and 2.5 mL of phosphate buffer (pH 6.6). The experiment was kept at 50°C for 20 min. After 20 min, 2.5 mL of 20% trichloroacetic acid was mixed, halting the reaction. The mixture was centrifuged at 3,000 rpm for 10 min. After combining 2.5 mL of the centrifuged material with 0.5 mL of ferric chloride (0.1%), the absorbance at 700 nm was measured. Increasing the reducing power was shown by the mixture's increased absorbance. Ascorbic acid was used as the standard. As a control, phosphate buffer (pH 6.6) was employed.

3.4.3 Nitric oxide scavenging activity

The preparation of 10 mm sodium nitroprusside in phosphate buffer (pH 7.4). 0.5 mL of sodium nitroprusside solution was combined with 1 ml of various leaf extract concentrations. 180 minwere spent incubating the reaction mixture at 25°C. 0.5 mL of the incubated solution was combined with 0.5 mL of the Griess reagent (1% sulphanilamide, 2% H_2PO_4, and 0.1% N-1-Naphthyl Ethylenediamine Dihydrochloride) after 180 min. As a control, the reagent without extract was employed. At 546 nm, the absorbance was measured. The standard is utilized as ascorbic acid. The percentage of inhibition was computed using the following formula (Patel, Patel, & Patel, 2010).

$$\% \text{ Scavenging activity} = \left(\frac{A_C - A_t}{A_C}\right) \times 100 \qquad (2)$$

Where, A_c is the optical density of the ascorbic acid, and A_t is the optical density of the tested sample.

3.4.4 Phosphomolybdenum assay

Using Prieto's approach, the phosphomolybdenum activity of methanolic leaf extract was measured. Each test tube held 3 mL of distilled water and 1 mL of Molybdate reagent solution, and different doses (100-1000 µg/mL) of methanolic leaf extract were added to each one separately. In order to make the molybdate reagent

solution, 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added to 20 mL of distilled water. The remaining 50 mL of distilled water was then added. 90 minwere spent incubating the tubes at 95°C. After incubation, the reaction mixture's absorbance was measured at 695 nm while the tubes were left at room temperature for 20-30 min (Synergy HT, Multimode microplate Reader, BIOTEK). The standard employed was ascorbic acid.

3.4.5 Hydrogen peroxide reducing assay

This was analyzed with a few minor alterations from Patel's 2010 description. Phosphate buffered saline was used to prepare 20 mM of hydrogen peroxide (pH 7.4). 1 mL of various extract strengths (100–1000 µg/mL) were made in methanol. 2 mL solution of hydrogen peroxide was combined with the produced methanolic extract. The sample was incubated for 10 min. At 230 nm, the optical density was examined. Both ascorbic acid and hydrogen peroxide solution without extract were employed as positive controls, respectively.

$$\% \text{ Scavenged } (H_2O_2) = (A_c - A_t / A_c) \times 100 \quad (3)$$

Where, A_c is the absorbance of the control, and A_t is the absorbance of the test.

3.5 Estimation of total phenolic content

The assay was followed by the method prescribed by Singleton, Orthofer, and Lamuela-Raventós (1999), who used the Folin-Ciocalteu reagent to quantify the phenolic concentration of the extract. 2.5 mL of 20% sodium carbonate and 0.5 mL of folin's reagent were added to 1 mL of the sample. The mixture was thoroughly shaken and then distilled water was used to dilute it to 10 mL. After allowing the reaction mixture to stand for two hours, the absorbance at 765 nm was measured. The standard curve was calibrated using gallic acid (0–250 µg/mL). Milligrams of gallic acid equivalent (mg GAE)/g of dehydrated mass of plant matter was used to measure the total phenolic content.

3.6 Estimation of total flavonoid content

The technique of Chang, Yang, Wen, and Chern (2002) was used to determine the total phenol content of the methanolic leaf extract. 0.5 mL of the sample was added to 1.5 mL of methanol. 0.1 mL of 1% aluminium chloride and 0.1 mL of potassium acetate (1M) were added. 2.8 mL of distilled water was added to the mixture to reach 5 mL. The mixture was vigorously shaken. At 415 nm, the absorbance measurement was made. Through the calibration curve, the total quantity of flavonoid was expressed in milligrams of rutin equivalents per gram (m RE/g).

3.7 FTIR analysis

FTIR analysis was used to identify the functional groups that were present in the leaf extract of *S. auriculata*. When new compounds are discovered in plants, spectroscopy can also be an effective tool for contributing to structural elucidation. The crude methanolic leaf extract was subjected to FTIR analysis, and thus, the extract was loaded in an FTIR spectroscope (FTIR-8400S, Shimadzu) with a scan range from 500 to 4000 cm^{-1}.

3.8 GC-MS analysis

The following settings were used for the GC-MS investigation of the methanolic fraction of *S. auriculata* using the GCMS-QP 2010 Plus Shimadzu system: Column Elite-1 fused silica capillary column (30 m x 0.25 mm ID x 1 mm of the capillary column, made entirely of dimethyl poly siloxane), operating at 60.0°C, 270.0°C for injection, 230.0°C for the ion source and 73.3 kPa for pressure. The oven temperature was programmed to start at 60°C (hold time for 2 min) and end at 280°C, hold for 18 min. Percentage by peak area was used to express the composition of crude extract.

The National Institute of Standards and Technology (NIST) database and the WILEY8 Library were used to identify and interpret phytocompounds on mass spectrum GC-MS. The unknown components' spectra were compared to the spectra of known compounds stored in the NIST library. Component names, molecular formulas and structures were discovered.

3.9 Data analysis

Means and standard errors of the mean for the zones of inhibition were calculated, and antioxidant activity was measured for the two sets of experiments in each case. SPSS statistical software was used to perform an Analysis of Variance (ANOVA) on the obtained data, using a completely randomized design, to determine the Least Significant Difference (LSD) at $P<0.05$. All

results were presented as means and standard deviations. For selected sample comparisons, the independent-sample t-test was used.

