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Antioxidant and Antimicrobial Activities Along with Physico-Chemical Studies of Medicinal Plant *HYGROPHILA AURICULATA*

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Abstract: India has a significant opportunity to contribute to the growing global demand for medicinal and aromatic plant products. Plants have been used for long as medicine by humans, seeing back to prehistoric eras. Among the ancient civilization's, India have been known to be rich renowned as a rich source of medicinal herbs. Ayurveda, Siddha, Unani and Folk lore (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in world wide. Green plants have been synthesized and preserved a variety of secondary metabolites and phytochemical products. The medicinal plant *Hygrophila auriculata* belonging to Acanthaceae family and generally presents in aquatic area. It is a rare and endemic species all over the countries; the plant has been used to treat various diseases and disorders. Extracts and bioactive compounds from the plant have been found to possess antimicrobial, anthelmintic, antitermite, nephroprotective, hepatoprotective, central nervous system protective, antitumour, antidiabetic, anticataract, antioxidant, haematopoietic, diuretic, antinociceptive, anti-inflammatory, antipyretic, antimotility, aphrodisiac, neuroprotection, anti-endotoxin and anti-urolithiatic activities. In this study focused the preliminary tests are antibacterial activity for MIC, antioxidant scavenging activity using DPPH, FRAP, ABTS and Hydrogen peroxide activities, and qualitative and quantitative analysis phytochemicals and physical parameter evaluation for study plant.

Keywords: Aromatic plant, *Hygrophila auriculata*, Acanthaceae, Phytochemicals, Antioxidant, Diseases and Disorders

1. Introduction

On a global scale, the role of traditional medicines in resolving health problems is invaluable. In both modern and traditional medicine, medicinal plants continue to provide valuable therapeutic agents [1]**.** Acanthaceae is a normally utilized wild herb found on the riverbanks, irrigation channels, and agricultural land throughout India [2]. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Oxidative stress, described as an imbalance between oxidants and antioxidants, causes a variety of biochemical changes and is a major contributor to a number of chronic diseases in humans [3], including atherosclerosis and cardiovascular disease, mutagenesis and cancer, several neurodegenerative disorders, and the ageing process [4]. The plant *Hygrophila auriculata* a perennial angiosperm (K. Schum) Heine, (Acanthaceae) medicinal plant traditionally used to inflammation, ache, urinary infection, edema, gout, diuretic, Stomachache, headache, diabetic, cancer activities and its phytocompounds are regarded as the most important entity to treat various human maladies [5-7]. The plant *Hygrophila auriculata* labeled in Ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha or Indian Cuckoo. *Hygrophila auriculata* has been shown to possess hypoglycemic activity in human subjects [8], hepatoprotective activity against paracetamol and thioacetamide intoxification in rats [9] and CCl4-induced liver dysfunctions [10], antitumor [11], anabolic and adrogenic activities [12, 13]. The aim of the study is to determine the Qualitative analysis of phytochemical compound, antioxidant, antimicrobial and minimum inhibitory concentration activity.

Taxonomy of Hygrophila auriculata
Kingdom: Plantae
Division: Angiospermae
Order: Personales

Family: Acanthaceae
Genus: Hygrophila
Species: *Spinosa*
Vernacular names: Gokulakanta, Talimkhana, Nirmulli.

2. Experimental Details

2.1 Collection of plant leaves

The five selected medicinal plants for the present study were collected from the foot hills of Vallimalai Murugan Temple, Vellore District. The plants from the collection sites were immediately kept in perforated polythene bags to prevent the drying of fresh leaves by evaporation then brought to the laboratory, therefore the leaves alone handpicked from the stems and branches and washed in running tap water to remove debris, dust and insects adhere on the leaves. The dried plants were taken to mechanical grinder for made into fine powder. This fine powder was stored in a well cleaned dried plastic bottle. The taxonomic identification of the fresh plant was done and the voucher herbarium specimens were deposited in the Post Graduate & Research Department of Botany & Microbiology, Institute of herbal science, Plant anatomy research Centre, Chennai, Tamil Nadu. The identification specimen number is (PARC\2021\4574).

2.2 Extractive value estimation

The extractive value determination is one of the significant techniques to predict the amount of active phyto-constituents from plant and to confirm the suitable solvent for maximum extraction of phytocompounds. 5g of the air dried leaf powder were taken in a 50ml conical flask along with 100ml of different solvents (acetone, chloroform, distilled water, ethanol, ethyl acetate, hexane and methanol) were added. The top of the conical flask was tightly closed to use sterile cotton and aluminium foil. The mixture was shaking frequently, after the 24hrs the extract was filtered using Whatman No-1filter paper. Finally, the filtrate was transferred into pre–weighed petri plate and the solvent leave it to complete evaporate in atmospheric air temperature. After the complete removal of moisture content the petri plate was again weighed with dried extract.

2.3 Physical Parameters Determination:

Total ash:

The known weight of plant was incinerated at the temperature possible to remove all of the carbon by resulting of changes of carbonates to oxides. 2gm of leaf powder was precisely weighed and taken in a silica crucible and kept in a furnace. The temperature was set to up to 650°C until the plant materials free from carbon and leaf ash containing crucible was cooled and weighed.

$$\text{Percentage of total ash} = \frac{\text{Weight of dried leaf powder}}{\text{Weight of ash}} \text{ X100}$$

Water soluble ash estimation

The total amount of ash which was got from boiled with 25ml of water and plant sample at 5min. Thereafter, they were filtered through filter paper, the insoluble materials adhered on the paper again incinerated in a furnace at a temperature not exceeding 450°C and finally the ash weight of insoluble ash was weighed accurately.

In soluble ash= Weight of total ash- weight of insoluble ash

Acid-insoluble ash:

25ml of 2N HCl for 5min soaked and filtered. The insoluble residues present in filter paper was washed with boiled water, ignited, cooled in a desiccator and weighed. The proportion of acid-insoluble ash with reference to the dried drug was evaluated.

