

## Radical Scavenging, Antimicrobial and Insecticidal Efficacy of *Parmotrema cristiferum* and *Dirinaria applanata*

Prashith Kekuda T.R<sup>1</sup>, Dhanya Ramesh<sup>1</sup>, Dhatri Ramesh<sup>1</sup>, Sunita C. Mesta<sup>1</sup>, Onkarappa R<sup>1</sup> and Vinayaka K.S<sup>2\*</sup>

<sup>1</sup>Department of Studies and Research in Microbiology, Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga-577203, Karnataka, India

<sup>2</sup>Department of Botany, Kumadvathi First Grade College, Shimoga Road, Shikaripura, Karnataka, India

### Abstract

Lichens are self-supporting symbiotic association of mycobiont and photobiont. The present study was conducted to investigate antimicrobial, insecticidal and radical scavenging potential of methanol extract of two macrolichens viz., *Parmotrema cristiferum* (Taylor) Hale and *Dirinaria applanata* (Fée) D.D. Awasthi. Antibacterial activity was tested against a panel of 5 bacteria by Agar well diffusion assay. Minimum inhibitory concentration was determined against 2 bacteria by broth dilution method. Antifungal efficacy was determined against 5 molds by Poisoned food technique. Free radical scavenging activity was screened by DPPH radical scavenging assay. Total phenolic content was estimated by Folin-Ciocalteu reagent method. Insecticidal activity was determined against 2<sup>nd</sup> instar larvae of *Aedes aegypti*. Among lichen extracts, *D. applanata* exhibited stronger inhibition of bacteria as evidenced by wider zones of inhibition and low MIC values. Extract of *D. applanata* suppressed the mycelial growth of test fungi to higher extent when compared to extract of *P. cristiferum*. The scavenging of DPPH radicals was also marked in case of *D. applanata* when compared to *P. cristiferum*. The content of total phenolics was also higher in *D. applanata* than that of *P. cristiferum*. Lichen extracts showed concentration dependent larvicidal effect. *D. applanata* exhibited stronger larvicidal effect than *P. cristiferum*. Overall, *D. applanata* displayed marked bioactivities when compared to *P. cristiferum*. This could be mainly due to high phenolic content. The macrolichens of this study appear to be promising sources of bioactive agents.

### Article Information

#### Article History:

Received : 18-01-2015

Revised : 19-03-2015

Accepted : 24-03-2015

#### Keywords:

*Parmotrema cristiferum*

*Dirinaria applanata*

Agar well diffusion Poisoned

food technique DPPH

Total phenolic content

#### \*Corresponding Author:

Vinayaka K.S

E-mail:

[ks.vinayaka@rediffmail.com](mailto:ks.vinayaka@rediffmail.com)

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### INTRODUCTION

Lichens are nonvascular cryptogams and represent stable, self-supporting symbiotic association of photobiont (alga or cyanobacterium) and mycobiont (fungus). Lichens are one of the most fascinating organisms on earth. They grow on rocks (saxicolous), tree trunks (corticulous), leaves (foliicolous), undisturbed soil (terrocolous) etc. Lichens are able to grow in diverse climatic conditions including harsh environmental conditions and are often considered to be the primary colonizers of terrestrial ecosystem. Lichens grow slowly and take several years to get established in nature. The thallus of lichens lack specialized organs such as roots, leaves and stem as seen in case of higher plants. Lichens occur in different growth forms viz., crustose, foliose and fruticose. Lichens are considered to be one of the useful organisms to monitor air quality. Lichens are valuable resources of medicine, food, fodder, perfume, spices and dyes and are consumed as food in some parts of the world especially during famine. Several lichen species are used as spice and flavoring agents in the preparation of some foods. Some lichens are traditionally used worldwide to treat several diseases/disorders such as dyspepsia, bleeding

pile, bronchitis, scabies, stomach disorders and many disorders of blood and heart. Lichens produce a number of secondary metabolites such as depsides, depsidones, depsones, and dibenzofurans (lichen substances) which seldom occur in other organisms. These compounds are produced mainly by mycobiont and are important in lichen taxonomy. Recently, much attention has been paid to biological activities of lichen metabolites. The lichen extracts and the purified metabolites are known to exhibit a wide array of bioactivities such as antibacterial, antifungal, antioxidant, antitumor, antiherbivore, phytotoxic, analgesic, antipyretic, wound healing, antitermite, enzyme inhibitory, anti-inflammatory, insecticidal and others (Awasthi, 2000a; van Dobben *et al.*, 2001; Agelet and Valles, 2003; Oh *et al.*, 2006; Upreti and Nayaka, 2008; Luo *et al.*, 2010; Kumar *et al.*, 2010a; Kambar *et al.*, 2014a; Wang *et al.*, 2014). India represents a rich centre of lichen biodiversity, contributing nearly 15% of the 13,500 lichen species so far recorded in the world. Among various regions, Himalaya harbors largest number of species followed by Western Ghat region of India. Lichens are used as medicine, spice, dye and incense