4. Result and discussion

4.1 Phytochemical screening of *S. auriculata*

The secondary metabolites of medicinal plants are responsible for most of their pharmacological activities. Secondary metabolites, such as alkaloids, phenols, essential oils, steroids, lignins, and tannins, have been successful sources of potential drugs (Cavazos, Gonzalez, Lanorio, & Ynalvez, 2021). Table 1 lists the phytochemicals found in the methanolic leaf extract of *S. auriculata*. Several other studies have found carbohydrates, protein, alkaloids, terpenoids, tannins, phlobatannins and phenolic flavonoids in *S. auriculata*. Murugan, Wins, and Murugan (2013) noticed carbohydrates, proteins, alkaloids, flavonoids, steroids, saponins and tannins in the methanolic leaf extract of *S. auriculata*. Medicinally active bioactive components like proteins, carbohydrates, terpenoids, phenols, flavonoids, and steroids were found in the phytochemical constituents of *C. auriculata* flowers in methanolic extract (Chaudhary, & Kumar, 2014). Rajkumar, Selvaraj, Suganya, Velmurugan, & Kumaresan (2013) examined the phytochemical analysis of *C. auriculata* ethanol flower extract. They revealed the presence of pentadecane, propenoic acid, dodecanoic acid and hexadecenoic acid, all of which have been previously reported for their biological activities, such as anti-diabetic, anti-viral and anti-cancer. Socrates, and Mohan (2019) revealed 16-Dehydropregnenolone acetate in *C. occidentalis* flowers for the first time, which is used in the preparation of anti-cancer agents. The high levels of alkaloids, flavonoids, phenols, tannins and saponins were found in the methanolic leaf fraction of *C. auriculata*. The presence of various bioactive compounds was revealed by GC-MS analysis, which confirmed the activity.

4.2 Antibacterial activity

The methanolic leaf extract of *S. auriculata* was found to have strong antibacterial activity against all of the bacteria tested, including *A. hydrophila, E. coli, B. subtilis, P. vulgaris, V. cholera, S. typhimurium, K. pneumonia, C. glutamium, S. aureus* and *P. aeruginosa*. Figure 2 depicts the antimicrobial effect of methanolic leaf extract of *S. auriculata*. The formation of a zone around the well was used to measure activity. The leaf extract was active against all organisms tested, with the strongest activity against *A. hydrophila*. The activity level was highly dose-dependent (Alex et al., 2020). The antibacterial activity of *S. auriculata* methanolic leaf extract may be attributed to phytoconstituents: saponins, flavonoids, alkaloids, terpenoids, tannins and phenols, as well as the solvent methanol's ability to extract such antimicrobial phytocompounds from the leaf (Nigussie, Davey, Legesse, Fekadu, & Makonnen, 2021). Methanol outperformed the other solvents tested in extracting effective antimicrobial substances from the medicinal plants *S. auriculata* and *A. indicum* (Devi, Santhi, Kannagi, & Shobana, 2014). The presence of phenolic constituents in *C. auriculata* extract may explain its antimicrobial properties (Bargah, Kushwaha, Tirkey, & Hariwanshi, 2020). According to Anushia, Sampathkumar, & Ramkumar (2009), the methanolic leaf extract was very effective against *S. aureus* and *E. coli* at a concentration of 64 mg/mL. *C. auriculata* showed greater activity against *E. coli* and *S. aureus*, according to Samy, and Ignachimuthu (2000). Flavonoids are hydroxylated phenolic substances that plants produce in response to microbial infection, and they have been shown in vitro to be potent antibacterial substances against a wide range of microorganisms. Their activity is most likely due to their ability to interact with extracellular and soluble proteins and target cell walls (Othman, Sleiman, & Abdel-Massih, 2019).

4.3 DPPH radical scavenging activity

One of the most effective methods for determining antioxidant activity is DPPH (1, 1-diphenyl-2-picrylhydrazyl) analysis. It is a strong oxidant that receives a positive charge from an antioxidant to transform into a stable magnetic material (Siddartha, 2020). The discoloration of the DPPH confirmed the extract's radical scavenging activity (violet to pale yellow color). The radical scavenging activity of DPPH could be attributed to its reducing actions, which could donate hydrogen to a free radical, converting it to a nonreactive species (Neupane, & Lamichhane, 2020). In the current study, the methanolic leaf extract of *S. auriculata* exhibits strong anti-radical activity at higher concentrations. Figure 3 depicts the outcome. The free radical scavenging capabilities of leaf extract were dose-dependently increased (100-1000 µg/mL). The presence of phenols and

flavonoids in *S. auriculata* leaf extract may explain its antioxidant activity. However, the activity of leaf extract was lower than the standard (Ascorbic acid). Methanolic and ethanolic extracts of *C. auriculata* have a considerable DPPH scavenging effect, which increases with concentration from 50 to 250 µg/mL, but the scavenging activity of all extracts is lower than that of standard (Veerachari, & Bopaiah, 2011). The high content of total flavonoids and total phenols in the methanolic extract of *C. auriculata* and *C. siamea* flowers may be attributed to their high antioxidant activity (Deepa Priyadarshini, & Sujatha, 2011) *S. auriculata* L flowers had greater polyphenol content than other plants. *S. auriculata* L flower water extract was found to reduce oxidative stress-mediated complications in streptozotocin-induced diabetic rats (Jeyashanthi, & Ashok, 2010).

4.4 Reducing power activity

The antioxidant agent causes the ferricyanide complex to be reduced to a ferrous ion form. Reducing power is linked to antioxidant capacity and may be a good predictor of antioxidant activity. Compounds with reducing power are electron givers that can diminish oxidized precursors in the oxidative damage process (Jyothi, Chavan, & Somashekaraiah, 2012). Depending on the compound's reducing power, the test solution's color shifts from yellow to different hues of green and blue. Dietary antioxidants like ascorbic acid are employed as a point of comparison. In this study, concentration increased the methanolic leaf extract's reducing ability. Figure 4 depicts the outcome of the methanolic leaf extract of *S. auriculata*. Similar findings were reported by Jyothi et al. (2012), who found that ascorbic acid and the floral extract of *C. auriculata* L. both had greater reducing potential at higher concentrations. The reducing power of the *C. auriculata* flower extract in methanol increases with concentration (Veerachari, & Bopaiah, 2011).

