Lichen collections from Nilgiris of Western Ghats in Tamil Nadu and screening for antimicrobial, antioxidant efficacy of some selected species

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Abstract

Lichens are unique synergestic relationship between heterotrophic mycobionts (fungal partner) and phototrophic phycobionts (algal partner). Tamil Nadu recorded the ample number of lichens represented by 785 taxa in India. One reason for the luxuriant lichen population in the state is due to the lengthy stretch of Western Ghats and substantial biodiversity hotspots such as Nilgiri, Anamallais, Nilgiris, Palani and Shevory hills. Due to the fact that lichens are harvested in huge amounts for commercial marketing which threaten their diversity. However, the lichen diversity reports in many parts of Western Ghats are out of date. Therefore, the present study was intended to reassess the lichen diversity in this region and survey study has triggered with Nilgiris (Udhagamandalam). The chosen study area in Nilgiris has rich in lichen diversity and some of the Parmelioid lichens are available in huge quantity. The following are the major objectives of the present study such as reassessment of lichen diversity in Nilgiris hills, phytochemical total phenolic compound analysis and Bioprospection studies or biomedical application on selected lichens, by antimicrobial, and in vitro anti-oxidant assay. The lichen survey in seven localities of Nilgiris resulted in 39 species belonging to 23 genera and 9 families. The locality Doddabetta situated between 2250 – 2637 m has the richest lichen species with 10 species. Among the different genera Parmotrema with 13 species exhibited their dominance in the study area. The studies on antimicrobial assay of acetone extracts of four foliose and two fruticose lichen species resulted to show that a better pathogenic control was observed in acetone extracts of P.austrosinense with 13 mm zone of inhibition against Candida albicans and 10.67 mm dia against Staphylococcus aureus. The extracts of Ramalina conduplicans of fruticose lichen showed better antibacterial activity against Staphylococcus aureus. It was inferred that all the chosen lichen extracts except Usnea stigmatoides showed significant antimicrobial activity against clinical test strains. The Parmotrema tinctorum, P.austrosinense, and P.reticulatum species exhibited strong antioxidant activity.A higher absorbance value was recorded for P.tinctorum and P.austrosinense with the phenolic concentrations of 447.2339 ug/mL and 405.8596 ug/mL in total phenolic content assay.

Key words: Antibacterial, Antioxidant, lichen extract, P.austrosinense, P.reticulatum, Ramalina conduplicans, and Usnea stigmatoides.

INTRODUCTION

The Tamil Nadu part of Western Ghats has 3 National Parks and 5 wildlife sanctuaries. The Nilgiris is one of the high ranges (2637 m) bounded by the state of Karnataka in the north and Kerala in the western corner of Tamil Nadu in south India. The Ghats covers 10% of total land surface in India with diverse vegetation with enormous species diversity. In Tamil Nadu part of the Nilgiris possesses a Wild life Sanctuary (Mudumalai national park and Nilgiris Bisophere reserve and a National Park (Silent Valley National Park). The Nilgiris district possess flowering plants rich district in Tamil Nadu. The earliest lichenological study on Himalaya was explored by Quraishi (1928) and Chopra (1934) in India. The father of Indian Lichenology, D.D. Awasthi (1957) laid the strong foundation for lichenologists in India to study them in a systematic way and his findings of lichen collections from Western Ghats were published in 1957. The first review on the diversity distribution of lichen species of Western Ghats was consolidated by Singh (1964) followed by Awasthi (1965). The school of lichenology, Lucknow has explored lichen diversity distribution from Nilgiris and Palani hills. Nayaka and Upreti (2011) enumerated a total number of 657 taxa from Nilgiris and Palani hills of Western Ghats (Nayaka and Upreti, 2005). A total of five new crustose species additions to Western Ghats namely *Lecanora galactiniza, Normandina pulchella, Placynthiella icmalea, Trapelia placodioides,* and *Trapeliopsis flexuosa* were updated by Upreti *et al*. (2008). It has been estimated that there are 1096 lichen species belongs to 188 genera and 50 families in Western Ghats. The estimation that accounted around 47% of total lichen populations are confined to India (Bhat *et al*., 2011). Tamil Nadu recorded maximum diversity of *Usnea* species with the total of 33 out of 38 species followed by Kerala, Karnataka and Maharashtra states of Western Ghats in South India (Mesta *et al*., 2015). However, the lichen status in Nilgiris hills has not been documented in recent years. In addition, the Nilgiris is very delicate and flora and fauna often experiences sudden extinction due to the influence of natural disasters such as flash floods, landslides, various renovation activities etc.. Thus, documentation of lichen diversity in Nilgiris is today's urgent need. Therefore, this study has triggered us to explore qualitative assessment on representative lichen collections from Nilgiris of Western Ghats and establish their associated medical applications based on the *in vitro* experiments such as antimicrobial and antioxidant properties prior to planning the conservation strategies of existing lichen biodiversity in the forest reserve. With this conviction, the overall objective of the present study intends to assess the diversity and distribution of various lichen species in variety of habitats found from the foot to higher elevations of Nilgiris hills and antimicrobial properties of lichen compounds.

MATERIALS AND METHODS

Experimental Site

The Nilgiris reserve lies between 76°- 77°15'E and 11°15' - 12°15'N and stretches over an expanse of 5 520 sq. Km with elevation ranges from 1000 to 2637 Km. Mean annual rainfall is between 500 and 4500 mm and major reservoir Bhavani river. It is located at the junction of Eastern and Western Ghats of Tamil Nadu, India. Over the period of eight months during October 2018 – June 2019, intensive and extensive field explorations were carried out in the various sites (Fig. 1.1.) of Nilgiris hills. Many factors enable the lichens to be classified since they are substrate specific and in some respects they are dynamic in their form, family and altitude. Thus, the lichens were collected from different substratum such as the bark (corticolous) of trees, twigs at reachable height, rock (saxicolous) and soil (terricolous) samples. Foliose and fruticose lichens were scraped out of substratum while crustose lichens were collected along with the substratum with the help of chisel. In order to avoid damages to the bark of trees, only superficial layer was scrapped while collecting lichens. The collected lichens were dried and preserved in herbarium packets indicated with appropriate labels. All the specimens and a set of representative of some selected lichens utilized for antimicrobial and antioxidant studies were also stored at Biomedical Research Lab (BRL) Bharathiar University, Coimbatore. The average height of hills was about 2130 meters. The representative lichen samples were identified by studying their morphological, anatomical and their chemical features. The morphology of lichen specimen was examined under a stereomicroscope while hand cut sections of the thallus, apothecia and spore structures were studied under BX compound microscope. Colour tests were carried out by applying reagents to lichen thallus with 10% KOH (K), commercial bleaching powder (C) and 1% of phenylene di amine. The lichen compounds were detected by TLC methods (Orange *et al*., 2001) using solvent system A on precoated silica gel plates. Recent floras and literature were followed for identification from Awasthi (1988, 1989, 1991, 2007). The identification of specimens was confirmed by matching them with well identified herbarium specimens and type material stored at National Botanical Research Institute, Lucknow (LWG).

The physiographic localities of lichensin Nilgiris include **Coonoor, Doddabetta, Gudalur, Hindustan Photo Film (HPF), Kateri, Manjoor, and Valley view** showed altitude of height above 830 m with abundant lichens. The survey requirement in the expanded range showed support from Tribals, Hill Guard, forest conservation and department guards.

Preparation of extracts/fractionates:

The required amount of lichen sample was brushed to clean manually to separate aerosol and soil debris. The sample was washed in flowing tap water followed by rinsing with sterile distilled water and then dried in hot air oven to obtain a constant weight. Cold percolation method using Soxhlet apparatus was followed for solvent extraction by approximately, five grams of oven dried sample was packed in fat free thimble. The fat content was first extracted by

siphoning petroleum ether. After petroleum ether extraction, the lichen compounds were extracted by siphoning with acetone. The acetone was removed under reduced pressure to get only the powdered lichen compound. Attempts were made to identify the active compounds of lichen extract from the lichen species through chromatographic methods (TLC) (Orange *et al*., 2001; Cordeiro *et al*., 2004).

