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In vitro antimicrobial potentials of endolichenic fungi isolated from thalli of *Parmelia* lichen against some human pathogens

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ABSTRACT

This study was undertaken to isolate and evaluate antimicrobial potentials of endolichenic fungi colonizing lichen thalli of *Parmelia* sp. A total of 19 distinct endolichenic fungi were obtained from surface sterilized fragments of *Parmelia* thalli. The dominant fungi belonged to genera *Phomopsis*, *Aspergillus*, *Penicillium* and mycelia sterilia. The colonization frequency of mycelia sterile (47.4%) was found to be the highest. The result indicated that 10.52% of the isolates showed antimicrobial activity against all the test pathogens in varying degrees, while 31.57% and 10.52% of the isolates displayed antibacterial and antifungal activity inhibiting all bacterial and fungal pathogens respectively. Among the isolates, species of *Aspergillus* and *Cytospora* and two sterile isolates showed considerable antimicrobial activity. These isolates were cultured in different media and incubation periods for optimum metabolites production. The crude metabolites of the isolates showed significant antimicrobial activity against all the test pathogens. Analyses of the crude metabolites by Thin Layer Chromatography and spectrophotometer study showed presence of bioactive components. The study suggests that endolichenic fungi colonizing lichen thalli may be a source of potential antimicrobial agents.

1. Introduction

There is an urgent need for new antimicrobial agents to combat drug resistance in bacteria and for effective treatment of systemic infection by fungi. Various antibacterial and antifungal agents have been explored, but the control of many of the bacterial and fungal diseases has not yet been achieved. Over the years, metabolites from plants and microbes are considered as important sources of drugs for therapeutic applications in several countries. Among them, 50–60% is produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, etc.)

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and 5% have a microbial origin (Demain and Sanchez, 2009). It is believed that the less explored microbial diversity might provide new and interesting metabolites with wide therapeutic applications. Therefore research is underway to study diverse and cryptic microorganisms from various habitats to obtain novel metabolites.

The symbiotic association between a fungus and an alga results into a new life form, called lichen. Lichens are widely distributed and conquered in the early developmental stages of biological communities that undergo primary and secondary successions (Logesh et al., 2012). Endolichenic fungi living in close association with the alga in the thalli of lichen are analogous to that of plant endophytes inhabiting the intercellular spaces of the hosts. They have been demonstrated to produce various antibiotics and natural bioactive compounds with multiple applications (Wu et al., 2011). However, relatively very few studies have been undertaken on endolichenic fungi, and the substances they produced have not been investigated in detail for their bioactivity and therapeutic potentials.

The objective of this present study was to evaluate the antimicrobial potentials of ethyl acetate extracts of some endolichenic fungi inhabiting the lichen thalli of *Parmelia* sp. collected from Similipal Biosphere Reserve, India, against some clinically significant human pathogens and to optimize and characterize the antimicrobial metabolites of some potent strains.

2. Materials and methods

2.1. Collection of samples and identification of lichen

The lichen thalli of *Parmelia* sp. were collected from the Joranda forest division of the Similipal Biosphere Reserve (SBR), India, located at 21° 16′ to 22° 08′ North latitude and 86 ° 4′ to 86° 37′ East longitude. The lichen thallus was light green and pale yellow in the upper cortex when dry. The lobes were round and smooth, measuring 3–8 mm, but quite often have a wrinkled appearance especially in older specimens. The lower surface is black except for a brown margin; rhizomes attached to the lower surface are black and unbranched. The thalli were mostly found associated on tree bark (Photoplate-1). Based on its morphological features the species resembles that of *Parmelia caperata*. The tree barks were ensured for the presence of the *Parmelia* sp., and healthy looking thalli were cut out from the tree bark with a sterile knife and placed into sterile plastic collection bags. The collected samples were brought to the laboratory in sterile polythene bags and processed within 24 hours of collection.

