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Research Article

GC-MS Analysis and Biological Activities of Medicinally Important Lichen: *Parmelia perlata*

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ABSTRACT

Parmelia perlata colloquially known as Chadeela or Shilapushp belongs to the family Parmeliaceae. In Ayurveda, it is used to treat wounds, infections, inflammation, skin diseases, diarrhoea, dysentery, cough, fever, seminal weakness, amenorrhoea and renal calculi. In view of its medicinal importance we have analyzed this plant using Gas Chromatography–Mass Spectrometry and screened it for its antimicrobial and antioxidant activities. GC-MS analysis of the petether, chloroform and acetone extracts revealed the presence of 49 compounds in each. D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-,D-Friedoolean-14-en-3-one, (+)-Usnic acid, D:A-Friedooleanan-3-one, 5-methyl-1,3-benzendiol,5-pentyl-1,3-benzenediol, atranorin, methoxyolivetol and Z-10-tetradecen-1-ol were identified as major compounds. The results of antibacterial study suggested that pet-ether extract is more active against *S. grieveces*, whereas $CHCl_3$ extract is found to be more active against *B. subtilis* and *E. coli*. Acetone extract of this plant showed moderate activity against *B. subtilis* and *E. coli*. The results of antifungal activities showed that pet ether and acetone extracts possesses potential activity against *P. funiculosam*. We have also examined the extracts for their antioxidant potential by DPPH and FRAP total reduction capability methods. Bioactivity assays showed that the acetone extract possess strong free radical scavenging activity (IC_{50}=28 µg) followed by pet ether (IC_{50}=31 µg) and chloroform (IC_{50}=48 µg) extracts. Pet ether extract of this plant also showed strong ferric reducing ability of plasma (O.D. =.340).

Keywords: *Parmelia perlata*, Chadeela, GC-MS analysis, antimicrobial activity, DPPH, FRAP, free radical scavenging activity.

INTRODUCTION

Natural products have very important role in the drug discovery for the curing of human, animal and plant diseases. New drug preparations of natural origin are in need due to the numerous side effects and resistance development through the continuous and uncontrolled use of synthetic drugs[1]. Bioactive natural compounds have positive influence on the whole organism and without side effects. In search for those products, many of research teams have focused attention on lichens.

A lichen is a stable, ecologically obligate, self supporting mutualism between an exhabitant fungus (the mycobiont) and one or more inhabitant, extracellularly located unicellular or filamentous photoautotrophic partners (the photobiont: alga or cyanobacterium)[2]. The beautiful hills of Uttarakhand are the best source of lichens in India. For a long time, these organisms have been used in traditional medicine in the treatment of numerous infectious diseases[3]. The use of lichens in medicine is based on the fact that they contain unique, relatively low molecular weight[4] and varied biologically active substances. Lichen forming fungi synthesize many diverse secondary metabolites through different biosynthetic pathways including polyketide path (e.g. depsidones, usnic acid, depsides, xanthones), shikimic acid path (e.g. cyclopeptides, pulvinic acid derivatives) and mevalonic acid path (e.g. diterpenes, triterpenes, steroids)[5,6]. In addition, lichens have also been identified as a source of biologically active enzymes, polysaccharides and fatty acids that may have pharmacological potential[7]. In general, organic extracts resulted to be more active than aqueous extracts, which seems to be related to the poor solubility of lichen compounds in water.

In recent time a good number of research investigations were carried out to establish the positive biological properties of lichen extracts and their metabolites in different assays like antimicrobial, antioxidant, cytotoxic, antiproliferative, antiviral, genotoxic, anticancer, antimycotic, antiinflammatory, analgesic, antipyretic, antiradiation, allelochemical, antiherbivore etc.[8]. Hence, lichens can be utilized as a natural bioresource for antioxidant and antimicrobial agent due to their promising biological activity and protective antistress function.

