Current Research in Environmental & Applied Mycology 5 (2): 92–100 (2015) ISSN 2229-2225

www.creamjournal.org **Article** CREAM

Online Edition

Doi 10.5943/cream/5/2/3

Assessment of fungicidal potential of lichen *Heterodermia leucomelos* (L.) Poelt against pathogenic fungi

Babiah PS [1,2], Upreti DK[1], John SA[2]

[1]Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh-226001, India
[2]Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad, Uttar Pradesh, India – 211007

Babiah PS, Upreti DK, John SA 2015 – Assessment of fungicidal potential of lichen *Heterodermia leucomelos* (L.) Poelt against pathogenic fungi. Current Research in Environmental & Applied Mycology 5(2), 92–100, Doi 10.5943/cream/5/2/3

Abstract

The present investigation focuses on the evaluation of fungicidal potential of a foliose lichen *Heterodermia leucomelos* (L.) Poelt against five strains of phytopathogenic fungi. Acetone, methanol and chloroform extracts of test lichen were screened against pathogens *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium solani, Colletotrichum falcatum* by the Disc – Diffusion Assay. MIC values of the extracts were determined by Broth tube dilution method. Among the three solvents acetone and methanol exhibited highest activities. Mean diameter of zones of inhibition for acetone and methanol extracts ranged from 18.0±0.0 to 28.6±0.3 mg/ml and 12.3±0.6 to 24.6±1.2 mg/ml. respectively with MIC values ranging from 0.19 to 1.56 mg/ml. Tukey's multiple comparison test provides significant differences (at $p<0.05$ and 0.01) in the activity of the extracts towards different phytopathogens. The results proved that the lichen holds high medicinal properties and can be a rich source of potential natural antimicrobial agents.

Keywords – Antifungal activity **–** disk Diffusion method **–** *Heterodermia leucomelos* (L.) Poelt **–** MIC **–** plant pathogenic fungi

Introduction

Plant diseases are the major topic of concern for researchers nowadays as it directly affects the yield, nutritional value and quality assurance of the plants including cash crops and grains which hampers the agricultural productivity qualitatively, quantitatively and economically. Evey year more than 10% food is lost in our country due to plant diseases (Hadian 2012). Among the microbial agents responsible for severe plant diseases, plant pathogenic fungi are the main infectious agents in plants causing alterations during developmental stages including post-harvest (Strange et al. 2005). Fungi causes the greatest impact with regard to diseases and crop production losses. In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens (Dellavalle et al. 2011). Especially, species of *A. niger* and *flavus* and species of *F. oxysporum* and *solani* are considered to be highly detrimental to the agricultural crops leading to high yield lossess. They are the causative agents of most fatal infectious diseases in the most common consumable crops, cash crops, cereals, grains, legumes, fruits and vegetables. *A. niger* is the causative agent of "Mold diseases" in onion,

Submitted 2 February 2015, Accepted 10 May 2015, Published online 22 May 2015
Corresponding Author: D.K Upreti – e-mail – upretidk@rediffmail.com

