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RESEARCH PAPER

Anti-lipoxygenase, radical scavenging and antimicrobial activities of lichen species of genus *Heterodermia* (Physciaceae)

Bhaskar C. Behera*, Mangesh V. Morey & Subhash B. Gaikwad

Bhaskar C. Behera*
e-mail: bcbehera2002@yahoo.co.in

Mangesh V. Morey
e-mail: mangeshbiotech@gmail.com

Subhash B. Gaikwad
e-mail: subhashkyk@gmail.com

* corresponding author

Agharkar Research Institute, G.G. Agarkar Road, Pune – 411004, India

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ABSTRACT

The aim of the research is to explore antilipoxygenase, radical scavenging and antimicrobial activity of the ethyl acetate extract of the selected lichen species of genus *Heterodermia* (Physciaceae). The species extract have shown concentration dependent lipoxygenase (LOX) inhibition. Fifty percent (IC_{50}) LOX inhibition was obtained by *H. diademata*, *H. flabellata*, *H. antillarum* and *H. incana* with 0.123, 0.153, 0.160, 0.150 mg/ml respectively; which is smaller than the synthetic antioxidant BHA and BHT. Only the extract of *H. diademata* showed IC_{50}, 0.123 mg/ml is equal to LOX inhibitor Indomethicin. The LOX inhibition kinetics with respect to the lichen extract resulted different mode of inhibition. Competitive inhibition was found towards LOX by *H. albicans*, *H. antillarum*; uncompetitive inhibition by *H. diademata* and noncompetitive inhibition showed by *H. angustiloba*, *H. flabellata*, *H. incana*, *H. isidiophora* and *H. pseudospeciosa*. These species extract have also showed ≤50 % radical scavenging activity. The ethyl acetate extract of *H. diademata*, *H. angustiloba*, *H. albicans* and *H. isidiophora* showed the strong antimicrobial activity against most of the tested microorganisms at concentration ranging from 0.232 mg/ml to 0.591 mg/ml. These findings suggest that these lichen species of genus *Heterodermia* can be used as new bioresources for the natural lipoxygenase inhibitor with antimicrobial and radical scavenging features.

Keywords: *Heterodermia*, lipoxygenase inhibition, antioxidant, antimicrobial activity

РЕЗЮМЕ

Бехера Б.С., Мори М.В., Гайквад С.Б. Антилипоксигеназа, радикальная очистка и антимикробная активность лишайников рода *Heterodermia* (Physciaceae). Цель исследования – антилипоксигеназа, радикальная очистка и антимикробная активность этилацетатного экстракта видов лишайников рода *Heterodermia* (Physciaceae). Экстрактам видов рода свойсвенно липоксигеназное ингибирование (LOX), зависящее от концентрации. Пятидесятипроцентное (IC_{50}) ингибирование LOX отмечалось у видов *H. diademata*, *H. flabellata*, *H. antillarum* и *H. incana* при концентрациях 0,123 0,153, 0,160 и 0,150 мг/мл соответственно; что меньше, чем у синтетических антиоксидантов BHA и BHT. Только экстракт *H. diademata* показал IC_{50} при концентрации 0,123 мг/мл равным LOX ингибитора индометацин. Кинетика LOX ингибирования экстрактов разных видов лишайников показывает разный режим ингибирования. Конкурентное ингибирование LOX отмечено у *H. albicans* и *H. antillarum*; неконкурентое – у *H. diademata*; отсутствие конкуренции характерно для *H. angustiloba*, *H. flabellata*, *H. incana*, *H. isidiophora* и *H. pseudospeciosa*. Экстракты этих видов также показали пятидесятипроцентную активность радикальной очистки. Этилацетатные экстракты *H. diademata*, *H. angustiloba*, *H. albicans* и *H. isidiophora* показали сильную антимикробную активность в отношении большинства протестированных микроорганизмов в концентрациях от 0,232 до 0,591 мг/мл. Результаты показывают, что виды лишайников *Heterodermia* могут быть использованы в качестве нового биоресурса природной липоксигеназы с антимикробными и очистительными свойствами.