4.5 Nitric oxide scavenging activity

Small free radicals like nitric oxide (NO) play crucial signaling roles in physiological and pathophysiological processes like synaptic signaling, immune response and blood pressure regulation (Tejero, Shiva, & Gladwin, 2019). Numerous pathological conditions are brought on by the promotion of nitric oxide. The methanolic leaf extract of *S. auriculata* was found to have the ability to block the effects of NO in the current study. At a 1000 µg/mL concentration, it recorded the highest percentage of nitric oxide activity, 81.79%. Figure 5 shows the methanolic leaf extract's nitric oxide-scavenging capacity. A dose-dependent reduction in nitric oxide production was caused by the leaf extract. Mandal, Hazra, Sarkar, Biswas, and Mandal (2011) reported a comparable outcome. A dose-dependent increase in nitric oxide scavenging activity may be attributed to the tannin and flavonoid content. Bandawane, Juvekar, and Juvekar (2011) also reported a similar output. Nitric oxide scavenging effect in the methanolic extract of *C. auriculata* seeds improved in a dose-dependent manner, according to Gaurav Doshi, Shahare, Aggarwal, Pillai, and Desai (2011).

4.6 Phosphomolybdenum scavenging activity

The phosphomolybdenum activity of leaf extract increased with increasing concentration. At a concentration of 1000 µg/mL, the greatest activity was noted. Figure 6 displays the *S. auriculata* methanolic leaf extract's phosphomolybdenum activity. According to Pamulaparthi, Prathap, Banala, and Nanna (2016), the phosphomolybdenum activity of the methanolic leaf extract of *S. alata* reaches 60% at high concentrations. Because phenols are thought to be a good antioxidant agent, plant's phosphomolybdenum activity may be caused by their total phenol and flavonoid content, as well as their antioxidant capacity, which takes a crucial part in the absorption and pacification of free radicals (Chang et al., 2002). According to Albayrak, Aksoy, Sagdic, and Albayrak. (2012), the methanolic leaf extract of *C. angustifolia* contained 170.05±0.2 mg AAE/g. The higher polyphenol content of the extract may be the cause of the extract's higher antioxidant potential.

4.7 Hydrogen peroxide activity

Hydrogen peroxide is a potent oxidizer. It can cross cellular membranes, be produced in vivo by various oxidizing enzymes, including superoxide dismutase, and may slowly oxidize several intercellular compounds (Radha et al., 2013). In this study, *S. auriculata* leaf extract in methanol exhibits potent antioxidant activity at higher concentrations (Figure 7). As the concentration increased, the activity increased as well. The extract concentration of 1000 µg/mL was found to have the highest activity. The phenolics in

extracts may be responsible for scavenging H_2O_2 by donating electrons to it and neutralizing it into water (Veerachari, & Bopaiah, 2011). Our results are consistent with those of other researchers who studied various Caesalpiniaceae plants. According to Vijay et al. (2017), *C. occidentalis* methanolic seed extracts had a hydrogen peroxide activity of 48.7±4% at 40 µg/mL concentration. Total phenol, flavonoid content and antioxidant activity of the plant extract are positively correlated. The aerial portion of *C. javanica* had a hydrogen peroxide activity of 49.73±0.29, according to Mehta, Parmar, Vadia, Patel, and Tripathi (2017). Kolar, Gogi, Khudavand, Choudhari, and Patil (2018) also demonstrated comparable outcomes.

4.8 Total phenolic content

According to the quantitative phytochemical estimation, flavonoids and phenolics are present in significant amounts in the methanolic leaf extract. Leaf extract had a phenolic content of 108.13 mg GAE/g (Figure 8). Polyphenols and other reducing substances are sensitive to the Folin-Ciocalteu reagent (Bibi Sadeer, Montesano, Albrizio, Zengin, & Mahomoodally, 2020). There is a linear relationship between total phenol content and plant antioxidant activity as phenols react with phosphomolybdic acid in the Folin-Ciocalteu reagent in an alkaline medium to produce a blue color complex that can be quantified spectroscopically (Aryal et al., 2019). The regression equation of the calibration curve ($y=0.0258x+0.0352$; $R^2=0.9979$) measures the phenol content of the extract as an mg gallic acid equivalent curve. The primary cause of phenolic antioxidant activity is redox potential, which enables it to function as singlet oxygen quenchers and hydrogen donors. The leaf of *S. alata* was extracted using a variety of organic solvents, including methanol, chloroform, aqueous, benzene and diethyl ether. Methanol extract had the highest amount of phenolic content (52.3±0.03) (Murugan et al., 2013). The polarity of the solvent used for extraction is the cause of the difference in phenolic acid concentration in the various solvents (Rafińska et al., 2019). Because of their polarity, many secondary metabolite compounds from plants can dissolve in methanol. According to Choudary et al., (2014), the stem of *C. fistula* has a significantly higher phenol content than the leaf, at a level of 78.58±1.74 compared to 07.72±0.13. Phenolic content affects the DPPH radical scavenging activity, and the antioxidant potential of plant tissue is influenced by the kinds of antioxidant compounds that are found there. Total phenolic content and free radical scavenging capacity are positively correlated. Due to their antioxidative capacity, the phenolic compound plays a significant role in stabilizing lipid peroxidation and directs antioxidative activity (Jin, & Mumper, 2010). According to the report of Veerachari, & Bopaiah (2011), *C. auriculata* had the highest range of phenol content among the 12 species of *Cassia*, ranging from 61.77 to 174.34 mg GAE/g.

4.9 Total flavonoid content

An mg rutin equivalent curve is used to express the extract's flavonoid content (Neupane, & Lamichhane, 2020). The regression equation of the calibration curve shown in Figure 9 ($y=0.0012x+0.0185$, $R^2=0.9986$) calculates the entire flavonoid content of the leaf extract. *S. auriculata* leaf extract in methanol contained 61.25 mg RE/g of flavonoids. The polarity of the solvents used in preparing the plant extracts affects the concentration of flavonoids in those extracts (Truong et al., 2019). The methanolic extract demonstrates greater flavonoid compound extraction power. According to Pamulaparthi et al. (2016), the methanolic leaf extract of *S. alata* contains a significant amount of flavonoids. The extraction process and solute used for extraction affect the total flavonoid content in plants (Anushia et al., 2009). According to Poojitha (2017), the whole plant of *C. angustifolia* was extracted using a variety of solvents, including methanol, water, hexane and acetone. The polar solvent extract of the plant has a considerable quantity of flavonoids, while the nonpolar solvent extract has a much smaller amount. The findings of Choudary et al., (2014), Hazra, Devgan, Ramaiah, and Sarkar (2016) and Hamad, Darwish, Abu-Serie, and El Sohaimy (2017) are in agreement with our findings.