Determination of Swelling Index

1gm of plant material was soaked in 25ml water. This was shaken thoroughly in every 10 min ant stand for 3hrs at room temperature. Finally, the volume measured in ml occupied by plant material and calculated the mean value of individual determination, related to 1gm of crude plant material.

Foaming Index Determination

1gm of leaf powder was accurately weighed and soaked into 100ml of water, boiled for 30min, cooled and filtered and made the volume with water. The decoction obtained from the leaf was poured into 10 stoppered test tubes in serially from 1 to 10ml. The content of all test tubes were uniformly made up to 10ml with water and shaken them in lengthwise motion for 15sec, it stand for 15min and measured the height of the foam. The results were evaluated as follows: If the height of the foam in every tube was less than 1cm the foaming index was considered less than 100. If the height of the foam was higher than that of 1cm in every test tube, the foaming index was over than 1000. This experiment was repeated several times by using a new series of dilutions of decoction in order to obtain the result.

2.4 Qualitative Phytochemical Screening

To assess the phytochemicals present a qualitative phytochemical screening was performed on methanolic leaf extract of *Hygrophila auriculata* using a conventional technique [14]. The extracts (5mg) were dissolved in 50cc of the appropriate extraction solvents. Alkaloids (Mayer's reagent), flavonoid (NaOH test), tannin and phenol ($FeCl_3$), saponin (Foam test),

terpenoid and steroid (Salkwski method), cardiac glycosides (fehling test) and triterpenes (H_2So_4) were all identified in the solution.

2.5 Quantitative Phytochemical Screening

Alkaloid quantification:

1ml of leaf extract, 5ml of pH 4.7 phosphate buffer, and 5ml of BCG solution were added to a 25ml volumetric flask and the mixture was thoroughly mixed with 4ml of chloroform. The resulting volume was then diluted with chloroform to modify the volume. Following that, the complex's absorbance in chloroform was measured at 470nm in comparison to a blank made as described above but without the leaf extract. The test was compared to Atropine equivalents using Atropine as a reference material.

Flavonoid quantification:

1ml of leaf extract and 4ml of water were taken in a 25ml volumetric flask. After being left as is for 5min, 0.3ml (5%) of sodium nitrite and 0.3 ml (10%) of aluminium chloride were added. 2ml of 1M Sodium hydroxide was added and the mixture had been incubated for 6mins at room temperature. With distilled water, the volumetric flask's ultimate volume was increased to 10 ml. At 510 nm, the reaction mixture's absorbance was measured against a blank. The standard utilized was catchins. The amount of flavonoids was represented as mg of catechin per g of dried extract.

Saponins quantification:

1ml of leaf extract, 2ml of Vanillin in ethanol and the 2ml of 72% sulphuric acid solution was added and the solution was heated in a water bath for 20min at 600c. Finally, the absorbance was taken at 544nm against blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

Steroids quantification:

1ml of leaf extract, 2ml of 4N Sulphuric acid (2ml), and 2ml of (0.5% w/v) iron (III) chloride were added to a 25ml volumetric flask. Along with, 0.5ml 0.5%(w/v) potassium hexacyanoferrate (III) solution was added. This combination was temperature for 30 minutes in a water bath at 70 to 80°C. The final volume was increased to 10ml and measured at 780nm against a blank reagent.

Phenolic Compounds quantification:

Different concentrations of leaf extracts were taken in separate test tubes. In each test tube, 0.4ml FCR (diluted 1:10 v/v) was mixed equally. After a few minutes, 4ml of sodium carbonate solution was mixed, and the final amount was increased to 10ml by adding distilled water. This mixture was rest for 90 minutes at room temperature. The absorbance of the mixture was measured at 750 nm against a blank. The total phenolic content of the extract was reported in terms of mg/gram of dry weight and the standard graph using catechol solutions as the reference.

FTIR and GCMS study

The Fourier Transform Infrared Spectrophotometer (FTIR) is the most effective tool for determining the types of chemical bonds and functional groups found in plant substances. The wavelength of light absorbed defines the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. For FTIR analysis, dried powders of various solvent extracts of each plant material were utilised. To make translucent sample discs, 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet. Each plant specimen's powdered sample was placed into an FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan) with a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm-1. The methanol leaf extract of the study plant was taken to Vellore Institute of Technology for GCMS analysis. Gas chromatography-mass spectroscopy (GC-MS) is a hyphenated analytical technique mostly used to separate the components of a mixture and characterise each of the components individually. The GCMS (Thermo Scientific Co,) T(GC-17A Ver.3), GCMS-QP5050A) instrument both qualitatively and quantitatively evaluates the phytocompounds present in the leaf extract (Fig. 3).

DPPH Radical Scavenging Estimation

DPPH radical scavenging activity was done by the method followed by Sharma and Bhat, (2009) [15]. Take six test tubes and add 1ml, 2ml, 3ml, 4ml, 5ml and 6ml of plant extract, along with each test tube 1ml of 200µmol/L DPPH solution added and agitated well. The test tubes are incubated at 30°C for 30min and the mixture was measured at 517 by a spectrophotometer (Uvi Line 9100-94000UV/Vis). Instead of plant extract, the control test tube has 3 ml of methanol and 1 ml of 200 mol/L DPPH. As an alternative to plant extract, a test tube containing a solution of common antioxidants such ascorbic acid, Trolox, and BHT is referred to as a positive control.

ABTS Radical Scavenging Estimation

The method was estimated according to the method of Re, *et al*., (1999). 2.45mm of potassium persulfate and ABTS solution were made and mixed in a ratio of 1:0.5. This solution was to stand at 30°C in the dark for 12–16 hours. The solution was diluted with ethanol until (0.70 ± 0.02) absorbance at 734nm. Plant extract was taken respectively 1ml, 2ml, 3ml, 4ml, 5ml and 6ml in separate test tubes and added 3ml of ABTS solution. The absorption of this mixture in each test tube was measured at 734nm. The antioxidant activity is stated in terms of the Trolox equivalent capacity (gTE/mgE), and a Trolox calibration curve was created over the range of 5-400 g/ml.