Antimicrobial test

The lichens used for the antimicrobial and antioxidant screening study are presented in Fig. 1.2. A Gram-positive bacterium, *Staphylococcus aureus*, a yeast *Candida albicans* and clinical test strains of Gram negative bacteria such as *Escherichia coli, Kelbsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa and Salmonella typhi* were obtained from multi specialty clinic in Coimbatore. Clinical test strains of bacterial cultures were grown in MHA broth and incubated at 37°C for 24 hrs while fungal cultures were grown in Potato dextrose broth and left at room temperature for 24 hrs. A standard concentration of 10 mg/mL of the lichen extract was prepared using sterile Di Methyl Sulphoxide (DMSO) at a concentration of 20% in sterile distilled water. About 10 ml of the pure culture was centrifuged washed twice by using sterile physiological saline, then the suspension was adjusted to optical density value of 0.1 at 600 nm, equivalent to a cell population of 10^6 cells/mL standard. The suspension was kept in the test tube and stored at 4°C until used.

The assay for antibacterial activities of each extract was determined by disc diffusion method (Kirby *et al*., 1957; Bauer *et al*., 1959, 1966; Stoke and Ridgway, 1980; Chauhan and Abraham, 2013). Bacterial and fungal cultures were seeded into Mueller Hinton agar and Sabourad agar plates. Sterile disc measuring 6 mm diameter was purchased from Hi Media laboratories Mumbai. About 20 µL of the extract was introduced into sterile discs using micropipette and allowed them to dry. The discs were loaded on to MHA and PDA plates previously swabbed with overnight culture. The plates were kept for overnight in an incubator at 37ºC for bacterial test strains and 25ºC for fungal culture. Extracts with zone of inhibition at this concentration were regarded as having antimicrobial properties while other with no zone of inhibition at this concentration were disregarded as explained by Hirasawa *et al.*(1999). The experiments were carried out in triplicates. The sterile disc impregnated with DMSO was used as negative control while chloramphenicol and flucanazole (30mg/ml) added disc was used as positive control for bacteria and fungi respectively. The zones of inhibition produced were measured in millimeters (mm), while those in with no zone of inhibition were regarded as negative (no activity).

***In vitro* antioxidant activities:**

In order to study the antioxidant activity of lichen metabolites, studies were to carried out with acetone extracts of selected lichens against stable 2, 2 diphenyl 2 picrylhydrazyl hydrate (DPPH) as described by Prior *et al.*(2005). Different concentration of bioactive compounds were prepared at 25, 50, 75 and 100 µg/mL and tested against 2.0 mL of DPPH in methanolic solution. The reaction mixture was maintained in dark room at 25°C. After incubation, the percentage of

inhibition was measured using UV Visible Spectrophotometer at 517 nm. DPPH radical-chelating activity was calculated by the following formula

$$\text{Radical chelating Activity (\%)} = 100 - \left(\frac{Tc - Ts}{Tc} \right) X\ 100$$

Where T_C = the absorbance of the test control and T_S = the absorbance of test sample (in the lichen Extract).

Determination of Total phenolic content

The total phenolic content of the lichen acetone extracts was carried out by Folin–Ciocalteu method, as described by Slinkard and Slingleton (1997) which is based on the abilitiy of phenolic compounds of lichen extracts to take part in redox reactions to form a phosphotungstic/ phosphomolybdenum complex indicated by blue chromophore. Higher the abosrbtion indicates higher the concentration of phenolic compounds. The lichen compounds were diluted by dissolving 1 mg concentration of lichen extracts in 1 mL of acetone from which varying concentrations such as 0.025, 0.050, 0.075 and 0.100 mg/mL extracts was taken and added to 2.5 mL Folin-Ciocalteu reagent (1:1) and 2 mL Na CO (2%). The absorbance was read at 760 nm using a UV–Vis spectrophotometer, and the standard gallic acid was used.

Statistical analysis

DPPH radical scavenging test was carried out in triplicate. The required amount of lichen extract found to inhibit DPPH concentration by 50%, IC_{50} was graphically determined using Ms-Windows based graphpad Instat (version 3) software. Results were expressed as graphically / mean± standard deviation.

RESULTS AND DISCUSSION

Distribution of lichen diversity in Nilgiris

The diversity of lichen study at seven different sites around Nilgiris in Western Ghats of Tamil Nadu, India is represented by the occurrence of totally thirty nine species belongs to 22 genera and 9 families (Table 1.1). Among 39 species, foliose form was leading with 32 (82.05%) species followed by fruticose and crustose lichens (Fig. 1.2). There were four fruticose lichen species detected from Gudalur, Coonoor and Doddabetta (1072 – 2250 m). Similar study conducted by Nayaka and Upreti (2011) reported that crustose lichen was more prevalent in Western Ghats with a total number of 618 taxa than foliose and fruticose that represented 269

and 62 taxa respectively. The predominant family diversity showed that the Parmeliaceae was leading by 61% found at an altitude above 1850 m MSL (Fig. 1. 3). The elevation at above 2250 m MSL majority (25.64 %) of lichen diversity has been detected from Doddabetta. Similarly, about 23.08% of lichen diversity was found to be better adapted to survive at 2209 m MSL at H.P.F-Thalaikunda in Nilgiris. In Manjoor, lichen species were found at 1831 m with 6 (15.38%) species. The data investigated in our present study is in accordance with the general conclusion to prove that lower the elevation fewer would be the lichen species to occur by showing 0.25% prevalency at lowest point 830 m MSL from Kateri in Nilgiris (Fig. 1.4; 1.5.). The distribution of lichen substrate specificity showed that corticolous and saxicolous were predominant with the mean average of 46.15% each. Joseph *et al.* (2011) reported 5 new macro lichens additions in Nilgiris were such as *Heterodermia microphylla, Parmotrema indicum, Parmotrema melanothrix, Pyxine cocoes,* and *Usnea eumirtrioides*. Thin layer chromatographic identification test results are presented in (Fig. 1.6).

Determination of antimicrobial test

The antibacterial potency of acetone extracts of lichen four *Parmotrema* sp.. one single *Ramalina conduplicans* and *Usnea stigmatoide*s against the test microorganisms is shown in Table 1 (Fig. 3). The acetone extracts of all the tested lichen species were highly significant in inhibiting the growth of test strains. Acetone extracts of *P. austrosinense* showed the maximum antimicrobial activity to arrrest the growth of *Candida albicans* with the inhibition zone of 13.67±0.58 mm while the standard antibiotic showed 26.00±0.56 mm followed by the acetone extract of *P.tinctorum* with the value of 10.33±0.58 mm. Our results are in accordance with those of Ganesan *et al*. (2015) reported that benzene extract of *P. austrosinense* showed higher activity against *E.coli* and *S. typhimurium* with the zone of inhibition measured was 15.27 mm and 12.23 mm respectively. The lichen P.nilgherense collected from Nainital, Kumaun Himalaya by Sati and Savitha (2011) reported that the chloroform extract showed potential antibacterial activity against *E.coli* followed by Ethanol and Methanolic extract with the zone of inhibition of 29±1.3, 15±0.3, and 19±1.3 respectively. Our results showed that the acetone extract of *P.austrosinense* significantly inhibited *E.coli, and S.typhii* with the inhibition zone of 11.33±0.58 and 10.67 ±0.58 respectively. Chloroform and acetone of weak and moderate polar solvents respectively are thought o be the main causes of differences in the antimicrobial potency of lichen extracts. The key factor was that in the weak polar solvent more the number of bioactive compounds greater the antimicrobial activity the study suggested *by* Sati and Savitha (2011). Similarly, the extract of *P.austrosinense* was equally effective against both Gram positive and Gram negative bacteria such as *Pseudomonas aeruginosa, Salmonella typhii and Staphylococcus aureus*. The result of the present study was found to be near to the antibiotic standard. Therefore, the lichen compound provides an effective alternative agent to pharmaceutical drugs for the treatment of human diseases. The results of the present study showed acetone extracts of *P. austrosinense* has highest antibacterial activity.