4.10 FTIR analysis

Over the past ten years, Fourier transform infrared (FTIR) spectroscopy has undergone significant development, promising simpler, quicker and more accurate diagnoses (Fadlelmoula, Pinho, Carvalho, Catarino, & Minas, 2022). The results of FTIR studies have shown that the methanolic leaf extract of *S. auriculata* contains a number of different chemical components (Figure 10). Alkyl halides were assigned the peak at 465.78

cm^{-1}, while the peak at 3438.84 cm^{-1} was assigned to O-H stretching of alcohol compounds, the peak at 2925.81 cm^{-1} was assigned to C-H stretching of alkane compounds, the peak at 1615.27 cm^{-1} was assigned to aromatic compounds, the peak at 1025.10 cm^{-1} was assigned to alcohols, esters, and carboxylic acids, and the peak at 1454.23 cm^{-1} was assigned to amines. Alkane C-H stretching is represented by the peak at 1376.12 cm^{-1}, and amide N-H stretching is represented by the peaks at 1514.98 cm^{-1} and 1615.27 cm^{-1}. The methanolic flower extract of *C. auriculata* exhibits the functional group of C=O, C=N and S=N stretch, according to Soundharajan, and Ponnusamy (2014). According to Ashokkumar, and Ramaswamy (2010), the methanolic leaf extract of *S. auriculata* contained phytoconstituents that could serve as a source of antibiotics, including C-O, OH, C=C, and C-H stretching. Lacking free OH groups, flavonoids exhibit increased antimicrobial activity (Nadia, & Rachid, 2013).

4.11 GC-MS *analysis*

Using GC-MS analysis, 31 bioactive compounds from the methanolic extract of *S. auriculata* leaf exhibiting various phytochemicals were discovered. Figures 11, 12 and Table 2 show the retention time, peak area, biological activities and percentage peak area of various bioactive compounds. Resorcinol (3.31), 1,2-Benzenedicarboxylic acid, ester (0.06), Mome Inositol (79.63), Hexadecanoic acid, Methyl ester (0.04), n-Hexadecanoic acid (0.46), Phytol (0.30), Methyl stearate (0.03), Octadecanoic acid (0.04), 1,2-Benzenedicarboxylic acid (0.18), Benzamide, 2-Amino-N-(4-methoxyphenyl) (0.18), Solanesol (0.11), Squalene (0.54), Vitamin E (1.07), Stigmasterol (0.32) and gamma.-Sitosterol (0.60) are the major compounds present in the extract. Figure 11 provides the structures of the main bioactive compounds. All of the identified bioactive substances are found to have antimicrobial, antioxidant, anticancer and anti-inflammatory properties.

Table 1 Phytochemical screening of methanolic leaf extract of *S. auriculata*

Test	Reagents/ chemicals used	Inference	Result
Carbohydrate	Benedict's reagent	Bluish-green color	+
Protein	Millon's reagent	White precipitate turns red	+
Alkaloids	Dragendroff's reagent	Turbid orange color	+
Saponin	Distilled water	Foam formation	+
Flavonoids	Sodium hydroxide	Golden yellow color	-
Terpenoids	Chloroform Con. Sulphuric acid	Reddish-brown color formation	+
Tannins	Ferric chloride	Dark green color	+
Phlobatannins	1% Aqueous HCl	Red precipitation	+
Phenolic flavonoids	10% lead acetate	Brown precipitate	+