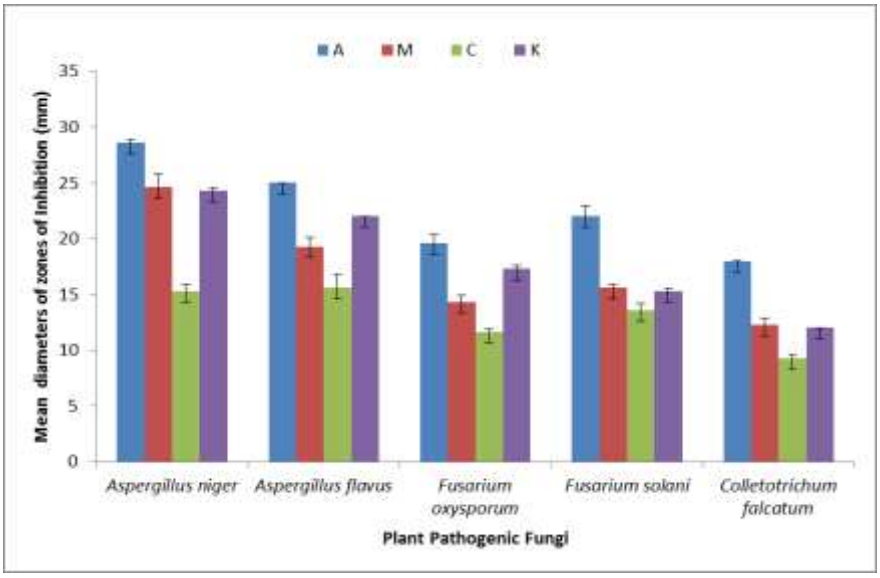

Fig. 1 – The data represents the comparitive Antifungal activity of *H. leucomelos* with the commercial synthetic standard Ketoconazole. The results are mean values ± SE of three independent replicates**.**

garlic, maize, peanuts, grapes and is a common contaminant of food (Sharma 2012). *A. flavus* is responsible for causing severe damaging "Rot diseases" in cereal grains and legumes. The affected crops are rice, wheat, barley, corn, grains, legumes. *F. oxysporum* causes "Fusarium wilt" in tomato, tobacco, sweet potato, banana, cucurbits (Hadian et al. 2012) and *F. solani* causes "Root Rot diseases" in potato, tomato, brinjal etc and *C. falcatum* is the responsible for causing most fatal disease of "Red Rot of sugarcane" in Sugarcane which causes huge loss of sugarcane crops all over the world (Singh & Singh 1989). Management of these pathogens is required, as these pathogens affect a wide variety of hosts of economic value.

These phytopathogenic fungi are generally controlled by synthetic fungicides, but the intensive use of fungicides have resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment and also in the built-up of resistance in pathogens (Thakur et al. 2014). So, the use of these is increasingly restricted due to their harmful effects on human health and environment (Harris et al. 2001). Hence, an environmental friendly approach is required and the synthetic chemicals need to be replaced by safe, biodegradable products. In this regard natural plant products are gaining much attention of scientists worldwide. Such products are relatively broad-spectrum, bioefficaceous, economical, biodegradable and environmentally safe. Similar to higher plants lichens also serve as potential natural product against various diseases since ancient times.

Lichens are symbiotic organisms comprised of a fungal partner (mycobiont) and an algal partner (photobiont). The photobiont can be green algae, cyanobacteria or both (Nash, 1996). Other than primary metabolites, they comprise of unique secondary metabolites which are specific and unknown in other plant sources. Their flexibility in habitat enables them to synthesize naturally occurring potential metabolites which are different in their chemical structures as well as in the biological activities. Because of these secondary metabolites the lichens are known to possess manifold biological activities such as antimicrobial, antibacterial, antiviral, antiprotozoal, antioxidant, enzyme inhibitory, insecticidal, wound healing and anti-inflammatory (Ingolfsdottir K et al 1997, Muller K 2001, Halama P & van Haluwin C 2004, Behera et al. 2005). Since traditional times numerous researchers have studied and proved the antimicrobial potential of lichen extracts. However, studies related to their potential usage as fungicidal agrochemicals are very few and is yet to be explored to a great extent. So far, the antifungal properties of lichens have been studied by

Land and Lundstrom 1998, Shahi et al 2003, Rankovic et al 2007, Marijana et al 2010, Sati S.C. et al 2011, Tiwari et al 2011.

H. leucomelos (L.) Poelt (Physciaceae) is a foliose lichen growing luxuriantly on rocks and trees of Himalayan regions and Western Ghats of India (Awasthi 2007). The first antifungal activity of aqueous extract of *H. leucomelos* was studied by Shahi et al (2001) against some pathogenic fungi. Hence, the present study is aimed to investigate the fungicidal potential and biocontrol efficacy of different solvent extracts of lichen *H. leucomelos* in order to determine possible inhibitory effects against the selected important plant pathogenic fungi and this is the first study targeting the antifungal activity of a lichen against plant pathogenic fungi *Colletotrichum falcatum* which is the causative agent of "Red rot of sugarcane" disease.