Determination of MIC

The MIC values of the extract related to the tested bacterial strains varied between 25 and 200 µg/mL. The MIC of the *Parmotrema austrosinense* lichen extract against *Candida albicans , E.coli, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus* was 500 µg/mL while the MIC value against *Klebsiella aerogenes and Proteus mirablilis* was found to be 1000 µg/mL (Fig. 4). Similarly, acetone extract of *P.nilgherense* was effective against *E.coli* at a lowest concentration of 250 µg/mL. The acetone extracts of lichen *Parmotrema reticulatum* showed its minimum inhibitory concentration value ranges between 500 and 2000 µg/mL. According to Jain *et al*. (2016) the acetone extract of *P.reticulatum* showed antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonasa eruginosa* with the minimum inhibitory concentration value of 100 µg/mL, 100 µg/mL, 200 µg/mL and 200 µg/ml respectively. The lichen extract of *P.tinctorum* was effective against all tested strains with the MIC value ranges between 500 and 1000 µg/mL. *The Ramalina conduplicans* extract was effective against all tested strains at 500 µg/mL. The acetone extracts of *Usnea stigmatoides* did not show antimicrobial activity. Similar study conducted by Srivastava *et al*. (2013) showed that the lichen extracts of *Usnea longissima, Everniastrum cirrhatum, Peltigera polydactylon and Sulcaria sulcata* were effective against tested microbial strains at a varying concentration ranges between 25 and 3.125 µg/mL. The lichen extracts of *Usnea longissima* and *Everniastrum cirrhatum* were effective to inhibit *Staphylococcus aureus and Pseudomonas aeruginosa* at a concentration of 6.25 µg/mL and 200 µg/mL respectively. Among the investigated lichen extracts, *P.nilgherense* showed the highest antibacterial activity against *E.coli* with MIC value of 250 µg/mL. Normally, a man use expensive pharmaceutical drugs which perform excellently at initial stages but a pathogen that cause diseases then develop resistance due to many reasons and hence, there is a growing demand for promising alternative drugs. The lichen compounds are natural, and effective against number of pathogens at low dosage.

Determination of antioxidant

The extract was subjected to investigate the antioxidant property using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Analysis of DPPH radical scavenging activity showed that the acetone extract of *P.tinctorum* and *P.austrosinense* exhibited strong antioxidant activity with the IC_{50} value of 18.10 and 20.09 µg/ml respectively (Fig. 5). Similar study conducted by Poornima *et al*. (2017) indicated that the value of half the inhibitory concentration in acetone extract of *P.tinctorum* which showed higher DPPH radical scavenging property with IC_{50} value of 17.52 µg/mL while *P.asutrosinense* and *P.reticulatum* showed moderate activity with the value of 26.39 and 37.45 µg/mL (Fig. 6) respectively. The results of the present study showed that the IC 50 value of *P. reticulatum* had exhibited 29.86µg/mL. The DPPH radical scavenging activity of *Ramalina conuplicans* and *Usnea stigmatoides* were found to be poor with the IC 50 value of 70.97 and 119.42 µg/mL respectively. The study has resulted to infer that the activity of L-ascorbic acid to capture DPPH radical was directly proportional to its concentrations. The

IC 50 value of *P.tinctorum* was 18.10 µg/mL was found near to standard ascorbic acid 15.78 µg/mL. Rajaram *et al*. (2016) inferred that the methanolic extract of *P.austrosinense* showed higher DPPH scavenging activity with the value of 2.430±0.0046 followed by acetone extracts with 2.770±0.0078.

Determination of total phenolic content

Total phenolic content was higher in acetone extracts of *P.tinctorum* with 447.234 mg gallic acid equivalent (GAE/g lichen extracts) followed by *P.asutrosinense* with the concentrations of 405.860 mg gallic acid equivalent (GAE/g) while *Usnea stigmatoides* showed the little phenolic content with the minimum free radical scavenging activity. Similar study conducted by Ganesan *et al*. (2015) reported that phenolic content was found to be more in benzene extracts (154.2 mg gallic acid equivalent (GAE)/g of lichen extracts) whereas least content was observed in the petroleum ether extract for *P. Tinctorum* (Fig. 3C). *T*he extracts of *P.nilgherrense* and *P.reticulatum* showed sufficient amount of phenolic contents with the value of 243.00 and 254.58 gallic acid equivalent (GAE)/g respectively. Ganesan *et al*. (2015) suggested that the phenolic content may play a vital role in antioxidant and antibacterial activities. Moreover, the study has revealed that antioxidant activity of extracts of *Parmotrema tinctorum* and *P.austrosinense* had the higher total polyphenol content (TPC) with the maximum free radical potency. Singleton *et al*. (1999) opined that the reason for the high or low phenolic content of lichen extract corresponding its antioxidant activity was probably due to the number of phenolic groups in their native structure. The acetone extracts of *P.tinctorum* and *P.austrosinense* exhibited maximum polyphenolic content corresponding to its free radical scavenging activity (Fig. 7).

CONCLUSION

Lichen diversity was recorded in seven different sites of Nilgiris hills in Western Ghats of Tamil Nadu, India. Lichen diversity of our present study was leading by macrolichens (fruticose, foliose form) with 36 species (92.30%) followed by crustose and leprose form with 3 species. The family Parmeliaceae with 24 (61.5%) species belonging to 10 genera was predominant in our study. The survey study from seven sites in Nilgiris offered some diversified substrates for the proliferation of lichens and there are about 39 lichens collected in our study in which 46.15% are corticolous, and another 46.15% are saxicolous 2.5% are terricolous. The optimum elevation shows the maximum number of lichens was found between 2250 – 2637 m. The World health Organization (WHO) has already hastened the industries to develop a new generation drugs as the results of microbial resistance are quite an eye opening and worrying to unveil that existing antibiotics are warned out. The situation is not only sparked by the resistance of some pathogens to existing pharmaceuticals but also the parallel increase in number of diseases, costs of pharmaceuticals world-wide, and increased side effects and hence the search for new and alternative natural drugs is inevitable. Medicinal lichens have been identified both scientifically and traditionally as an alternative source of treatment for various diseases over pharmaceuticals. In the present study, following lichen compounds such as Atranorin, Lecanoric acid, Salazinic acid, Alectorinic acid, and Usnic acid, have been identified to establish their antimicrobial and DPPH radical scavenging efficiency.

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Table. 1: shows the list of lichen species with its Altitude, Family, Longitude, Substrate specificity, Form diversity and its location

No.of species	Lichen	Altitude	Family	Longitude	Substrate specificity	Location	Forms
1	*Bulbothrix tabacina*	1850 m	Parmeliaceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Foliose
2	*Caloplaca herbidulla*	1850 m	Teloschistaceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Crustose
3	*Chrysothrix candelaris*	1831 m	Chrysotrichaceae	76°38'43.577"E 11°16'17.086"N	Corticolous	Manjoor	Leprose
4	*Chrysothrix chlorina*	2637 m	Chrysotrichaceae	11°16'17.086"N	Saxicolous	Doddabetta	Foliose
5	*Everneastrum cirrhatum*	2600 m	Parmeliaceae	10.2692° N, 77.4811° E	Corticolous	Doddabetta	Foliose
6	*Flavoparmelia caperata*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
7	*Graphis sp.*	1831 m	Graphidaceae	76°38'43.577"E 11°16'17.086"N	Corticolous	Manjoor	Crustose
8	*Heterodermia leucmelos*	2637 m, 2223 m	Physciaceae	11°24'08.7°N 76°44'12-2°E, 76°40'42.669"E 11°24'47.943"N	Saxicolous and Corticolous	Doddabetta, Valley view	Foliose
9	*Hypotrachyna brevirhiza*	1850 m	Parmeliaceae	11.3530° N, 76.7959° E	Saxicolous	Coonoor	Foliose
10	*Hypotrachyna excecta*	2600 m	Parmeliaceae	10.2692° N, 77.4811° E	Corticolous	Doddabetta	Foliose
11	*Hypotrachyna infirma*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose

