

# Phytochemical Characterization and Assessment of *Hygrophila auriculata* (Buch. -Ham) Leaves for Antioxidant and Antimicrobial Activity

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Ekambaram *et al.*: Characterization and Pharmaceutical Assessment of *Hygrophila auriculata* Leaves

*Hygrophila auriculata* is a thorny sub-shrub of the family Acanthaceae. It is a potent medicinal plant in the Indian systems of medicine with various therapeutic uses, from simple management of infections to treatment of diseases. The present study was conducted to investigate the phytochemical and pharmacological properties of the leaves of *Hygrophila auriculata*. Various phytochemicals were screened using a standard procedure, and the characterization was analyzed with spectral studies. The pharmacological investigation includes antioxidant and antimicrobial activities of the extract. Antioxidant activity was carried out using 2, 2-diphenylpicrylhydrazyl free radical scavenging assay and reducing power assay. Antimicrobial activity was done by the agar well diffusion method. The phytochemical screening detected the presence of alkaloids, flavonoids, terpenoids, tannins, carbohydrates and saponins. The 2,2-diphenylpicrylhydrazyl antioxidant assay reveals that the extract had a considerable scavenging capacity. It was found that the reducing power of different concentrations of the leaf extract was remarkable. Antimicrobial tests showed that the leaf extract was active against selected Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and few fungal species (*Aspergillus* sp.). These findings disclosed that the *Hygrophila auriculata* leaves have various phytotherapeutic activities and the compounds identified using the gas chromatography were responsible for the above actions. Thus, it would be helpful to isolate the compounds responsible for the bioactivities in the future for the treatment of various ailments.

**Key words:** *Hygrophila auriculata*, phytochemicals, antioxidant, antimicrobial

Millennia-old Traditional Medicines (TMs) offer empirical practices, including medication with natural compounds and focusing on overall wellness. The acceptance of TM by the scientific community is limited by the lack of ground-breaking scientific evidence of its benefits and efficiency, coupled with the ignorance of its inherent medical basis. Nearly 80 % of the world's population relies on TMs for primary health care, most involving plant extracts. This has resulted in using many medicinal plants with curative properties to treat various diseases<sup>[1]</sup>.

Indian medicinal plants have contributed much to academic curiosity, as apparent from the number of publications. Yet, the main reason for the scientific abandonment is unclear evidence for their mechanism of action. But at times, it has

been recommended that drug discovery should not permanently be restricted to a single molecule and the existing statement 'one disease-one drug' method might be indefensible. The rationally synthesized polyherbal formulations could also be evaluated as an alternative in multi-target therapeutics and prevention<sup>[2]</sup>.

The plants prevent the consequences of the oxidative stress-derived compounds with the assistance of essential substances known as antioxidants. The role of medicinal plants in disease prevention

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has been attributed to the antioxidant properties of their constituents. Therefore, the isolation of natural antioxidants mainly of plant origin has increased immensely during the last decade. The modes of action for curing diseases vary from plant to plant. The mixture form may exhibit enhanced activity than individual plants, known as synergistic action<sup>[3]</sup>.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing<sup>[4]</sup>. Antimicrobial compounds from potential plants should be explored to alleviate this problem. The side effects of these drugs are scanty, less toxic and cost-effective. They effectively treat infectious diseases while mitigating many side effects of synthetic antimicrobials<sup>[5]</sup>. Hence, in the present study, the same efforts are continued in searching for novel therapeutics.

*Hygrophila auriculata* (*H. auriculata*) (Buch.-Ham) is a thorny sub-shrub of the family Acanthaceae that grows widely throughout India, Sri Lanka, Myanmar, Indo-China, Tropical Africa and Malaya. It grows naturally in moist places on the banks of ditches and paddy fields. In Ayurvedic literature, it was described as Ikshura, Ikshugandha, Kokilaksha, or Indian Cuckoo, and in the Ayurvedic system of medicine as Seethaveryam and Mathuravipaka. Leaves of this shrub are whorled, linear-lanceolate, with undulating margins. Traditionally, the leaves were used as/in diuretic, jaundice, antibacterial, dropsy, rheumatism, diseases of the urinogenital tract, aphrodisiac, hypnotic, diarrhea, dysentery, urinary calculi, urinary discharge, anti-inflammatory, joint pain, eye diseases, anemia, anuria, cough, stomachic, lumbago, arthritis, gastric disorder and leucorrhoea<sup>[6]</sup>.