FRAP Scavenging Estimation

The FRAP scavenging activities were estimated and followed by the method Benzie and strain method (1996) [16]. 2.5ml of TPTZ solution (10 mM), 2.5ml of $FeCl_3$ solution (20 mM), and 25ml of sodium acetate (300mM at pH = 3.6) buffer solution are mixed to make FRAP solution. 3ml of FRAP solution was taken and mixed with different concentrations of plant extract taken in separate test tubes such as 2ml, 3ml, 4ml, 5ml and 6ml. The reaction mixture's absorbance was measured at 595nm. A Trolox calibration curve in the range of 5-50 g/ml was created, and the antioxidant activity is given in Trolox equivalent capacity (g TE/mgE).

Hydrogen Peroxide Scavenging Assay

The assay was estimated by method described by Ruch *et al*., (1989) [17]. In phosphate buffer saline (PBS), a solution of 40mM hydrogen peroxide was made (50mM, pH 7.4). 0.6ml of this H_2O_2 was taken and uniformly put in a test tube containing various proportions of plant extract, such as 1ml, 2ml, 3ml, 4ml, 5ml, and 6ml. The mixture's absorbance was measured at 230nm. A blank solution containing plant extracts and PBS without hydrogen peroxide absorbed similarly.

Antibacterial Activity

The antibacterial activities of *Hygrophila auriculata* against the bacterial species *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were determined using the agar disk diffusion method [18, 19]. The disks made from Whatman no. 1 were immersed in the leaf extract and allowed to absorb the plant extract. Simultaneously, 25 grams of Mueller-Hinton agar dissolved in 100ml of distilled water and covered with sterile cotton, aluminum foil. The medium was sterilized in heating a water bath at 115C° for 15 minutes. The microbes preserved in the slanting test tube culture containing MH agar were transferred to agar plate. The plates were allowed to dry. After completing the inoculation of bacterial species on agar plate, the leaf extract implemented sterile disc transferred using flamed but cooled forceps onto the surface of the seeded agar plates. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The extractive solvent (water) was used as a negative control. The plates with the organisms were incubated for 24 h. After incubation, the growth inhibition rings were quantified by measuring the diameter for the zone of inhibition to the nearest millimetre from the lower surface of the Petri dishes. As the diameter of the disk was 6 mm, inhibition zones of less than 7 mm were not evaluated [20]. Negative control disks contained the solvent. Standard antibiotic (ampicillin) was used as positive control for comparison.

Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentrations were determined by dilution method [21]. For this, Muller Hinton broth was used to grow the bacterial strains *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* up to the exponential phase with an A560 of 0.8, or 3108 CFU/ml. For this experiment, different leaf extract concentrations of 5, 10, 15, and 20 g/ml were taken in separate test tubes, each containing 4 ml of MH broth and 0.5 ml of bacterial suspension at a final concentration of 108 CFU/ml. 5 separate tests were conducted in duplicate to determine each MIC. The tubes containing 4.5ml of bacterial inoculates and 0.5 ml of 7% methanol used as bacterial control, 4.5 ml of uninoculated Mueller Hinton broth and 0.5 ml 7 % methanol served as a blank. All experimental and bacterial control blank inocula were incubated for 18 hours at 37°C. Finally, the absorbance at A560 nm was measured to determine the suppression of bacterial growth.

3. Results and Discussion

Numerous techniques are followed in the discovery of novel pharmacologically active phytocompounds with many successful medications, the most important of which are isolation, purification, and identification. The extractive value was estimated using different solvents in the current study, and the results were recorded in Table.1 and depicted in Fig.1, which showed that the highest yield of extractive value obtained maximum range 4.3% for the solvent methanol and the lowest range observed at 0.34% for the solvent chloroform. Other solvent extractive values observed for the following sequences are ethanol 3.9%, water 3.4%, ethyl acetate 1.2%, and hexane 0.93%. This finding suggests that the extraction yield increases even as the polarity increases.

The physico-chemical parameters are mainly used to judge the purity and quality of the plant extract [22]. The residue ash value left over after heating plant raw material in a muffle furnace 400 ± 25°C. It indicates the presence of inorganic salts, either naturally occurring or adhering to the plant material from the environment or purposefully added as an adulterant by humans [23]. For the results the total ash value is 13.22%, water soluble ash 1.63% and acid insoluble ash 2.24% were denoted in table.2. When an aqueous solution of plant material is agitated, the saponins in the plant material generate persistent foam. The results indicated the foaming index of the plant *Hygrophila auriculata* leaf powder was below than 100. Due to the existence of the diverse elements of pectin or hemicelluloses, mucilage, and gum among various medicinal herbs, the different medicinal plants have a distinct medicinal value, which results in varying swelling abilities of various herbal materials. The

outcome of the results has been suggests that the swelling index of the plant is 2.12%.

Table 1 Extractive value of the plant *Hygrophila auriculata*

% of extractive value		
SOLVENTS	DRY WEIGHT OF RAW LEAF POWDER(g)	*HYGROPHILA AURICULATA*
Water	1.0	3.4
Methanol	1.0	4.3
Ethanol	1.0	3.9
Petroleum ether	1.0	0.74
Chloroform	1.0	0.34
Hexane	1.0	0.93
Ethyl acetate	1.0	1.2

Table 2 Physical parameter analysis of the plant *Hygrophila auriculata*

PLANTS	PARAMETERS					
	TA%	WSA%	AIA%	SI (%)	FI	FB
Hygrophila auriculata	13.22	1.63	2.24	2.12	Below to the 100	Dark green
TA-Total ash, WSA-Water soluble ash, AIA-Acid-insoluble ash, SI-Swelling index, FI-Foaming index and FB-Fluorescence behavior						

Table 3 Qualitative analysis of phytocompounds of leaves of *Hygrophila auriculata*