Materials & Methods

Collection & Identification of Lichen samples

The lichen sample of *H. leucomelos* was collected from temperate Himalayan regions of Uttarakhand district, India at 31º05´56.36" N latitutde and 78º17´35.94" E longitude, on rock, bark and soil. The Identification was done morpho-anatomically and chemically with the help of colour tests and standard Thin Layer Chromatography (TLC) technique (Elix et al 1993 & Orange et al 2001) using Solvent system A having TDA (Toluene 180: Dioxane 60: Acetic acid 8). Identification was done using relevant literature (Table 1) (Divakar & Upreti 2005, Awasthi 2007). The voucher specimen of the selected lichen was deposited at the Lichen herbarium (LWG), CSIR-National Botanical Research Institute (NBRI), Lucknow, India.

Table 1 Morpho-anatomical and chemical characters of lichen *H. leucomelos* selected for study.

Habitat	Thallus	Colour Test	TLC
Corticolous or Terricolous	Loosely attached, dichotomously branched, pendulous, lobes tapering at apices, upper surface corticated, grey; lower surface canaliculate, white; rhizines marginal, black.	Medulla K+ yellow turning red, C − , P+ yellow.	Zeorin, Norstictic, Salazinic acids, Atranorin

Extraction from lichen samples

The selected lichen specimen was sorted, cleaned of substratum, washed with distilled water and dried for extraction. The air dried lichen sample was grinded mechanically to powder to a particle size of < 2.5 mm, and then extracted using Soxhlet Extractor equipped with a Reflux condenser (Harwood & Moody 1989, Bauer 1959) in 3 different solvents, Acetone, Methanol and Chloroform, differing in polarity. The solvent extraction was carried out at the specific boiling temperature of the solvents, i.e. (acetone- 56ºC, methanol- 65ºC and chloroform- 61.2ºC) for 48 hours for complete extraction of secondary compounds. The crude lichen extracts obtained were filtered through Whatmann No.1 filter paper and then concentrated to dryness in vacuo at 50ºC using Rotary vacuum Evaporator (Buchi Rotavapour R-200™). Extracts were stored at −80ºC for further assays.

Micro-organisms & Media

Five strains of plant pathogenic fungi were used as test organisms in the study, viz, *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium solani, Colletotrichum falcatum* which were obtained from mycological collection maintained by Babasaheb Bhimrao Ambedkar University, Lucknow and and Microbiology laboratory of Indian Institute of Sugarcane Research (IISR), Lucknow. The fungal cultures (slants) were maintained on Potato Dextrose Agar

(PDA) and were transferred to Sabouraud Dextrose Broth (SDB) for experimental purposes. All cultures were stored at -4ºC and subcultured every 15 days.

