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GC-MS analysis and antimicrobial activity of *Hygrophila auriculata*

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ABSTRACT

The genus *Hygrophila auriculata* contains about 43 species of tendril – bearing climbing herbs distributed in tropical India. The plant was collected at Thottiam village, Tiruchirapalli District, Tamil Nadu. The antimicrobial activity of plant extracts and preliminary phytochemical screening was evaluated with antibiotic susceptible and resistant micro-organisms. It was tested for antimicrobial activity by disc method. The extract of plant showed antimicrobial activity against both gram (+) and gram (-) bacteria. Maximal antibacterial activity was observed against *S.epidermidis* and maximal antifungal activity was against for *Hygrophila auriculata*. The ethanolic extract was subjected to GC – MS analysis. Alkaloids, tannins, glycosides, terpenoids, steroids, flavonoids, and saponins are found in the plant extracts.

Key words: *Hygrophila auriculata*, antimicrobial activity, phytochemical screening, Plant extract, therapeutic use.

INTRODUCTION

Plants have a great potential for producing new drugs of great benefit to mankind. Nowadays a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. The plant is an important medicinal herb widely distributed in India and used by local population for different medicinal purposes[1]. This revival of interest in plant – derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs many of which have adverse side effects. The need of the hour is to screen a number of medicinal plants for promising biological activity[2]. In recent years secondary plant metabolites have been extensively investigated as a source of medicinal agents[3]. Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action [4].

The demand for plant based medicines, health products, Pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices. India has lot of potential for producing world class herbal medicines. The Indian herbal Industry is on a roll and poised to grow in the coming years owing to its high demands for herbal products. The modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening. The use of synthetic compounds led to a decline in the use of plants in modern medicine however, synthetic medicine can cause side effects and as a result of people are more favourable to use natural compounds obtained from plants[5]. Medicinal plants are commonly used for the treatment of various ailments in India[14]. According to the world health organization, the traditional medicine

system will continue to play an essential role in health care system since over 80% of the population in the third world country relies on the use of traditional medicine[15].

The aim of the present study is to investigate phytochemicals constituents of *Hygrophila auriculata* by GC – MS and antimicrobial studies.

MATERIALS AND METHODS

Collection of plant materials

Fresh parts of plant *Hygrophila auriculata* whole plants were collected at Thottiam village in Tiruchirappalli District. The plant materials were identified by botanically. The plant materials were shaded and dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were grinded well using mechanical blender into fine powder and transferred into air tight container with proper labeling.

Preparation of plant extracts

Solvent extraction

Plant Powder was extracted successively with petroleum ether, diethyl ether, chloroform and alcohol using a soxhlet apparatus [6]. Crude plant extract was prepared by Soxhlet extraction method[7]. About 100g of powdered plant material was uniformly packed into a thimble and extracted with 250ml of diethyl ether solvent is used separately. The process of extraction continues for 18 hours and till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40 °C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4 °C.

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 μ Mdf, composed of 100% Di methyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used[8]. The dried extract was injected to in the GC – MS equipment and the sample was analyzed[9].

The antimicrobial activity was determined using disc diffusion method[5,12] by measuring zone of inhibition in mm and comparing with standard drugs *Ciproflaxin* (2μg/disc for bacteria) and *fluconozale* (10μg/disc for fungi)[9]. The plant was subjected to preliminary phytochemical screening for the detection of various plant constituents[5].

RESULTS AND DISCUSSION

Qualitative preliminary screenings of extracts are performed initially with different chemical reagents to detect the phytochemical constituents present in the extracts. The extracts show the presence of alkaloids, saponins, tannins, flavonids and phenolic compounds[5]. The obtained results are furnished in table – 1.

Antimicrobial studies : Diethyl ether extract of *Hygrophila auriculata(L)*. plant shows antimicrobial activity against the tested organisms in the order of *S.epidermidis*(22mm), *E.coli*(17mm), *Corneybacterium*(12mm), *vibriochlerace* (9mm), *E.fecalis*(20mm), *Salmonalla typi* (19mm). In case of fungi, antimicrobial activity against tested organisms was in the order of *C.albicans*(11mm) and *A.niger*(9mm). Maximal antibacterial activity is observed against *s.epidermidis* while maximal antifungal activity is against *Candida albicans* (Table–2).