With the above context, the present investigation aimed to evaluate *H. auriculata* for its potential use as a source of phytochemicals, antioxidant compounds and other biological agents, including those which may show antimicrobial activities.

## MATERIALS AND METHODS

### Plant collection and preparation of extract:

The fresh plant of *H. auriculata* was collected from Kuttanad wetlands, Alappuzha (Dist.), Kerala. The plant material was identified, and its authenticity was

confirmed at the herbarium of Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (BSI/SRC/5/23/2015/Tech-1422).

The fresh leaves were cleaned and dried for 5-7 d in the shade at room temperature. The dried samples were then powdered using an electric grinder and stored in screw cap bottles until further analysis. Ten grams of powder from the dried sample of *H. auriculata* leaves were taken, to which 100 ml of different solvents (chloroform, ethyl acetate, methanol, and water) were added, mixed and kept for 4 d. They were intermittently shaken with an electrical shaker and further concentrated by evaporation.

### Qualitative phytochemical analysis:

As the extractive index and the intensity of phytochemicals were shown to be high in the methanol extract of *H. auriculata*, the methanol extract was subjected to various spectral analyses to identify the chemical nature of the active components present in the plant.

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### Ultra Violet (UV)-visible Spectral analysis:

UV-Visible spectrum profile of the sample was detected with a Shimadzu UV-1700 spectrophotometer at a wavelength ranging from 200-800 nm (fig. 1).

### Fourier Transform Infrared (FT-IR) spectral analysis:

The powdered sample was loaded in an FTIR spectroscope (Shimadzu, IR AFFINITY-1, Japan), with a scan range from 800-4000  $\text{cm}^{-1}$ . The peaks obtained were plotted as percentage transmittance on the X-axis and wavenumber ( $\text{cm}^{-1}$ ) on the Y-axis (fig. 2).

### X-Ray diffraction analysis:

The X-ray diffraction pattern of the powder samples was recorded on a PANalytical X'PERT PRO X-ray diffractometer using Cu K-alpha radiation ( $\lambda=1.54060 \text{ \AA}$ ). The particle characterization was

done by measuring the crystallite size of the sample from the line broadening analyses using the Debye-Scherrer formula after accounting for instrumental broadening (fig. 3).

### Gas Chromatography–Mass Spectrometry (GC-MS) analysis:

Chromatographic analysis was conducted using thermo GC-Trace Ultra Ver: 5.0 GC-MS (Model Thermo MS DSQ II gas chromatograph). The GC

temperature program was as follows; the initial temperature was 75°, held for 2 min, increased to 150° at a rate of 2°/min, then to 220° at a rate of 3°/min, and finally to 260° at a rate of 6°/min and held for 10 min. The split ratio was 1:12, the injection temperature was 250°, the transfer line temperature was 270° and the mass spectrometer was operated at 70 eV in a run time of 29 min. The individual components were identified by computerized matching of their mass spectra of peaks with those