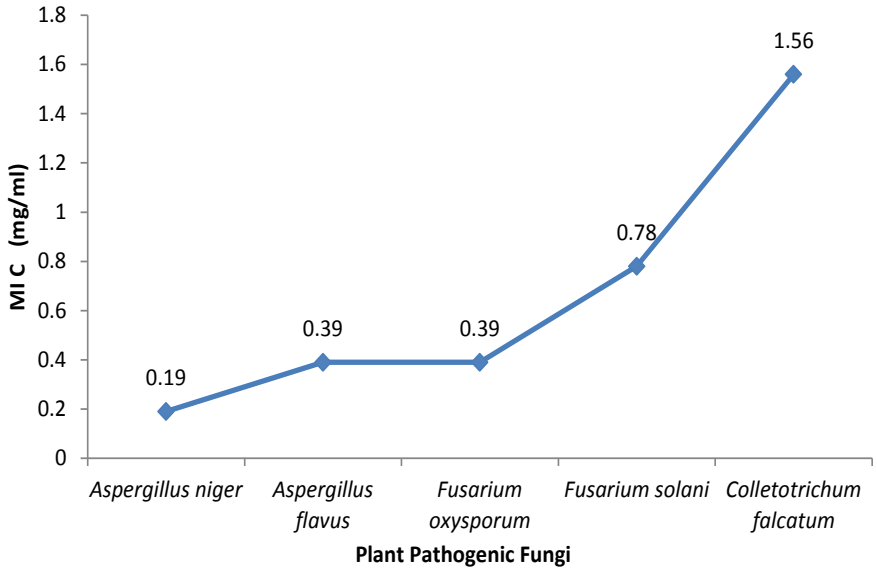

Fig. 2 – The values of Minimum Inhibitory Concentration (MIC in mg/ml.) of *H. leucomelos* against tested five fungal phytopathogens

Determination of Antifungal activity

The antifungal susceptibility test against pathogens was performed by employing Disc-Diffusion method of Kirby and Bauer recommended by the National Committee for Clinical Laboratory Standards (NCCLS 1993 & 2002).

The stock solutions of recovered lichen extracts were prepared in their respective solvents. The fungal pathogens were swabbed aseptically from their broth cultures onto sterile potato dextrose agar (PDA media) plates. Experimental paper discs (6 mm diameter) were soaked with 30-50µl of lichen extract and laid on the inoculated substrate. Commercially available synthetic standard antifungal drug Ketoconazole was used as a positive control. The plates were sealed and incubated for 3 days at 25ºC. The antifungal activity was evaluated by measuring the inhibition zone diameter around the disc. All the experiments were performed in triplicates.

Determination of Minimum Inhibitory Concentration (MIC value)

The minimal inhibitory concentration (MIC) was determined by the Broth tube dilution method (NCCLS 2002). A series of dilutions with concentrations ranging from 50 - 0.01 mg/ml was used in the experiment for every microorganism tested. The starting solutions of extracts with a concentration of 50 mg/ml were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. Two fold dilutions of extracts were prepared. Fungal inoculums were transferred into each test tube containing 5 ml of Sabouraud Dextrose Broth (SDB). About 50µl of inoculum was taken from fungal cultures and added in test tubes alongwith the respective concentration of lichen extracts prepared. All the test tubes were subsequently incubated at 25ºC for 48 hours. The boundary dilutions without any visible growth was defined as the minimal inhibitory concentration (MIC) for the tested micro-organism at the given lichen extract concentration. The last test tube carrying no visible growth of micro-organism was rechecked by Agar Plate method in triplicates.

Statistical Analysis

The results of experimental antifungal activities are expressed as Mean ± SE of three replicates determinations in each sample. Statistically significant differences among the activity of three solvent extracts and differences of their effects on different pathogens used for activity were measured using Tukey's multiple comparison test at $p<0.05$ and 0.01.

Table 2 Phytopathogenic fungi and its impact on crop plants

S.No.	Phytopathogenic Fungi	Affected Crops	Its Impact (Diseases)
1.	*Aspergillus niger*	Onion, garlic, peanuts, grapes, maize, some fruits and vegetables	Causes Mold diseases, like, "Black mold of fruits and vegetables"
2.	*Aspergillus flavus*	Rice, wheat, barley, corn, other cereal grains and legumes	Causes Rot diseases of cereal grains and legumes, like, "Ear rot of corn"
3.	*Fusarium oxysporum*	Tomato, tobacco, sweet potato, banana, cucurbits	Causes "Fusarium wilt"
4.	*Fusarium solani*	Potato, tomato	Causes "Fusarium Root rot"
5.	*Colletotrichum falcatum*	Sugarcane	Causes severe damaging "Red rot of sugarcane" disease