Ключевые слова: *Heterodermia*, липооксигиназное ингибирование, антиоксидант, антимикробная активность

Переведено редколлегией

INTRODUCTION

Lipoxygenase (Linoleate: oxygen 13-oxidoreductase, EC 1.13.11.12) (LOX) is widely distributed in nature and is found in all plants and animals (Serpen & Gokmen 2007). LOX catalyzes the oxidation of poly unsaturated fatty acids and forming hydroperoxides. The products of the degradation of poly unsaturated fatty acids and their derivatives can react with proteins, peptides, and amino acids; which can result in off-flavour production and rancidity of oils thereby lowering nutritional values of oil-based foods (Iassonova et al. 2009). Hence LOX is the main enzyme in off-flavor development. It is one of the most important quality parameters in foods. LOXs are important for food stuff and are also important in medical applications. LOX has

been implicated in the progression of certain cancers and health disorders (Kelvakar et al. 2000, 2001). Furthermore, the products of LOX catalyzed oxygenation [hydro peroxy eicosa tetra enoic acid (HPETE), hydroxyl eicosa tetra enoic acid (HETE), Leukotrines and Lipoxins) apparently are involved in the development of rheumatoid, arthritis, psoriasis, asthmatic responses (Sircar et al. 1983). LOX are sensitive to antioxidants as antioxidants are involved in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy- or lipidperoxy-radicals. This could lead to less availability of lipid hydroperoxide substrate required for LOX catalysis (Rakova et al. 2007).

Synthetic antioxidants Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisol (BHA) use as additives in food are efficient LOX inhibitors. However, their use is being restricted because of their possible toxic and carcinogenic effects (Barlow 1990). Recently, there is a great interest in substituting them with natural LOX inhibitors.

Bacterial resistance to antibiotics is becoming an increasing problem world wide. Due to risk of adverse effects encountered with the use of synthetic antibiotics, plants or other organisms may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. Natural products derived from these sources offer a new source of biologicals that may have a great impact on infectious disease and over all human health (Balandrin et al. 1985, Conner 1993, Govindappa 2011).

Natural products with diverse bioactivities are an important source of novel chemicals. Pharmaceutical industry is forced to continuous search and development of new pharmacological active molecules. Similar to higher plants, lichens are considered as potential source of novel biologically active compounds. Lichens are complex symbiotic associations between a fungus (mycobiont) and an alga (photobiont) with unique characteristics in plant kingdom. They are proven as the earliest colonizers of terrestrial habitats on the earth with a worldwide distribution from tropical to alpine and from the plains to the highest mountains (Taylor et al. 1995, Mitrovic et al. 2011). Lichens produces unique secondary metabolites, known as lichen substances are mostly small, but complex molecules. Structures for more than 1050 different lichen substances have been reported to date (Molnar & Farkas 2010, Stocker-Worgotter 2008). The lichen substances and lichen extracts exert a remarkable variety of biological effects; antibacterial, antiviral, antifungal, antiprotozoal, antiherbivore, antimutagenic, antioxidant, antitumor, antiulcerogenic, antinociceptive, antipyretic, anti-inflammatory, anti-proliferative and cytotoxic effects, cardiovascular protective, glucosidase inhibitory and probiotic stimulating activities (Muller 2001, Behera et al. 2005a,b, 2012, Rankovic 2007, Verma et al. 2011, 2012, Mahadik et al. 2011, Manojlovic et al. 2012, Gaikwad et al. 2014). Despite of these manifold activities of lichen metabolites been recognized, their therapeutic potential has not yet been fully explored and thus remains pharmaceutically unexploited (Crittenden & Porter 1991).

Therefore the objective of the study is to explore antilipoxygenase, antimicrobial, and antioxidant activity of the some of the lichen species of genus *Heterodermia* of family Physciaceae.