Parmelia perlata colloquially known as Chadeela or Shilapushp belongs to the family Parmeliaceae. *Parmelia perlata* (Foliace lichen) thallus is dirty white or grayish brown nearby 5-10 cm long. It is usually used as a spice to enhance the taste and flavor of the foods. It has folkloric repute of cosmetics for skin bleach and has been prescribed to for the management of diarrhoea, dyspepsia, spermatorrhoea, amenorrhoea, dysentery and wound healing[9,10]. It has also been reported to possess the

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antiemetic, analgesic, antipyretic, aphrodisiac and astringent activity[10,11]. Its smoke relives the headache, heal the wound[12]. From literature survey, It has been found that compounds like tridecyl myristate, 3-ketooleanane, icosan-1-ol, usnic acid[13], parmelanostene, permelabdone[14], atranorin, lecanoric acid, orcin, erythrolein, azolitmin and spaniolitmint[15], salazinic acid, proto-lichesteric acid[16] have been isolated from *P. perlata*. *In vitro* antimicrobial, antiviral, antioxidant, cytotoxic, enzyme inhibitory, hypoglycemic and *in* vivo protection against mercury toxic effect on organs like pharmacological activities of ether, acetone, ethanol, methanol, n-hexane, aqueous extracts and crude polysaccharide fractions have also been reported[16]. It contain acidic substance that has been used as an antibiotic in several countries as a topical antibacterial agent for human skin diseases[17].

To date, a large number of different compounds have been isolated in Parmeliaceae family, mainly by high-pressure liquid chromatography (HPLC) methods. These methods, however, need standards for compound identification or isolation of extract constituents and their structure elucidation. The economic factor of very expensive HPLC grade solvents and long analysis time should not be neglected. In addition, the volatile part of the extracts might represent a minor proportion of the constituents and consequently identification by HPLC could be difficult or even impossible. On the other hand, good software for searching various MS libraries enables constituent identification by GC–MS for many known compounds without isolation and standards. Direct GC–MS analysis of a solvent extract is not straight forward because of the non-volatile residue, which remains undetected and stuck in the chromatographic system. However, GC–MS gave good results in analysis of volatile constituents of selected parmeliaceae lichens[18].

To the best of our knowledge, there have been no attempts at creating a database of GC–MS profiles of *P. perlata* volatiles. Herein for the first time, the composition of the volatiles from the pet ether, chloroform and acetone extracts of whole lichen are reported. Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. It is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc. Atranorin, salazinic acid, lecanoric acid, usnic acid are well known constituents of lichens and are reported to possess strong antibacterial, antifungal, antitumor and antioxidant etc. biological activities. Keeping in view of presence of above bio active compounds, the petroleum ether, chloroform and acetone extracts of this lichen were analysed for their antimicrobial and antioxidant activities.

MATERIALS AND METHODS

GC-MS Analysis

Plant material

The plant material *Parmelia perlata* (lichen) was collected from the hills of Uttarakhand (India) and the authenticity of the lichen was confirmed by Incharge of Harberium, Department of Botany, University of Rajasthan, Jaipur.

Plant extraction

The fresh plant material was dried at room temperature. The shade dried thalli of investigated lichen was powdered in grinder. 500gm of finely powdered material was taken in round bottom flask for successively extraction in petroleum ether, chloroform and acetone based on the polarity for 72 hrs on water bath separately. The extracts were filtered hot and evaporated to dryness by using a vacuum distillation unit.

Preparation of Plant extract

The above collected extracts were filtered through Whatman No. 41 paper (Merck, Mumbai, India) to remove particulate matter and were concentrated. The concentrated extracts were then subjected to GC-MS analysis.

Instruments and Chromatographic Conditions

Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. In this study, 1.5 µl of petroleum ether, chloroform and acetone extracts of *Parmelia perlata* was used separately for the carrying out the GC-MS analysis for various phytochemical compounds present in the lichen. GC-MS technique was carried out at AIRF centre, JNU, New Delhi. Analysis was performed using a Gas chromatography unit Shimadzu GCMS-QP2010 Plus comprising AOC-20i+s autosampler and gas chromatograph interfaced to a Mass spectrometer (GC-MS)instrument employing the following conditions: equipped with the RTX-5 capillary column; helium was used as carrier gas and an injection volume of 1.5 µL was employed (split ratio of 20:1) with column flow rate 1.21 mL/min; Injection temperature 250ºC; Ion source temperature 230 ºC; Interface temperature 260°C; Pressure at column inlet 85.7 kPa. Initial column oven temperature was 80ºC, held for 2 min; finally programmed to 280 ºC at a rate of 10 ºC/min, then held for 28min, run time 60min. The method of electron-impact ionisation was applied. All data were obtained by collecting the full scan mass spectra with scan speed 1250 within the scan range 40 to 600m/z.

Identification of components

Various components were identified by different retention times which are detected by mass Spectrophotometer. The chromatogram a plot of intensity against retention time was recorded by the software attached to it. From the graph the compounds are identified comparing the data with the existing software libraries like WILEY8.LIB, NIST11.lib, NIST11s.lib, FFNSC 2.lib. and mass spectra of standard. The Name, Molecular weight and structure of the components of the test materials were ascertained.