Results

The fungicidal potential of Lichen *Heterodermia leucomelos* (L.) Poelt was evaluated in the present study against a broad panel of disease causing micro-organisms commonly found in the environment. Table 1 and Table 2 shows the details of the lichen selected in this study and the impact of selected pathogens on crop plants respectively. In the results obtained, a broad spectrum of antifungal activity was observed using different extraction solvents, i.e. acetone, methanol and chloroform extracts. The activity was determined by employing the Kirby-Bauer Disc-diffusion technique and its assessment was based on the measurement of diameters of zones of inhibition (mm) formed around the disc (Table 3). The differential extracts of lichen *H. leucomelos* demonstrated strong antifungal activity towards all the pathogenic fungi tested. The highest activity was shown by acetone extract followed by methanol and least activity was given by chloroform extract. All the three solvent extracts showed highest activity against *A. niger* and lowest activity against *C. falcatum*. The differential and moderate activities were observed against other pathogens but the results revealed that activity of lichen extract was greater in comparison to the synthetic standard Ketoconazole. The antifungal activity of extracts showed little variation and excellent reproducibility of zone of inhibition for all selected pathogens. In *Aspergillus* species, the largest inhibition zone diameter was found in *A. niger* with 28.6±0.3mm and then in *A flavus* with 25.0±0.0mm. On the other hand, largest diameter of inhibition zone among *Fusarium* species, was found in *F.solani* with 22.0±1.0mm followed by *F. oxysporum* with 19.6±0.8mm and lowest for *C. falcatum* with 18.0±0.0mm in acetone extracts. The results were compared with the synthetic standard Ketoconazole (Fig.1) for which the largest area of inhibition zone was observed against *A. niger* with 24.3±0.3 mm followed by *A. flavus* which was recorded to be 22.0±0.0 mm, *F. oxysporum* with 17.3±0.3 mm and *F. solani* with 15.3±0.2 mm which was further followed by *C. falcatum* with 12.0±0.0 mm. The acetone extract gave strong and promising results in comparison to the standard. The results also showed that different solvent extractions gave different results against the same pathogens. As seen in Table 3, the methanol extract was more potent to *A. niger* with growth inhibition of 24.6±1.2 mm which was higher than the standard. The other pathogens were also susceptible to the extract and showed equivalent inhibition as shown by the standard. The results of the antifungal activity of differential lichen extracts alongwith the standard Ketoconazole revealed that the intensity of pathogenic inhibition by the extracts was shown in following sequence : acetone > ketoconazole > methanol > chloroform.

Based on Tukey's multiple comparison test (Table 4) acetone was found best extraction solvent as antifungal activity in acetone was significantly different at $p<0.01$ with methanol and chloroform at $p<0.05$ with ketoconazole. Tukey's analysis showed that there was no any significant difference in antifungal activity when extract was prepared either in methanol or chloroform. Difference between the antifungal activity of *H. leucomelos* on *A. niger* and *A. flavus* was

negligible as Tukey's multiple comparison test shows non-significant difference at level $p<0.05$ and $p<0.01$. This indicates that *H. leucomelos* is capable to inhibit the growth of *A. niger* and *A. flavus* with same extent. Same effect was observed for *F. solani* and *F. oxysporum*. The antifungal activity of extract was found significantly lower in *C. falcatum* compared to *A. niger, A. flavus, F. solani, F.*

Table 3 Mean diameters of zones of inhibition (mm) of extracts of *H. leucomelos* against test pathogens

S.No.	Phytopathogenic fungi	*Heterodermia leucomelos* Extracts			Standard
		Acetone	Methanol	Chloroform	Ketoconazole
1.	*Aspergillus niger*	28.6±0.3	24.6±1.2	15.3±0.6	24.3±0.3
2.	*Aspergillus flavus*	25.0±0.0	19.3±0.8	15.6±1.2	22.0±0.0
3.	*Fusarium oxysporum*	19.6±0.8	14.3±0.6	11.6±0.3	17.3±0.3
4.	*Fusarium solani*	22.0±1.0	15.6±0.3	13.6±0.6	15.3±0.2
5.	*Colletotrichum falcatum*	18.0±0.0	12.3±0.6	9.3±0.3	12.0±0.0

(values are in arithmetic mean ± standard error)

oxysporum. Thus, it may be concluded that the extract showed very low antifungal activity in *C. falcatum* and is very effective for *A. niger, A. flavus, F. oxysporum, F. solani.* The statistical analysis proved that there was significant differences among the solvent extracts as well as their effect on different pathogens.