MATERIALS AND METHODS

Study material

Lichens species of genus *Heterodermia* (Physciaceae) were collected from different parts of Western ghats of India (Fig. 1). The identification of the species was done in the laboratory on the basis of morphological, anatomical and chemical characteristics. The presence of major lichen substances in the species was identified using Thin Layer Chromatography method described by Culberson & Kristinsson (1972). *Heterodermia albicans* voucher no. AMH-99.306 producing Atranorin, Zeorin, Salazinic acid; *H. angustiloba* voucher no. AMH-78.80 producing Atranorin, Zeorin, Norstictic, Salazinic acid; *H. antillarum* voucher no. AMH-74.1138 producing Atranorin, Zeorin, Salazinic acid and unknown substances; *H. diademata* voucher no. AMH-04.118 producing Atranorin, Zeorin, Chloroatranorin; *H. flabellata* voucher no. AMH-74.1194 producing Atranorin, Zeorin and unknown yellow pigment; *H. incana* voucher no. AMH-77.299 producing Atranorin, Zeorin; *H. isidiophora* voucher no. AMH-99.342 producing Atranorin, Zeorin; *H. pseudospeciosa* voucher no. AMH-04.170 producing Atranorin, Zeorin, Norstictic, Salazinic acid. The materials are deposited in Ajrekar Mycological Herbarium (AMH) at Agharkar Research Institute, Pune, India. Lichen specimens of *Heterodermia* producing biological active secondary compounds with their class is presented in Table 1.

Bacterial and fungal cultures were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. These bacterial cultures are namely *Bacillus subtilis* (NCIM 2063), *Streptococcus faecalis* (NCIM 5024), *Candida albicans* (NCIM 3471) and *Cryptococcus albidus* var. *diffluens* (NCIM 3371) were tested against lichen extract to ascertain their antimicrobial activity.

Extraction of lichens compounds

Eight lichen species *Heterodermia albicans*, *H. angustiloba*, *H. antillarum*, *H. diademata*, *H. flabellata*, *H. incana*, *H. isidiophora* and *H. pseudospeciosa* were selected for the study for different experiments. Approximately 300 mg biomass of each dry natural lichen thallus were extracted through soxhlet apparatus with solvent ethyl acetate. Extracting period was 72 hours at room temperature. Then these extracted compounds of the above mentioned lichens were weighed. Then this extract of each lichen thallus was taken and used in the testing of antilipoxygenase, antimicrobial and antioxidant activities.

Lipoxygenase inhibition assay

Lipoxygenase inhibition of lichen extracts were evaluated with the spectrophotometer methodology described by Shinde at al. (1999) and Theerakulkait & Barrett (1995), with minor modifications. The substrate solution was prepared by mixing 25 µl of pure linoleic acid and 15 ml of 0.1 M sodium borate buffer at pH 9.0. The solution was made transparent by adding 0.15 ml of 0.1 N NaOH and diluting to 25 ml with 0.1 M sodium borate buffer at pH 9.0 to a final concentration of 3 mM linoleic acid. The reaction mixture was then prepared which consisted of 3.0 ml of substrate solution with 0.15 ml of lichen extract concentra-

tion (5, 40, 80, 120, 200 μg/ml) and then 0.05 ml of LOX solution (20000 U ml-1) was added and incubated for 5 minutes at room temperature. The absorbance at 234 nm was recorded on a UV-Vis spectrophotometer (Shimadzu-1601 PC). The percentage LOX inhibition was calculated by the following equation:

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Lipoxygenase inhibition kinetics

The LOX inhibition kinetics was analysed by Lineweaver-Burk plot with inhibitor lichen extract concentration (10, 30, 90, 180 μg/ml). Reaction mixture without extract was used as negative control.