12	*Leptogium denticulatum.*	1850 m	Collemataceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Foliose
13	*Parmelia sp.*	1072 m	Parmeliaceae	11.5170° N, 76.2682° E	Corticolous	Gudalur-Cherambadi	Foliose
14	*Parmelina quercina*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
15	*Parmelinella chozoubae*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
16	*Parmelinella wallichiana*	1850 m	Physciaceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Foliose
17	*Parmelinopsis horrescens*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
18	*Parmotrema austrosinense*	1072, 2209 m	Parmeliaceae	11.5170° N, 76.2682° E,10.2107° N, 77.46°E,	Saxicolous and Corticolous	Gudalur-Cherambadi, HPF - thalaikunda	Foliose
19	*Parmotrema chinense*	830 m	Parmeliaceae	11.3530° N, 76.7959° E	Corticolous	Kateri	Foliose
20	*Parmotrema defectum*	1831 m	Parmeliaceae	76°38'43.577"E 11°16'17.086"N	Saxicolous	Manjoor	Foliose
21	*Parmotrema grayanum*	2637 m, 2209 m	Parmeliaceae	11°24'08.7°N 76°44'12-2°E	Saxicolous	Doddabetta, H.P.F-Thalaikunda	Foliose
22	*Parmotrema indicum*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
23	*Parmotrema melanothrix*	2250 m	Parmeliaceae	10.2692° N, 77.4811° E	Saxicolous	Doddabetta	Foliose
24	*Parmotrema nilgherrense*	2223 m	Parmeliaceae	76°40'42.669"E 11°24'47.943"N	Saxicolous	Valley view	Foliose
25	*Parmotrema permutatum*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
26	*Parmotrema pseudonilgherrense*	2600 m	Parmeliaceae	10.2692° N, 77.4811° E	Corticolous	Doddabetta	Foliose
27	*Parmotrema reticulatum*	2637 m, 2209 m	Parmeliaceae	11°24'08.7°N 76°44'12.2°E	Saxicolous	Doddabetta, H.P.F-Thalaikunda	Foliose

28	*Parmotrema sancti-angeli*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda	Foliose
29	*Parmotrema stuppeum*	2637 m, 2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	Doddabetta, H.P.F-Thalaikunda	Foliose
30	*Parmotrema tinctorum*	2209 m	Parmeliaceae	11.4461° N, 76.67411° E	Saxicolous	H.P.F-Thalaikunda,Valley view	Foliose
31	*Pertusaria colorata*	1831 m	Pertusariaceae	76°38'43.577"E 11°16'17.086"N	Corticolous	Manjoor	Foliose
32	*Physcia aipolia*	1850 m	Physciaceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Foliose
33	*Physcia stellaris*	1072 m	Physciaceae	11.5170° N, 76.2682° E	Corticolous	Gudalur-Cherambadi	Foliose
34	*Pseudocyphellaria aurata*	1831 m	Lobariaceae	76°38'43.577"E 11°16'17.086"N	Corticolous	Manjoor	Foliose
35	*Ramalina intermedia*	1072 m	Ramalinaceae	11.5170° N, 76.2682° E	Saxicolous and Corticolous	Gudalur-Cherambadi	Fruticose
36	*Rmalina pacifica*	1850 m	Ramalinaceae	11.3530° N, 76.7959° E	Corticolous	Coonoor	Fruticose
37	*Sticta orbicularis*	1831 m	Lobariaceae	76°38'43.577"E 11°16'17.086"N	Saxicolous	Manjoor	Foliose
38	*Teloschistes flavicans*	1850 m	Teloschistescea e	11.3530° N, 76.7959° E	Corticolous	Coonoor	Fruticose
39	*Usnea stigmatoides*	2250 m	Parmeliaceae	10.2692° N, 77.4811° E	Corticolous	Doddabetta	Fruticose

Table 1.2. shows the Antimicrobial activity of acetone extracts

Sl. No	*Phytochemical tests*	*Parmotrema austrosinense*			*Parmotrema nilgherense*			*Parmotrema reticulatum*			*Parmotrema tinctorum*			*Ramalina conduplicans*		*Usnea stigmatoides*	Standard
		100 µg	200 µg	250 µg	100 µg	200 µg	250 µg	100 µg	200 µg	250 µg	100 µg	200 µg	250 µg	200 µg	250 µg	200 µg	30mg /mL
1	*Candida albicans*	6.24 ±0.36	8.33± 0.58	13.67 ±0.58	1.10 ±0.08	4.66± 0.49	8.21± 0.24	1.20 ±0.04	3.04 ±0.21	6.50± 0.48	6.10 ±0.53	8.33 ±0.32	10.33 ±0.58	3.40± 0.24	6.50 ±0.87	0.40±0 .08	26.00 ±0.53
2	*Escherischia coli*	3.00 ±0.12	6.80± 0.13	11.33 ±0.58	8.30 ±0.03	9.90± 0.37	10.10 ±0.51	3.80 ±0.06	4.50 ±0.24	5.33± 0.32	4.20 ±0.10	5.93 ±0.11	6.67± 0.58	1.70 ±0.12	3.40 ±0.08	0.50±0 .03	20.33 ±0.58
3	*Kelbsiella pnemuoniae*	-	3.40± 0.22	6.00± 0.10	-	-	0.50± 0.03	-	-	0.30 ±0.21	-	0.80 ±0.16	1.80 ±0.13	-	1.10 ±0.20	-	6.66± 0.58
4	*Proteus vulgaris*	6.50 ±0.58	10.33 ±0.58 -	11.27 ±0.25	-	- 1.20± 0.08	2.60± 0.20	-	3.10 ±0.27	5.70± 0.11	-	3.00 ±0.24	4.20± 0.32	-	0.80 ±0.06	-	20.33 ±0.58

5	*Pseudomonas aeroginosa*	4.50 ±0.32	7.20± 0.37	8.67± 0.58	3.30 ±0.18	5.50± 0.27	8.33± 0.18	5.10 ±0.03	6.4± 0.08	8.33± 0.27	3.00 ±0.06	4.60 ±0.13	6.20± 0.48	0.30± 0.01	2.90 ±0.14	3.10±0 .49	20.10 ±0.08
6	*Salmonella typhi*	2.60 ±0.03	7.37± 0.58	10.67 ±0.58	1.90 ±0.08	2.30 ±0.22	4.10± 0.36	1.80 ±0.20	4.60 ±0.21	6.8±0 .34	2.60 ±0.32	4.80 ±0.27	6.67± 0.53	-	1.50 ±0.09	3.90±0 .10	19.80 ±0.06
7	*Staphylococcus aureus*	6.80 ±0.32	9.80± 0.21	10.33 ±0.58	6.80 ±0.22	9.20± 0.13	10.20 ±0.58	2.90 ±0.27	7.2± 0.26	10.33 ±0.71	2.80 ±0.20	6.50 ±0.87	7.2±0 .73	7.00± 0.51	9.60 ±0.42	10.66± 0.70	20.40 ±0.03

The value represented shows mean ± standard error of mean where n=3.