S.No.	SOLVENTS / PHYTOCOMPOUNDS	NH	EA	CH	AC	PE	WA	ET	MT
1.	Alkaloids	+	+	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+	-	+	+
3.	Tannins	+	+	-	-	-	-	+	-
4.	Saponins	-	-	-	-	-	-	-	-
5.	Phenol	+	+	-	-	-	+	+	+
6.	Terpenoids	+	+	+	+	+	-	+	+
7.	Steroids	+	+	+	+	+	-	+	+
8.	Glycosides	-	-	-	-	-	-	-	-
9.	Triterpenes	+	+	+	+	+	-	+	+

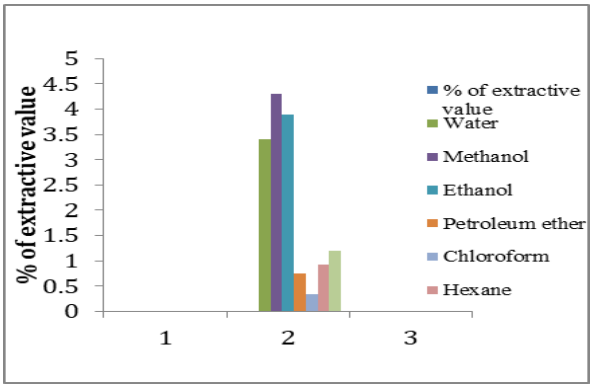

Figure 1 The graphs shows the Extractive value determination and phytochemical qualitative analysis of study plant.

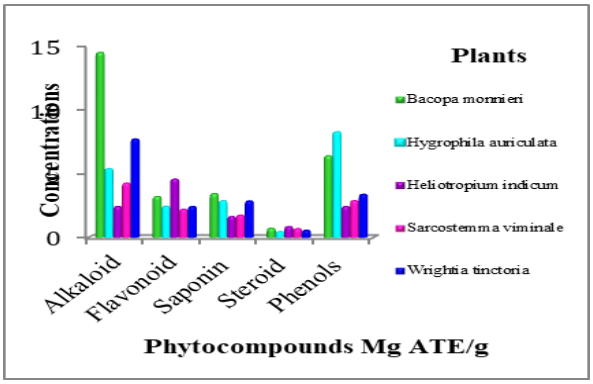

Figure 2 The graphs shows the phytochemical qualitative analysis of study plant.

Table 4 Quantitative analysis of phytocompounds in the methanolic leaf extract of study plant

PLANTS	PHYTOCOMPOUNDS				
	Alkaloid Mg ATE/g	Flavonoid Mg CAE/g	Saponin MgDIE/g	Steroid Mg DIE/g	Phenols Mg CAE/g
HA	5.36 ± 4.25	2.43 ± 0.04	2.85 ± 0.02	0.45 ± 0.02	8.25 ± 0.04
DIE*:* Diosgenin equivalents, ATE: Atropine equivalents, CAE: *catechin equivalents*					

Table 3 Qualitative analysis of phytocompounds of leaves of *Hygrophila auriculata*

S.No.	SOLVENTS / PHYTOCOMPOUNDS	NH	EA	CH	AC	PE	WA	ET	MT
1.	Alkaloids	+	+	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+	-	+	+
3.	Tannins	+	+	-	-	-	-	+	-
4.	Saponins	-	-	-	-	-	-	-	-
5.	Phenol	+	+	-	-	-	+	+	+
6.	Terpenoids	+	+	+	+	+	-	+	+
7.	Steroids	+	+	+	+	+	-	+	+
8.	Glycosides	-	-	-	-	-	-	-	-
9.	Triterpenes	+	+	+	+	+	-	+	+

Table 5 FTIR peak value, appearance, stretches and functional group for all solvent extract of *Hygrophila auriculata*

SOLVENTS								BONDING	FUNCTIONAL GROUP
Hexane	Ethyl acetate	Chloroform	Acetone	Petroleum ether	Water	Ethanol	Methanol		
606.17 712.35	606.17	655.60	606.17	614.41	614.41	622.65	671.16	C-l stretching	Halo Compounds
-	-	819.45	-	653.48 889.64	720.59	770.02	794.73	C–Br stretch, C–H bend	Alkyl Halides, Alkynes,
926.54	901.83	926.54	926.54	926.54	951.25	934.78	959.49	O–H bend	Carboxylic Acids
1132.49 - 1321.05	1099.54-1386.95	1050.11-1321.05	1107.78 1378.71	1041.87-1345.76	1107.78 1206.63	1058.35 -1395.19	1050.11-1297.25	C–N stretch C–O stretch	Aliphatic Amines, Alkyl Aryl Ether, Alcohol, Sulfonyl Chloride, Aromatic ester
1411.67 1477.57 1559.95	1494.05	1411.67-1559.95	1485.81	1461.09-1485.81	1436.38	1502.28	1411.67 1510.52	N–O symmetric stretch	Nitro Compounds, Alkane, Aromatics
1650.57	1634.09	1650.57	1642.33	1634.09	1634.09	1643.09	1634.09	N–H bend	Alkene, Amines
1807.09	1798.85	1864.75	1782.37	1700 1732.95	1798.85	1732.95 1798.85	1815.33	C=O	Aldehyde, Esters, Aromatic compound
2119.22	-	2110.98	-	-	-	-	2119.22-	C≡C Stretch	Alkynes (Monosubst)
2678.48 - 2991.53	2876.20 2925.62 2991.53	2374.59-2991.53	2925.62	2358.12-2917.39	-	2358.12-2983.29	2983.29	CH_3 and CH_2, C-H stretch	Alkanes, Aliphatic Compounds, Alkenes
3427.23 - 3872.08	3460.18	3410.75 3740.27	3451.94	3008.09-3863.84	-	3427.23-3847.36	3427.23	-OH stretch	Alcohols and Phenols