* + indicates Present, - indicates absent

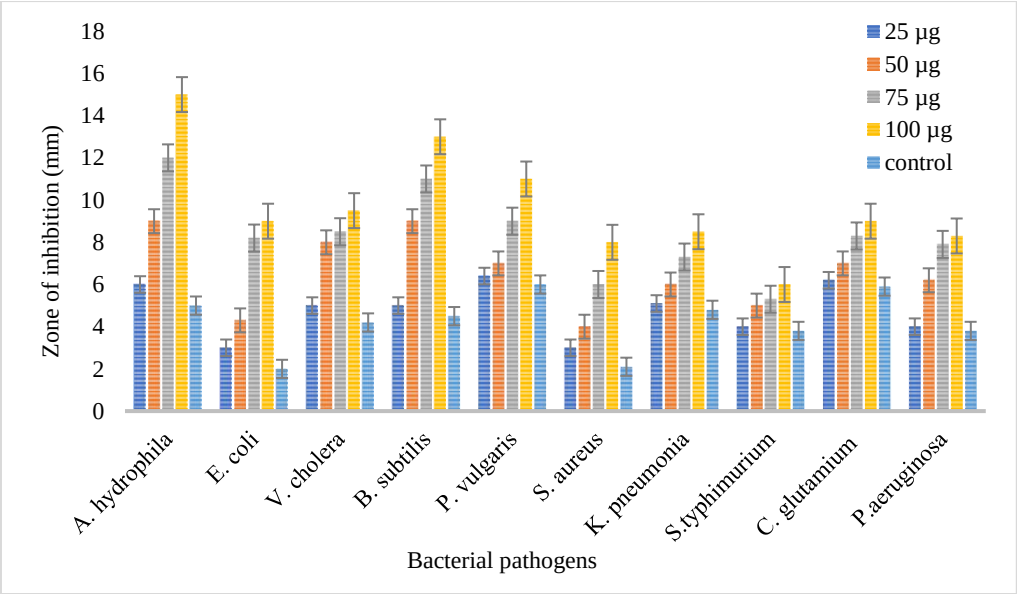

Figure 2 Antibacterial activity of methanolic leaf extract of *S. auriculata*

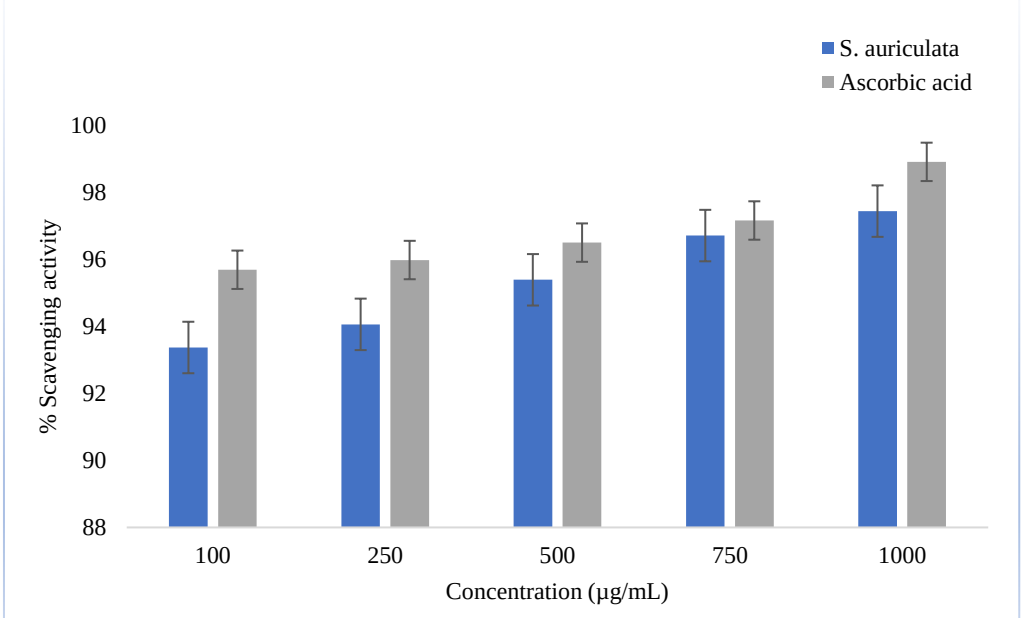

Figure 3 DPPH radical scavenging activity of methanolic leaf extract of *S. auriculata*

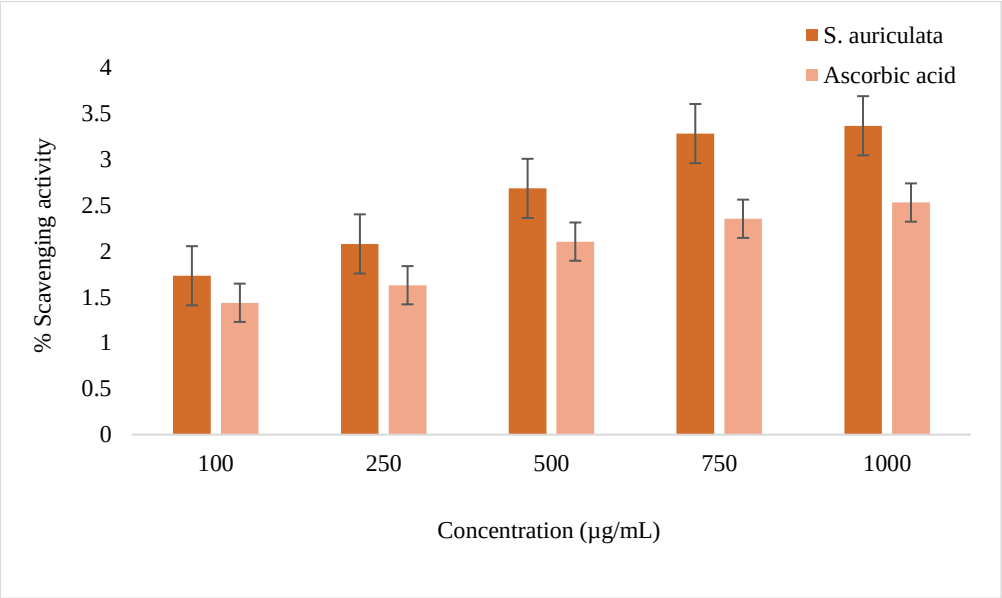

Figure 4 Reducing power activity of methanolic leaf extract of *S. auriculata*

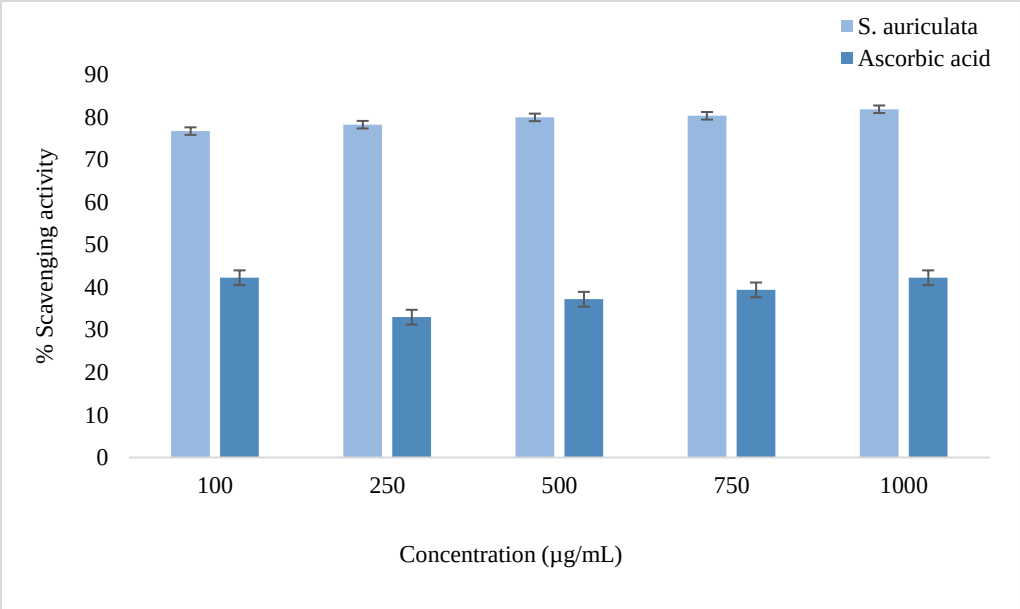

Figure 5 Nitric oxide reduces the power activity of methanolic leaf extract of *S. auriculata*