Antioxidant assay

Antioxidative activity of eight lichen species of genus *Heterodermia* were determined in terms of free radical scavenging (DPPH) following the methodology described by Blois (1958) and for trolox equivalent activity capacity (TEAC) following by Miller et al. (1995). The detailed descriptions with minor modifications of the procedure for these antioxidative assays were reported in our previous papers (Verma et al. 2008, Behera et al. 2005). Synthetic antioxidant Butylate Hydroxy Anisol (BHA) and Butylate Hydroxy Toluene (BHT), Trolox (water soluble vitamin E analogue) were used as positive standard compound for comparison with lichen extracts antioxidant activity.

Calculation of 50% inhibition concentration (IC_{50})

IC_{50} value of the lichen extract and the standard compound BHA, BHT and Indomethicin for the 50 % lipoxygenase inhibition were calculated by extrapolation from concentration/effect regression line obtained from different concentrations (5, 40, 80, 120, 200 μg/ml).

Antimicrobial assay

Antimicrobial potential of eight lichen species of *Heterodermia* genus were determined by standard disk diffusion method approved by the National Committee for Clinical Laboratory Standards (NCCLS, 1993), and Minimum Inhibitory Concentration (MIC) of lichen extract were determined with the help of method described by Rankovik et al. (2007). Erythromycin, Tetracyclin, Streptomycin were used as positive control. The details of the procedure were described earlier in our previous report (Mahadik et al. 2011).

All chemical reagents used in the assays carried out were of analytical grade obtained from different agencies; e.g. Sigma (USA), Hi-media, Qualigens (India).