materials in India. Lichens have been used in Ayurveda and Unani systems of medicine in India (Pinokiyo *et al.*, 2008; Upreti and Nayaka, 2008; Kumar *et al.*, 2010a; Tiwari *et al.*, 2011; Vinayaka *et al.*, 2012). The present study was performed to evaluate antimicrobial, insecticidal and radical scavenging activity of two foliose macrolichens viz., *Parmotrema cristiferum* (Taylor) Hale (Parmeliaceae) and *Dirinaria applanata* (Fée) D.D. Awasthi (Physciaceae).

## MATERIALS AND METHODS

### Collection and Identification of Lichens

The corticolous macrolichens *P. cristiferum* and *D. applanata* (growing on barks of areca trees) of the present study were collected in and around Shikaripura, Shivamogga district, Karnataka, India. The identification of these lichens was done by performing morphological, anatomical and chemical tests. Color tests were carried out on cortex and medulla by using 10% potassium hydroxide (K), Steiner's stable paraphenylenediamine solution (P) and calcium hypochlorite solution (C). Secondary metabolites were detected by Thin layer chromatography (TLC) using solvent system which consisted of Benzene:1,4-Dioxane:Acetic acid in the ratio 90:25:4 (Culberson and Kristinsson, 1970; Culberson, 1972; Awasthi, 2000b).

### Preparation of Lichen Extracts

The lichen materials were powdered using a blender. A known quantity (25g) of each lichen powder was transferred into separate conical flasks and 100ml of methanol was added. The mouth of the flasks was closed and the content of the flasks was mixed well. The flasks were left for two days with regular stirring. The content of flasks was filtered through muslin cloth followed by sterile Whatman No. 1 filter paper. The filtrates were subjected to evaporation at 40°C in an oven in order to get the condensed extract (Kambar *et al.*, 2014a).

### Antibacterial Activity of Lichen Extracts

Antibacterial effect of lichen extracts was tested by Agar well diffusion assay against two Gram negative bacteria viz., *Vibrio cholerae* and *Enterobacter aerogenes* and three Gram positive bacteria viz., *Staphylococcus aureus*, *S. epidermidis* and *Bacillus subtilis*. 24 hours old Nutrient broth cultures of test bacteria were swabbed uniformly over sterile Nutrient agar plates followed by punching wells of 8mm diameter using sterile cork borer. The wells were labeled and were filled with lichen extracts (20mg/ml of 25% Dimethyl sulfoxide [DMSO]), standard antibiotic (Streptomycin, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile distilled water). The plates were incubated at 37°C for 24 hours in upright position. Zone of inhibition formed around the wells was measured using a ruler after incubation (Kambar *et al.*, 2014a).

### Minimum Inhibitory Concentration of Lichen Extracts

The Minimum Inhibitory Concentration (MIC) of lichen extracts was evaluated by the broth dilution method. In brief, a series of extract dilutions (ranging from 20 to 0.0mg/ml) was used against *E. aerogenes* and *S. epidermidis*. Two-fold extract dilutions were prepared in Nutrient broth. Test tubes containing different concentrations of lichen extracts were inoculated with 100µl of 24 hours old Nutrient broth cultures of test bacteria. The tubes were incubated at 37°C for 24 hours. The MIC was determined by observing the visible growth of test bacteria after incubation. The dilution tube showing

no visible growth was considered as the MIC (Kosanic and Rankovic, 2010).

### Antifungal Activity of Lichen Extracts

Inhibitory effect of lichen extracts was tested against a panel of 5 fungi which included *Colletotrichum capsici* (isolate from anthracnose of chilli) and four fungi viz., *Helminthosporium* sp., *Alternaria* sp., *Fusarium* sp., and *Curvularia* sp (isolates from moldy grains of sorghum) by Poisoned food technique (Kambar *et al.*, 2014a). The test fungi were inoculated at the centre of control (without extract) and poisoned plates (2mg of extract/ml of Potato dextrose agar). The control and poisoned plates were incubated at 28°C for 5 days. Using a ruler, the diameter of fungal colonies on control and poisoned plates was measured in mutual perpendicular directions. The potential of lichen extracts was assessed in terms of inhibition of mycelial growth of test fungi using the formula:

$$\text{Mycelial growth inhibition (\%)} = (C - T / C) \times 100,$$

where C and T is colony diameter of test fungi on control and poisoned plates respectively.