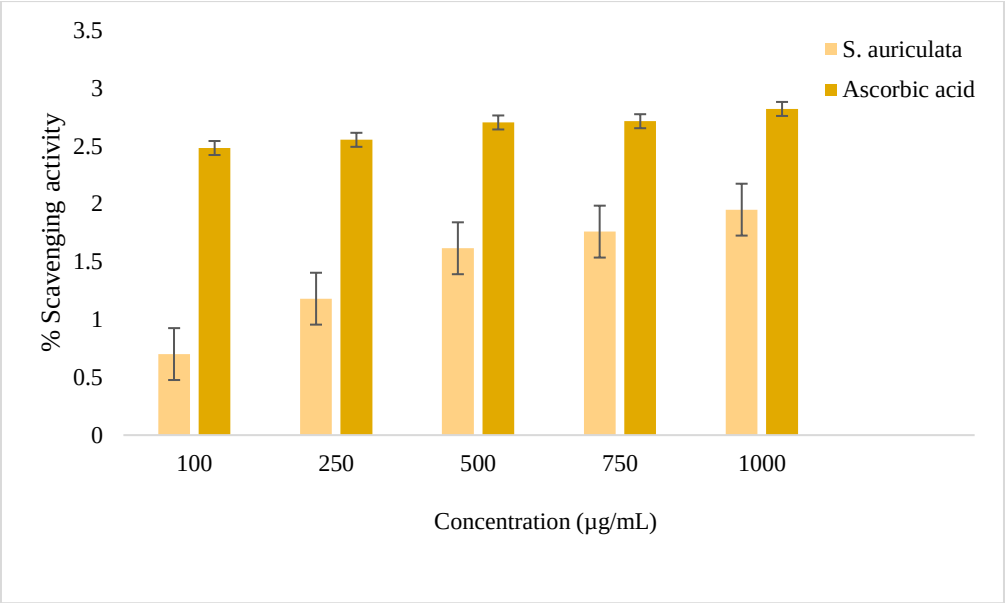

Figure 6 Phosphomolybdenum activity of methanolic leaf extract of *S. auriculata*

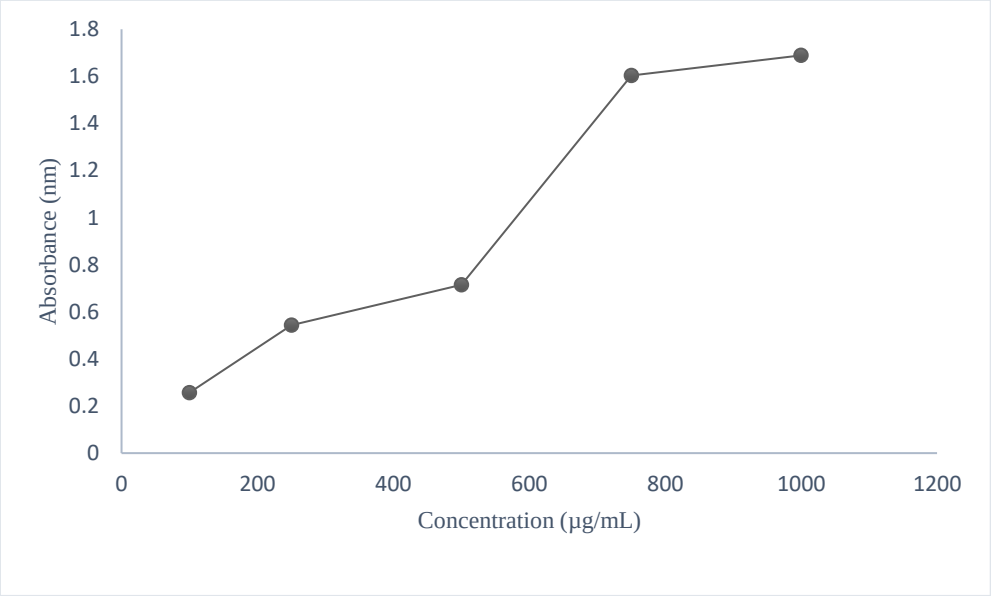

Figure 7 Hydrogen peroxide activity of methanolic leaf extract of *S. auriculata*