GC – MS studies : GC – MS studies are reported that the Diethyl ether extract of the whole plant of *Hygrophila auriculata(L)*. The various plant phytochemical components are found in the plant of *Hygrophila auriculata(L)*. They are listed in table - 3

The peak is found at RT 120 with a peak area of 100%. It shows Ellipticine. This compound is a Alkaloid with a molecular formula is $C_{17}H_{14}N_2$ and molecular weight is 246.31. Ellipticine shows antimicrobial activities. Alkaloid play some metabolic role and control development in living system[5].

The peak at RT 104 with a peak area of 45% corresponds to the compound is 2-Furan carboxaldehyde hydroxyl methyl. The molecular formula and a molecular weight of this compound is $C_6H_6O_3$ and 126.12 respectively. 2-

Furan carboxaldehyde hydroxyl methyl compound belongs to aldehyde group. It shows antimicrobial and preservative[11].

The peak at RT 280 and peak area 25% is Quercetin which is a Flavonoid compound. The molecular formula and the molecular weight of this compound is $C_{15}H_{10}O_7$ and 302.23, respectively. This flavonoid is known to be most of medicinal activities like antidiabetic, anti-inflammatory, antidermatic, antileukemic and anticancer activities. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process[12].

The hydroxyl group of compound 2-hydroxycyclopentadecane is observed at peak at RT 162 with a peak area of 18%. This compound has the molecular formula $C_8H_{10}O_3$ and molecular weight is 154.16. It shows antimicrobial activities[13].

The peak at RT 309 with peak area 10% is 2,3 Dihydro benzofuran nature of the compound is methylene group. The molecular formula is $C_{11}H_{15}NO$ and molecular weight of this compound is 177.24. This compound has less antimicrobial activities.

TABLE-1: Preliminary phytochemical analysis of *Hygrophila auriculata*

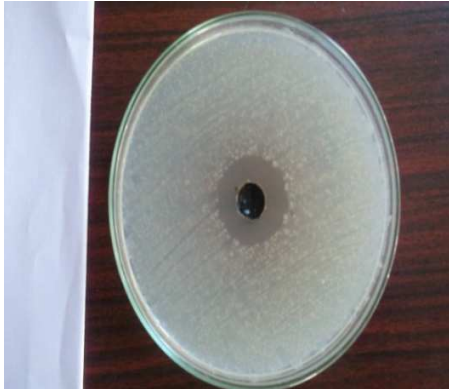
Name of the test	Phytochemical constituents	Ether Extract	Aqueous Extract
Mayer's test		+	+
Dragendroff test	Alkaloids	+	+
Wagner test		+	+
Molish test		-	-
Fehling test		-	-
Benedict's test	Carbohydrates	+	-
Form test	Saponins	+	+
Lead acetate test	Tannins	+	+
Ferric chloride test	Pseudo tannins Condensed Tannin	+	+
Ammonia test	Chlorogenic acid	-	+
Salkowski test	Steroidal Glycosides	+	-
H ₂ SO ₄ test	Anthocyanin	-	-
Liebermann's Burchard test	Steroidal Glycosides	++	+
H ₂ SO ₄ test	Saponins glycosides	+	+
Ammonia test	Flavonoids	+	+
Shinoda's test	Flavones	-	-
Ferric chloride test	Phenols	++	+
Sodium chloride test	Coumarin	+	+
Borntrager's test	Anthracene Glycoside	-	-

High : +++ Moderate : ++ Present : + Absent : -

TABLE-2: Antibacterial activity of *Hygrophila auriculata*

Organism	Zone of inhibition (mm)
<i>S.epidermidis</i>	22
<i>Escherichia coli</i>	17
<i>Corneybacterium.sp</i>	12
<i>Vibriocholerace</i>	09
<i>E. fecalis</i>	20
<i>Salmonalla typhi</i>	19
<i>Candida albicans</i>	12
<i>Aspergillus niger</i>	10
Control	20