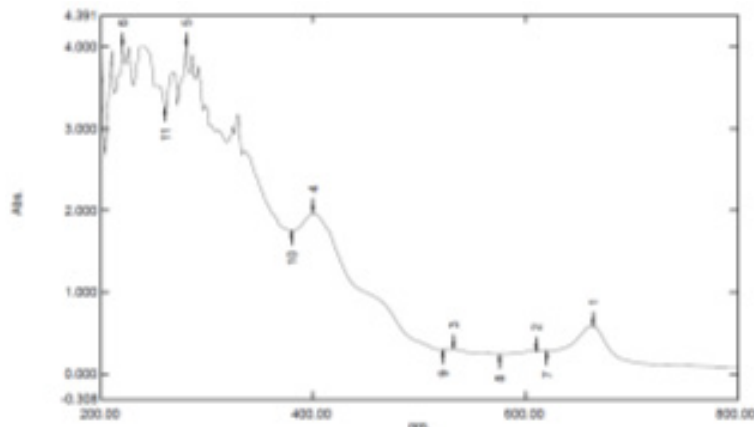


Fig. 1: UV-Vis Spectra of MEHA

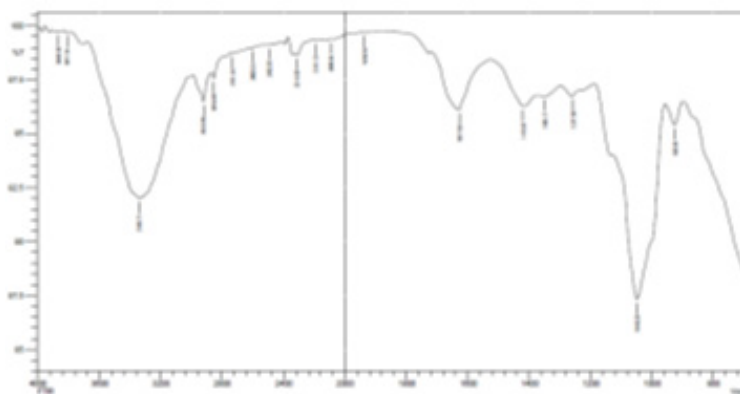


Fig. 2: FTIR Spectra of MEHA

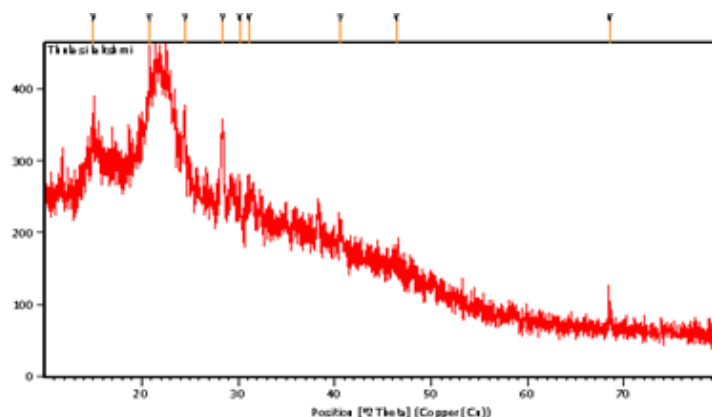


Fig. 3: XRD Spectra of MEHA

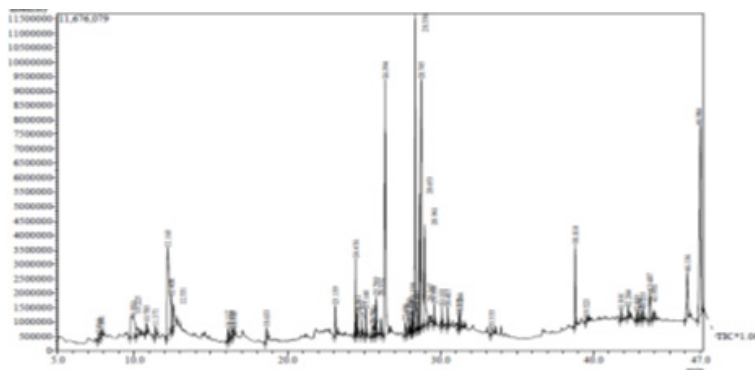


Fig. 4: Mass spectra of *H. auriculata* leaves

gathered in the NIST 08 and WILEY 8-Mass Spectral library of the GC-MS data software system (fig. 4).

#### Antioxidant studies:

**2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) assay<sup>[8]</sup>:** The diluted working solutions of the test extracts were prepared in methanol. About 3 ml of graded concentration (10-50 µg/ml) of extracts were taken in different test tubes. 1 ml of 0.3 mM DPPH methanol solution was added to these test tubes and shaken vigorously. Methanol served as the blank, and DPPH in methanol, without the leaf extracts, was the negative control, whereas ascorbic acid was a positive control. After 30 min incubation of samples at 25° in the dark, the absorption was measured at 517 nm. The change in absorbance of each sample was determined and the following equation calculated the scavenging activity.