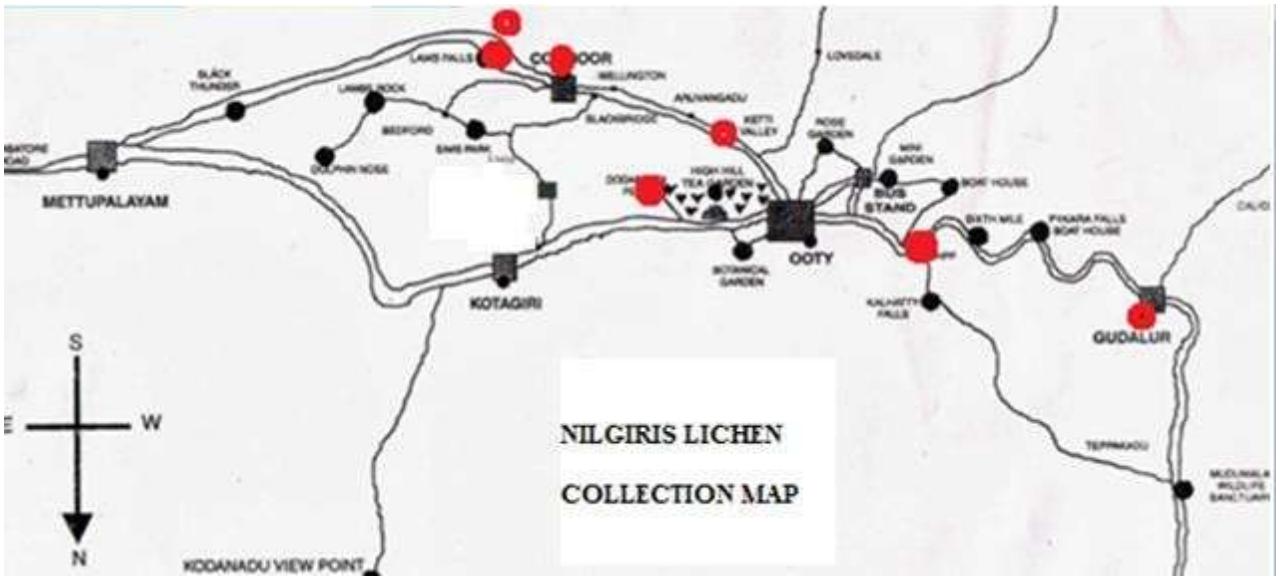

Fig: 1.1 A map showing the study area

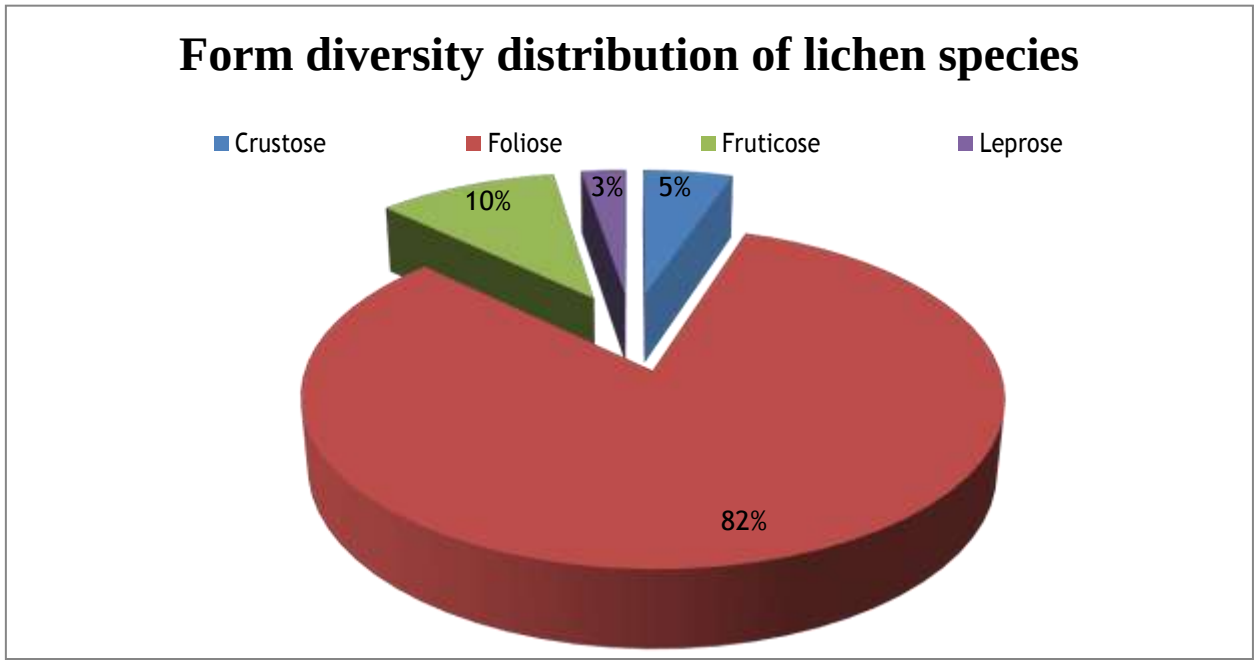

Fig 1.2 Graph shows the form diversity distribution of lichen species

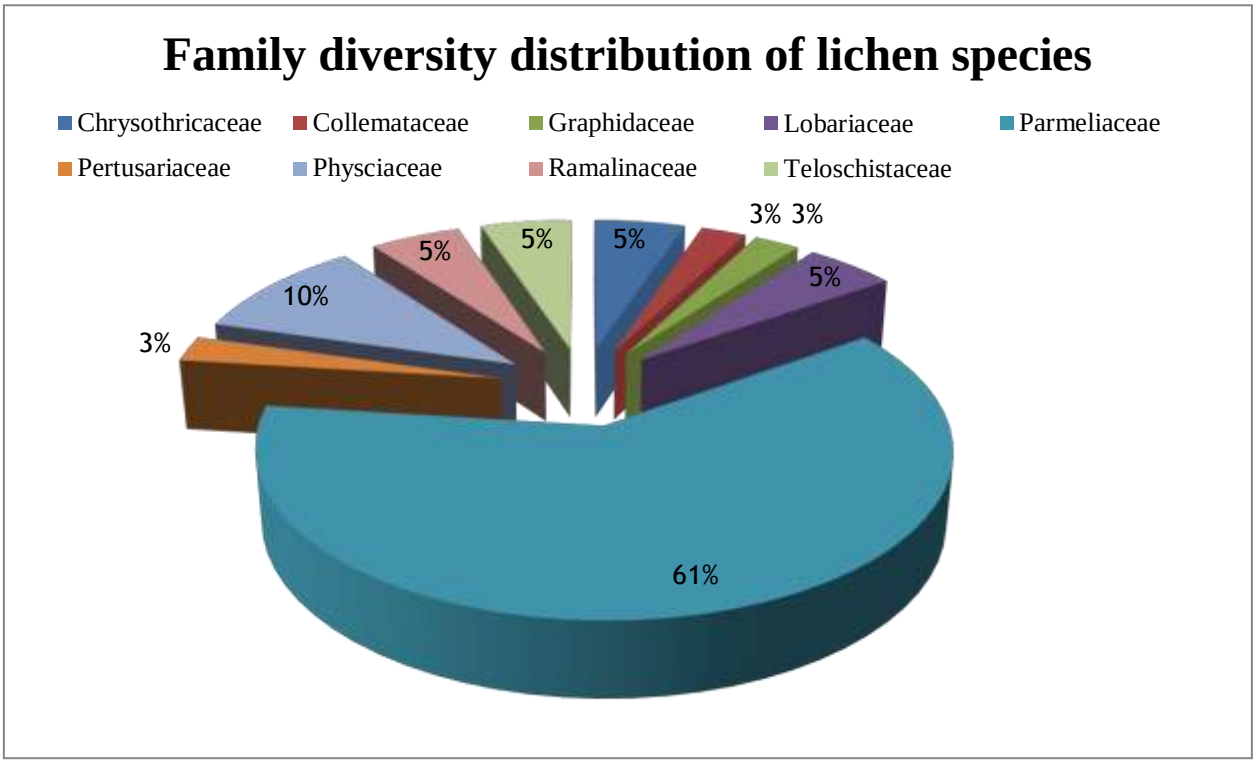

Fig. 1.3 Graph shows the family diversity distribution of lichen species

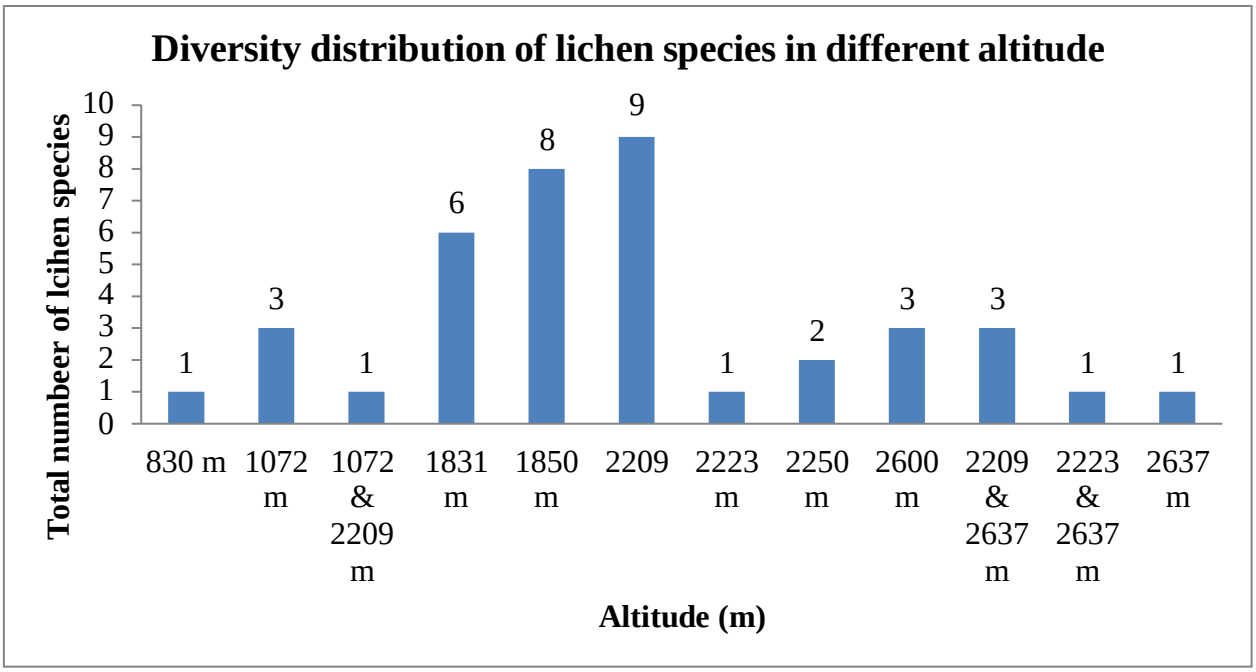

Fig1.4. Graph shows the diversity distribution of lichen species in different altitude

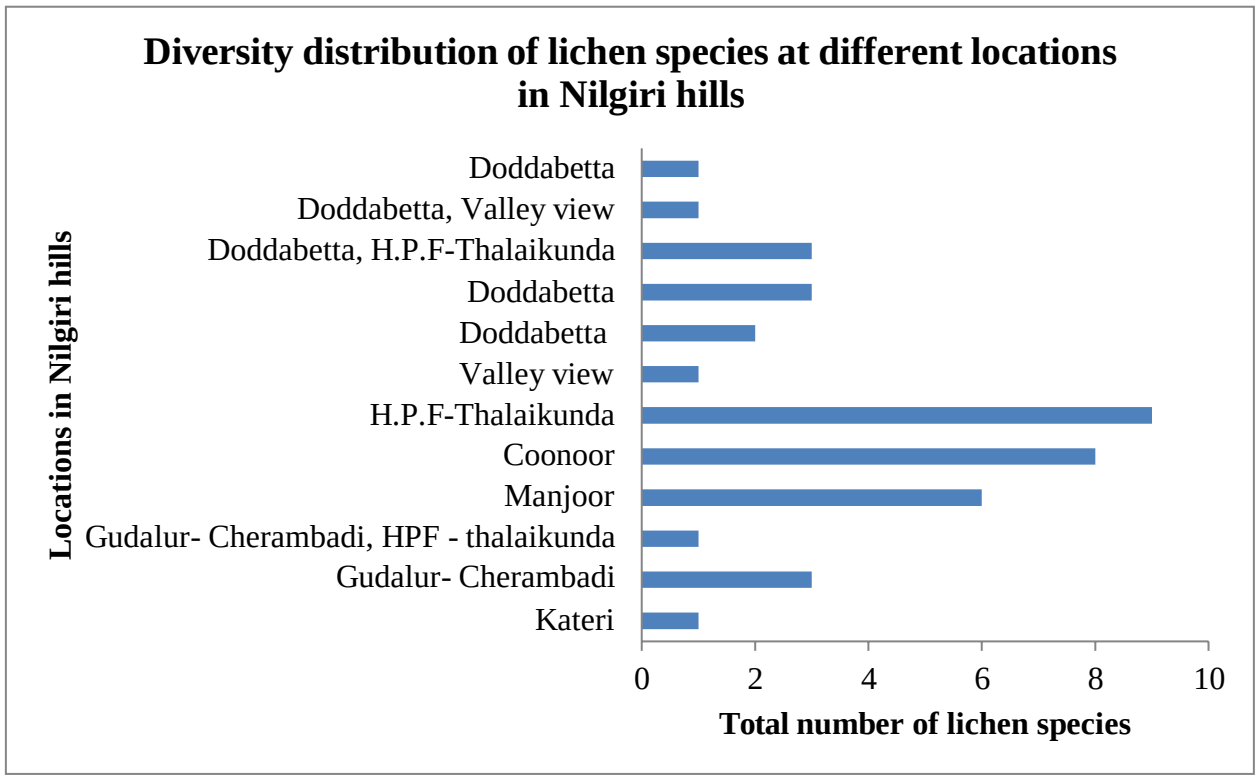

Fig. 1. 5. Graph showing the diversity distribution of lichen species in different localities of Nilgiris

A

B

C

D

E

F

