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Phytochemical Evaluation, Gas Chromatography -Mass Spectroscopy (Gc-Ms) And Anti Microbial Activity Studies From *Parmelia Perlata*

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ABSTRACT

Phyto compounds have constituted an alternative source of antimicrobial agents. Qualitative phytochemical screening was carried out on the documented procedures. Antimicrobial activity was assessed using modified disc diffusion method. The chemical constituents of the herbal drugs were assessed using gas chromatography mass spectrophotometry (GCMS) The preliminary phytochemical screening revealed the presence of alkaloids, phenols, saponins, tannins, steroids, glycosides. The GC-MS analysis revealed the following major bioactive compounds: 25 Compounds were identified in lichen *Parmelia perlata* powder .The ethyl acetate and aqueous extract was inhibited the growth of selected Gram positive bacterial strain *Staphylococcus aureus* in the range of 16 mm and 14.5 mm by comparing standard Linezoid 15.5 mm, and *Bacillus subtilis* 15mm and 15mm by comparing standard Tetracycline 14 mm .Gram negative bacterial strain *Escherichia coli* in the range of 17mm and 13 mm comparing standard Sulbactum 16 mm and *Proteus vulgarius* in the range of 15.5 mm and 14 mm by comparing standard Cefotaxim 34 mm respectively (Table 5). The antifungal activity of *Candida albicans* in the range of 16.5 mm and 11.5 mm by comparing standard Fluconazole 17mm. The ethyl acetate extract of *Parmelia perlata* in all gram positive and gram negative microorganisms showed maximum good inhibition and also the anti fungal activity maximum good inhibition in ethyl acetate extract of *Parmelia perlata*.

Keywords: Phytocompounds, GC-MS , Anti microbial activity, Anti fungal activity.

INTRODUCTION

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness[1]. It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources into developing medicines by medicinal plants[2].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [3,4].

Tannins, alkaloids, saponins, flavonoids and Phenols have been reported to exhibit antibacterial activity against Salmonella Typhi [5]. In the recent years, medicinal plants are gaining increasing popularity globally as drugs, complementary and alternative medicines, food supplements, cosmetics [6]. Herbal drugs are increasingly used worldwide during the last few decades as seen in the rapidly growing global and national markets of herbal drugs[7]. The workhorse of contemporary plant metabolite profiling is no doubt gas chromatography coupled to mass spectrometry (GC-MS)[8]. Therefore, in this study the preliminary phytochemical screening, GCMS analyses and antimicrobial activity of two herbal mixtures was carried out to profile the chemical constituents in them that are responsible for their antimicrobial activity and other medicinal properties.

MATERIALS AND METHODS

COLLECTION OF MATERIALS

The lichen *Parmelia perlata* is obtained from local store, Annanagar, Madurai, Tamilnadu. Sample was cleaned under running tap water and dried at room temperature.

PROCESSING OF EXTRACT PREPARATION

200g of lichen was ground using mortar and pestle to make fine powder and sieved. Powdered sample was extracted in 500 ml ethyl acetate for 72 hours at room temperature under cold maceration method and the same time another 200g of lichen was extracted in 500 ml water by the same method. The beaker is covered with aluminium foil. Extraction was continued for 7 days. After 7 days the aluminium foil was removed and the extract was filtered with Whatman No. 1 filter paper and the ethyl acetate filtrate was concentrated using hot plate to get a brownish green liquid extract.

The percentage yield of extract and the colour of the extract was noted in Table 1.

TABLE 1: PERCENTAGE YIELD AND THE COLOUR OF THE EXTRACT OF LICHEN *PARMELIA PERLATA* POWDER

S.NO	EXTRACT	PERCENTAGE YIELD	COLOUR
1	Ethyl acetate extract	13.5%	Brownish green
2	Aqueous extract	12.7%	Dark brown

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

Phytochemical analysis reveals the occurrence of alkaloids, anthracene derivatives, flavonoids, phenolics, phytosterols, saponins, tannins, triperpenoids and volatile oils. (Table 2)

Table 2: Phytochemical analysis of lichen *Parmelia perlata* powder

S.NO	PHYTO CONSTIUTENTS	ETHYL ACETATE EXTRACT	AQEOUS EXTRACT
1	Alkaloids	+	+
2	Carbohydrates	-	-
3	Tannins	+	+
4	Saponins	+	+
5	Triterpenoids	+	+
6	Proteins and aminoacids	-	-
7	Phenolic compounds	+	+
8	Gums and mucilages	-	-
9	Fixed oil and fats	-	-
10	Glycosides	+	+
11	Triterpenes	+	+
12	Phytosterols	+	+

+ = presence of active constituents
- = absence of active constituents

GAS CHROMATOGRAPHY -MASS SPECTROSCOPY (GC-MS)

GC-MS analysis of ethanol extract was carried out using the equipment GC Clarus 500 Perkin-Elmer system comprising a AOC–20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: Equipped with a column Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1µm df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as carrier gas at a constant flow rate of 1.20 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature is set at 250 °C, and the ion-source temperature is 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase for 10 °C/min, to 200°C/ min, then 5°C/ min to 280 °C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70

eV; a scan-interval of 0.5 seconds, and fragments from 45 to 450 Da. Total GC running time was 36 min[9].