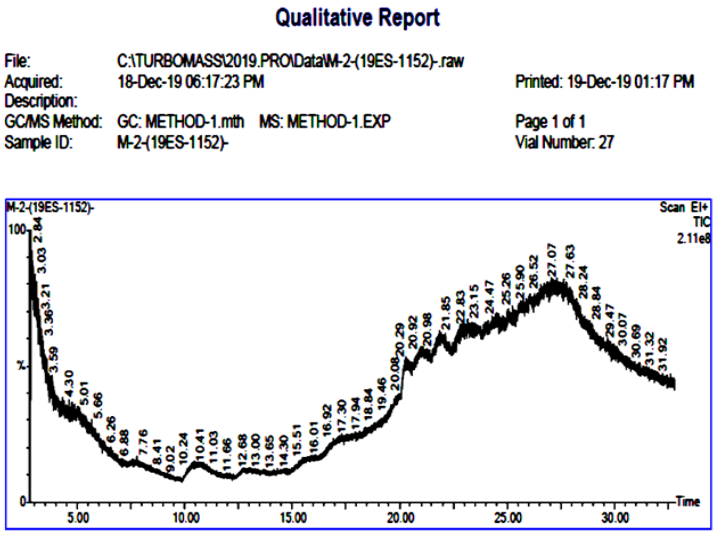

Figure 3 GCMS Qualitative report of methanol extract of *Hygrophila auriculata.*

The phytocompound screening in the Hexane, Ethyl Acetate, Chloroform, Acetone, Petroleum ether, Water, Ethanol and Methanol extract of *Hygrophila auriculata* showed the presence of alkaloids in all extract but compound flavonoids was observed in all the extract except water extract. The phytochemical tannins were exhibited in only Hexane, Ethyl Acetate and Ethanol extracts. The phytocompounds saponins and glycosides were not observed in any extracts (Table 3). The phenols were present in Hexane, Ethyl Acetate, Water, Hexane, Ethyl Acetate extract out of eight extracts. The phytocompounds terpenoids, steroids and triterpenes were observed in seven extract such as Hexane, Ethyl Acetate, Chloroform, Acetone, Petroleum ether, Ethanol and Methanol except Water out of eight.

Phytochemical alkaloids were found in all of the *Hygrophila auriculata* extracts tested, but compound flavonoids were found in all, exception of water extract. The only extracts that included phytochemical tannins were hexane, ethyl acetate, and ethanol extracts. There were no extracts found that included the phytochemicals saponins and glycosides [24]. Out of the eight extracts, the phenols were found in the hexane, ethyl acetate, water, ethanol, and methanol extract. Out of eight extracts, terpenoids, steroids, and triterpenes were found in seven of them, including 7 but not in water. The secondary plant metabolites acts as a defensive mechanism against herbivores, insects, and other microorganisms [25, 26] (Table 4 and Fig. 2).

The quantitative phytochemical results obtained from the plant *Hygrophila auriculata,* Phenol reported the highest value 8.25±0.04 Mg CAE/g comparing other phytocompound and steroid reported minimum range 0.45±0.02 for the study plant. The other plant shows the values are alkaloid is 5.36±4.25, flavonoid 2.85±0.02, saponin is 2.43±0.04 respectively. The multiplication of many cancer cells is stopped by the natural anti-cancer drug saponin, which interacts with the cholesterol-rich plasma membrane of the cancer cells [27]. Alkaloids and flavonoids have a range of therapeutic effects including analgesic, anti-oxidant properties anti-inflammatory, antioxidant, and astringent action [28].

The IR spectrum for the plant *Hygrophila auriculata* made on hexane, ethyl acetate, chloroform, acetone, petroleum ether, water, ethanol and methanol. The FTIR results based on absorption of peaks shows in a graphical view [29]. The hexane leaf extract revealed the strong peaks at 606.17 cm^{-1}, 1132.49 cm^{-1}, 1206.63 cm^{-1}, 1321.05 cm^{-1}, 1411.67 cm^{-1}, 1477.57 cm^{-1}, 2119.22 cm^{-1}, 2876.20 cm^{-1}, 2975.05 cm^{-1} and 3427.23 cm^{-1} indicated the presence of halo compounds (C-Cl stretching), aliphatic ether. The presence of carboxylic acid (O-H bend) is indicated by the prominent wide peaks at 1050.11 cm^{-1} and 2678.48 cm^{-1} (O-H stretching). 926.54cm^{-1}, 1650.57cm^{-1}, and 2851.48 cm^{-1} for Carboxylic acids (O-H bend), alcohol (O-H bend), cyclic alkene (C=C stretching), and aldehyde (C-H stretching). The ethyl acetate extract revealed the presence of 12 peaks at 606.17 cm^{-1}, 901.83 cm^{-1}, 1099.5 cm^{-1}4, 1264.30 cm^{-1}, 1386.95 cm^{-1}, 1494.05 cm^{-1}, 1634.09 cm^{-1}, 1798.85 cm^{-1}, 2876.20 cm^{-1}, 2925.62 cm^{-1}, 2991.53 cm^{-1} Secondary alcohol C-O stretching, alkyl aryl ether C-O stretching O-H Stretch, H-Bonded of Alcohols, Phenols, N-O Asymmetric Stretch of Nitro Compounds, C-C Stretch (In-Ring) of Aromatics, C-H Bending of Aromatic Compound, Broad N-H Stretching of Amine Salt, and O-H Stretch of Phenol, respectively [30]. The chloroform extract of *Hygrophila auriculata* leaf extract indicates 655.60 cm^{-1}, 819.45 cm^{-1}, 926.54 cm^{-1}, 1050.11 cm-1, 1157.20 cm^{-1}, 1190.96 cm^{-1}, 1256.06 cm^{-1}, 1321.05 cm^{-1}, 1411.67 cm^{-1}, 1477.57 cm^{-1}, 1559.95 cm^{-1}, point to the presence of halo compounds, aliphatic ethers, ester, aromatic esters, sulfones, nitrogen compounds, isothiocyanate, and carboxylic acid, alkene, carboxylic acids for single and double bond stretching. The acetone extract of the leaves of *Hygrophila auriculata* revealed the presence of spectral peak at 606.17 cm^{-1}, 926.54 cm^{-1}, 1107.78 cm^{-1}, 1214.87 cm^{-1}, 1378.71 cm^{-1}, 1485.81 cm^{-1}, 1642.33 cm^{-1}, 1782.37 cm^{-1}, 2925.62 cm^{-1}, and 3451.94 cm^{-1} serves as several phyto-constituents, including halo compounds (C-Cl stretching), carboxylic acids (O-H bend), aliphatic ethers (C-O stretching), vinyl ethers (C-O stretching), phenols (O-H bends), nitrogen compounds (N-O asymmetric stretch) and alkenes (C=C stretching). Peaks are observed at 614.41 cm^{-1}, 720.59 cm^{-1}, 926.54 cm^{-1}, 1041.87 cm^{-1}, 1206.63 cm^{-1}, 1280.77 cm^{-1}, 1345.76 cm^{-1}, 1485.81 cm^{-1}, 1634.09 cm^{-1}, 1700 cm^{-1}, 2358.12 cm^{-1} 2876.20 cm^{-1}, 2917.39 cm^{-1}, 3008.09 cm^{-1}, 3468.42 cm^{-1}, 3699.08 cm^{-1}, and 3863.84 cm^{-1} in petroleum ether extract [31] (Table 5).