Antimicrobial Activity

Microorganism used

Clinical laboratory bacterial isolates viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Streptomyces grieveces*, *Escherichia coli* and fungal isolates viz. *Aspergillus niger*, *Trichoderma reseei*, *Fusarium oxysporium* and *Penicillium funiculosam* were collected from the stock cultures of Microbiology laboratory, SMS Medical college, Jaipur, India.

Preparation of extract solution

In vitro, experiments were performed by dissolving the crude extracts in dimethylsulfoxide (DMSO). The pet ether, chloroform and acetone extracts were diluted in 100% DMSO at the concentration of 5 mg/ml for antimicrobial sensitivity test.

Culture and Maintenance of Bacteria

Above mentioned pure cultures were used as indicator organisms. These bacteria were grown in nutrient agar medium prepared by autoclaving 8% Nutrient agar (Difeco-Laboratories, Detroit, USA) in distilled water at 15 lbs psi for 25-30 min and incubating at 37ºC for 48h. Each bacterial culture was maintained on the same medium after every 48 h of transferring. A fresh suspension of test organism in the saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude Pet ether, chloroform and acetone extracts was studied against gram +ve and gram -ve bacterial strains by the agar well diffusion method[19]. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The Mueller Hinton agar was melted and cooled to 48-50ºC and a standardized inoculum (1.5x108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test extracts (40 µL) were introduced in the well (6 mm). The plates were incubated overnight at 37ºC. At the end of the incubation period, the antimicrobial spectrum of extracts was determined for the bacterial species in terms of zone size around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics streptomycin. For each bacterial and fungal strain controls were maintained where pure solvents were used instead of the extracts. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean ± SD are presented for evaluating the antibacterial activity of the extracts.

Determination of Antifungal Assay

Agar well diffusion method was used for the assessment of antifungal activity of the experimental plant extracts[20].The yeasts and saprophytic fungi were subcultured onto Sabourand's Dextrose agar, SDA (Merck, Germany) medium and respectively incubated at 37 ºC for 24h and 25 ºC for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffered saline) and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension was rolled on the surface of agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 40 µL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37 ºC, After incubation of 24 h bioactivity was determined by measuring the diameter of inhibition zone (in mm) around the each well. All experiments were made in triplicate and mean ± SD are presented for evaluating the antifungal activity of the extracts.

The standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi) was used (at concentration of 1mg/mL) in order to compare the results at mid well of plates. The results showed that the standard antibiotics had stronger activity than the extracts.

Activity index can be measured by applying following formula

ZOI for Sample/ ZOI for standard

Antioxidant Activity

In present work, we have carried antioxidant activity by employing DPPH free radical scavenging activity and Ferric reducing antioxidant power methods.

Chemicals

The stable free radical DPPH, TPTZ (2, 4, 6-tris (2pyridyl)-*s*-triazine) were obtained from Sigma chemicals (Sigma-Aldrich GmbH, Sternheim, Germany).

DPPH Assay

The different extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical $DPPH^{\cdot}$ (2, 2-diphenyl-1-picrylhydrazyl)[21]. 0.75 mL of a methanolic solution of the extract at different concentrations ranging from 1 to 500 µg mL^{-1} (methanol as a control) was mixed with 1.5 mL of a DPPH methanolic solution (20 mg L^{-1}). The absorbance was measured at 517 nm after 20 min of reaction. The percent of DPPH decoloration of the sample was calculated according to the formula

$$\% \text{ Decoloration} = [1 - (Abs_{\text{SAMPLE}}/Abs_{\text{CONTROL}})] \times 100$$

The decoloration was plotted against the sample extract concentration and a logarithmic regression curve was established in order to calculate the IC_{50} (inhibitory concentration 50), which is the amount of sample necessary to decrease by 50% the absorbance of DPPH. The results are expressed as antiradical efficiency (AE), which is 1000-fold inverse of the IC_{50} value ($AE = 1000/IC_{50}$)[22].

FRAP Assay (Ferric reducing ability of Plasma)

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. The Fe++ interacts with TPTZ providing a strong absorbance at 593 nm. The method was performed by the protocol of Varga *et al*, 1998[23].