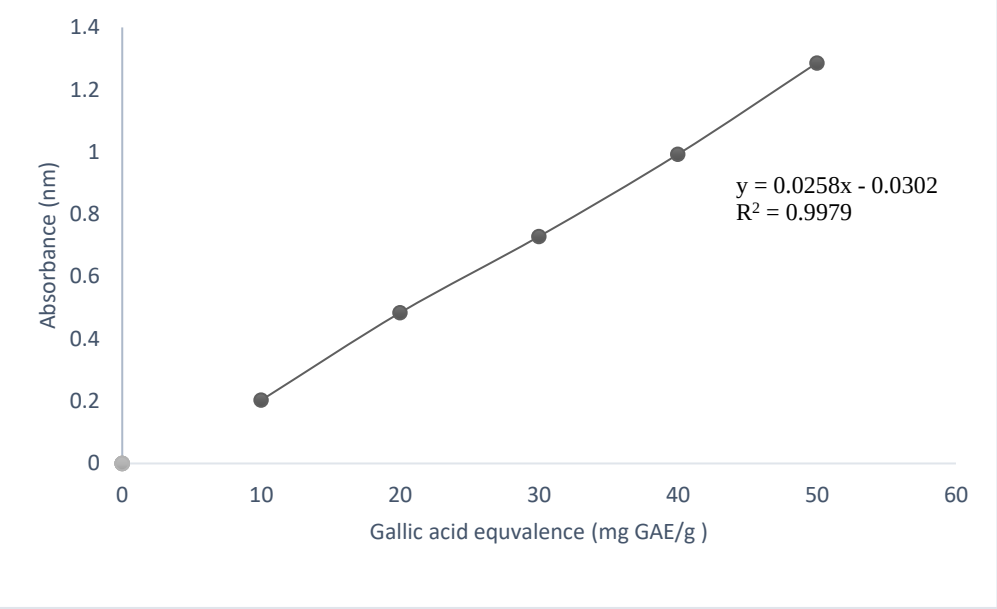

Figure 8 Total phenolic content of methanolic leaf extract of *S. auriculata*

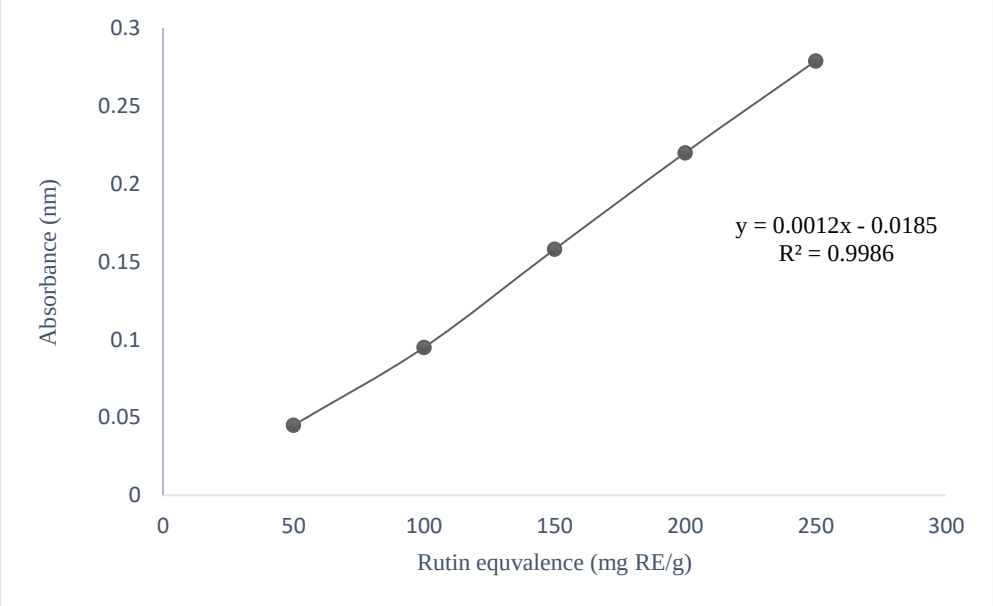

Figure 9 Total flavonoid content of methanolic leaf extract of *S. auriculata*

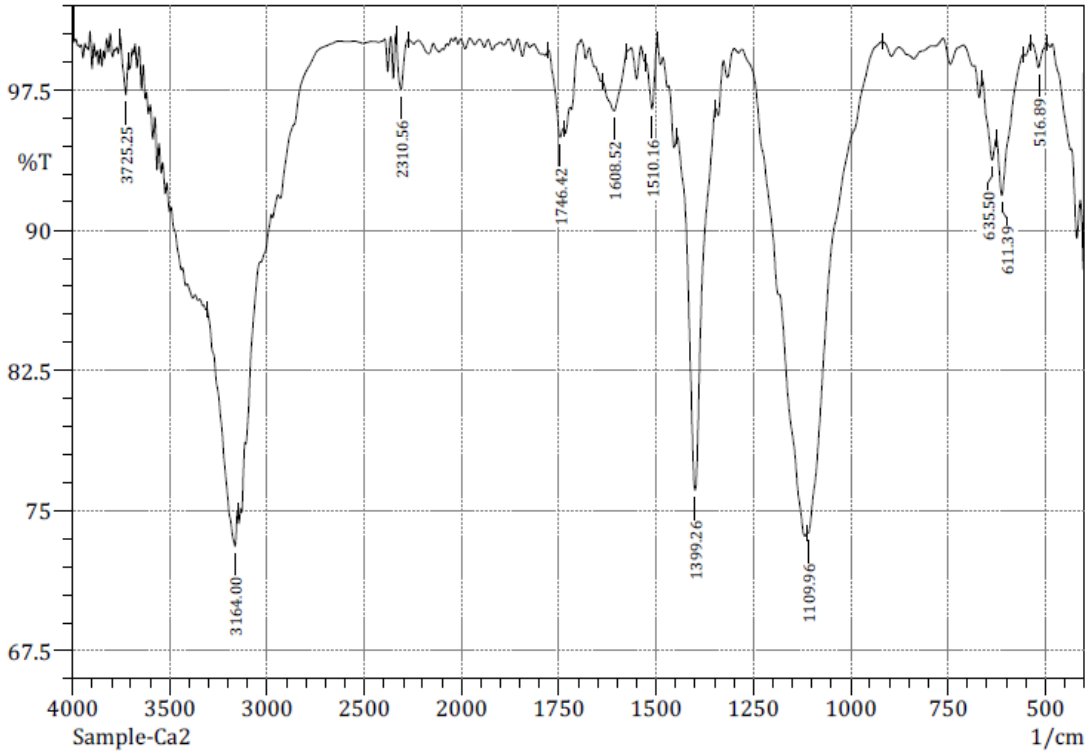

Figure 10 FTIR analysis of methanolic leaf extract of *S. auriculata*

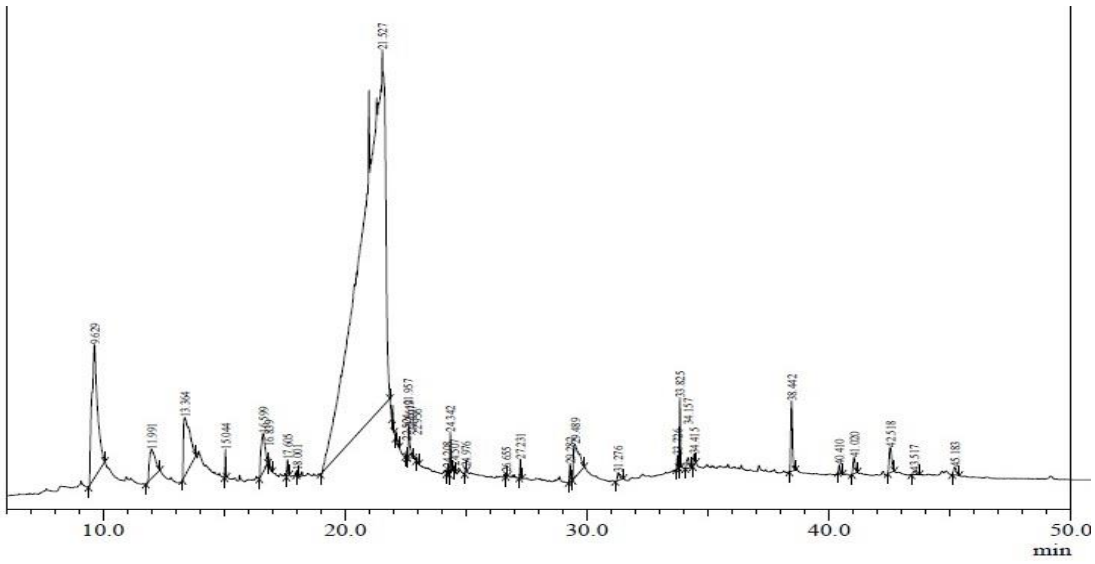

Figure 11 GC-MS analysis of methanolic leaf extract of *S. auriculata*

Table 2 Biological activity of the compounds present in GC-MS analysis of the leaf extract *S. auriculata*