### Radical Scavenging Activity of Lichen Extracts

The radical scavenging ability of lichen extracts was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. In brief, 2ml of different concentrations of lichen extracts (6.25-400µg/ml of methanol) were mixed with 2ml of DPPH radical solution (0.004% in methanol) in clean and labeled test tubes. The tubes were incubated in dark for 30 minutes followed by measuring absorbance (optical density) at 517nm. The absorbance of DPPH control (extract replaced by methanol) was noted. Ascorbic acid was used as reference standard. The radical scavenging efficacy of lichen extracts was calculated using the formula:

$$\text{Radical scavenging effect (\%)} = (A_c - A_t / A_c) \times 100,$$

where  $A_c$  and  $A_t$  are absorbance of DPPH control and absorbance of DPPH in presence of extract/standard respectively. The  $IC_{50}$  (Inhibitory Concentration) value for extracts/standard was calculated.  $IC_{50}$  represents the concentration of extract/standard required to scavenge 50% of DPPH free radicals (Vivek *et al.*, 2014a).

### Total Phenolic Content of Lichen Extracts

Folin-Ciocalteu reagent method was employed to estimate the content of total phenolics in the lichen extracts. Here, a dilute concentration of lichen extract (0.5ml) was mixed with 0.5ml of Folin-Ciocalteu reagent (1:1) and 2ml of sodium carbonate (7%). The tubes were left for 30 minutes and the absorbance was read at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000µg/ml). The total phenolic content was estimated as µg Gallic acid equivalents (GAE) from the graph (Vivek *et al.*, 2014a).

### Insecticidal Activity of Lichen Extracts

Insecticidal activity of lichen extracts was assessed in terms of larvicidal effect on 2<sup>nd</sup> instar larvae of *Aedes aegypti* mosquito (Kumar *et al.*, 2010a). The extracts were prepared in DMSO. Twenty 2<sup>nd</sup> instar larvae were transferred into separate conical flasks containing 100ml of water with different concentrations of lichen extracts (0.5, 1.0 and 2.0mg/ml). Flask containing DMSO (1% in water) served as control. After 24 hours, the number of dead larvae was counted. Death of larvae was confirmed when the larvae failed to move even after probing with a

needle in siphon or cervical region. The larvicidal effect in terms of larval mortality (%) was calculated using the formula:

$$\text{Larval mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

**Statistical analysis**

All data were expressed as Mean±Standard deviation (n=3). IC<sub>50</sub> values were calculated using Origin 6.0 software.

**RESULTS**

**Characteristics of selected macrolichens**

The thallus characteristics and results of color tests and secondary metabolites of selected lichens are shown in Table 1.

**Antibacterial Activity of Lichen Extracts**

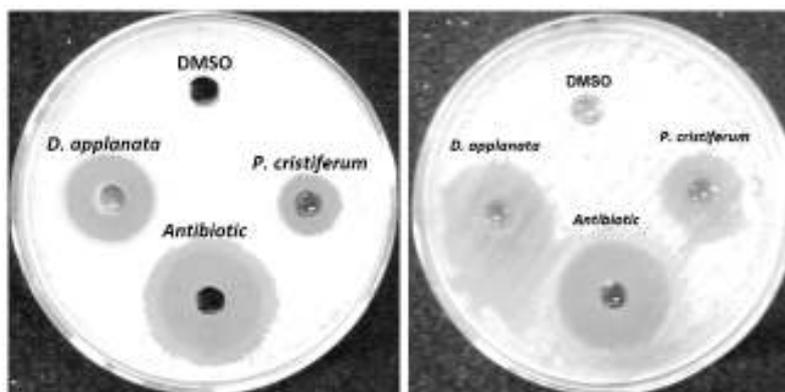
The lichen extracts were effective in inhibiting the test bacteria as evidenced by the presence of zone of inhibition. Among lichen extracts, extract of *D. applanata* caused marked inhibition of test bacteria. *S. epidermidis* and *E. aerogenes* exhibited higher susceptibility to extract of *P. cristiferum* and *D. applanata* respectively. Overall, *V. cholerae* was demonstrated least susceptibility to lichen extracts. *E. aerogenes* and *S. epidermidis* were inhibited to higher extent among Gram negative and Gram positive bacteria respectively. Standard antibiotic displayed higher inhibitory activity when compared to lichen extracts. DMSO did not cause inhibition of any test bacteria (Table 2 and Figure 1). The MIC against test bacteria was found to be lesser in case of *D. applanata* when compared to *P. cristiferum* (Table 3).