2.2. Isolation and identification of endolichenic fungi

The isolation procedure of endolichenic fungi was carried out following the method described by Li et al. (2007) with slight modification. Lichen thalli were washed thoroughly with Milli-Q water to remove dirt or debris from surfaces and then surface sterilized by immersing sequentially in 10% of 30% hydrogen peroxide (H_2O_2) solution for 4–5 min followed by rinsing with 70% alcohol for 5 s and 1% of 4% sodium hypochlorite (NaOCl) for 6 min and washed with sterile distilled water. The lichen thalli were then dried by placing over sterile filter papers and dissected aseptically to a fragment of 1 mm^2 size. The efficiency of sterilization process was verified by rubbing the sterilized fragment over Potato dextrose agar medium and incubated for growth of contaminant if any. Each fragment was then inoculated onto Potato Dextrose Agar (PDA) medium. The plates were sealed with parafilm and incubated in BOD incubator at 30 °C until growth of endolichenic fungi. The plates were observed once a day for fungal growth. After several days of incubation the colonies growing out of surface sterilized fragments were immediately isolated, sub-cultured and maintained at 4 °C in PDA slants. The isolates were identified based on their morphological and reproductive characters using the standard identification manuals (Barnett and Hunter, 1998; Gilman, 1971). The cultures that failed to sporulate were categorized as sterile mycelia.

2.3. Screening of for antimicrobial activity

Pure cultures of endolichenic fungi were cultivated on Potato Dextrose Broth (PDB) by placing agar blocks of actively growing culture (3 mm in diameter) in 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated in BOD shaking incubator for 14 days at 29 ± 1 °C with periodic shaking at 150 rpm. The cultures were filtered through sterile cheesecloth to remove the mycelial mats. The liquid broth were collected and extracted with equal volume of ethyl acetate in a separating funnel by vigorous shaking for 15 min. The cell masses were separated, and the ethyl acetate extracts were collected for each isolate. Ethyl acetate was evaporated, and the resultant compounds were dried individually with magnesium sulfate ($MgSO_4$) and concentrated to yield the crude extracts. The crude extracts were then dissolved in 15% Dimethyl sulfoxide (DMSO) for antimicrobial bioassay. Antimicrobial activities of the crude metabolites isolated from the endolichenic fungi were determined by agar cup diffusion method against six bacterial pathogens, *Bacillus subtilis* (MTCC 736), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426), *Shigella flexneri* (MTCC 1457), and *Klebsiella pneumonia* (MTCC 3384), and three pathogenic fungi, *Candida albicans* (MTCC 227), *Candida krusei* (MTCC 9215), and *Trichophyton mentagrophytes* (MTCC 8476). The test pathogens were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Muller Hinton Agar (MHA) plates were inoculated with overnight culture of each bacterial suspension. Similarly for the fungal pathogens, Sabourad Dextrose Agar (SDA) plates were inoculated with each fungal suspension. The plates with the inoculated organisms were evenly spread out with sterile cotton swabs. Agar cups were prepared by scooping out the media with a sterile cork borer (7 mm diameter). The cups were then filled with 100 µl of the crude metabolites that was dissolved in DMSO to get a concentration of 1 mg/ml. The plates were then incubated at 36 ± 1 °C for 24 h and 48 h for bacterial and fungal pathogens respectively. Antimicrobial activity was determined as growth inhibition of the target organism around agar cup as appearance of clear zones.

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2.4. *Effect of culture media and incubation period on metabolites production*

Based on preliminary antimicrobial assay, four endolichenic fungi were selected for optimum metabolites production in different media and incubation time. The selected isolates were separately cultivated in four different media, namely, Potato Dextrose Broth (PDB), Yeast Extract broth (YEB), Malt Extract Broth (MEB) and Czapek Dox Broth (CZB), and antimicrobial activity was determined up to 21 days at 30 °C with period shaking at 120 rpm in a rotary incubator shaker.

2.5. *Partial characterization of crude metabolites*

The crude metabolites were separated by preparative Thin Layer Chromatography (TLC) on pre-coated TLC plates with silica gel HF_{254} (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) with Hexane:Ethyl acetate (9:1) as solvent system. Visualization of the compounds was undertaken by UV irradiation at 254 and 366 nm. The bioactive metabolites dissolved in ethyl acetate were calibrated for λ-max indices using a UV spectrophotometer (SPECORD 210).