Plant sample (1g) were cut into small pieces and mashed with a cool mortar and pestle using quartz sand and 9 mL cool 0.1M phosphate buffer was added. (pH 7.6, containing 0.1mM EDTA). Each of the test mixture was filtered through a filter paper and centrifuged at 15,000 rpm for 10 min. The supernatant was used for the measurements of OD (optical density) at 593nm after make up to 5mL volume.

Calculation

The relative activities of samples were assessed by comparing their activities standard curve of ferrous sulphate. Ferrous sulphate was dissolved in distill water and different concentrations (100-1000µM/L) was used for the measurement of OD. Standard FRAP reagent was used as blank.

Table 1

Solvents	Peak #	R. Time	Name of compound	Molecular formula	Molecular weight	Structure	Nature of compound
Pet-ether		12.208	4-hydroxy-3-methoxy-benzaldehyde	$C_8H_8O_3$	152		Phenolic
		13.697	Methoxyolivetol	$C_{12}H_{18}O_2$	194		Phenolic
		14.016	Benzoic acid, 3-formyl-2,4-dihydroxy-6-methyl-, 3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenyl ester (Atranorin)	$C_{19}H_{18}O_8$	374		Depside
		14.421	1,3-Benzenediol, 5-pentyl- (Olivetol)	$C_{11}H_{16}O_2$	180		Polyphenolic
		16.357	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256		Fatty acid
		16.606	3,6-Dipropyl-2,5-dimethylpyrazine	$C_{12}H_{20}N_2$	192		Benzopyrans
		18.097	Hexadec-(9Z)-enal	$C_{16}H_{30}O$	238		Unsaturated Carbonyl
		18.254	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	$C_{18}H_{32}O_2$	280		Polyunsaturated ω-6 fatty acid
		21.509	9-Octadecenoic acid (Z)- (Oleic Acid)	$C_{18}H_{34}O_2$	282		Unsaturated ω-6 fatty acid
		22.809	2,4-Pentanedione, 1-(7-acetyl-4,6-dihydroxy-3,5-dimethyl-2-benzofuranyl)- (Decarbousnic acid)	$C_{17}H_{18}O_6$	318		Benzofuran
		23.340	(+)-Usnic acid	$C_{18}H_{16}O_7$	344		Dibenzofuran
		32.034	D-Friedoolean-14-en-3-one	$C_{30}H_{48}O$	424		Triterpene
		32.728	Lupeol	$C_{30}H_{50}O$	426		Triterpene

	35.497	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-(Simiarenol)	$C_{30}H_{50}O$	426		Triterpene
	39.835	D:A-Friedooleanan-3-one (Friedelin)	$C_{30}H_{50}O$	426		Triterpene
Chloro-form	12.233	Pyrimido[1,2-a] azepine, 2,3,4,6,7,8,9,10-octahydro-	$C_9H_{16}N_2$	152		Amidine
	14.230	Ethyl 2,4-dihydroxy-6-methylbenzoate (*o*-Orsellinic acid, ethyl ester)	$C_{10}H_{12}O_4$	196		Hydroxybenzoic acid derivative
	14.351	Benzoic acid, 3-formyl-2,4-dihydroxy-6-methyl-, methyl ester (Methyl haematommate)	$C_{10}H_{10}O_5$	210		Hydroxybenzoic acid derivative
	14.764	5,7-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$	192		Coumarin
	18.142	9,12-Octadecadienoic acid, methyl ester (Linoleic acid, methyl ester)	$C_{19}H_{34}O_2$	294		Polyunsaturated fatty acid
	20.099	Benzoic acid, 2,4-dihydroxy-6-(2-oxoheptyl)-, 4-carboxy-3-hydroxy-5-pentylphenyl ester	$C_{26}H_{32}O_8$	472		Depside
Acet-one	3.411	(R)-(-)-2,2Dimethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$	132		Alicyclic
	6.923	Dodec-1-ene	$C_{12} H_{24}$	168		Alkene
	9.218	Butanal, 3-methyl-	$C_5H_{10}O$	86		Aldehyde
	9.892	5-Methyl-1,3-benzenediol (Orcinol)	$C_7H_8O_2$	124		Poly-phenolic
	12.207	Benzaldehyde, 2,4-dihydroxy-6-methyl-(*o*-Orsellinaldehyde)	$C_8H_8O_3$	152		Hydroxy benzal-dehyde
	12.319	Pentadecane <n->	$C_{15} H_{32}$	212		Alkane
	13.158	1,13-Tetra-decadiene	$C_{14}H_{26}$	194		Alkene
	14.239	Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester	$C_9H_{10}O_4$	182		Polyphenolic
	16.563	Hexadecane <n->	$C_{16} H_{34}$	226		Alkane
	18.173	Z-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254		Ester
	18.406	Octacosane	$C_{28}H_{58}$	394		Alkane