GC-MS ANALYSIS OF SAMPLES

The given lichen *Parmelia perlata* powder sample was extracted with ethyl acetate and ethanol and analysed through GC-MS for identification of different compounds.

IDENTIFICATION OF COMPOUND IN GC-MS ANALYSIS

25 Compounds were identified in lichen *Parmelia perlata* powder by GC-MS analysis. The active principles with their retention time (RT), Molecular weight (MW) and concentration are presented in the compound of various extract is given below the table:

Table 3: COMPOUNDS IDENTIFIED IN *PARMELIA PERLATA* POWDER OF ETHYL ACETATE EXTRACT

S.No	R.T	Name of the compound	Molecular Formula	Molecular Weight
1	15.556	orcinol	$C_7H_8O_2$	124
2	17.519	1,3-diphenyl-1-((trimethylsilyl)oxy)-1(z)-heptene	$C_{22}H_{30}OSi$	338
3	18.124	6-(hydroxymethyl)-2,2'-bipyridine	$C_{12}H_{10}N_2O_2$	214
4	19.252	benzaldehyde, 2,6-dihydroxy-4-methyl-	$C_8H_8O_3$	152
5	19.326	1,3-benzenediol, 5-pentyl-	$C_{11}H_{16}O$	180
6	19.836	1,2-benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
7	19.938	1,2-benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
8	20.76	1-(3,4-ditrimethylsiloxyphenyl)-2-isopropylaminoethanol	$C_{19}H_{34}O_3Si_2$	366
9	21.346	methyl 2,6-dihydroxy-4-methylbenzoate	$C_9H_{10}O_4$	182
10	21.407	benzoic acid, 3-formyl-2,4-dihydroxy-6-methyl-, methyl ester	$C_{10}H_{10}O_5$	210
11	22.22	benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	$C_{10}H_{12}O$	196
12	22.291	ethyl 2-hydroxy-4-methoxy-6-propylbenzoate	$C_{13}H_{18}O$	238
13	22.743	Ethyl 2,4-dihydroxy-6-methylbenzoate	$C_{10}H_{12}O_4$	196

14	22.898	Ethyl hematommate	$C_{11}H_{12}O$	224
15	23.181	benzoic acid, 4-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-2-hydroxy-6-methyl-, 4-carboxy-3-hydroxy-5-methylphenyl ester	$C_{25}H_{22}O_{11}$	498
16	23.744	ethyl 2-hydroxy-4-methoxy-6-propylbenzoate	$C_{13}H_{18}O$	238
17	24.222	2h-1-benzopyran-2-one, 4,7-dihydroxy-	$C_{10}H_8O$	178
18	26.481	6-chloromethyl-2,3-dihydro-benzo[1,4]dioxine	$C_9H_9ClO_2$	184
19	26.58	hexadecanoic acid	$C_{16}H_{32}O_2$	256
20	29.257	(z,z)-heptadeca-8,11-dien-1-yl bromide	$C_{17}H_{31}Br$	314
21	29.346	(z)-cis-9,10-epoxyheptadec-6-ene	$C_{17}H_{32}O$	252
22	35.586	1,2-cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{44}O_4$	396
23	37.4	2-isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	$C_{30}H_{33}ClO_6$	525
24	38.288	6,10-dodecadien-1-ol, 3,7,11-trimethyl-, [s-(z)]-	$C_{15}H_{28}O$	224
25	39.357	dodecane, 1,1'-oxybis-	$C_{24}H_{50}O$	354

ANTI MICROBIAL ACTIVITY

Microorganisms

The bacteria used as the test organisms in the study were *Staphylococcus aureus* and *Bacillus subtilis* (Gram +) *Escherichia coli* and *Proteus vulgarius* (Gram-). The fungus used was *Candida albicans*. The bacterial cultures were taken from Plant Secondary Metabolite Laboratory at Madurai. All bacterial cultures were maintained on Nutrient Agar/ Broth .*C. albicans* culture was maintained on Yeast Peptone Dextrose media.