**Table 1:** Characteristics of selected macrolichens

Features	<i>D. applanata</i>	<i>P. cristiferum</i>
<b>Thallus characteristics</b>	Thallus 5-10 cm wide, adnate and lobed; lobes flabellate, plicate and confluent; upper side greyish white, sorediate; soralia globose, capitate with farinose or granular soredia	Thallus coriaceous; lobes eciliate; upper side grey, centrally brownish, cracked, soralia marginal on lateral lobules in central part, crescent shaped or confluent; soredia granular; lower side centrally black, wide marginal zone brown, nude; medulla white; apothecia rare, imperforate
<b>Color tests (Cortex)</b>	K+ yellow, C-, KC-, P+ yellow	K+ yellow, C-, KC-, P-
<b>Color tests (Medulla)</b>	K -, C -, KC -, P -	K + yellow turning red, C -, KC -, P + orange-red
<b>Secondary metabolites (TLC)</b>	Atranorin, Divaricatic acid	Atranorin, Salazinic acid, Consalazinic acid

**Table 2:** Antibacterial activity of lichen extracts

Test bacteria	Zone of inhibition in cm			
	DMSO	Antibiotic	<i>P. cristiferum</i>	<i>D. applanata</i>
<i>V. cholerae</i>	0.0±0.0	2.80±0.00	1.52±0.04	2.20±0.00
<i>E. aerogenes</i>	0.0±0.0	3.01±0.09	2.00±0.00	2.82±0.04
<i>S. aureus</i>	0.0±0.0	3.40±0.00	1.60±0.00	2.20±0.00
<i>S. epidermidis</i>	0.0±0.0	2.73±0.04	2.31±0.09	2.60±0.00
<i>B. subtilis</i>	0.0±0.0	3.46±0.09	1.70±0.00	2.42±0.04



**Figure 1:** Inhibition of *S. aureus* (left) and *E. aerogenes* (right) by lichen extracts

**Table 3:** MIC of lichen extracts

Test bacteria	MIC (mg/ml)	
	<i>P. cristiferum</i>	<i>D. applanata</i>
<i>E. aerogenes</i>	1.250±0.000	0.312±0.000
<i>S. epidermidis</i>	0.625±0.000	0.312±0.000

**Antifungal activity of lichen extracts**

The result of inhibitory effect of lichen extracts against mycelial growth of test fungi is shown in Table 4 and Figure 2-7. Both lichen extracts were effective in inhibiting the growth of test fungi. A drastic reduction in the mycelial

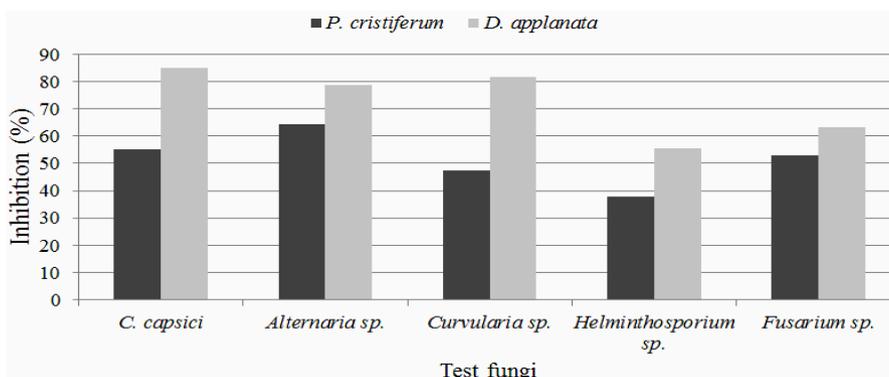
growth of test fungi was observed in poisoned plates when compared to control plates. When compared to extract of *P. cristiferum*, marked inhibition of test fungi was caused by extract of *D. applanata* (>50% inhibition). *P. cristiferum* inhibited *Alternaria* sp. to higher extent

(64.28% inhibition) while *C. capsici* was inhibited to higher extent (85%) among test fungi by extract of *D. applanata*.

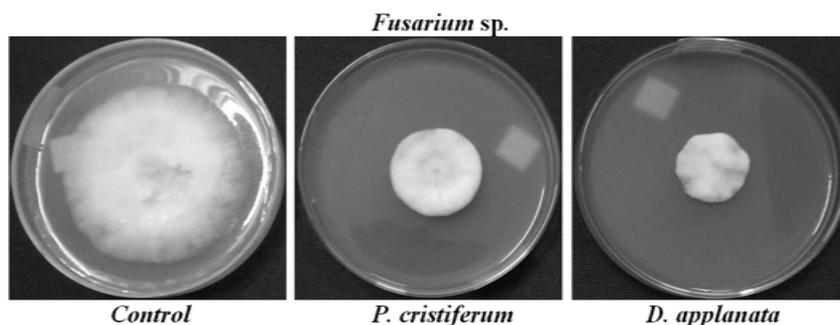
Among fungi, *Helminthosporium* sp. was inhibited to least extent by both lichen extracts.