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$$

Where  $\text{Abs}_{\text{control}}$  is the absorbance of DPPH radical with methanol and  $\text{Abs}_{\text{sample}}$  is the absorbance of DPPH radical with sample extract.

**Reducing power assay<sup>[9]</sup>:** Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (1 %), and varying concentrations of extracts (10-50 µg/ml). After the reaction, mixtures were incubated at 50° in the water bath for 30 min, cooled at room temperature (28°), and 2.5 ml of 10 % Trichloroacetic Acid (TCA) were added to each reaction mixture and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube, and 2.5 ml of distilled water was added. This was further added with 0.5 ml Ferric chloride ( $\text{FeCl}_3$ ) (1.0 %) and allowed to react for 10 min at room temperature.

Ascorbic acid served as the control. The absorbance was measured at 700 nm.

**Antimicrobial activity:** The antimicrobial sensitivity assay was carried out against six bacterial strains (*Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Klebsiella* sp., *Salmonella typhi* (*S. typhi*) and *Proteus* sp.) and four fungal strains *Aspergillus fumigatus* (*A. fumigatus*), *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*) and *Trichoderma* sp. by the agar well diffusion method by Barry *et al.*<sup>[10]</sup>. Petri plates containing 20 ml Muller-Hinton medium were swabbed with the 24 h bacterial culture and similarly, the fungal cultures to be tested were evenly spread over Rose Bengal chloramphenicol agar plates. The wells were bored into the plate. Each well was filled with different doses of methanolic extract of *H. auriculata* (10, 15, 20, 25 and 30 µg/ml) and the last well with standard antibiotics (Chloramphenicol for bacteria and nystatin for fungi). The bacterial plates were subjected to incubation at 37° for 24 to 48 h and the fungal plates were kept at room temperature for 3-5 d to assess the fungal activity. After incubation, the results were observed and the zones of inhibition thus developed were measured with the scale to the nearest in mm. The experiment was done in triplicates and the mean values were presented.

## RESULTS AND DISCUSSION

Therapeutic properties of the herbs are closely related to their chemical components, which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins and tannins, where the effectiveness of those as herbal remedies depends upon their solubility in various solvents<sup>[11]</sup>. The present study was designed to document the pharmacological properties of *H. auriculata* in association with its phytochemicals.

In the present investigation, among the four extracts analyzed, the methanolic extract showed the maximum concentration of phytochemicals such as alkaloids, flavonoids, terpenoids, phenols, tannins, carbohydrates, cardiac glycosides and saponins as represented in Table 1. This attributes in utilizing the Methanol Extract of *H. auriculata* (MEHA) for further analysis. Alkaloids were absent in the aqueous extract but present in all the other three extracts. The absence of alkaloids in the aqueous extract might be due to the relative insolubility of alkaloids in water compared to organic solvents<sup>[12]</sup>. These results corroborate with Rastogi *et al.*<sup>[13]</sup> who reported high intensity of phytochemicals in the MEHA. Further, many previous reports reveal the presence of specific isolated compounds such as flavonoids, alkaloids, triterpenes, sterols, and other compounds that belong to minerals, aliphatic esters, amino acids, fatty acids, and essential oils<sup>[14]</sup>. Also, the results reveal the occurrence of cardiac glycosides which might prevent the pump of Na<sup>+</sup>/K<sup>+</sup>, consequently raising the availability of calcium ions for the heart muscles contraction, reducing congestive heart failure, cardiac arrhythmia and cardiac arrhythmia and distension of the heart<sup>[15]</sup>.