19.229	Lauraldehyde 2,4-dinitrophenylhydrazone	$C_{18}H_{28}N_4O_4$	364		Phenylhydrazone
20.895	Tetracosyl heptafluoro-butyrate	$C_{28}H_{49}F_7O_2$	550		Ester

Table 2: Frap assay of *Parmelia perlata* lichen extracts

Lichen extract	Absorbance (OD) at 593nm	Frap value (μm/l/g)
Petroleum ether	0.340	430
Chloroform	0.442	540
Acetone	0.500	600

RESULTS AND DISCUSSION

GC- MS analysis was carried out on petroleum ether, chloroform and acetone extracts of *P. perlata* whole lichen and 34 phytoconstituents were detected. The active principles with their retention time (RT), molecular formula, molecular weight, structure and nature of compounds are presented in Table-1. The petroleum ether, chloroform and acetone extracts chromatograms showed the presence of 15, 16 and 21 major peaks respectively.

The GC/MS profiles of pet-ether showed the presence of Simiarenol (27.84%), Friedelin (18.41%), Atranorin (13.26%), Methoxyolivetol (7.88%), 4-hydroxy-3-methoxy-benzaldehyde (4.11%), (+)-Usnic acid (4.06%), D-Friedoolean-14-en-3-one (4.02%), Hexadec-(9Z)-enal (3.13%), Oleic Acid (1.92%), Olivetol (1.58%), Palmitic acid (1.20%), Decarbousnic acid (1.09%), Linoleic acid (1.01%), Lupeol (.84%), 3,6-Dipropyl-2,5-dimethylpyrazine (.82%). Other constituents were <0.80%.

The detailed tabulation of the GCMS analysis of all the 3 extracts revealed that in case of chloroform and acetone chromatogram's most of the peaks are of similar nature to that of petroleum ether extract, except for few phytocompounds which were extracted only in chloroform are Pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro- (3.91%), *o*-Orsellinic acid, ethyl ester (1.33%), Methyl haematommate (5.06%), 5,7-Dihydroxy-4-methylcoumarin (1.43%), Linoleic acid, methyl ester (2.32%), Benzoic acid, 2,4-dihydroxy-6-(2-oxoheptyl)-, 4-carboxy-3-hydroxy-5-pentylphenyl ester (1.11%). similarly in case of acetone thirteen compounds (R)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol (3.66%), Dodec-1-ene (2.58%), Butanal, 3-methyl- (1.67%), Orcinol (17.76%), *o*-Orsellinaldehyde (1.64%), Pentadecane <n-> (2.86%), 1,13-Tetradecadiene (1.26%), Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester (1.04%), Hexadecane <n-> (3.31%), Z-10-Tetradecen-1-ol acetate (3.95%), Octacosane (1.37%), Lauraldehyde 2,4-dinitrophenylhydrazone (1.47%), Tetracosyl heptafluorobutyrate (1.51%) were different rest all other peaks were of similar in nature to the peaks of pet-ether and chloroform extracts.

In present study, Significant antibacterial activity (Table 3) screened towards *B. subtilis*, whereas chloroform extract of lichen produces a large ZOI of 18±2.0 as compared to its acetone extract (12±1.0) and pet ether extract (6±0.3) while the standard antibacterial substance Streptomycin produces ZOI of 20mm. Against *E. coli* also observed moderate activity of chloroform, acetone and pet ether extracts expressing inhibition zone of 10±2.0, 8±1.0 and 6±0.5 respectively. There was also a considerable activity towards *Streptomyces grieveces* and *Staphylococcus aureus*.

On the other hand, the results of antifungal activities (Table 4) showed that pet ether and acetone extracts possesses potential activity against *P. funiculosam* exhibiting inhibition zone of 10±0.5 and 6±0.2 respectively while no inhibition observed around chloroform well. Against *A. niger* and *F. oxysporium* observed moderate antifungal activity of extraxs. The standard antibiotic ketoconazole produces ZOI of 20mm. No fungal inhibition screened towards *T. reseei* strain by any extracts under experimental conditions.

The free radical ($DPPH^{\cdot}$) scavenging activity expressed as AE ranged from 20.83 to 35.71 as shown in table (5). Acetone extract demonstrated a very potent activity with IC_{50} value of 28 μg mL^{-1} as compared to pet ether (31 μg mL^{-1}) and chloroform (48 μg mL^{-1}) extracts.