The FTIR analysis of the water extract from the leaves of *Hygrophila auriculata* revealed peaks are 614.41 cm^{-1}, 951.25 cm^{-1}, 1107.78 cm^{-1}, 1206.63 cm^{-1}, 1436.38 cm^{-1}, 1634.09 cm^{-1}, and 1798.85 cm^{-1}, which could be attributed to the C-Cl stretching of halo compounds, the O-H bend of carboxylic acids and C-O stretching of secondary alcohol. For ethanol sample the wavenumbers are 622.65 cm^{-1}, 770.02 cm^{-1}, 934.78 cm^{-1}, 1058.35 cm^{-1}, 1107.78 cm^{-1}, 1165.44 cm^{-1}, 1264.30 cm^{-1}, 1395.19 cm^{-1}, 1502.28 cm^{-1}, 1643.09cm^{-1}, 1732.95 cm^{-1}, 1798.85 cm^{-1}, 2358.12 cm^{-1}, 2884.43 cm^{-1}, 2925.62 cm^{-1}, 2983.29 cm^{-1}, 3427.23 cm^{-1}, 3723.79cm^{-1} and 3847.36 cm^{-1} respectively, it indicates the functional phytocompounds such as Alkene, carboxylic acids, sulfoxide, Secondary alcohol, tertiary alcohol. The intensely high peaks at 2876.20 cm^{-1}, 2917.48 cm^{-1}, 2975.05 cm^{-1}, 2991.53 cm^{-1}, and 3427.23 cm^{-1} might all be attributed to wide amine salt, alcohol and phenol [32].

Table 6 GCMS analysis showed the retention time related bioactive phyto-compound identified for the methanol extract of leaf of *Hygrophila auriculata.*

RETENTION TIME	NAME OF THE COMPOUND	MOLECULAR FORMULA	W
20.291	Benzoxazol 3-Carboxylic Acid, 2,3-Dihydro-2-Oxo-6-Nitro-, Pentyl Ester	$C_{13}H_{14}O_6N_2$	94
21.096	2-O-P-Toluenesulfonylarabinose	$C_{12}H_{16}O_7S$	04
21.846	2-Naphthalenecarboxylic Acid, 4,4'-Methylenebis[3-Methoxy	$C_{25}H_{20}O_6$	16
23.152	Hexane, 1-(1-Ethoxyethoxy)-	$C_{10}H_{22}O_2$	74
24.512	7-Hydroxy-3-(1,1-Dimethylprop-2-Enyl)Coumarin	$C_{14}H_{14}O_3$	30
27.318	3,6-Methano-8h-1,5,7-Trioxacyclopenta[Ij]Cycloprop[A] Azulene-4,8(3h)-	$C_{15}H_{18}O_6$	94
28.629	Androstane-11,17-Dione, 3-[(Trimethylsilyl)Oxy]-, 17-[O-(Phenylmethyl)Oxime], (3.Alpha.,5.Alpha.)-	$C_{29}H_{43}O_3NSi$	81

Table 7 Free radical scavenging activities of methanol extract of *Hygrophila auriculata* at different concentrations.

CONCENTRATIONS (µg/ml)	FREE RADICAL SCAVENGING ACTIVITIES					
	DPPH %	ABTS%	FRAP%	Hydroxyl radical	Superoxide	Nitric oxide
25	08.15±0.26	15.32±1.1	05.170.43	28.61±1.7	41.15±2.4	10.89±0.8
50	17.22±1.13	24.75±1.8	13.25±1.28	36.25±2.5	65.34±3.2	18.27±1.2
100	29.31±1.9	33.12±2.3	24.48±1.7	57.18±3.2	74.12±5.7	31.52±2.4
250	43.15±2.6	47.54±2.9	32.27±2.1	68.13±3.5	81.04±3.8	45.66±4.0
500	68.22±3.1	53.08±3.8	59.44±3.6	79.04±5.1	94.45±4.1	51.12±3.1

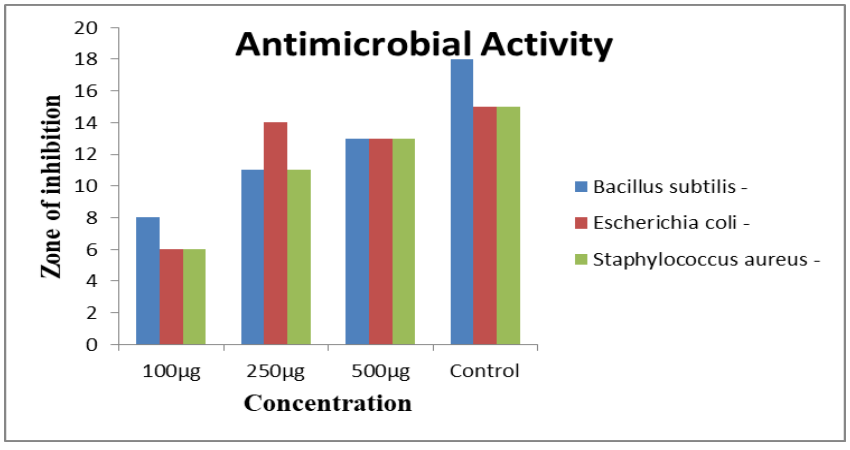

Figure 4 Different bacterial strains at different concentration of methanol extract of *Hygrophila auriculata.*