3. Results and discussion

Fungi are highly diverse and found colonized in varied environments. Lichen thalli provide an unexplored ecological niche for a wide variety of microorganisms including fungi. Fungal symbionts resembling endophytes also inhabit inner healthy lichen thalli forming symptomless infections and are termed as endolichenic fungi (Arnold et al., 2006). In the present study, a total of 19 endolichenic fungi were obtained from surface sterilized tissue fragments of three different thalli of *Parmelia* sp. Identification of the fungi species were carried out by their colonial morphology, microscopic observations and nature of the spores referring standard fungi identification manuals. The isolates that did not sporulate in culture were categorized as mycelia sterilia and conventionally classified as morphotypes. The thalli were found colonized with fungal species belonging to genera *Phomopsis* (15.7%), *Aspergillus* (10.5%), *Penicillium* (10.5%), *Trichoderma* (5.2%), *Fusarium* (5.2%), *Cytospora* (5.2%) and *mycelia sterilia* (47.4%). The frequently isolated fungi were mycelia sterilia followed by *Phomopsis*, *Aspergillus* and *Penicillium* species. The distribution of endolichenic fungi seems to be ubiquitous among lichens since many workers have also reported their association in several lichen thalli (Li et al., 2007; Petrini et al., 1990; U'Ren et al., 2010). The isolates were obtained in pure culture and evaluated for preliminary antimicrobial activity against some clinically significant human pathogens that consisted of six bacteria and three fungal pathogens. The result indicated that 10.52% of the isolates showed antimicrobial activity against all the test pathogens in varying degrees, while 31.57% and 10.52% of the isolates displayed antibacterial and antifungal activity inhibiting all bacterial and fungal pathogens respectively (Table 1). Several endolichenic fungi have been reported to produce new and interesting bioactive molecules with antimicrobial, antitumor, antiviral and antioxidant activity (Ding et al., 2009; He et al., 2012; Wang et al., 2010, 2012). It is believed that production of bioactive metabolites by these fungi may be similar to the mechanism of fungal endophytes

Table 1 – Preliminary antimicrobial activity of endolichenic fungi against test pathogens.

	Zone diameter (mm)								
Endolichenic	*Bs*	*Sa*	*Pa*	*Pv*	*Sf*	*Kp*	*Ca*	*Ck*	*Tm*
Fungi									
Phomopsis sp.1	–	–	–	–	+	+	–	–	–
Phomopsis sp.2	–	–	–	–	–	–	–	–	–
Phomopsis sp.3	–	–	–	–	–	–	–	–	–
Aspergillus sp.1	++	++	+	++	++	++	–	–	+
Aspergillus sp.2	++	++	+	++	++	++	++	+	++
Trichoderma sp.	+	–	–	–	+	–	–	–	+
Fusarium sp.	–	–	–	++	++	–	+	–	–
Penicillium sp.1	++	++	++	++	++	++	–	–	+
Penicillium sp.2	++	–	–	–	++	–	++	–	++
Cytospora sp.	++	++	++	++	++	++	–	++	++
Morphotype1	–	–	–	–	–	+	–	–	–
Morphotype 2	–	–	–	+	–	+	–	–	+
Morphotype 3	–	–	–	–	–	–	–	–	–
Morphotype 4	++	++	++	++	++	++	++	++	–
Morphotype 5	–	–	–	–	–	–	–	–	++
Morphotype 6	–	–	–	–	–	–	–	–	++
Morphotype 7	–	–	–	–	–	–	–	–	++
Morphotype 8	++	–	–	–	–	–	–	–	–
Morphotype 9	++	++	+	++	++	++	++	+	++

(+) indicates zone of inhibition < 15 mm; (++) indicates zone of inhibition > 15 mm; (–) indicates empty cells. The isolates used for further study are marked bold.