In FRAP assay, frap values were calculated after measuring OD for each extract. The values are directly correlated with antioxidant activity as the higher the FRAP value the greater is the antioxidant activity. The frap values ranged from 430 μm/l/g to 600 μm/l/g as shown in table (6). Highest activity was observed in acetone extract (600 μm/l/g) and lowest in pet ether extract (430 μm/l/g).

The extracts exhibiting the highest radical scavenging activity were those with the highest phenolic content. Thus, highest level of phenolics found in acetone extract which exerts strongest antiradical activity. Above results are better than methanolic extract (IC_{50} value 54 μg mL^{-1}) that has been reported in literatures[1].

The ferric reducing antioxidant potential (FRAP) assay is a simple and inexpensive procedure that measures the total antioxidant levels in a sample. FRAP measures the ability of the extract to donate electron to Fe (III). Subsequently, the Fe++ formed may interact with TPTZ providing a strong absorbance at 593 nm.

All the obtained chromatograms of the examined extracts showed only a few prominent peaks corresponding to components present on significant amount. The observed mono-aryl compounds in the lichen extracts could be both considered as authentic lichen metabolites but also as possible products of hydrolysis, transesterification and decarboxylation of depsides during the extraction and analysis processes[24].

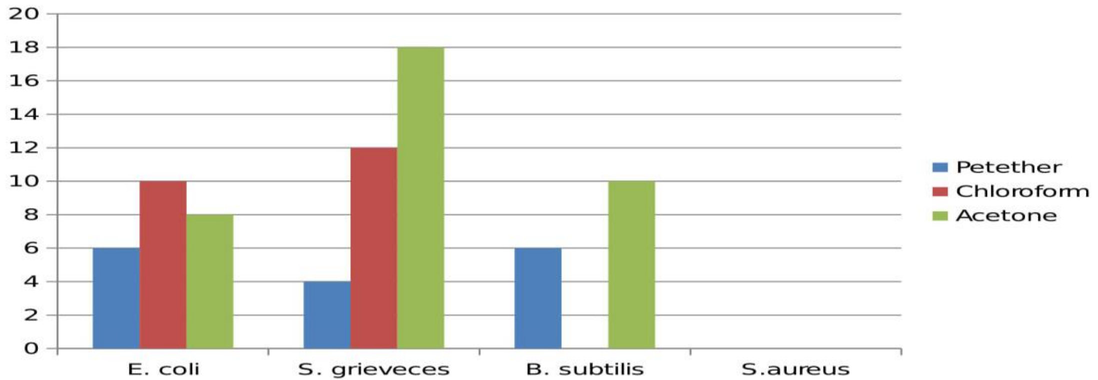

Figure 1: Antibacterial activity of extracts of *P. perlata*

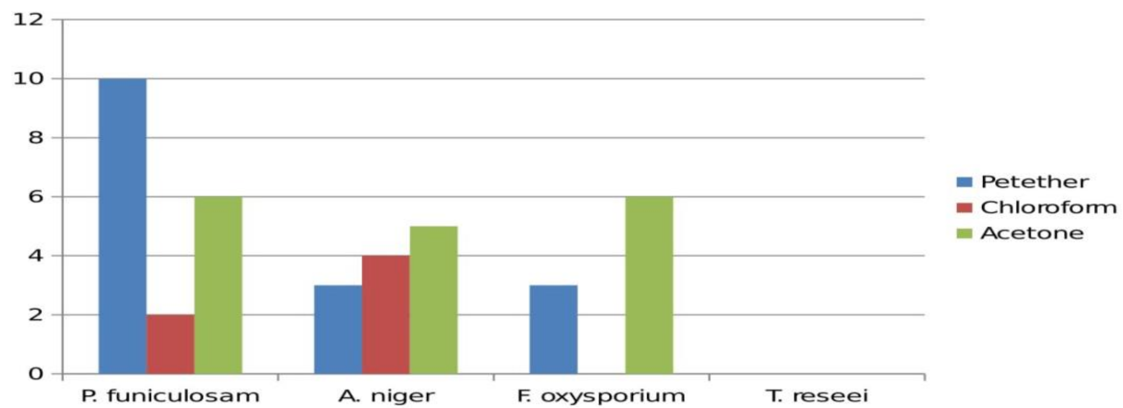

Figure 2: Antifungal activity of extracts of *P. Perlata*

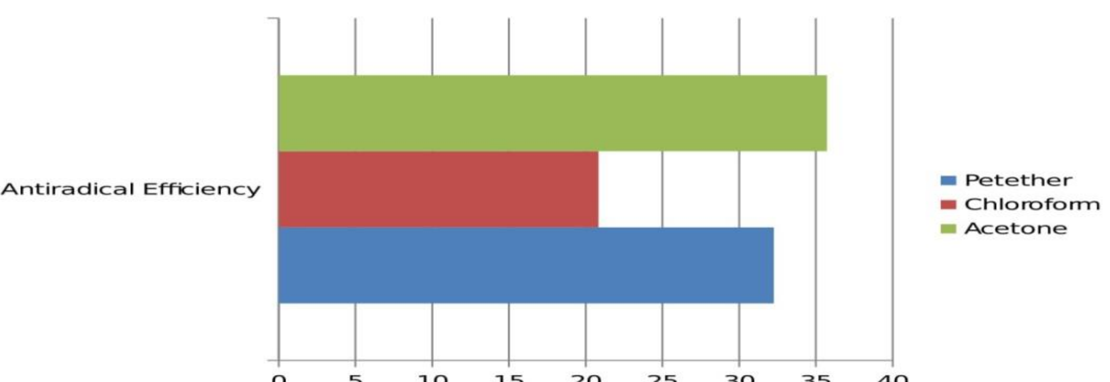

Figure 3: Antiradical efficiency of different extracts of *P. perlata*

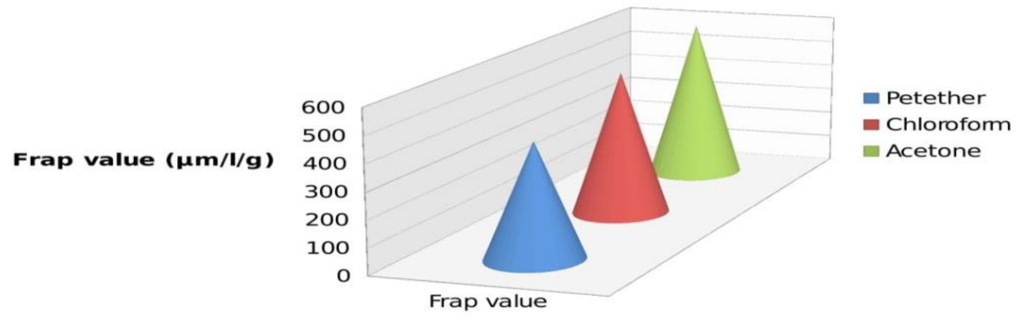

Figure 4: Frap assay of *P. perlata* lichen extracts

The high contents of 5-alkyl resorcinols (orcinol and olivetol) in the examined extracts indicate that the related carboxylic acids (orsellinic and olivetonic acid) readily decarboxylate, contrary to methyl β-orcinolcarboxylate, which is stable under the above-specified experimental condition[18].