Fig. 1.6. The six macrolichen samples tested for antibacterial avtivity; DPPH radical scavening activity and Total phenolic content examination. (A) P.reticulatum (Hoffm.) Shaer., (B) *Parmotrema austrosinense* (Zahlbr.) Hale, (C) *Parmotrema tinctorum* (Despr. ex Nyl.) Hale (D),*Phaeophyscia hispidula* (Ach.) Moberg. (E) *Ramalina conduplicans* Vain

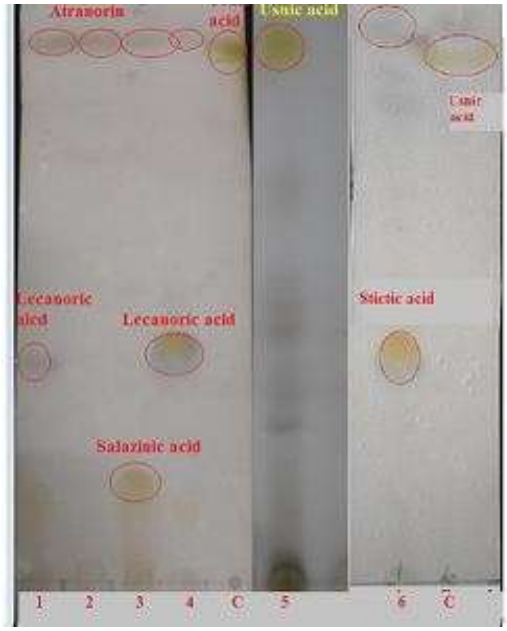

Fig. 2 Identification of lichen substances using Thin layer chromatography.

Fig. 2. Thin layer chromatography profile of lichen species in solvent system A. 1, *P. austrosinense* with lecanoric acid 2 *P. nilgherrense* with atranorin 3, *P. reticulatum* with salazinic acid 4, *P. tinctorum* with lecanoric acid, 5, *Ramalina conduplicans* with usnic acid 6, *Usnea stigmatoides* with stictic acid C, control Usnic acid.