Bs, Bacillus subtilis (MTCC 736), *Sa, Staphylococcus aureus* (MTCC 737), *Pa, Pseudomonas aeruginosa* (MTCC 424), *Pv, Proteus vulgaris* (MTCC 426), *Sf, Shigella flexneri* (MTCC 1457), *Kp, Klebsiella pneumonia* (MTCC 3384), *Ca, Candida albicans* (MTCC 227), *Ck, Candida krusei* (MTCC 9215) and *Tm, Trichophyton mentagrophytes* (MTCC 8476)

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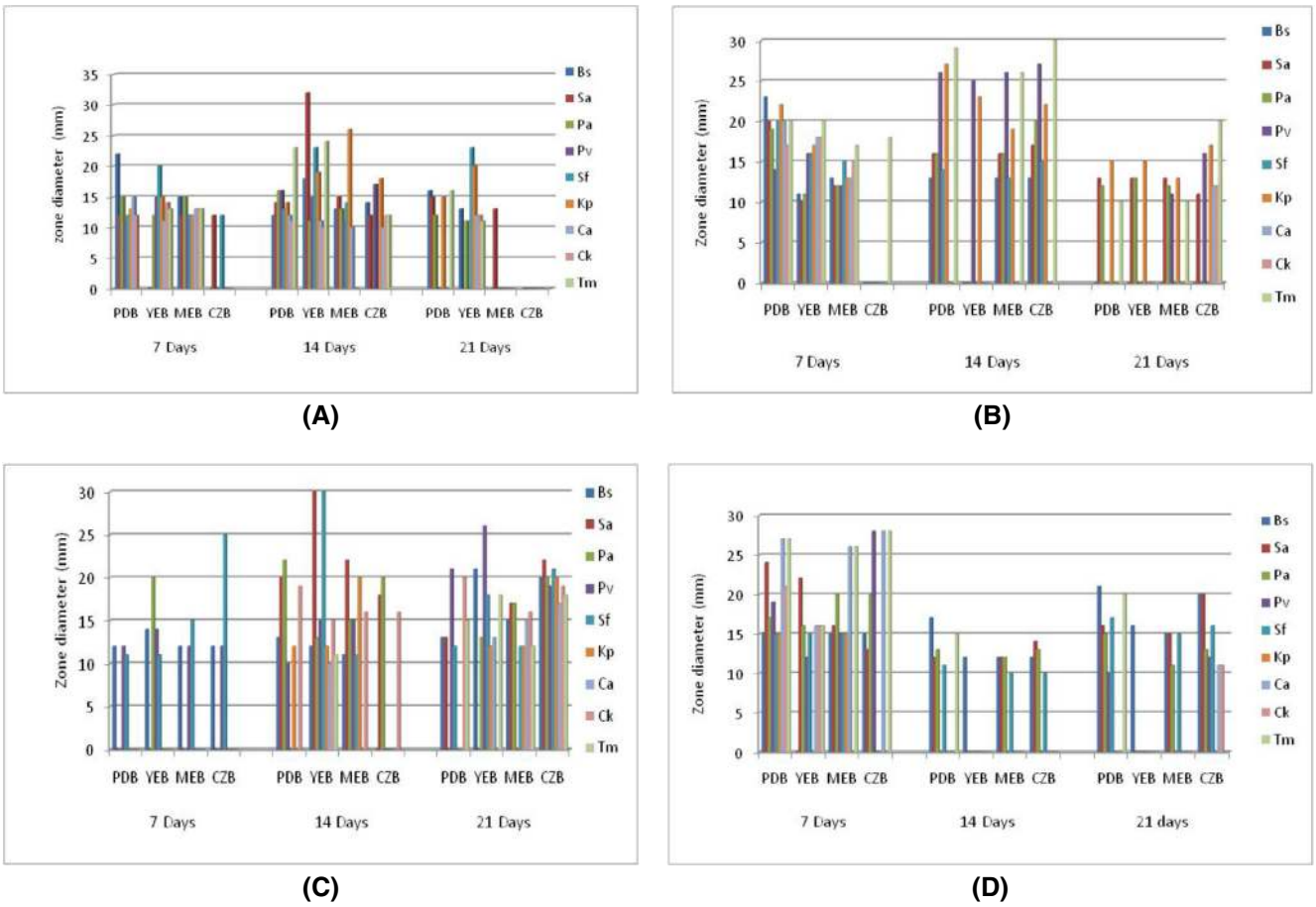