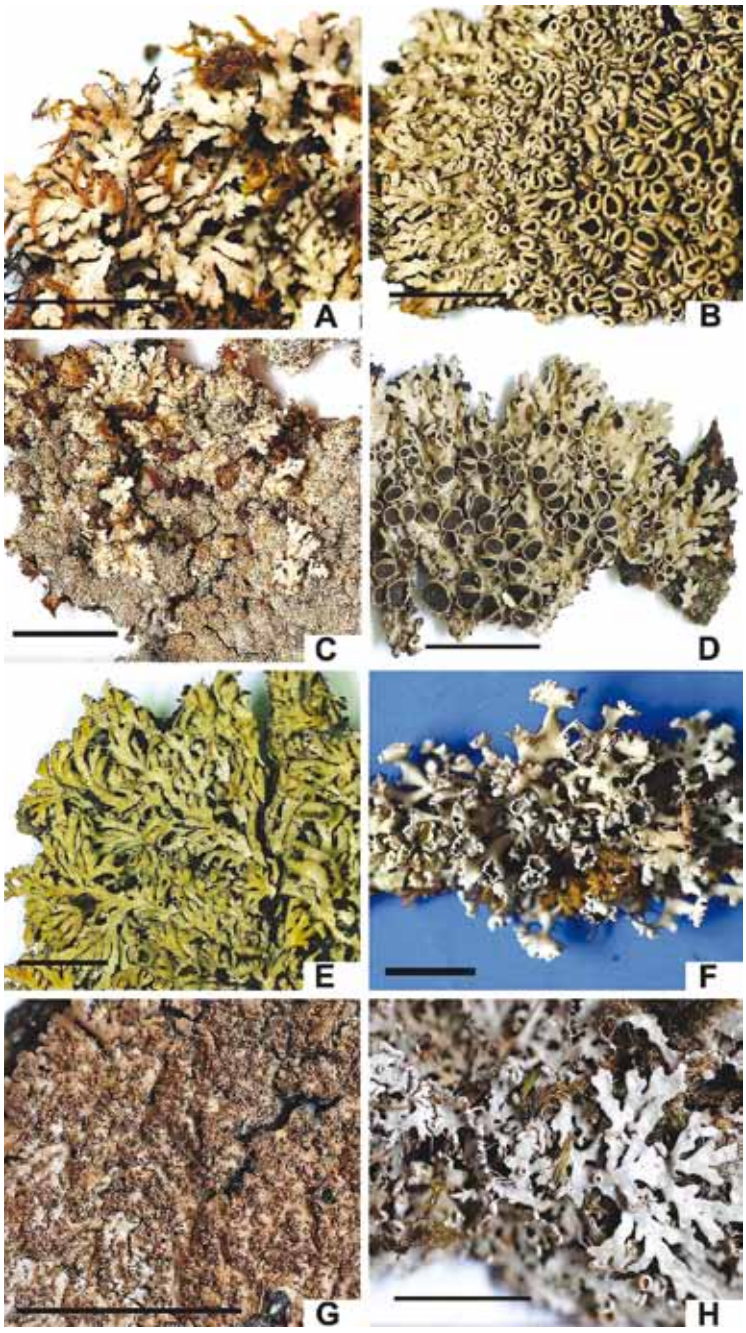

Figure 1 Natural thalli of lichens: A: *Heterodermia albicans*, B: *H. angustiloba*, C: *H. antillarum*, D: *H. diademata*, E: *H. flabellata*, F: *H. incana*, G: *H. isidiophora*, H: *H. pseudospeciosa* (scale = 10 mm)

RESULTS AND DISCUSSION

Lipoxygenase (LOX) inhibition by the extract of eight lichen species of genus *Heterodermia* was studied. The inhibition of LOX by the ethyl acetate extract of lichen species are presented in Table 2. All the species extract have shown increasing trend in LOX inhibition, when the extract concentration increased from 5.0 to 200 μg/ml. This result indicated that the LOX inhibition is inhibitor concentration dependent. However, variation in LOX inhibition was found among the species with respect to the extract concentration. *H. diademata* and *H. albicans* showed LOX inhibition between 69.2 % and 86.64 % at extract concentration between 120

Table 1. Lichen specimens producing biological active secondary compounds with their class

Lichen species	Compounds with Class
Heterodermia albicans	Atranorin: Depsides Salazinic acid: Depsidones Zeorin: Terpenoid
Heterodermia angustiloba	Atranorin: Depsides Norstictic acid: Depsidones Salazinic acid: Depsidones Zeorin: Terpenoid
Heterodermia antillarum	Atranorin: Depsides Salazinic acid: Depsidones Zeorin: Terpenoid
Heterodermia diademata	Atranorin: Depsides Chloroatranorin: Depsides Zeorin: Terpenoid
Heterodermia flabellata	Atranorin: Depsides Zeorin: Terpenoid
Heterodermia incana	Atranorin: Depsides Zeorin: Terpenoid
Heterodermia isidiophora	Atranorin: Depsides Zeorin: Terpenoid
Heterodermia pseudospeciosa	Atranorin: Depsides Norstictic acid: Depsidones Salazinic acid: Depsidones Zeorin: Terpenoid

and 200 µg/ml. *H. angustiloba*, *H. antillarum* and *H. incana* varies from 52.11 to 62.32 % at 120 to 200 µg/ml extract. Other species showed upto 43 % LOX inhibition even at higher concentration of extract at 200 µg/ ml. Indomethicin a standard LOX inhibitor had from 72.64 % to 81.13 % LOX inhibition at concentration range between 80 and 200 µg/ml. Comparing with the species extract and with the standard inhibitor, *H. diademata* and *H. albicans* showed LOX inhibition are equivalent to the Indomethicin at 200 µg/ml conc. 75 % to nearly 80 % LOX inhibition was achieved by synthetic antioxidant BHA and BHT at the same concentration. As far as IC_{50} value of the lichen extract for 50 % LOX inhibition is concerned, the IC_{50} value of *H. diademata*, *H. flabellata*, *H. antillarum*, *H. incana* are found 0.123, 0.153, 0.160, 0.150 mg/ml respectively. The IC_{50} value of the mentioned species are found less than the standard antioxidant BHA (0.198 mg/ml) and BHT (0.186 mg/ml). Only IC_{50} value of *H. diademata* was found to be equal to IC_{50} of Indomethicin (Table 3).