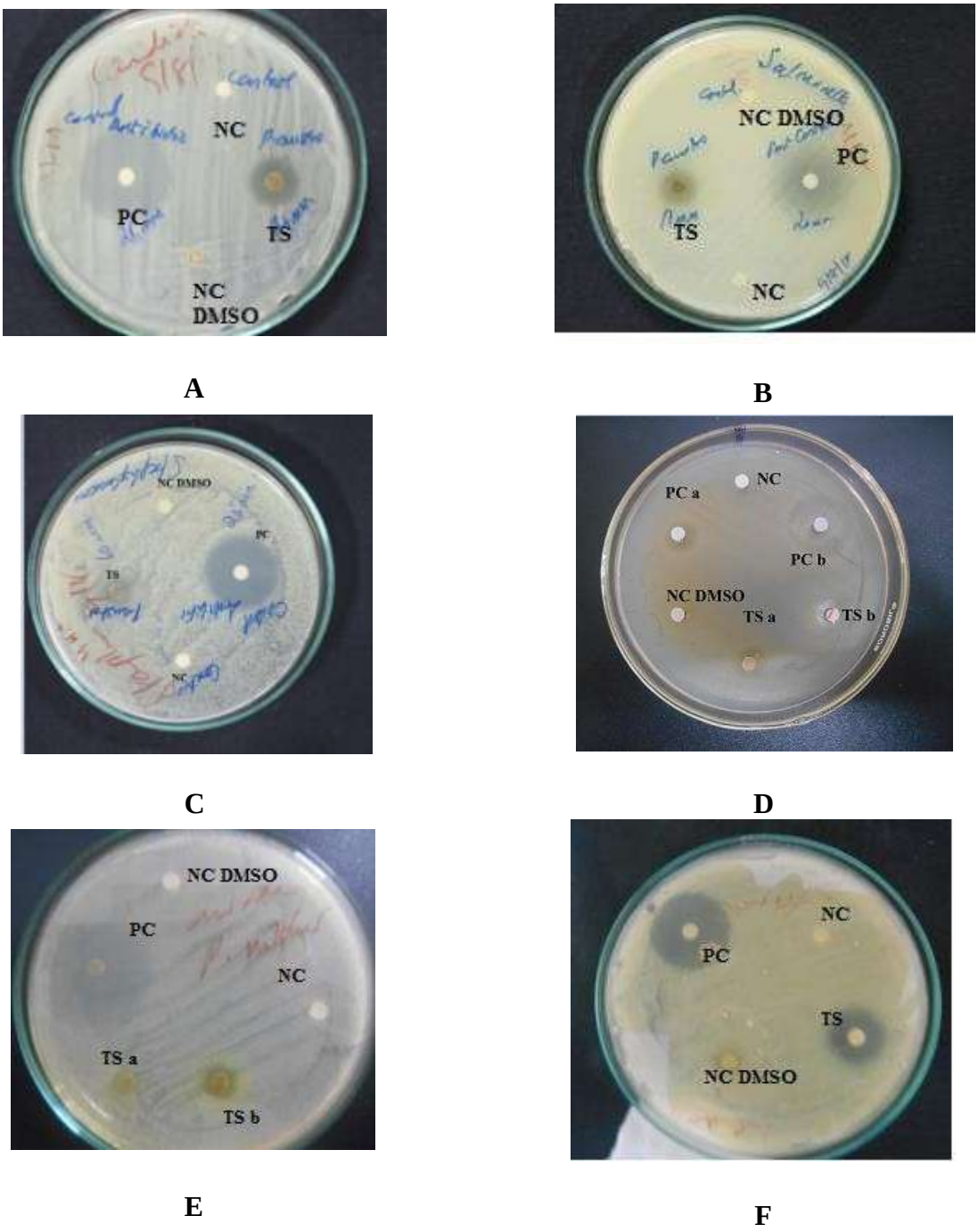

Fig. 3. Antimicrobial activity of lichen extracts.

Fig. 1. (A) – *Parmotrema austrosinense*; *against Candida albicans ;* (B) – *Salmonella typhii ;* (C) against *Staphylococcus aureus* , (D); *Parmotrema nilgherrense* acetone extract against *Staphylococcus aureus* ;(E) *Parmotrema nilgherrense* acetone extract against *Candida albicans* ; (F). *P.tinctorum* against *Staphylococcus aureus*,; (NC) - Negative control – Disc with water ; (NC DMSO) Negative control with Di Methyl Sulfoxide ; (PC) Positive control with corresponding antibiotic (PC a) - Positive control – with Antibiotic 10 µL ; (PC b) Positive control with antibiotic 20 µL (TS) Test sample (TS a) Test sample lichen extraxt with 200 µg/ mL ; (TS b) Test sample lichen extract with the concentration of 250 µg/mL