The lowest concentration of growth inhibition of pathogen was determined by Broth tube dilution method, which was defined as the minimal concentration at which no growth of micro-organism was observed after incubation which was considered as the MIC value (Fig 2). For all the micro-organisms tested, the results were obtained at the concentration ranging from 0.19 to 1.56 mg/ml. Highest MIC value of 0.19 mg/ml was observed against *A. niger* and lowest value of 1.56 mg/ml against *C. falcatum.* The lower the MIC value, the more active is the extract at the particular concentration.

Table 4 Tukey's multiple comparison test for zones of inhibition (mm) of differential extracts of *H. leucomelos* against selected pathogenic micro-organisms

Extract	***Acetone***	***Methanol***	***Chloroform***
Methanol	5.18**	x	
Chloroform	6.43**	2.28^{NS}	x
Ketoconazole	3.84*	1.89^{NS}	2.70*

Effect on Pathogen	***A. niger***	***A. flavus***	***F. oxysporum***	***F. solani***
A. flavus	1.54^{NS}	x		
F. oxysporum	3.81*	5.48*	x	
F. solani	3.47*	5.60*	1.73^{NS}	x
C. falcatum	5.40*	10.50**	6.74**	17.15**

** = Significant differences among the values of the extracts at $p<0.05$ and $p<0.01$,

* = Significant difference among the values at $p<0.05$,
NS = non-significant differences between antifungal activity.

Discussion

The results obtained in the present study indicate that lichen *Heterodermia leucomelos* possess biocidal properties. The antifungal activity with varying zones of inhibition reveals the fungicidal potency of this lichen, where the extracts were able to inhibit the growth of all the pathogens. The activity could be ascribed to the presence of a broad spectrum of bioactive compounds from extracts of the studied lichen. The differential extracts of *H. leucomelos* showed conspicuous degrees of antifungal activity, where the acetone extract exhibited strong inhibitory effect on pathogens in comparison to other solvent extracts. Also its results were higher than that of standard, which suggests it to be a potential natural biocontrol agent.

Following this the methanol extract gave good results where its activity was higher than the standard for *A. niger*, which reveals that the differential extracts of this selected lichen are able to combat this particular pathogen strongly. For other pathogens the activity of methanol extract was good and effective but less than the standard. The chloroform extracts showed the inhibitory activity against all the pathogens but the results were non-significant in comparison to others. This may be due to the difference in polarities of different solvents. Since bioactive components of any medicinal plant have different solubility in different extracting solvent (Oloke & Kolawole 1998). All selected fungal species were susceptible to the extract, but variations occurred depending on the type of extracting solvent, on the type of lichen species and pathogenic microorganisms selected. As acetone and methanol extracts were more effective in the inhibition of pathogenic growth as compared to the chloroform extracts, it is suggested that the polar solvents acetone and methanol are most efficient in extracting secondary metabolites responsible for antimicrobial property than non-polar solvents (Banso et al. 2007) from lichen *H. leucomelos.*

The antifungal potential revealed from the present study is in accord with numerous previous reports. Shahi et al (2001) studied the broad spectrum of antifungal properties of lichen *H. leucomelos* and found similar results, where the lichen was able to inhibit the growth of *A. flavus* and *F. oxysporum.* Rankovic et al (2009) studied the strong antifungal effect of acetone and ethanol extracts some lichen species against *Aspergillus* and *Fusarium* species. Halama & Haluwin (2004) obtained similar results in studying the antifungal activity of lichen extracts against phytopathogenic fungi. Land & Lundstrom (1998) studied the inhibition of fungal growth by extracts from lichen *Nephroma articum.* Recently Shivanna et al (2014) reported the strong antifungal effect of acetone, methanol and ethyl acetate extracts of some lichen species against *F. oxysporum.* These similarities are a positive evidence towards the findings of the present study.