S.EPIDERIMIS



Zone of inhibition measured – 22mm

ESCHERICHIA COLI



Zone of inhibition measured-17mm

CORNEYBACTERIUM



Zone of inhibition measured-12mm

V.CHOLERA



Zone of inhibition measured-09mm

E.FECALIS

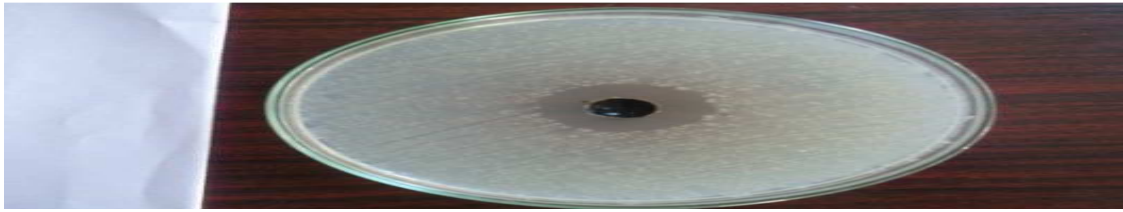


Zone of inhibition measured-20mm

SALMONALLA TYPI

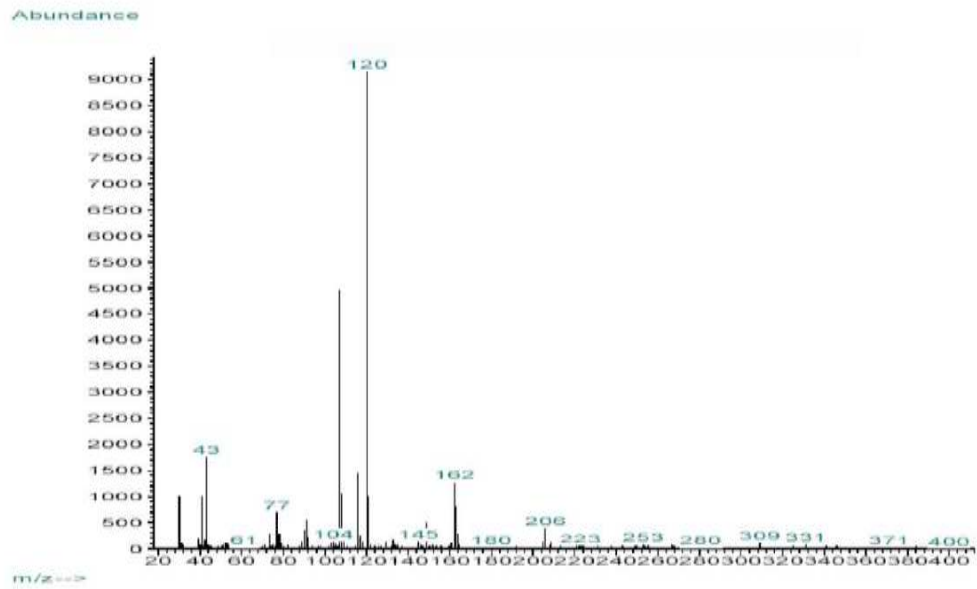


Zone of inhibition measured-19mm



Control (Ciproflaxin) 20 mm

Figure : GC – MS Chromatogram



Column: 12m x 0.15mm ID Initial Temp.: 140°C, 0 min

Program Rate: 25°C/min Scan Range: 50:500 amu

Injection Mode: Split 50:1 Sample 3

TABLE-3 *Phytocomponents identified in the Ether Extract of Hygrophila auriculata by GC-MS*

S. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak area %	Nature of the Compound
1	43	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.371	1.8	Saturated fatty acid
2	77	Diundecylphthalate	C ₃₀ H ₅₀ O ₄	474.72	0.6	Esters of phthalic acid
3	104	2-furan carboxaldehydes hydroxyl methyl	C ₆ H ₆ O ₃	126.12	4.9	Aldehyde Group
4	120	Ellipticine	C ₁₇ H ₁₄ N ₂	246.31	9.7	Alkaloid group
5	162	2 hydroxycyclo Pentadecane	C ₈ H ₁₀ O ₃	154.166	1.0	Hydroxyl group
6	206	1 – Octadecene	C ₁₈ H ₃₆	252.48	0.29	Alkenes
7	280	Queretin	C ₁₅ H ₁₀ O ₇	302.236	0.01	Flavonoid
8	309	2-3 Dihydrobenzofuran	C ₁₁ H ₁₅ NO	177.242	0.2	Methylene Group

CONCLUSION

In the present study Eight chemical constituents have been identified from ether extract of the whole plant of *Hygrophila auriculata* by GC-MS analysis. The same plant extract is effective against both gram positive, gram negative bacteria as well as fungi. Therefore it can be concluded that antimicrobial activity of *Hygrophila auriculata* against bacteria and fungi shows its medicinal value and supports the widespread use of the plant as local remedy for a variety of ailments. The presence of various bioactive compounds justifies the use of whole plant for various ailments by traditional practitioners.

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