Fig. 1 – Antimicrobial activity of *Cytospora* sp. (a), *Aspergillus* sp. (b), Morphotype 4 (c) and Morphotype 9 (d) in different media and incubation periods against test pathogens respectively. *Bs, *Bacillus subtilis*, Sa, *Staphylococcus aureus*, Pa, *Pseudomonas aeruginosa*, Pv, *Proteus vulgaris*, Sf, *Shigella flexneri*, Kp, *Klebsiella pneumonia*, Ca, *Candida albicans*, Ck, *Candida krusei* and Tm, *Trichophyton mentagrophytes*.

that colonize higher plant species. Altogether four endolichenic isolates (*Cytospora* sp., *Aspergillus* sp.2, Morphotype 4 and Morphotype 9) were selected for further study based on their preliminary antimicrobial activity. The fungi were cultivated in different mycological media, namely, PDB, YEB, MEB and CZB, and effect of metabolites production was observed up to 21 days of incubation. The result indicated that metabolites production varied among the isolates in different media and incubation time. In *Cytospora* sp., the metabolites obtained on 14 days of incubation period showed antimicrobial activity against all the test pathogens, and highest activity was observed in YEB medium (Fig. 1A). Similarly, *Aspergillus* sp.2 showed optimum metabolites production on 7 days of incubation in PDB medium (Fig. 1B). However, metabolites produced on 14 days of incubation also showed significant antimicrobial activity but not against all the test pathogens. Considerable antimicrobial activity was also observed in two isolates of mycelia sterilia. The sterile fungus, morphotype 4, showed significant activity against all the test pathogens on 21 days of incubation on CZB medium (Fig. 1C). Nevertheless, good antimicrobial activity was also observed on 14 days of incubation in YEB medium but only against two bacterial pathogens, *Staphylococcus aureus* and *Shigella flexneri*. The metabolites produced by another sterile fungus, morphotype 9, showed maximum antimicrobial activity against all the test pathogens on 7 days of incubation in PDB medium, but thereafter metabolites production decreases significantly (Fig. 1D). However, moderate activity was also seen on 7 and 21 days of incubation in other media. The fungi were cultured in BOD shaking incubator at 29 ± 1 °C with periodic shaking at 150 rpm in their respective optimized medium and incubation periods.

The crude metabolites of the fungi were extracted, and antimicrobial activity was tested against a panel of clinically significant human pathogens. The result indicated that the crude metabolites considerably inhibited all the test pathogens in varying degree (Table 2). Significant activity was observed against *C. albicans* and *T. mentagrophytes*. Among the isolates, crude metabolites of *Aspergillus* sp.2 and *Cytospora* sp. were found to be highly effective in inhibiting all the test pathogens. Further the metabolites of three endolichenic fungi, i.e. *Aspergillus* sp.2, *Cytospora* sp. and sterile isolate Morphotype 9, showed strong antifungal activity against *C. albicans*, *C. krusei* and *T. mentagrophytes*. Lichens are known to produce a number of secondary metabolites that may protect them against physical stresses or biological attack (Kahng et al., 2004). Some lichen species and their compounds have also been utilized for

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Table 2 – Antimicrobial activity of the crude metabolites of four endolichenic fungi.

Test pathogens	Zone diameter (mm)			
	Cytospora sp.	*Aspergillus* sp.	Morphotype 4	Morphotype 9
B. subtilis	12 ± 0	18.3 ± 1.52	12.3 ± 1.52	10 ± 0
S. aureus	16 ± 0	19.3 ± 0.57	15 ± 0	10.6 ± 0.57
P. aeruginosa	14.3 ± 0.57	16 ± 0	22 ± 1	14 ± 0
P. vulgaris	13.3 ± 0.57	15.3 ± 1.15	14.3 ± 0.57	10 ± 1
S. flexneri	14.6 ± 1.15	16 ± 1	11.6 ± 1.15	11 ± 1
K. pneumonia	10.6 ± 0.57	15 ± 0	12 ± 0	10 ± 0
C. albicans	23.3 ± 1.15	21.3 ± 1.15	16 ± 0	21.6 ± 0.57
C. krusie	16.3 ± 1.15	16 ± 1	11.6 ± 1.52	16.6 ± 0.57
T. mentagrophytes	18.6 ± 0.57	20 ± 0	14.3 ± 0.57	19.3 ± 0.57

Values are means of three replicates ± Standard Deviation.