Interestingly, the extracts of *H. leucomelos* in the present study were also found effective against *C. falcatum,* which is a deadly pathogen of sugarcane and many researches are in progress to combat this pathogen and this is the first study regarding the investigation of lichen, as antifungal agent against this pathogen and no antifungal study is reported against this.

It is also observed that the plant extracts inhibit the growth of various microorganisms at different concentrations (Esimone et al. 1999), therefore, the minimum inhibitory concentration was obtained in a range of 0.19 to 1.56 mg/ml against different pathogens by the standard Broth Tube Dilution method (NCCLS, 2000). The minimal inhibitory concentration is the lowest concentration of a material which inhibits the growth of an organism. The minimal fungicidal concentration was found against *C. falcatum* and highest against *A. niger* which is in agreement with the results shown by disk-diffusion technique against different pathogens.

Thus, the study suggests that lichen have unique secondary metabolites, known as “lichen substances” which have antibiotic, antimicrobial and medicinal properties responsible for showing strong potency to combat various pathogenic micro-organisms because of which lichens have also been used in traditional medicinal system for centuries (Dayan & Romagni 2002) and hence the same is proved for lichen *H. leucomelos* against plant pathogenic fungi in this investigation study.

This study revealed the importance of natural extracts of lichen *H. leucomelos* as a potential antifungal agent, since, in comparison to the synthetic standard it gave effective and significant results which suggests that it is a strong alternative as a natural drug to synthetic drugs, as nowadays, researchers are imperative to identify natural antimicrobial compounds and formulate new and more potent antimicrobial drugs of natural origin which are safe for environment as well as for well – being.

Conclusion

It may be concluded from the present study that the investigated extracts of lichen *H. leucomelos* manifested strong antifungal activity. This suggests the expedience of isolation of the active components from different extracts of the investigated lichen for possible use in treating many diseases caused by plant micro-organisms.

Acknowledgements

The authors are thankful to The Director, CSIR-National Botanical Research Institute, Lucknow, India for providing necessary laboratory facilities to carry out this work.
Financial support of GAP 3304 is gratefully acknowledged.

References

Awasthi DD. 2007 – A compendium of the Macrolichens from India, Nepal and SriLanka. Bishen Singh Mahendra Pal Singh. Dehra Dun, India.

Banso. 2007 – Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea.* African Journal of Biotechnology 6, 1785–1787.

Bauer AW, Perry DM, Kirby WMM. 1959− Single disc antibiotic testing of *Staphylococci.* AMA Arch. Intern. Med. 104, 208–216.

Behera BC, Verma M, Sonone A, Makhija U. 2005 – Antioxidant and antimicrobial activities of lichen *Usnea ghattensis* in vitro. Biotechnology Letters 27, 991–995.

Dayan FE, Romagni JG. 2002 – Structural diversity of lichen metabolites and their potential for use. In Advances in Microbial Toxin Research and Its Biotechnological Exploitation.

Dellavalle PD, Cabrera A, Alem D, Larranaga P, Ferreira F, Rizza MD. 2011 – Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria sp.

Divakar PK, Upreti DK. 2005 – Parmelioid Lichens in India: A Revisionary study. Bishen Singh Mahendra Pal Singh. Dehradun. pp488.

Elix JA, Ernst Russel JL. 1993 – A catalogue of standardized thin layer chromatographic data and biosynthetic relationships for lichen substances. Australian National University, Canberra.

Esimone CO. 1999 – Antimicrobial activity of the cytotoxicity of *Ramalina farinaceae.* Fitoterapia 7, 428–431.

Hadian S. 2012 – Antifungal activity of some plant extracts against some plant pathogenic fungi in Iran. Asian Journal of Exp. Biological Sciences. 3(4),714–718.