**Table 4: Growth of test fungi on control and poisoned plates**

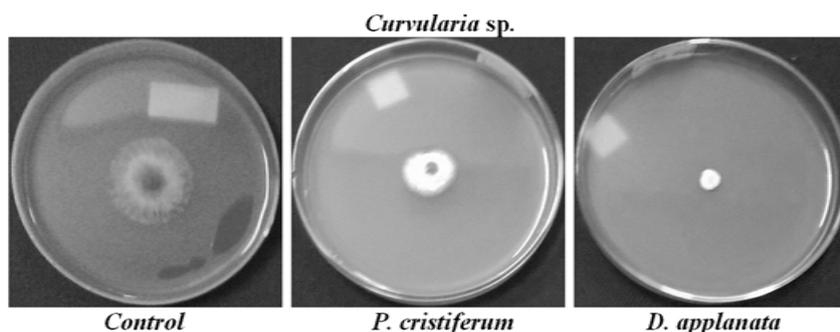
Test fungi	Colony diameter in cm		
	Control	<i>P. cristiferum</i>	<i>D. applanata</i>
<i>C. capsici</i>	4.03±0.10	1.80±0.00	0.60±0.00
<i>Alternaria</i> sp.	4.20±0.00	1.50±0.00	0.90±0.00
<i>Curvularia</i> sp.	3.80±0.00	2.00±0.00	0.70±0.00
<i>Helminthosporium</i> sp.	4.51±0.10	2.81±0.10	2.00±0.00
<i>Fusarium</i> sp.	6.84±0.20	3.20±0.00	2.56±0.10



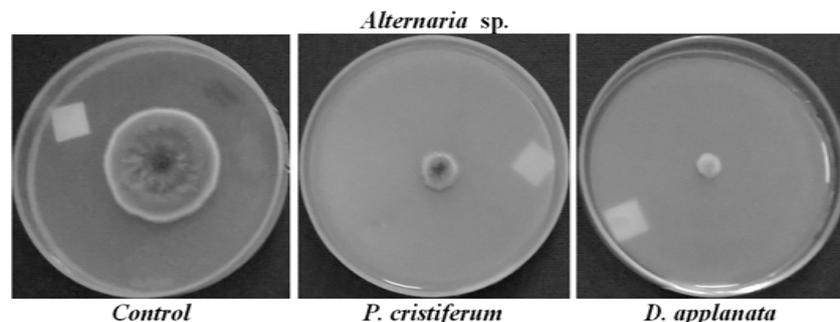
**Figure 2: Inhibition of test fungi (%) by lichen extracts**