Researchers are bound to discover the curative properties of medicinal plants and authenticate them to be nontoxic using analytical tools and experiments. This is an essential quality criterion for reporting efficacy and safety. This type of authentication focuses on identifying and recording the profiles of the secondary metabolic fingerprints by chromatography and spectroscopy which provides valuable information about the qualitative

and quantitative pattern of the composition of plant compounds<sup>[16]</sup>. In the present study, the UV-Visible spectrum profile of MEHA was chosen at a 200-800 nm wavelength. The profile showed the peaks at 664, 610, 532, 400, 280 and 220 nm with absorption of 0.585, 0.280, 0.302, 1.967 and 4.00 respectively. MEHA showed significant and minor peaks indicating the presence of wide range of phytochemicals. UV-Vis spectra had absorption utmost in the region of 200-400 nm. Generally, the flavonoid spectra comprise two absorption bands maxima in 230-285 nm and 300-350 nm<sup>[17]</sup>. Hence the band occurring at 280 nm typically represents flavonoid groups. The FTIR spectra produce the sample profile, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. The FT-IR spectrum revealed mainly hydroxyl groups, implying Alcohols, Alkanes, Amines and Carboxylic acid compounds in MEHA (Table 2). It is discovered through the literature survey that the FTIR spectra for the MEHA have not been reported previously. The bands identified in this research are similar to those found by Mohammed *et al.*<sup>[18]</sup>, who studied the Methanolic Extract of Selected Medicinal Plant of *Anethum graveolens* and *Plantago major*. Additionally, the results of the present study corroborate with the findings of Bharathi *et al.*<sup>[19]</sup>, who reported the presence of functional groups such as amines and Carboxylic acid through FTIR characterization of aqueous extract of *H. auriculata*. X-ray powder Diffraction (XRD) technique utilizes

**TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS OF *H. auriculata***

S. no.	Constituents	Solvents			
		Chloroform	Ethyl acetate	Methanol	Aqueous
1	Alkaloids	+	+	+	-
2	Flavonoids	+	+	+	+
3	Sterols	-	-	-	+
4	Phenols	-	-	+	-
5	Saponins	-	-	+	-
6	Tannins	+	+	+	-
7	Quinones	-	-	-	-
8	Proteins	+	+	-	+
9	Carbohydrates	-	-	+	-
10	Cardiac glycosides	-	+	+	+
11	Terpenoids	-	-	+	-

**TABLE 2: PEAK VALUES AND FUNCTIONAL GROUPS PRESENT IN MEHA**

Peak value	Functional group
3340.71	Alcohols
2924.09	Alkane
2854.65	Alkane
1627.92	Amine
1049.28	Amine
925.83	Carboxylic acid

the X-ray scattering phenomenon to determine the nature of the materials as crystalline or amorphous. Powder diffraction data of the present study showed nine prominent diffraction peaks at 20 positions of 14.9, 20.8, 24.51, 28.38, 30.16, 31.12, 40.60, 46.42 and 68.48.

The Braggs reflections indicated the presence of sets of lattice planes and further. The presence of broad peaks in the spectrum of extract suggests the predominance of amorphous nature. The broadening and noise of the observed peak were probably indicating the presence of macromolecules present in the plant powder. This information can be used to establish parameters for developing of Phytotherapeutics products, helping to ensure their quality, safety and efficacy.

Mass spectrometric examination plays a vital role in the phytochemical analysis and chemotaxonomic investigation of medicinal plants containing biologically active components. Many researchers have applied GC-MS analysis to identify the possible bioactive components present in the plant extracts and herbal preparations, which might be helpful for the identification of lead compounds for the development of new pharmaceutical drugs. In the present study the results of the GC-MS analysis exhibited the presence of twenty pharmacologically important compounds from MEHA with nine major peaks (Table 3). Among all, hexadecanoic acid, octadecanoic acid and phytol were previously reported to have antimicrobial activities in the plant *Carduus pycnocephalus* L.<sup>[20]</sup>. Also, these compounds have been shown to possess many aspects of ideal chemo preventive agents, such as availability, low cost, oral bioavailability, and low toxicity, making it feasible to consider them for chemoprevention testing. Ravisankar *et al.*<sup>[21]</sup> reported compounds such as 2-Furancarboxaldehyde, 2,6,10-Trimethyl,14-Ethylene-, Tetradecanoic acid in *H. auriculata*, which was similar to the present study. The compound phytol was found to

have antimicrobial, antioxidative and antidiabetic properties<sup>[22]</sup>. Also, n-Hexadecanoic acid possesses antioxidant, antimicrobial and anti-inflammatory properties<sup>[23]</sup>. Likewise, stigma sterol has some reports regarding its anticancer efficacies<sup>[24]</sup>.