Halama P, van Haluwin C. 2004 – Antifungal activity of lichen extracts and lichenic acids. Biocontrol 49, 95–107.

Harris CA, Renfrew MJ, Woolridge MW, 2001 – Assessing the risk of pesticide residue to consumers: Recent & future developments. Food additives & contamination 18,1124–1129.

Harwood LM, Moody CJ. 1989 – Experimental organic chemistry: Principles and Practice (Illustrated edition ed.), pp. 122−125.

Ingolfsdottir K. 1997 – *In vitro* susceptibility of *Helicobacter pylori* to protolichesterinic acid from *Cetraria islandica.* Antimicrobial agents chemotherapy 41, 215–217.

Land CJ, Lundstrom H. 1998 – Inhibition of fungal growth by water extracts from the lichen *Nephroma articum.* Lichenologist 30, 259–262.

Marijana 2010 – Antioxidant properties of some lichen sp. Journal of Food Sci Technol. 48(5), 584 –590.

Muller K. 2001 – Pharmaceutically relevant metabolites from lichens. App. Microb. Biotech. 56, 9 –16.

Nash TH. 1996 – Lichen Biology. Cambridge University Press, Cambridge. 303 pp.

NCCLS (National Commitee for Clinical Laboratory Standards). 1993 – Performance Standards for antimicrobial disk susceptibility tests, Approved Standard. NCCLS documentM2-A5, Valionova, PA, USA.

NCCLS (National Commitee for Clinical Laboratory Standards). 2002 – Performance standards for antimicrobial susceptibility testing. Twelfth informational supplement. NCCLS document M100–S12. NCCLS, Wayne, Pa.

Oloke JO, Kolawole DO. 1998 – The antibacterial and antifungal activities of certain components of *Aframomum melleguetea* fruits. Fitoterapia 59, 384–388.

Orange A, James PW, White FJ. 2001 – Microchemical methods for the Identification of Lichens. London, British Lichen Society.

Rankovic B, Misic M, Sukdolak S, Milosavljevic D. 2007 – Antimicrobial activity of the lichen *Aspicilia cinerea, Collema cristatum, Ochrolechia androgyna, Physcia aipolia, Physcia caesia.* Italian Journal of Food Science 4, 461–469.

Rankovic B, Misic M, Sukdolak S. 2009 – Antimicrobial activity of extracts of lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes, Umbilicaria polyphylla.* Biologia 64 (1), 53–58.

Shahi SK, Shukla AC, Dikshit A, Upreti DK. 2001 – Broad spectrum antifungal properties of lichen *Heterodermia leucomela*. Lichenologist. 33, 177–179.

Sharma R. 2012 – Pathogenecity of *Aspergillus niger* in plants. Cibtech Journal of Microbiology. 1(1), 47–51.

Shivanna R., Garampalli R.H. 2014 – Efficacy of lichen extracts as biocontrol agents against *Fusarium oxysporum* F. sp. capsici. Adv. Appl. Sci. Res. 5(5), 273–277.

Singh K, Singh RP. 1989 – Red Rot, In: Ricaud CG, Egan BT, Gillaspie AG, Hughes CG (eds.) Sugarcane diseases of the world: Major diseases, pp: 69–88. Elsevier, Amsterdam, the Netherlands.

Strange RN, Scott PR. 2005 – Plant diseases: A threat to global food security. Annual Review of Phytopathology, 43, 83–116.

Thakur S, Harsh NSK. 2014 – Efficacy of volatile metabolites of phylloplane fungi of *Rauwolfia serpentine* against *Alternaria alternate.* Current Research in Environmental and Applied Mycology 4(2), 152–156.

Tiwari P, Rai H, Upreti DK, Trivedi S, Shukla P. 2011 – Assessment of antifungal activity of some Himalayan foliose lichen against plant pathogenic fungi. American Journal of Plant Sciences. 2, 841–846.