**Figure 3: Inhibition of *Fusarium* sp. by lichen extracts**



**Figure 4: Inhibition of *Curvularia* sp. by lichen extracts**



**Figure 5: Inhibition of *Alternaria* sp. by lichen extracts**

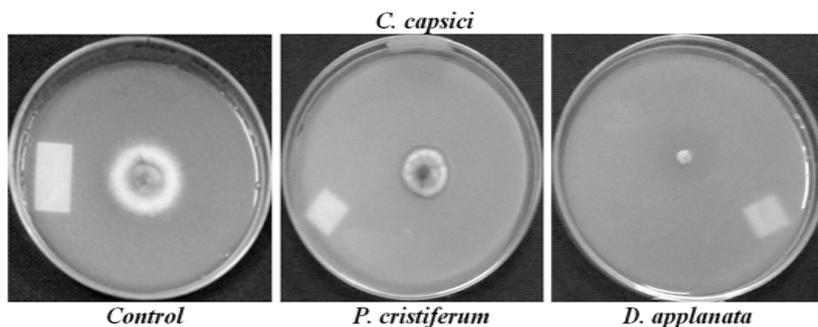


Figure 6: Inhibition of *C. capsici* by lichen extracts

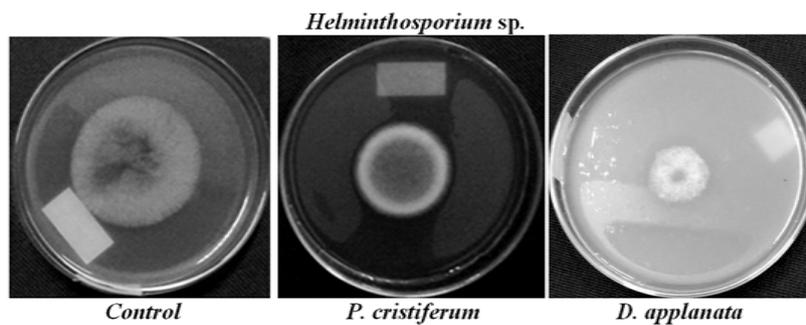


Figure 7: Inhibition of *Helminthosporium* sp. by lichen extracts

**Radical Scavenging Activity of Lichen Extracts**

The result of DPPH free radical scavenging efficacy of lichen extracts is shown in Figure 8. Lichen extracts scavenged radicals in a dose dependent manner. Among extracts, marked scavenging effect was observed in case of *D. applanata* (IC<sub>50</sub> 205.14µg/ml) when compared to *P. cristiferum* (IC<sub>50</sub> 365.23µg/ml). However, ascorbic acid exhibited stronger scavenging activity (IC<sub>50</sub> 1.63µg/ml) when compared to lichen extracts.

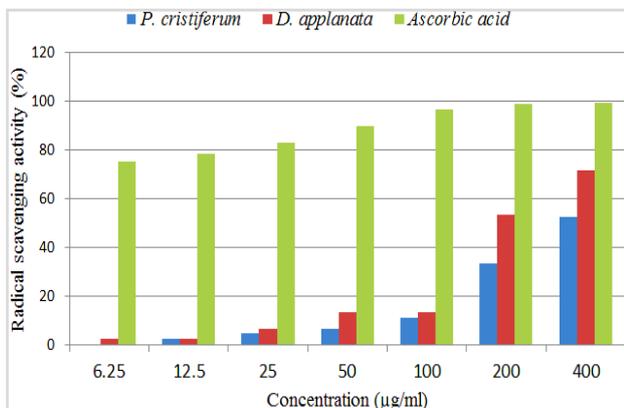


Figure 8: DPPH radical scavenging activity of lichen extracts

**Total Phenolic Content of Lichen Extracts**

The content of total phenolics in the lichen extracts was evaluated by FCR method. Extract of *D. applanata* contained high phenolics when compared to extract of *P. cristiferum* (Table 5).

Table 5: Total phenolic content of lichen extracts

Extract	TPC (mg GAE/g)
<i>P. cristiferum</i>	31.55±1.24
<i>D. applanata</i>	42.18±1.60

**Insecticidal Activity of Lichen Extracts**

The result of killing effect of lichen extracts against 2<sup>nd</sup> instar larvae of *A. aegypti* is shown in Table 6. The

extracts showed dose dependent mortality of larvae. Pronounced activity was seen in case of extract of *D. applanata* when compared to *P. cristiferum*. No mortality of larvae was observed in control (1% DMSO).

Table 6: Larvicidal effect of lichen extracts

Concentration (mg/ml)	Mortality of larvae (%) / Lichens	
	<i>P. cristiferum</i>	<i>D. applanata</i>
0.0 (control)	00.00±00.00	00.00±00.00
0.5	00.00±00.00	43.33±05.77
1.0	46.66±09.42	80.00±00.00
2.0	90.00±00.00	100.00±00.00

**DISCUSSION**

**Antibacterial Activity of Lichen Extracts**

The discovery of antibiotics has been the major milestone in the development of clinical medicine as their use saved countless lives and resulted in prevention and control of infectious diseases. However, antibiotic resistance and spread of resistance have been observed in pathogens the last decade of the 20<sup>th</sup> century and the first decade of the 21<sup>st</sup> century and resulted in failure of antibiotic therapy leading to death of patients. A gradual increase in resistance rates of pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococci*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* poses a serious threat to public health. These resistant pathogens are troublesome in both community and hospital settings (Wright *et al.*, 2003; Oancea and Stoia, 2010; Lee *et al.*, 2013). Natural products can be effectively used as promising alternates to combat drug resistance. It has been shown that extracts and purified components from lichens exhibit promising antibacterial activity with activity even against drug resistant pathogens (Esimone *et al.*, 2002; Elo *et al.*, 2007; Sharma *et al.*, 2012; Vivek *et al.*, 2014a). In the present study, we evaluated antibacterial activity of extract of *P. cristiferum* and *D. applanata* against two Gram negative and three Gram positive bacteria. *D. applanata* caused marked inhibition of test bacteria when

compared to *P. cristiferum* as evidenced by wider zones of inhibition and lower MIC values. Extracts inhibited *E. aerogenes* and *S. epidermidis* to higher extent among Gram negative and Gram positive bacteria respectively. In an earlier study, extracts of *P. tinctorum*, *P. grayanum* and *P. praesorediosum* were shown to inhibit Gram positive and Gram negative bacteria (Vivek *et al.*, 2014a) and uropathogenic bacteria (Kambar *et al.*, 2014b) in a dose dependent manner. Other species of *Parmotrema* such as *P. nilgherrense* (Javeria *et al.*, 2013), *P. pseudotinctorum* (Kumar *et al.*, 2010b) and *P. sancti-angelii* (Verma *et al.*, 2011) were also shown to exhibit antibacterial activity.