We have further investigated the lipoxygenase inhibition kinetics with respect to the various lichen species extract analyzing by lineweaver burk plot. The Km and Vmax value obtained by lichen extract as inhibitor against LOX are presented in Table 4. The lichen extract concentration as inhibitor used were 10, 30, 90 or 180 µg/ml. Linoleic acid concentration 0.2, 0.5, 1.0, 2.5 mg/ml used as substrate. Without lichen extract was used as -ve control. *H. antillarum* extract at low concentration (10 µg/ml) showed Km 3.75 µM with Vmax 5.263 µmol/min. Increased concentration of *H. diademata* extract showed the Km value ranging from 0.227 µM to 0.43 µM with a Vmax up to 1.01 µMol/min. *H. angustiloba*, had Vmax 5.0 µMol/min only at extract concentration of 10 µg and at higher concentration decreasing trend in Vmax and inconsistent Km value was obtained. *H. albicans* followed the same trend with respect to Km

Table 2. Lipoxygenase inhibition by the ethyl acetate extracts concentration of various species of *Heterodermia* genus of lichen family Physciaceae. The values presented are mean of 3 consecutive reading of the assay in every five minute interval

Lichen species	Extract concentration (µg.ml^{-1})				
	5	40	80	120	200
	Lipoxydase inhibition (%)				
H. diademata	7.0	38.5	47.86	69.2	86.64
H. angustiloba	11.75	38.31	47.04	52.49	53.44
H. albicans	9.2	28.76	52.33	77.39	85.44
H. flabellata	2.7	19.57	27.44	38.78	65.17
H. antillarum	6.18	21.57	36.02	58.77	62.14
H. isidiophora	11.23	28.16	36.52	39.27	43.64
H. incana	2.42	13.78	33.26	52.11	66.32
H.pseudospeciosa	5.2	14.28	21.44	37.51	43.09
Standard antioxidant					
BHA	23.44	37.53	48.16	63.79	79.42
BHT	16.33	29.04	53.17	60.08	74.52
Standard lipoxygenase inhibitor					
Indomethicin	10.44	23.51	72.64	75.27	81.13

Table 3. IC_{50} value of lichen species extract for 50 % lipoxygenase inhibition. Determination of antioxidant activity in terms of TEAC and DPPH free radical of the lichen extract carried out with 100 µl of 1mg extract / ml was used. TEAC value is expressed as mM of Trolox solution having the antioxidant equivalent to a 0.1 % (w/v) extract solution. The values presented are mean of the 3 consecutive reading of the assay in every five minute interval

Lichen Species	Lipoxygenase inhibition IC_{50} (mg/ml)	Antioxidant activity	
		TEAC (mM)	DPPH (% inhibition)
H. diademata	0.123	11.9	44.47
H. angustiloba	0.187	12.36	39.29
H. albicans	0.187	12.56	37.33
H. flabellata	0.153	12.96	36.35
H. antillarum	0.160	11.96	31.65
H. isidiophora	0.229	12.92	25.92
H. incana	0.150	12.78	21.56
H. pseudospeciosa	0.232	12.9	51.77
Standard antioxidant			
BHA	0.198	-	84.72
BHT	0.186	-	83.49
Trolox	-	3.77	-
Lipoxygenase inhibitor			
Indomethacin	0.123	-	-

value, but Vmax 1.667 µMol/min was obtained at extract concentration 90 µg/ml, beyond that Vmax was decreased. *H. incana* showed equal value in Km (2.5) and Vmax 2.5 µmol/min at 30 µg extract. *H. flabellata* had no significant increase in Km and Vmax value even after increasing the extract concentration up to 180 µg *H. isidiophora*, *H. pseudospeciosa* followed the same trend with respect to their Km and Vmax as observed in *H. flabellata*. The results suggest that extract concentration beyond 30 µg/ml acts as antagonistic effects on lipoxygenase inhibition.