The antimicrobial activity of the lichen was examined in several studies in various ways and with different results. This may be due to many factors such as, different quantity of the same active component in lichen extracts, different components involved in antimicrobial actions, different locations of lichen sampling, and different sensitivity of tested microorganisms or different methods of testing, different bacterial strains. Ranković and colleagues observed the dependence of the level of the antimicrobial activity of the lichen on the solvent used in extraction[25].

Many species of genus *Parmelia* exhibited strong antimicrobial activity[26]. There have already been many reports about the antibacterial and antifungal activities of different extracts of above lichen[11,27-29]. Ethanolic extract of *P. perlata* is reported to be active against *Staphylococcus aureus* and the efficiency of the extract increased in the presence of colloidal silver[29]. The solvent system used in this study for extraction of lichen has not been tried before for antmicrobial activity against the test bacteria *Streptomyces grieveces, Bacillus subtilis* and fungas *Penicillium funiculosam*. Further research needs to be done to determine the compounds that are responsible for antimicrobial activity against used pathogens. Thippeswamy et al. found the compounds which are responsible for its antimicrobial property[28].

The better antimicrobial activity of the *P. perlata* was attributed to the presence of some of its major components atranorin (antibacterial), ethyl orsellinate (antifungal), methyl hematommate (antifungal), usnic acid (antibacterial, antifungal), friedelin (antifungal, antibacterial), taraxerone (antifungal). The synergistic or antagonistic activity between some components may affect the observed antimicrobial activity[30], which exerts its toxic effects against microorganisms through the disruption of bacterial and fungal membrane integrity[31].