#### Antifungal activity of lichen extracts

Current approaches for controlling mycotic diseases of plants are based mainly on genetic resistance in host plant, environmental management and the use of synthetic fungicides. The use of fungicides is an extensively used approach for plant disease control. However, the extensive use of these synthetic fungicides suffer from drawbacks such as high cost, effect on non-target organisms, residual effect and development of resistance in pathogens. Hence, alternatives to several synthetic fungicides currently in use are needed. It has been shown that lichen and lichen forming fungi exhibit marked antifungal activity against a wide range of phytopathogenic fungi (Shahi *et al.*, 2001; Oh *et al.*, 2006; Wei *et al.*, 2008; Goel *et al.*, 2011; Tiwari *et al.*, 2011; Kambar *et al.*, 2014a). In the present study, we screened the potential of extract of *P. cristiferum* and *D. applanata* to inhibit fungi. Marked inhibitory effect against all test fungi was observed in case of *D. applanata* as the extract caused high suppression of fungal growth (as evidenced by drastic reduction in the size of the fungal colonies when compared to fungal growth in control plates). Among fungi, *Alternaria* sp., and *C. capsici* were inhibited to higher extent by extract of *P. cristiferum* and *D. applanata* respectively. Both lichen extracts caused >50% inhibition of *C. capsici* (an isolate from chilli anthracnose). In a previous study, Kekuda *et al.* (2014) screened the inhibitory efficacy of three *Parmotrema* species *viz.*, *P. tinctorum*, *P. grayanum* and *P. praesorediosum* against *C. capsici*. Extract of *P. tinctorum* had high inhibitory effect followed by *P. grayanum* and *P. praesorediosum*. Extract of *P. cristiferum* and *D. applanata* were effective in inhibiting fungi from moldy sorghum grains. *Alternaria* sp. and *Curvularia* sp. were inhibited to higher extent by *P. cristiferum* and *D. applanata* respectively. In an earlier study, Vivek *et al.* (2014b) showed antifungal effect of *P. tinctorum*, *P. grayanum* and *P. praesorediosum* against *Aspergillus flavus*, *Helminthosporium* sp. and *Alternaria* sp. from moldy sorghum grains. Strong inhibitory activity against mycelial growth of fungi was observed in case of *P. praesorediosum* when compared to other two lichens. More recently, Kambar *et al.* (2014a) showed antifungal efficacy of solvent extracts of *Ramalina conduplicans* against fungi from moldy sorghum grains.

#### DPPH Radical Scavenging Activity of Lichen Extracts

Among various *in vitro* radical scavenging assays, DPPH assay is the most popular and widely used assay. In alcoholic solution, DPPH radical shows maximum absorption at 517nm. On accepting an electron or hydrogen atom from donor (antioxidant), DPPH radical is converted into a non-radical DPPHH and the purple color changes to yellow. The scavenging of DPPH free radicals is used to assess radical scavenging potential of various kinds of substances including lichen extracts. This assay

is advantageous as it is simple, rapid, sensitive and requires only small amount of sample (Chung *et al.*, 2006; Kaviarasan *et al.*, 2007; Kekuda *et al.*, 2011; Sharma and Kalikoty, 2012; Kekuda *et al.*, 2013; Vivek *et al.*, 2014a). In the present study, we evaluated radical scavenging effect of extract of *P. cristiferum* and *D. applanata* by DPPH assay. We determined the absorbance of DPPH radical solution at 517nm in the presence of varying concentration of lichen extracts. The lichen extracts exhibited concentration dependent scavenging of radicals. Among extracts, *D. applanata* displayed stronger scavenging effect than that of *P. cristiferum* as revealed by lower IC<sub>50</sub> value. The scavenging effect of lichen extracts was weaker when compared to reference standard. Similar results were observed in the study of Sharma and Kalikoty (2012) and Vivek *et al.* (2014a) where extracts of *Parmotrema* species displayed low scavenging potential when compared to ascorbic acid. Although the scavenging effect of lichen extracts of this study was lesser than that of reference standard, it is evident that the extracts exhibit hydrogen donating ability and could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al.*, 2006).