Antioxidative activity in terms of Trolox Equivalent Antioxidant Capacity (TEAC) and scavenging of DPPH

Table 4. The kinetics for lipoxygenase reaction Km and Vmax was determined using Lineweaver-Burk plot. Linoleic acid as substrate (0.2, 0.5, 1.0, 2.5mg/ml) was used with various concentration of extract (10, 30, 90 or 180 µg/ml) of different species of lichen genus *Heterodermia*

Lichen species	0		10 µg		30 µg		90 µg		180 µg	
	Km (µM)	Vmax (µmol/min)	Km (µM)	Vmax (µmol/ min)	Km (µM)	Vmax (µmol/ min)	Km (µM)	Vmax (µmol/min)	Km (µM)	Vmax (µmol/ min)
H. didemata	0.4	0.625	0.227	4.0	0.4	4.0	0.4	1.01	0.435	0.909
H. anguistiloba	0.4	0.625	0.44	5.0	0.385	1.25	0.444	0.741	0.345	0.476
H. albicans	0.4	0.625	0.526	-	0.333	1.333	0.588	1.667	0.455	0.769
H. flabellate	0.625	0.625	0.625	1.25	0.5	0.833	0.529	0.8	0.714	0.833
H. antillarum	0.625	0.625	3.751	5.263	0.667	1.429	0.588	1.0	0.455	0.667
H. isidiophora	0.164	0.667	0.1	1.333	0.172	0.8	0.147	0.364	0.238	0.308
H. incana	0.617	0.606	0.909	1.282	2.5	2.5	0.819	0.667	0.714	0.5
H. pseudospeciosa	0.625	0.625	0.819	0.833	0.455	0.5	0.714	0.345	0.625	0.263

free radicals of extract of the species of lichen genus *Heterodermia* are evaluated and the data are presented in Table 3. The extract of all the lichen species showed consistent TEAC value from 12 to 13 mM concentration. The TEAC value is expressed as the milimolar concentration of Trolox solution having an antioxidant equivalent to a 0.1 % (w/v) extract solution. This indicates higher the TEAC value of the sample, the stronger the antioxidant activity. As far as DPPH radical scavenging by the lichen extract is concerned only *H. psedospeciosa* showed nearly 52 % scavenging of radical with 100 µl of 1 mg extract/ml in assay system. However other species showed 22 % to 44 % scavenging of radicals at same concentration of extract. The result on the basis of TEAC indicates that all the species have antioxidant potential.

Antimicrobial effects of the extract of species of lichen genus *Heterodermia* was determined and the data are presented in Table 5. The microbes *B. subtillis*, *C. albidus* and *C. albicans* were found to be very sensitive to the extract of lichen species *H. didemata* with lower MIC 0.232 mg/ml; *H. angustiloba* with MIC 0.267 mg/ml; *H. albicans* with 0.514 mg/ml; *H. isidiophora* with MIC 0.591 mg/ml; *H. flabellata* with MIC 2.670 mg/ml; *H. antillarum* with 3.944 mg/ml. Only *H. incana* and *H. pseudospeciosa* had shown effects on *S. faecalis* with MIC 1.624 mg/ml and 3.246 mg/ml respectively. On comparing with the MIC value of standard antibiotics Streptomycin, Erythromycin and Tetracycline, it was found that lichen extract activity against the bacteria and fungus is higher.

Traditional use of lichens has clues to unknown potential novel drug molecules in the form of secondary metabolites. Many lichen extract or their isolated secondary metabolites have been recorded by many authors for various biological activities (Muller 2001, Rankovic 2007, Molnar & Farska 2010, Shah 2014).