The strong peaks were obtained for methanol solvent at 671.16 cm^{-1} and 852.40$cm^{-1}$for halo compound, 959.49 cm^{-1} for alkene, 1050.11 cm^{-1} for primary alcohol, 1157.20 cm^{-1} for aliphatic ether, 1198.39 cm^{-1}. The weak peaks at 2119.22 cm^{-1} and strong wide bands at 3427.23 cm^{-1}, 3751.58 cm^{-1} (O-H Stretching) of alcohol, as well as the medium peaks (C=C Bending) of alkene and 2843.24 cm^{-1}, 2925.62 cm^{-1}, and 2983.29 cm^{-1} (C-H Stretching) of alkane [33]. The wax of many different species contains alkane molecules protecting it from water loss, bacteria, dangerous insects, fungus, and rainwater leaching of vital minerals [34]. non-aspirin pain reliever ibuprofen is also a carboxylic acid [35] and The hydroxyl groups in flavonoids intervene in the strengthening of cells and chelating metal ions [36, 37].

The methanol extracts of *Hygrophila auriculata* leaves contain the following compounds such as Benzoxazol 3-Carboxylic Acid, 2,3-Dihydro-2-Oxo-6-Nitro-, Pentyl Ester, 2-O-P-Toluene sulfonylarabinose, 2-Naphthalene carboxylic Acid, 4,4'-Methylenebis [3-Methoxy], Hexane, 1-(1-Ethoxyethoxy)-, 7- Hydroxy-3-(1,1-Dimethylprop-2-Enyl) Coumarin, 3,6-Methano- 8h-1,5,7- Trioxacyclopenta [Ij] Cycloprop[A] Azulene-4,8(3h)-, Androstane-11,17-Dione, 3-[(Trimethylsilyl)Oxy]-, 17-[O-(Phenylmethyl) Oxime], (3.Alpha.,5.Alpha.). Among these, the compound 3,6-Methano-8h-1,5,7- Trioxacyclopenta[Ij] Cycloprop[A] Azulene-4,8 (3h)- was observed in the fruit of plant *Persea americana* [38] in plant *Cleistanthus collinus* (Roxb.) [39].

The compound Androstane-11,17-Dione, 3-[(Trimethylsilyl)Oxy]-, 17-[O- (Phenylmethyl)Oxime], (3.Alpha.,5.Alpha.)- observed in the present study has the antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic activities [40] (Fig. 3 and Table 6).

Antioxidant activity of the plant extract of *Hygrophila auriculata* is effective against DPPH, ABTS, FRAP, hydroxyl radicals, superoxide, and nitrous oxide. The first-highest free radical scavenging activity is 94.454.% against superoxide, followed by the second- and third-highest scavenging activities, 79.045 and 68.223 respectively, at the maximum dosage of plant extract, 500mg. At 500 g of leaf extract, the scavenging activities of additional free radicals like ABTS, FRAP, and nitrous oxide exhibited more or less comparable results, at 53.08 %, 59.44 %, and 51.12 %, respectively. With increasing leaf extract concentration, free radical scavenging was consistently shown to rise, reaching a maximum at 500g. The presence of antioxidant components such flavonoids, phenols, tannins, and terpenoids in *T. officinale* might clarify the extracts' strong antioxidant potential [41] (table 7).

At the antimicrobial activity of the plant extract of *Hygrophila auriculata* is effective in higher concentration. At a concentration of 50g, the plant extract *Hygrophila auriculata* likewise showed no inhibition of *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [42, 43].

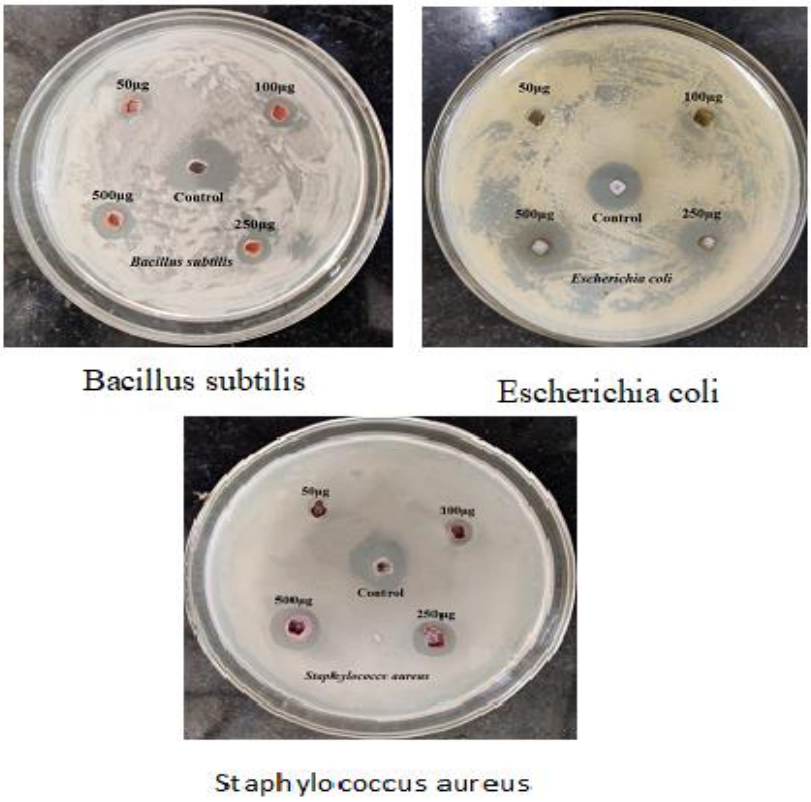

Figure 5 Antimicrobial activity of Bacillus subtilis, Escherichia coli and Staphylococcus aureus.