medicinal and industrial purposes (Muller, 2001). Considering the rationale suggested by Strobel and Daisy (2003), there is a great chance of obtaining novel bioactive metabolites from endolichenic fungi since these fungi would be producing similar compounds as their respective host. Lichens are also frequently ignored by pharmaceutical industries because of their slow growth. So, once the microbial source of bioactive metabolites would be available, it would eliminate the need to harvest the compounds from slow growing lichens. However, very few works are being carried out to screen the bioactivities of endolichenic fungi isolated from lichen species. Therefore, in this investigation, the endolichenic fungal isolates were screened for their antimicrobial activity against some clinically significant human pathogens.

Out of the total isolates, four endolichenic fungi demonstrated considering antimicrobial activity against a panel of human pathogen comprising gram positive and gram negative bacteria and pathogenic fungi. It is generally believed that secondary metabolites, also known as lichens substances, are produced mainly by the fungus and secreted onto the surface of the lichen's hyphae either in amorphous forms or as crystals (Molnar and Farkas, 2003). Therefore, it can be assumed that the bioactivity of any lichen species might be due to metabolites secreted by the fungal partner and the endolichenic fungi that live within the interior of healthy lichens. Since the endolichenic fungi that showed antimicrobial activity were isolated from thalli of *Parmelia* spp. which are also used in treatment of wound infections, inflammation, skin diseases, diarrhea, dysentery, cough and fever (Thippeswamy et al., 2013), we rationale that the medicinal properties of *Parmelia* might be due to its associated endolichenic fungi or host–symbionts interaction. Some of the isolates showed promising antifungal activity against fungal pathogens like *C. albicans*, *C. krusei* and *T. mentagrophytes*, indicating that the metabolites could be exploited as antifungal agents. Over the years, invasive fungal infections have emerged as an important cause of morbidity and mortality in immune compromised patients. Although several new antifungal drugs have been licensed in recent years, antifungal drug resistance is becoming a major concern during treatment of such patients. Among pathogenic fungi, majority of nosocomial fungal infections are caused by *Candida* species, with *Candida albicans* being the most common etiological agent of fungal bloodstream infections (Perea and Patterson, 2002). Besides, there are very few antifungal agents that are used for treatment of dermatomycoses caused by *T. mentagrophytes* and others. Such information suggests the need for new and effective antifungal agents. Our study therefore indicates that metabolites from endolichenic fungi inhabiting *Parmelia* thalli could be alternative agents to control such fungal diseases. This may help to reduce the dependence on chemically synthesized antimicrobials and overcome the problem of the emergence of fungi being resistant to antifungal chemicals.

The isolates were also found to be effective against both gram positive and gram negative pathogenic bacteria. Since most of bacterial pathogens used in this present study are responsible for causing secondary and opportunistic infections, the metabolites could also be used as antibacterial agents. This indicates the metabolites to be broad spectrum in nature. Out of four potent strains, two of the isolates were found to be fungi belonging to genera *Aspergillus* sp. and *Cytospora* sp. while the other two isolates could not be identified due to their sterile nature and therefore classified as morphotype 4 and morphoype 9. Mycelia sterilia have been isolated as endophytes from a wide range of plant species (Arnold et al., 2000; Frohlich et al., 2000). Since these non-sporulating mycelia sterilia cannot be provided with taxonomic names without reproductive structures in conventional classification, they are now generally categorized as "morphotypes" based on similar cultural characteristics (Promputtha et al., 2005). In many instances sterile endophytic fungal isolates have been reported to produce several interesting bioactive metabolites (Hussain et al., 2009; Meshram et al., 2013). Nevertheless, they are often overlooked because of their non-sporulating nature, and the compound that is produced remains understudied. Most of the endolichenic fungi represent lineages of Ascomycota that are distinct from lichen mycobionts, lichenicolous fungi or incidental fungi on thallus surfaces (Barnett and Hunter, 1998). The same was true to our isolates, as morphological and microscopic traits revealed to be fungi belonging to class ascomycetes. Filamentous fungi belonging to genera *Aspergillus, Penicillium* and *Cytospora* are known to be chemical factories due to their ability to produce numerous bioactive compounds. These species have also been isolated as endophytes from several plant species and demonstrated significant antimicrobial and anticancer activities (Abreu et al., 2010; Budhiraja et al., 2013; Kokubun et al., 2003; Shaaban et al., 2013; Singh et al., 2007). In this investigation, we isolated *Aspergillus* sp. as endolichenic fungus from *Parmelia*