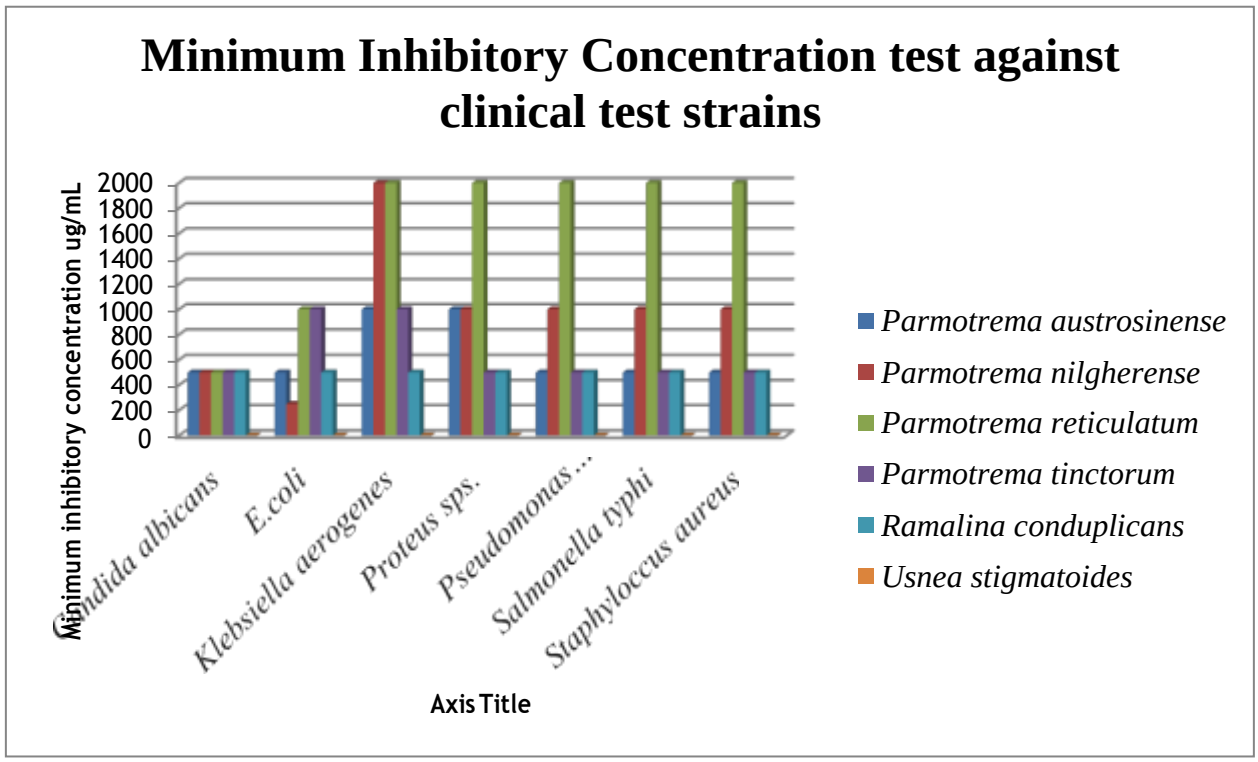

Fig. 4. Graph showing MIC of lichen extracts against clinical test strains

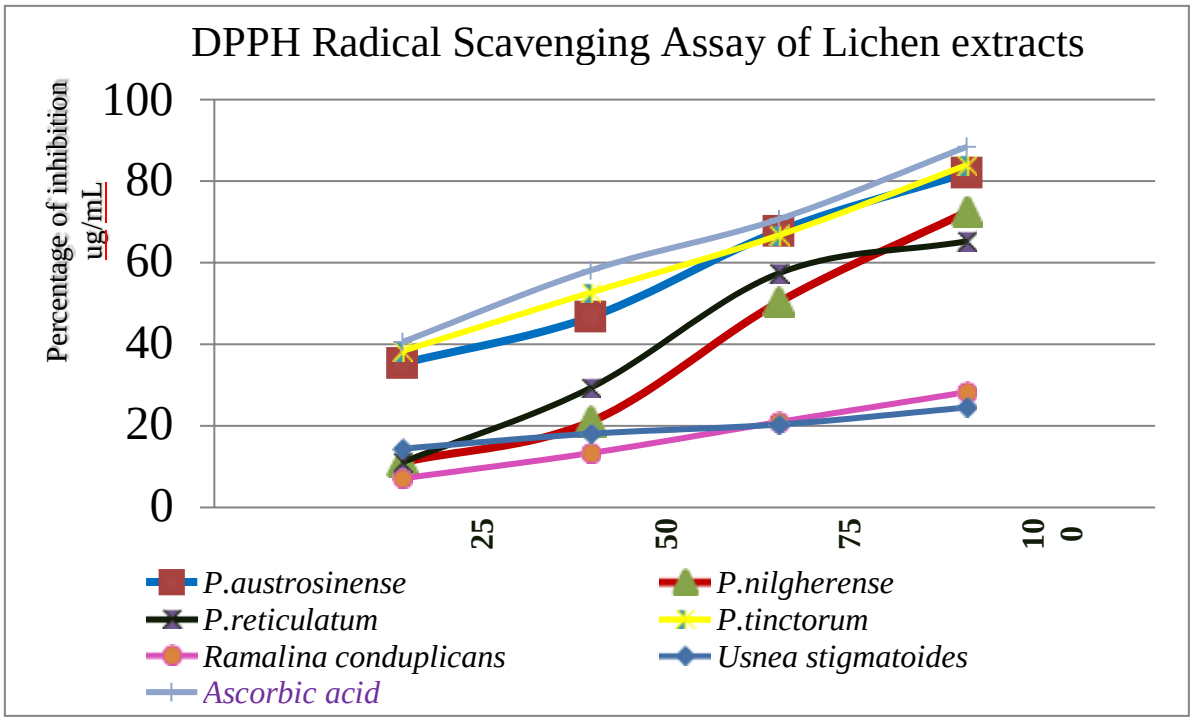

Fig. 5. Antioxidant activity - DPPH radical scavenging activity

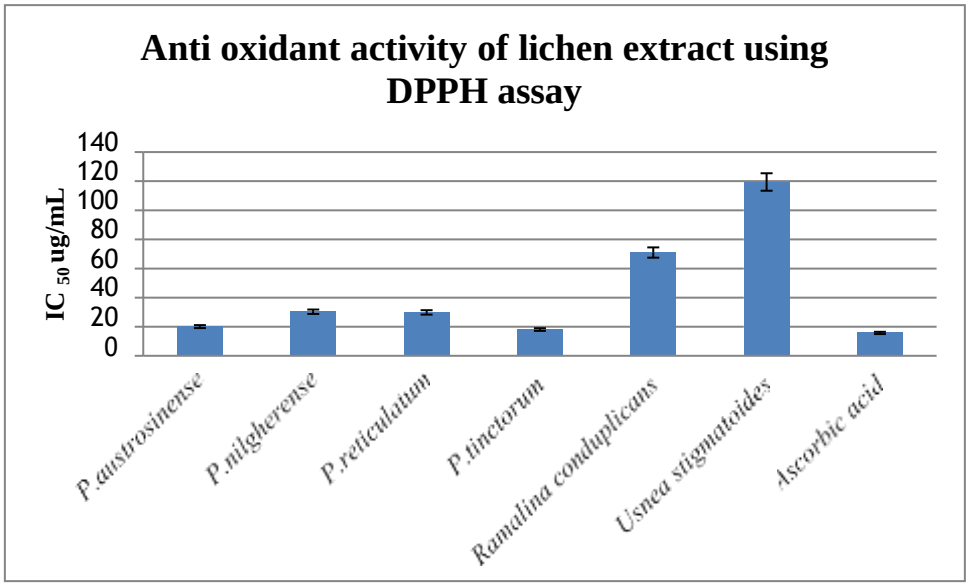

Fig. 6. Graph shows *in vitro* DPPH radical scavenging activity (IC $_{50}$ µg/mL) of Lichen extracts

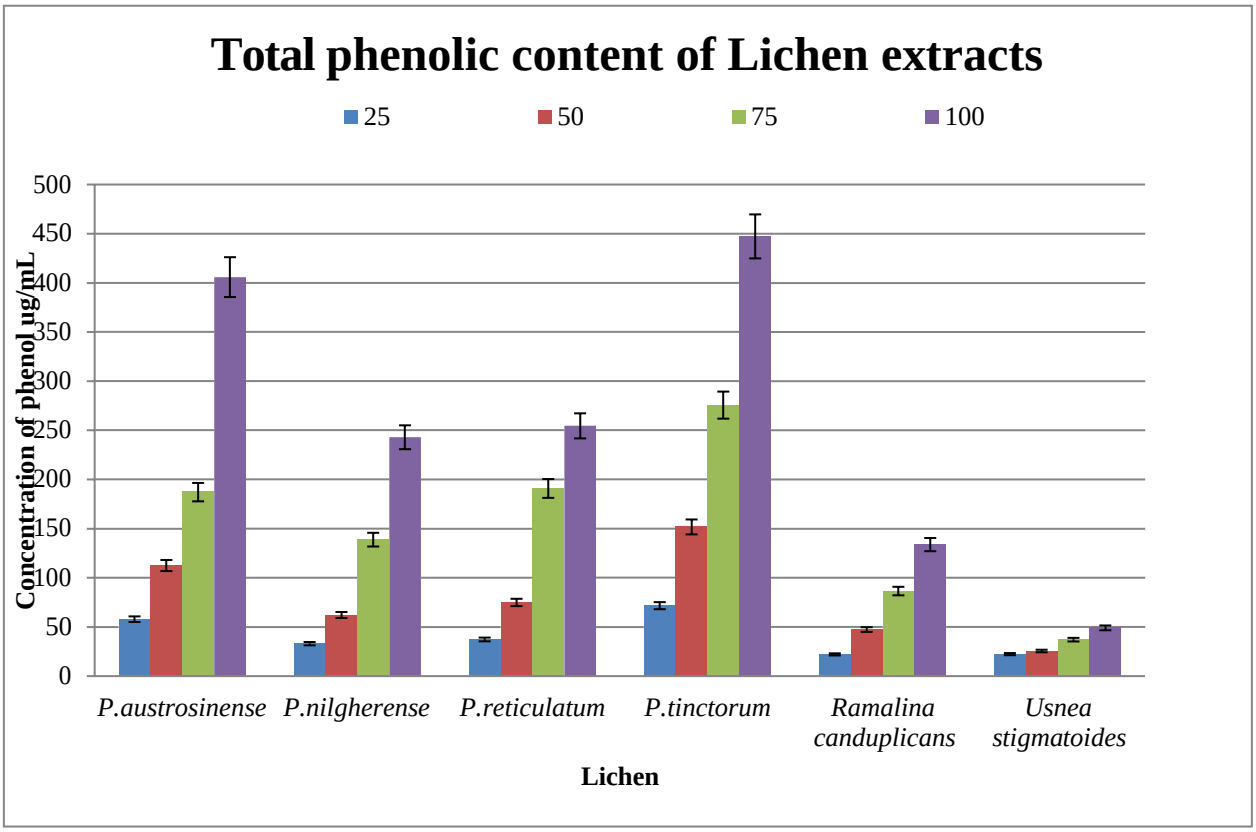

Fig. 7 Total phenolic content of lichen samples