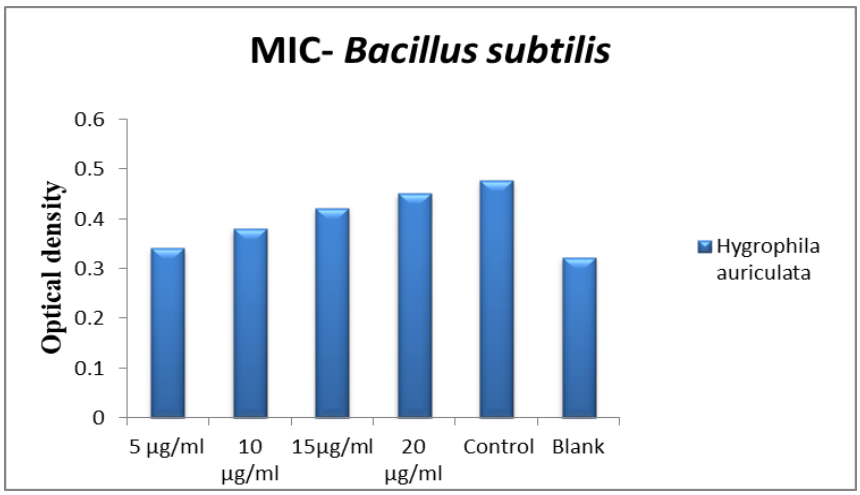

Figure 6 MIC in *Bacillus subtilis.*

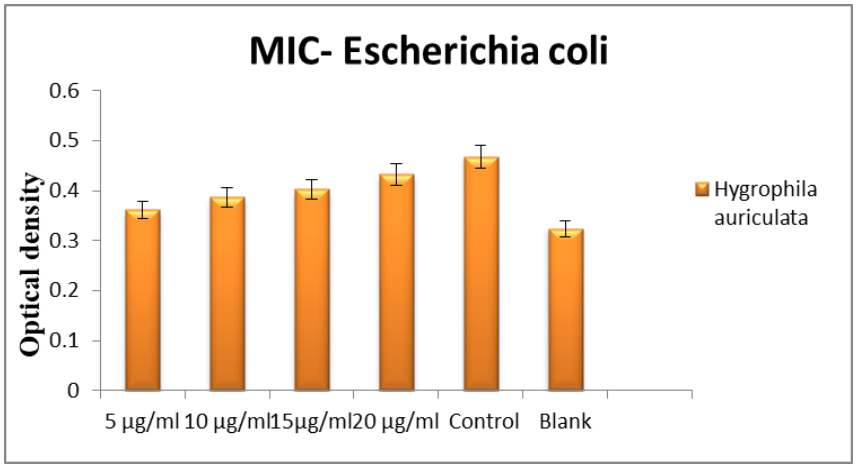

Figure 7 MIC in *Escherichia coli.*

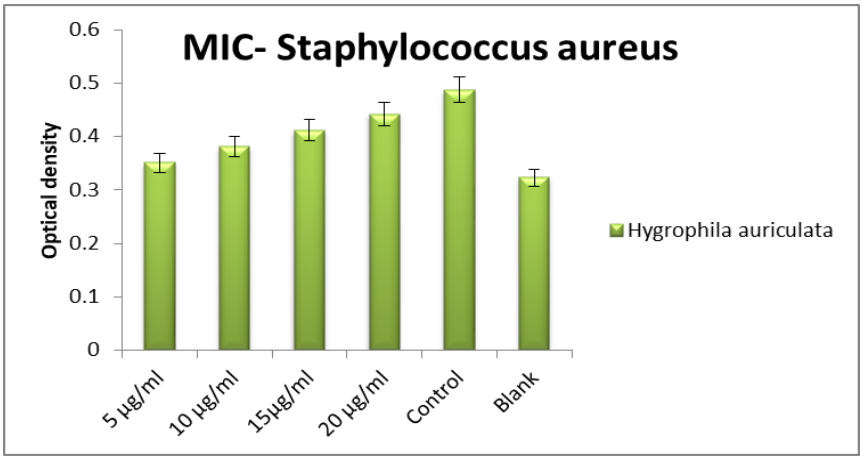

Figure 8 MIC in Staphylococcus aureus.

Fig.4 shows the different concentration 100g, 250g, and 500g, this extract had a significant zone of inhibition, with measurements of 8mm, 6mm, and 6mm; 11mm, 14mm, and 11mm; and 13mm, 13mm, and 13mm respectively. The zone of inhibition shown at the high concentration of 500g was close to the zones of inhibition seen by synthetic compound cephalexin against all bacterial species at 18mm, 15mm and 15 mm and Fig.5 shows in the antimicrobial activities to against resistant in the methanol extract of *hygrophila auriculata* and the microorganism are *bacillus subtilis*, *Escherichia coli* and *staphylococcus aureus*. The fig.6, 7 and 8 shows on the MIC against in the methanolic extract of *hygrophila auriculata* and inhibit the three different bacterial strains.

4. Conclusions

Hygrophila auriculata is widely distributed throughout India and is used to treat inflammation, ache, urinary infection, edoema, gout, diuretic, stomachache, headache, diabetic, cancer activities, bacterial infection, and other conditions. The plant Hygrophila auriculata contains a variety of phytochemicals, including alkaloids, flavonoids, phenol, triterpenes, terpenoids, and steriods. *Hygrophila auriculata* has a high antibacterial activity. As a result, we can isolate some pure phytochemicals, which can then be used as lead molecules to synthesise novel agents with good therapeutic activity. In the future, we will work on antibiotics and drug development.

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Conflict of interest

The Authors have no conflicts of interest to declare that they are relevant to the content of this article.

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