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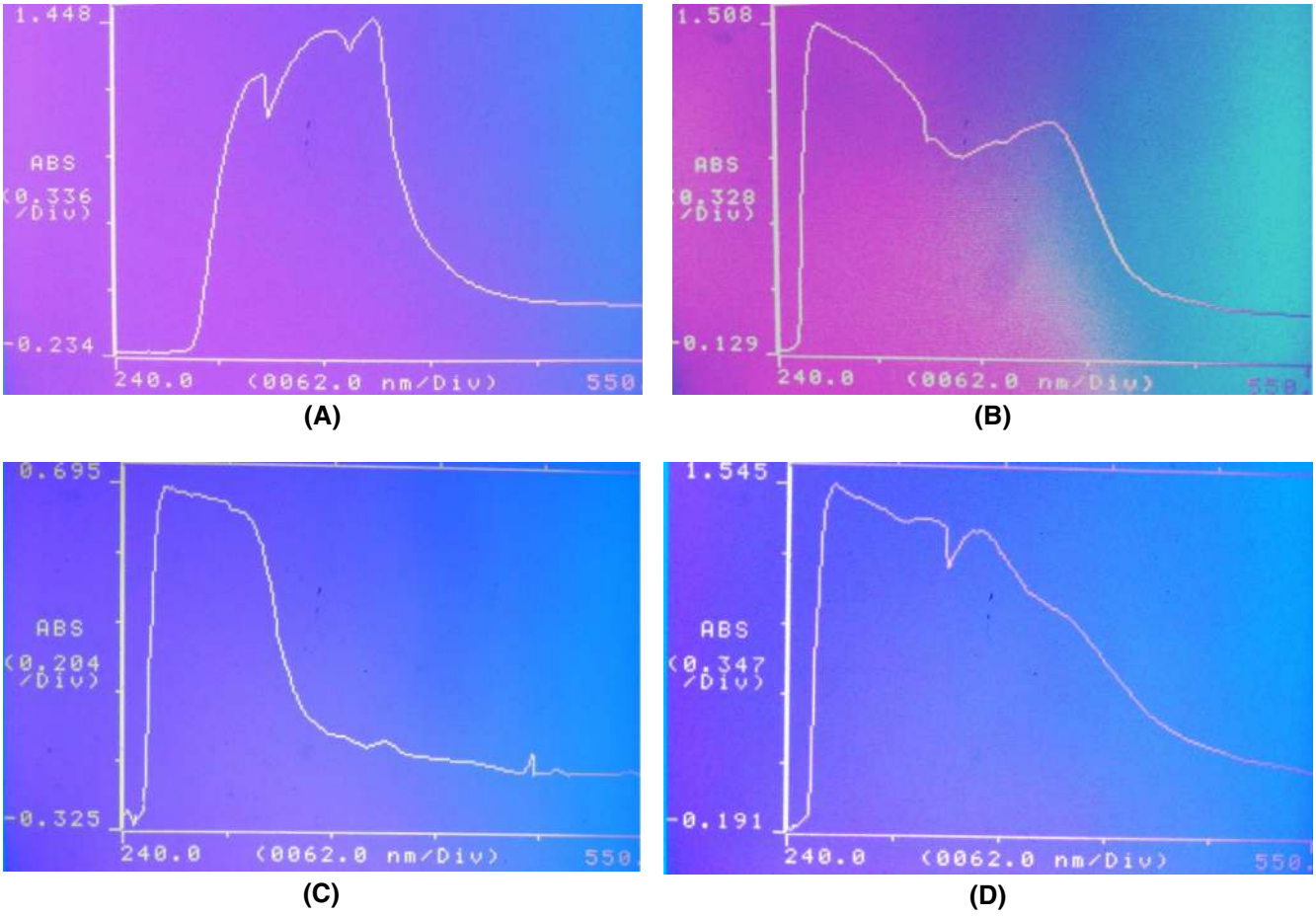