Previously, numerous lichen compounds were screened for antimicrobial activity in search of the new antimicrobial agents[32-34]. Antibacterial activity was stronger than antifungal. This observation is in accordance with other studies[35,36] focused on the antimicrobial activity which have demonstrated that bacteria are more sensitive to the antimicrobial activity than the fungi due to differences in the composition and permeability of the cell wall.

There are many different antioxidant components in plants and it is relatively difficult to measure each antioxidant component, separately. Owing to the complexity of the oxidation and anti-oxidation processes, no single testing method was found to be capable of providing a comprehensive picture of the antioxidant profile. Therefore, in the present investigation a multi-method approach was necessary to assess the antioxidant activity of biological sample[37].

DPPH Assay has also been used to quantify antioxidants in complex biological systems in recent years. Free radicals are reactive species with an unpaired electron. Antioxidants are able to reduce free radicals by donating an electron or hydrogen atom to the free radical. The hydrogen atom transfer (HAT) activity of plant extracts was studied using the DPPH free radical and its reaction with a phenolic antioxidant can be written as:

$$DPPH\bullet + ArOH \longrightarrow DPPH_2 + ArO\bullet$$

The DPPH radical, which has a deep violet color, reacts with hydrogen donor species such as phenolics, flavonoids and upon receiving a proton loses its color and becomes yellow. In fact, number of previous studies found that the lichens where found the higher content of phenols exert stronger antioxidant activity[38,39], which means that phenols are important antioxidants. It has been reported that the antioxidant activity of phenolics was mainly due to their redox properties, hydrogen donators and single oxygen quenchers[40].

The reducing power of the extracts increased in a concentration-dependent manner[41]. Therefore, reducing power evaluation might be taken as important parameter for the assessment of antioxidant activity.

Antioxidant effect of some other lichen compounds was also studied by other researchers. Jayaprakasha and Rao previously reported that methyl orsenillate, orsenillic acid, atranorin and lecanoric acid are all good antioxidants[42]. As per earlier reports it was observed that the presence of chemical constituents such as atranorin, lecanoric acid, salazinic acid, stictic acid, usnic acid also resulted in antioxidant and antimicrobial properties[16,43,44]. Hafizur Rahman et al. found antioxidant activity in the methanol extracts of *P. perlata* using free radical scavenging method[1].

Various researchers have found high correlations between antioxidant activity and phenolic content[45,46]. Interestingly, Odabasoglu *et al.*[46] reported that in some lichen extracts there was no correlation between total phenolic content and antioxidant activity, suggesting that the antioxidant activity of different lichens may also depend on other, non-

Table 3: Antibacterial activity of extracts of *P. perlata* at 40µl 5mg/ml concentration

S.No.	Strains	Lichen extracts					
		Petroleum ether		Chloroform		Acetone	
		ZOI±SD in mm	AI	ZOI±SD in mm	AI	ZOI±SD in mm	AI
1.	*Escherichia coli*	6±0.31	0.3	10±.97	0.5	8±.77	0.4
2.	*Streptomyces grieveces*	4±0.15	0.2	–		–	
3.	*Bacillus subtilis*	6±0.21	0.3	12±0.98	0.6	18±1.0	0.9
4.	*Staphylococcus aureus*	–		–		10±.67	0.5

Table 4: Antifungal activity of extracts of *P. perlata* at 40µl 5mg/ml concentration

S.No.	Strains	Lichen extracts					
		Petroleum ether		Chloroform		Acetone	
		ZOI±SD in mm	AI	ZOI±SD in mm	AI	ZOI±SD in mm	AI
1.	*Penicillium funiculosam*	10±0.55	0.5	–		6±0.22	0.3
2.	*Aspergillus niger*	3±0.15	0.15	2±0.03	0.1	5±0.21	0.25
3.	*Fusarium oxysporium*	3±0.25	0.15	4±0.02	0.2	6±0.17	0.3
4.	*Trichoderma reseei*	–		–		–	

Table 5: Antiradical Efficiency (AE) of *Parmelia perlata* extracts using DPPH Radical Scavenging Assay

Lichen extract	Petroleum ether	Chloroform	Acetone
Antiradical Efficiency	32.25	20.83	35.71

phenolic components. The present results showed that standard antioxidants had stronger activity than the tested extracts, probably because the former contain more purified compounds than the latter.

CONCLUSION

Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many researchers[47-49]. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important[50].It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects[51].

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