#### Total Phenolic Content of Lichen Extracts

Polyphenols including flavonoids are a large group of naturally occurring chemicals having diverse biological activities including antioxidant activity. These compounds exhibit antioxidant activity because of their redox potential and act as reducing agents (free radical terminators), hydrogen donors, metal chelators and singlet oxygen quenchers. FCR method is the oldest and most commonly used method for estimating the content of total phenolics in a variety of samples including lichen extracts. The phenolic compounds react with FCR only under basic conditions and form blue complex displaying absorption maxima near 750nm. This assay is convenient, simple, and reproducible (Chung *et al.*, 2006; Kaviarasan *et al.*, 2007; Choi *et al.*, 2007; Coruh *et al.*, 2007; Kekuda *et al.*, 2011; Rekha *et al.*, 2012; Vivek *et al.*, 2014a). In the present study, we estimated the content of total phenolics by FCR method. The phenolic content was found to be highest in case of extract of *D. applanata* when compared to *P. cristiferum*. Many studies have revealed direct correlation between antioxidant activity and phenolic content (Coruh *et al.*, 2007; Rekha *et al.*, 2012). In the present study also, similar observation was noticed as the extract of *D. applanata* having high phenolic content displayed higher scavenging activity. In a similar study by Vivek *et al.* (2014a), a direct correlation between antioxidant activity and phenolic content of *Parmotrema* species was observed. In another study, Pavithra *et al.* (2013) showed a direct correlation between total phenolic content and antioxidant activity of solvent extracts of the lichen *Usnea pictoides*.

#### Insecticidal Activity of Lichen Extracts

Among various vectors transmitting dreadful diseases, mosquitoes remain the most important single group of insects. Mosquitoes spread a number of diseases such as dengue fever, chickungunya, yellow fever, malaria, filariasis, Japanese encephalitis and others in tropical and subtropical areas. Among various approaches, targeting and killing of larvae is one of the most important approaches used for mosquito control. It is a successful method for reducing the mosquito densities before they reach adult stage. Killing mosquito larvae mainly depends on the use of synthetic chemicals which have drawbacks such as high cost, environmental problems and

development of resistance in mosquitoes. The use of natural products including lichen extracts and their bioactive compounds seems to be an alternative strategy for insect control. The natural products are selective in action, have little or no adverse effect on non-target organisms and environment (Emmerich *et al.*, 1993; Cetin *et al.*, 2008; Swathi *et al.*, 2010; Kumar *et al.*, 2010a; Yildirim *et al.*, 2012). In the present study, we evaluated insticidal activity of lichen extracts against 2<sup>nd</sup> instar larvae of *A. aegypti*. It was observed that the lichens had dose dependent larvicidal potential. Here also, extract of *D. applanata* exhibited potent activity in terms of causing larval mortality to higher extent than *P. cristiferum*. Earlier studies have shown that the lichens possess inhibitory activity against larvae of *A. aegypti*. The study of Vinayaka *et al.* (2009) revealed dose dependent inhibitory effect of lichen extracts against 2<sup>nd</sup> instar larvae. Extract of *Everniastrum cirrhatum* was more effective against 2<sup>nd</sup> instar larvae when compared to 3<sup>rd</sup> instar larvae (Swathi *et al.*, 2010). Extract of *Ramalina hossei* exhibited stronger larvicidal effect against 2<sup>nd</sup> instar larvae when compared to *R. conduplicans* (Kumar *et al.*, 2010a). Methanolic extract of *P. pseudotinctorum* exhibited stronger insecticidal activity than other extracts against 2<sup>nd</sup> instar larvae (Vinayaka *et al.*, 2010).

## CONCLUSIONS

Non-vascular plants including lichens have been traditionally used worldwide for various purposes such as food, medicine and others. In this study, both macrolichens exhibited antimicrobial, insecticidal and radical scavenging activity. When compared to antimicrobial and insecticidal activity, the radical scavenging activity of lichen extracts was low. Overall, *D. applanata* displayed marked bioactivities when compared to *P. cristiferum*. This may be ascribed to high phenolic content and bioactive secondary metabolites. These lichens appear to be promising resources and may be used for the development of bioactive agents. Further studies on recovery of active components from lichen extracts and their bioactivity determinations are to be carried out.

## Acknowledgements

Authors are thankful to Chairman, Dept. of Studies and Research in Microbiology and Principal, Sahyadri Science College (Autonomous), Kuvempu University for providing all facilities and moral support to conduct work.

## Conflict of Interest

All the Authors declared no conflict of interest.

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