No	Retention Time	Name of the compound	Nature of the compound	Biological activity
1	13.364	Resorcinol	Benzenediol	Antimicrobial activity and Antioxidant activity
2	17.605	1,2-Benzenedicarboxylic acid, diethyl ester	Diethyl Phthalate	Antimicrobial activity, alpha-Glucosidase inhibition and the in vitro hypoglycemic effect
3	21.527	MOME INOSITOL	Inositol	Antialopecic, Anticirrhotic, Antineuropathic, Cholestrolytic, Lipotropic and Sweetener
4	22.107	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	Enzyme inhibitor, Antihyperlipidemic, Nematicide, Herbicide, Antimutagenic and 5-Alpha reductase inhibitor
5	22.619	n-Hexadecanoic acid	Palmitic acid	Oxidizing agent, cardioprotective, herbicides, emollients, antiandrogenic, flavoring, cytotoxicity, 5-alpha reductase inhibitor, antipsychotic and effective mosquito larvicide
6	24.342	Phytol	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and antimalarial
7	24.507	Methyl stearate	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and Anti proliferative activity
8	24.976	Octadecanoic acid	Stearic acid	Antimicrobial activity
9	29.282	1,2-Benzenedicarboxylic acid	phthalic acid	Antimicrobial and Antifouling activity
10	29.489	Benzamide, 2-Amino-N-(4-methoxyphenyl)	Benzamide	Antimicrobial, antifungal
11	33.726	SOLANESOL	Terpene alcohol	Antibacterial, Antiviral, Antifungal, Anticancer, Anti-inflammatory and Antiulcer activities
12	33.825	Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide Diuretic and Neutralizes different xenobiotics
13	38.442	Vitamin E	Vitamin compound	Anticarcinogenic, Neuroprotective, Hypoglycaemic, Muscle relaxer, Anti-inflammatory, Immunomodulator, Hepatoprotective; Hypocholesterolemic and Hypoglycemic
14	41.020	Stigmasterol	Steroid	Antioxidant, hypoglycemic, and thyroid inhibiting properties, the precursor of progesterone, antimicrobial, anticancer, anti-arthritic, anti-asthma, anti-inflammatory, diuretic activity and Anti HIV-reverse transcriptase
15	42.518	gamma.-Sitosterol	Sterol	Anti-diabetic, Anti-angiogenic, Anticancer, Antimicrobial, Anti-inflammatory, Antidiarrhoeal and Antiviral

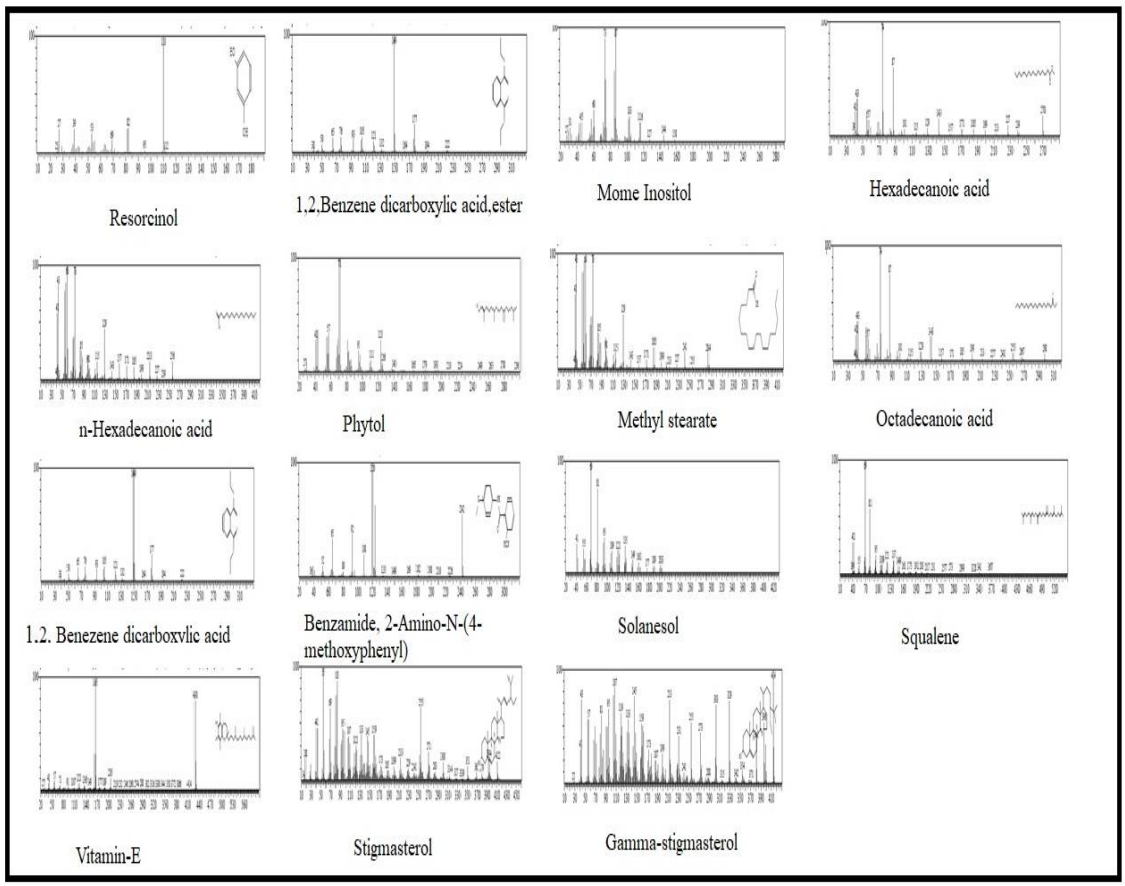

Figure 12 Mass spectra of identified compounds from methanolic leaf extract of *S. auriculata*

5. Conclusion

The current study found that S. auriculata leaf extract has antimicrobial activity against various human pathogens, implying that it could be beneficial for treating infectious diseases associated with microorganisms. Moreover, S. auriculata leaf extract is discovered to be high in total phenolic and total flavonoids. Similarly, the extracts demonstrated significant antioxidant activity in various tests, including DPPH, H_2O_2, phosphomolybdenum, reducing power activity and nitric oxide scavenging activity. Overall, the findings support the plant's antimicrobial, antioxidant and anti-inflammatory properties. As a result, the plant's positive qualities in terms of conventional biomedical application have been confirmed. Plant-based medicines are beneficial to human health and serve as a source of novel therapeutic compounds. According to the findings of the study, this plant has tremendous potential for use in the field of pharmacy and is a prospective source of valuable drugs. Numerous bioactive substances found in plants are crucial to our well-being and are also used to enhance our society. However, additional research, including in vitro and in vivo studies, is required to demonstrate their antimicrobial and antioxidant properties identify and isolate the bioactive components, and explicate their pharmacological properties. As a result, additional fractionation and purification of the compound will reveal which component is responsible for the reactions.

Declarations

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Conflicts of interest: Authors declare that there is no conflict of interest in this research.

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