The major groups of the phytochemicals obtained from the plant showed antioxidant activities and were known to prevent degenerative diseases. There is a growing interest in natural antioxidants that might attenuate oxidative stress and paves the way for safe therapeutics<sup>[25]</sup>. Medicinal plants are believed to be a potential source of reactive oxygen species scavenger molecules<sup>[26]</sup>. Antioxidants might work either alone or in association with each other against different types of free radicals.

DPPH is a stable free radical, which has been widely used in phytomedicine to assess the scavenging activities of bioactive compounds. The DPPH scavenging activity was shown in the Table 4. MEHA showed a significant free radical scavenging capacity. The inhibition percentage of DPPH radical increases with an increase in concentration. The antioxidant capacity ranged from 43 % to 70 %, which was relatable to the standard. The antioxidant effect is proportional to the disappearance of the purple color of DPPH in test samples. Antioxidant molecules present in the extracts can quench DPPH free radicals by providing hydrogen atoms or electrons and then forming a colorless stable molecule<sup>[27]</sup>. Similar significant results were reported by Anusha *et al.*<sup>[28]</sup> and Raaman *et al.*<sup>[29]</sup> for various concentrations using the MEHA. The reducing power activity of the MEHA of different concentrations was remarkable. As the concentration gradually increased, the absorbance of each concentration was found to rise. Maximum absorbance in 50 µg/ml, was shown in Table 5. In the reducing power assay, the yellow color of the test solution changed to multiple shades of green and blue depending on the reducing power of MEHA and showed excellent

reducing power ability. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with reductions, which break the free radical chain by donating a hydrogen atom<sup>[30]</sup>. These effects of the extract could be attributed to the presence of bioactive compounds in MEHA. Several synthetic antibiotics and drugs are employed to treat microbial infections and infectious diseases, but microbial pathogens develop resistance to synthetic antibiotics. The increasing incidence of antibiotic resistance and its side effects on organ systems functioning necessitate finding substitutes for antibiotics<sup>[31]</sup>. In the present study, the same efforts are continued in searching for novel therapeutics against antibiotic activity. The methanol leaf extract of *H. auriculata* had promising activity against selected Gram-positive bacteria such as *B. subtilis*, *S. aureus*, *E. coli*, *Klebsiella* sp., *S. typhi* and *Proteus* sp. (Table 6). The zone of inhibition increased with increased concentration of the extracts in the well. This showed the concentration-dependent activity. The highest concentration showed maximum inhibition activity against species such as *B. subtilis*

(18.0±1.0), *S. aureus* (17.66±1.52), moderate activity against *E. coli* (17.3±0.57), *Klebsiella* (17.0±1.52), but mild activity against *S. typhi*. (16.6±0.57) and *Proteus* sp. (15.8±1.0) when compared with the standard antibiotic. The results reveal variability in the inhibitory nature of the extract against specific bacteria. The inhibition of bacterial growth was dose-dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains, as evidenced by the higher zone of inhibitions at higher concentrations of MEHA.

In the antifungal activity, the MEHA was also more active against *Aspergillus* species than *Trichoderma*. The MEHA significantly inhibited the growth of all the *Aspergillus* species (*A. fumigatus*, *A. niger* and *A. flavus*) and *Trichoderma* species in a concentration-dependent manner (Table 7). The maximum inhibition was exhibited against the *A. fumigatus* (23.8±2.3) followed by *A. niger* (22.2±2.3) and minimum inhibition was found against *A. flavus* (21±1.0) followed by *Trichoderma* sp. (18.8±2.2). The bioactive compounds present in *H. auriculata*