LOX inhibition by the species under different concentration of the extract, only two species *H. didemata* and *H. albicans* showed from 77 % to 87 % inhibition of LOX at higher concentration of extract 120–200 µg/ml with IC_{50} values 0.123 and 0.187 mg/ml. Whereas *H. flabellata*, *H. antillarum* and *H. incana* showed 39 % to 66 % at the same concentration with IC_{50} from 0.15 to 0.16 mg/ml. IC_{50} value of standard antioxidant BHA, BHT 0.198, 0.186 mg/ml showed 50 % LOX inhibition was found to be more or less equivalent than the IC_{50} for LOX inhibition by seven species except *H. didemata* (IC_{50} 0.123 mg/ml). Our results are in agreement with those reported antioxidants such as BHA and BHT, which acts as free radical quenchers, may also act as LOX inhibitors (Zhou et al. 2005, Banerjee 2006).

The inhibition kinetics of the reaction by the species extract analysed by Lineweaver-Burk plot revealed that *H. albicans* showed competitive inhibition towards LOX. *H. antillarum*, *H. didemata* showed uncompetitive inhibition. *H. angustiloba*, *H. flabellata*, *H. incana*, *H. isidiophora*, *H. pseudospeciosa* showed non competitive inhibition. The differential mode of lipoxygenase inhibition might be attributed to the inconsistent Km and Vmax obtained are because of increasing substrate concentration with respect to the species extract concentration that resulted differential intercept with different slopes. Furthermore, it was also observed that beyond 30 µg extract per ml, it acts as antagonistic effects on lipoxygenase inhibition.

The result of antibacterial effects of species extract revealed on the basis of MIC value, the bacteria *B. subtilis*, *C. albidus* and *C. albicans* are sensitive to the extract of *H. didemata*, *H. angustiloba*, *H. albicans* and *H. isidiophora* with concentration ranging from 0.232 mg/ml to 0.591 mg/ml.

Table 5. Minimum Inhibitory Concentration (MIC) values of the lichen species of the genus *Heterodermia* and the MIC value of standard antibiotics Streptomycin, Erythromycin, Tetracyclin against bacterial strain

Lichen species	Extract (mg/ml)	Bacterial strain	Standard antibiotics (µg/ml)		
			Erythromycin	Tetracyclin	Streptomycin
H. didemata	0.232	*B. subtills*	5.16	5.17	5.21
H. angustiloba	0.267	*C. albicans*	5.33	5.32	7.37
H. albicans	0.514	*C. albidus*	5.11	5.13	5.15
H. flabellata	2.670	*B. subtills*	5.29	5.33	5.47
H. antillarum	3.944	*C. albicans*	5.34	5.36	6.71
H. isidiophora	0.591	*C. albicans*	5.24	5.26	6.12
H. incana	1.624	*S. faecalis*	5.6	5.74	7.51
H. pseudospeciosa	3.246	*S. faecalis*	5.6	5.67	7.51

Other species, *H. flabellata* and *H. antillarum*, showed no inhibition or showed ≤ 1cm zone of inhibition. *H. incana* and *H. pseudospeciosa* showed effects on *S. faecalis* with zone of inhibition of ≤ 2.5cm with an MIC value 1.624 mg/ml and 3.246 mg/ml respectively. On comparing the potential of studied lichen species extract against tested microorganisms revealed that the MIC value of the extract was found to be very high than the standard antibiotics. Our results are in agreement with the Rankovic et al. (2007a, b) who reported that the bacterial inhibition can vary within the lichen extract, solvent used for extraction and bacteria tested.

CONCLUSION

The selected lichen species of *Heterodermia* genus had shown very mild to moderate activity in LOX inhibition, radical scavenging and antimicrobial activity. The crude extract of the species undertaken for the study contains lichen substances along with other accessory pigments. In this study at present, it is difficult to say which component of the extract had contributed towards the particular biological activities. In order to know lipoxygenase inhibition, radical scavenging and antimicrobial activity potential of the species, further studies would require to isolate and purification of the lichen substances possesses by the species should be tested. Probably this could explain the mode of action of the activities and usefulness of the lichen species and their metabolites for the use in pharmaceutics or may confirm its traditional use for several ailments.

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