Antimicrobial activity[10-14]

The antimicrobial activity of the *P. perlata* extracts against *the microbial pathogens* was determined by Kirby and Bauer disc diffusion method. Mueller- Hinton agar plates inoculated with 1 ml of bacterial and fungal suspension of 1×10^6 CFU/ml according to 0.5 Mc Farland standards was used for the antibacterial assay. Sterile filter paper discs of 6 mm dia were impregnated with 50 µl of crude extracts of lichen of varying concentrations such as 1 mg/ mL, 2 mg/mL and 5 mg/ml and after complete evaporation were placed on the surface of the inoculated agar plates. These plates were incubated at 37°c for 48 h. A t the end of the incubation period, the antimicrobial activities were evaluated by measuring the zone of inhibition. In

this assay, negative control was the respective pure solvent and the positive control was for *Bacillus subtilis* (Tetracycline), *Staphylococcus aureus* (Linezoild) , *Escherichia coli* (Sulbactum),*Proteus vulgarius* (Cefatoxim), *Candida albicans* (Fluconazole) .All the tests were performed in triplicates.

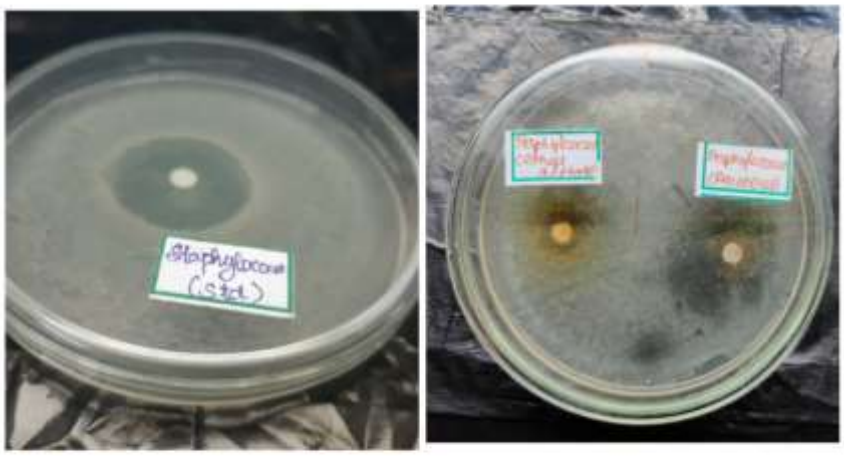
Table 4: Anti microbial activity of ethyl acetate and aqueous extract of *Parmelia perlata*

S.NO	ORGANISMS	STANDARD	ETHYL ACETATE EXTRACT	AQUEOUS EXTRACT
1	**Anti bacterial**			
	Gram (+)			
	Staphylococcus aureus	15.5	16	14.5
	Bacillus subtilis	14	15	15
2	**Gram (-)**			
	Escherichia coli	16	17	13
	Proteus vulgarius	14	15.5	15
3	**Anti fungal**	17	16.5	11.5
	Candida albicans			

Anti bacterial activity

Gram negative organisms

Gram positive organisms



Antifungal activity

RESULTS AND DISCUSSION

GAS CHROMATOGRAPHY -MASS SPECTROSCOPY (GC-MS)

25 Compounds were identified in lichen *Parmelia perlata* powder by GC-MS analysis.

ANTI MICROBIAL ACTIVITY

The results of the antimicrobial activity by Disc diffusion method of *Parmelia perlata* ethyl acetate and aqueous extract. The ethyl acetate and aqueous extract was inhibited the growth of selected Gram positive bacterial strain *Staphylococcus aureus* i*n* the range of 16 mm and 14.5 mm by comparing standard Linezoid 15.5 mm, and *Bacillus subtilis* 15mm and 15mm by comparing standard Tetracycline 14 mm . Gram negative bacterial strain *Escherichia coli* in the range of 17mm and 13 mm comparing standard Sulbactum 16 mm and *Proteus vulgarius* in the range of 15.5 mm and 14 mm by comparing standard Cefotaxim 34 mm respectively (Table 4). The antifungal activity of *Candida albicans* i*n* the range of 16.5 mm and 11.5 mm by comparing standard Fluconazole 17mm. The ethyl acetate extract of *Parmelia perlata* in all gram positive and gram negative microorganisms showed maximum good inhibition and also the anti fungal activity maximum good inhibition in ethyl acetate extract of *Parmelia perlata*. So it is proved that the ethyl acetate extract of *Parmelia perlata* has good anti microbial as well as good anti fungal activity.

CONCLUSION

Parmelia perlata is traditional medicinal plants and represent rich source of compounds possessing antimicrobial properties. Till now, little work has been carried out on their biological properties and hence extensive research is required to explore and identify the potential biological compounds of medicinal importance. The results of the present study revealed that the *Parmelia perlata* ethyl acetate and aqueous extract of lichen powder showed the presence of 20 compounds in GC-MS (Gas chromatography -Mass spectroscopy) analysis and also it could be used as powerful antimicrobial agents for the prevention of many diseases. Further study can be extended to check their ant-oxidative properties.

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