**TABLE 3: PHYTOCOMPONENTS IDENTIFIED IN THE LEAVES OF *H. auriculata* BY GC-MS**

S. no	RT	Compound name	Molecular formula	Area %
1	28.74	9,12,15-Octadecatrienoic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	14.27
2	46.98	Stigma sterol	C <sub>29</sub> H <sub>48</sub> O	12.92
3	26.39	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	11.48
4	12.16	2-Furancarboxaldehyde	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	9.03
5	28.35	Phytol	C <sub>20</sub> H <sub>40</sub> O	8.60
6	28.65	9,12-Octadecadienoic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	4.99
7	28.96	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	4.43
8	9.92	Cyclopentanone	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub>	3.47
9	48.13	Ergost-5-En-3-01, (3. Beta.,24R)	C <sub>28</sub> H <sub>48</sub> O	2.91
10	38.81	2,6,10,14,18,22-Tetracosahexaene	C <sub>30</sub> H <sub>50</sub> O	2.34
11	12.53	Nonionic acid	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	1.70
12	24.45	2,6,10-Trimethyl,14-Ethylene-	C <sub>20</sub> H <sub>38</sub>	1.67
13	43.68	Cholesterol	C <sub>27</sub> H <sub>46</sub> O	1.40
14	10.22	1,2,6-Trimethyl-hexane	C <sub>9</sub> H <sub>20</sub> O <sub>3</sub>	1.05
15	28.48	Octadecanoic acid, Methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	1.03
16	23.13	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	1.00
17	25.79	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	0.96
18	28.19	9,12,15-Octadecatrienoic acid	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	0.80
19	25.14	3,7,11,15-Tetramethyl-2-hexadecen	C <sub>20</sub> H <sub>40</sub> O	0.61
20	25.55	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	0.60

**TABLE 4: DPPH RADICAL SCAVENGING ACTIVITY OF *H. auriculata***

Drug	Concentration ( $\mu\text{g/ml}$ )	Inhibition (%)
Sample	10	43 $\pm$ 2.22
	20	56 $\pm$ 1.43
	30	60 $\pm$ 1.22
	40	65 $\pm$ 2.34
	50	70 $\pm$ 1.77
Ascorbic acid	10	48 $\pm$ 1.21
	20	53 $\pm$ 1.86
	30	60 $\pm$ 1.43
	40	64 $\pm$ 1.25
	50	73 $\pm$ 2.72

Note: Values are mean $\pm$ Standard Deviation (SD) of four replicates

**TABLE 5: REDUCING POWER ACTIVITY OF *H. auriculata***

Concentration ( $\mu\text{g/ml}$ )	Absorbance at 517 nm	
	Standard	Sample
10	0.32 $\pm$ 0.01	0.29 $\pm$ 0.005
20	0.38 $\pm$ 0.04	0.31 $\pm$ 0.15
30	0.41 $\pm$ 0.02	0.32 $\pm$ 0.017
40	0.46 $\pm$ 0.01	0.38 $\pm$ 0.005
50	0.48 $\pm$ 0.03	0.39 $\pm$ 0.01

Note: Values are mean $\pm$ SD of four replicates

**TABLE 6: DIAMETER OF ZONES OF INHIBITION (MM) OF MEHA AGAINST MICROORGANISMS**

Test organisms	Zone of inhibition in mm					Control*
	10 ( $\mu\text{l}$ )	15 ( $\mu\text{l}$ )	20 ( $\mu\text{l}$ )	25 ( $\mu\text{l}$ )	30 ( $\mu\text{l}$ )	
<i>B. subtilis</i>	13.0 $\pm$ 0.57	14.3 $\pm$ 1.15	15.3 $\pm$ 0.57	16.3 $\pm$ 1.0	18.0 $\pm$ 1.0	21.6 $\pm$ 1.15
<i>S. aureus</i>	12 $\pm$ 1.0	13.6 $\pm$ 0.57	14.6 $\pm$ 1.52	15.0 $\pm$ 1.73	17.66 $\pm$ 1.52	20.0 $\pm$ 1.0
<i>E. coli</i>	13.3 $\pm$ 1.52	14.0 $\pm$ 1.0	15.7 $\pm$ 1.0	16.5 $\pm$ 1.73	17.3 $\pm$ 0.57	20.3 $\pm$ 1.52
<i>Klebsiella</i> sp.	13.0 $\pm$ 2	14.0 $\pm$ 1.0	15.0 $\pm$ 1.0	16.3 $\pm$ 1.0	17.0 $\pm$ 1.52	21.0 $\pm$ 1.73
<i>S. typhi</i>	11.0 $\pm$ 1.0	12.33 $\pm$ 0.57	13.3 $\pm$ 1.52	14.0 $\pm$ 5	16.6 $\pm$ 0.57	21.3 $\pm$ 2.30
<i>Proteus</i> sp.	11.30 $\pm$ 1.52	12.0 $\pm$ 1.0	13.3 $\pm$ 1.15	14.6 $\pm$ 0.57	15.8 $\pm$ 1.0	20.6 $\pm$ 2.08