Fig. 2 – Calibrated λ-max of the crude metabolites obtained from *Cytospora* sp. (A), *Aspergillus* sp. (B), Morphotype 4 (C) and Morphotype 9 (D) by spectrophotometer study.

thalli. The crude metabolites showed considerable antifungal and antibacterial activity against some clinically significant human pathogens. Recently, Mathan et al. (2013) have also reported this fungus as endophyte from seaweed *Codium decorticatum* with broad spectrum *in vitro* antimicrobial activity. The antimicrobial property of this fungus may be due to production of active metabolites which was detected in our TLC and spectrophotometric analyses, where the major component showed sharp signaling at wavelength of 240–260 nm. The isolate also showed strong antifungal activity. Such activity may be due to presence of small antifungal substance, polyketide terrain, which is known to be produced by *Aspergillus* species since 1935 (Ghisalberti et al., 1990). Another endolichenic fungus, *Cytospora* sp., isolated from the same lichen thalli, also demonstrated moderate antibacterial and strong antifungal activity. Similarly, Singh et al. (2007) have reported that endophytic *Cytospora* sp., isolated from a buttonwood tree, produced compounds that inhibited the growth of gram-positive bacteria, including antibiotic-resistant strains. In our present study, the crude metabolites obtained from *Cytospora* sp. indicated a strong peak at 380–450 nm ranged with maximum absorbance (λ-max) of 1.448 and R_f value of 0.92 (Fig. 2A), indicating the presence of active compound. This fungus has been reported to produce compounds like Cytoskyrin A, Cytosporon D, Cytosporon E and 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin which are known to inhibit gram positive bacteria, *Escherichia coli*, yeast and fungi (Kokubun et al., 2003; Singh et al., 2007). Similarly, crude extract obtained from *Aspergillus* sp.2, showed the presence of two UV-visible components. The major component showed a strong peak at 240–260 nm ranged with maximum absorbance (λ-max) of 1.508 (Fig. 2B). The relative front (R_f) of the compound was found to be 0.93. Similarly, The other two, unidentified mycelia sterilia (Morphotype 4 and Morphotype 9) showed the presence of three and four UV-visible components respectively. The crude metabolite of Morphotype 4 also showed strong peak at 240–260 nm ranged but with maximum absorbance (λ-max) of 0.695 and R_f value of 0.60 (Fig. 2C). Again the crude metabolites of Morphotype 9 showed strong peak at 240–260 nm ranged with maximum absorbance (λ-max) of 1.545 and R_f 0.77 (Fig. 2D). The details of λ-max index, wave length range and R_f value of the major components are presented in Table 3. This also indicates presence of some active compounds in the crude metabolites. Further analysis may result into yield of new or interesting substances or might give suitable lead molecules so as to develop into effective antimicrobial agents. The study suggests that endolichenic fungi colonizing thalli of medicinally used lichen may be a source of potential antimicrobial metabolites. This is the first report of occurrence of *Cytospora* sp. as endolichenic fungi with antimicrobial activity isolated

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Table 3 – Characterization of major fraction present in the crude metabolites of potent endolichenic fungi.

Fungal strain	Relative font (R_f)*	λ-max	Wavelength range (nm)
Cytospora sp.	0.92	1.448	380–450
Aspergillus sp.	0.93	1.508	240–260
Morphotype 4	0.60	0.695	240–260
Morphotype 9	0.77	1.545	240–260

* R_f values were calculated using the formula, Distance moved by solute/Distance moved by solvent.

from *Parmelia* lichen thalli. Currently, characterization and structure elucidation of the active antimicrobial metabolites are being investigated. Efforts are also being made to characterize the sterile strains.

4. Conclusion

Endolichenic fungi are understudied and less explored groups of microbial symbiont. Studies have indicated that they produce potent bioactive metabolites with wide therapeutic applications. Considering the diminishing plant diversity which harbors maximum lichen flora research priority should be directed to study them especially in developing countries like India, because once lichens get extinct so will be the associated endolichenic fungi. The current study is an endeavor in this direction, and our study suggests that endolichenic fungi could be a potential source of antimicrobial agents.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.bjbas.2015.11.006.

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