**TABLE 7: ANTIFUNGAL ACTIVITY OF *H. auriculata***

16.3 $\pm$ 1.0	Zone of inhibition in diameter (mm)					Control (Nystatin)
	10 ( $\mu\text{g}$ )	15 ( $\mu\text{g}$ )	20 ( $\mu\text{g}$ )	25 ( $\mu\text{g}$ )	30 ( $\mu\text{g}$ )	
<i>A. fumigatus</i>	17 $\pm$ 1.0	18.5 $\pm$ 1.0	19 $\pm$ 1.0	21 $\pm$ 1.0	23.8 $\pm$ 2.5	27.6 $\pm$ 3.2
<i>A. niger</i>	16.6 $\pm$ 1.5	18 $\pm$ 1.0	19 $\pm$ 3.3	21.3 $\pm$ 1.2	22.2 $\pm$ 2.3	29.3 $\pm$ 1.5
<i>A. flavus</i>	16 $\pm$ 1.0	17.5 $\pm$ 1.5	19.6 $\pm$ 1.5	20.5 $\pm$ 1.0	21 $\pm$ 1.0	26.3 $\pm$ 1.5
<i>Trichoderma</i> sp.	14 $\pm$ 1.0	15 $\pm$ 1.0	16 $\pm$ 1.0	17 $\pm$ 2.5	18.8 $\pm$ 2.2	24.3 $\pm$ 2.4

Note: Values are mean $\pm$ SD of triplicates



showed the highest promising activity profile against *B. subtilis*, *S. aureus*, *E. coli*, and *Aspergillus* sp. MEHA has established a moderate inhibition zone over standard antibiotics, chloramphenicol, and nystatin.

Previous reports on the antibacterial activities of *H. auriculata* showed selective inhibition against Gram-positive bacteria. Usually, Gram-positive bacterial strains are more susceptible to plant extracts than Gram-negative bacteria. This is mainly attributed to the difference in cell wall composition and thickness. The peptidoglycan layer in Gram-positive bacteria makes them more sensitive to plant extracts, which are permeable to the extracts<sup>[32]</sup>. The presence of phytochemicals such as flavonoids, alkaloids, tannins and phenols might have interrupted the bacterial cell wall and complexed with the extracellular proteins, thus inhibiting growth. Tannins precipitate the microbial proteins that lack healthy proteins for microbial growth<sup>[33]</sup>. Likewise, fungus development might have been suppressed due to phenols in the extract. The phenols swell the hyphae, causing plasma seeping and leakage, distortion, abnormal branching or fusion, and wrinkling<sup>[34]</sup>. The present work reveals various phytochemical constituents in the plant extract that contribute to the antioxidant and antimicrobial properties, which provides scientific validation for its use in folk medicine. Other progressive research activities on the separation of the compounds from the plant extract can be explored.

To summarize, the methanol leaf extract of *H. auriculata* exhibited high antioxidant activities and selective inhibition against Gram-positive bacteria and few fungal strains. These findings suggest the leaf extract as a potential source for the isolating bioactive compounds for future therapeutics. Also, it is worthwhile to test the extract using human cell lines.

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#### Conflict of interests:

The authors declared no conflict of interests.

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