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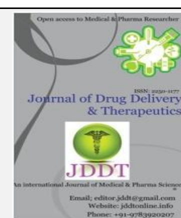

Research Article

In vitro study on screening antimicrobial and anti-oxidant potential of *Ramalina fastigiata*

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ABSTRACT

The aim of the present study is to investigate the antimicrobial and anti-oxidant potential of lichen *Ramalina fastigiata* collected from Kolli hills, Eastern Gahts of Tamil Nadu, India. Phytochemical study revealed that acetone extract of *Ramalina fastigiata* confirmed the presence of flavonoids, glycosides and phenols. Acetone extract of *Ramalina fastigiata* was tested against human pathogens, which exposed antimicrobial activity against *Klebsiella pneumonia* and *Candida krusei* with the inhibition rate of 2.1 mm and 1.3 mm respectively. The acetone extract of lichen *Ramalina fastigiata* exhibited significant antioxidant activity as well. Radical scavenging ability of *Ramalina fastigiata* was reported in terms of 61.53 % inhibition.

Keywords: Lichen, *Ramalina fastigiata*, acetone extract, antimicrobial activity, anti-inflammatory potential

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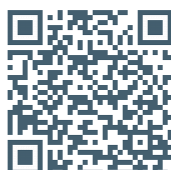

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INTRODUCTION

Lichens are two different organisms living together in a symbiotic relationship. The major partners in this symbiotic relationship are fungal-mycobiont and algae- photobiont (Lina *et al*., 2015; Karthikadevi et al., 2011). It was reported that about 300 genera and 25000 species lichens are recognized world-wide. They can produce variety of unique secondary metabolites under living conditions (Igor *et al.,* 2011). Lichens are slow growing (Bisht *et al*., 2014), they adapt to extreme conditions such as temperatures, high concentrations of salinity, air pollution and highly nitrified environments and lichens are the first colonizers of terrestrial environments (Rashmi *et al*., 2014). India represents about 10 % of lichens in world lichen population (Adriana *et al*., 2015). Lichens produce a wide range of organic compounds that can be divided into primary and secondary metabolites.

The diversity of lichen species in Kollihills was done by Kumar et al. (2011) wherein they spotted 48 lichen species belong to 23 genera and 12 families. The study evidenced that Kollihills is the good reservoir of lichens. They reported that lichen genera *Heterodermia, Parmotrema* and *Pertusaria* dominated in Kollihills. *Ramalina* lichens generally habitat on tree bark, rocks and soil (Ankith *et al.,* 2017). *Ramalina* species were found to have usnic acid, lecanoric acid, evernic acid and atranorin as bioactive secondary metabolites which in turn responsible for various activities such as cytotoxic, antimicrobial and antioxidant effect (Luo et al., 2010; Xia et al., 2015; Gulluce et al., 2006). Methanol and acetone extracts of *Ramalina* species were already reported to have biological activities. The methanolic extracts of *Ramalina farinacea*, *Ramalina fastigiata* and *Ramalina fraxinea* had remarkable antibacterial activity, further *Ramalina fastigiata* was found as the most effective antimicrobial agent (Sahin *et al.,* 2015). The present study focusses the antimicrobial activity of *Ramalina fastigiata* acetone extract against human pathogens and its anti-oxidant potential.

MATERIALS AND METHODS

Collection of sample

The lichen *Ramalina fastigiata* sample was collected from Arecanut trees in foot place of Kolli hills, Tamil Nadu for the current study. The sample was dried and preserved for further work (Nayaka, 2014). The colour tests (K-test, C-test and KC-test) were performed and the morphological characters were identified under stereo microscope.

Preparation of lichen extracts

The various solvent extracts of the lichen sample was prepared by cold maceration method. The solvents in various polarity such as acetone, benzene and water were used in this study. The lichen sample of approximately 5 g was soaked in 25 mL of solvents and kept in rotary shaker for 1 week and the contents were filtered by Whatman No. 1 filter paper. The filtrate was used for further analysis (Revathy *et al*., 2015; Rashmi et al.2014).

Phytochemical evaluation

The different solvent extracts were used to qualitatively test the presence of various phytochemical constituents (Rashmi et al., 2014). Phyto chemical constituents such as alkaloids **(**Dragondroff's test), flavonoids (Sodium hydroxide solution test), glycosides (Keller-Kilani test), phenols (Ferric chloride test), proteins (Xanthoproteic test), saponins (Frothing test) and triterpenoids (Salkowski Test) were evaluated.

In vitro antimicrobial activity

The human pathogenic microbial cultures such as *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumonia* and *Candida albicans* were used to test the efficacy of lichen extract. Agar well diffusion method (Bauer *et al*., 1966; Gayathri *et al*., 2012) was used in this study. The bacterial and fungal cultures were inoculated on nutrient broth and Potato dextrose agar for antibacterial and fungal tests were stored at room temperature for 24 hours. Then the plates were welled to load the lichen extracts of two different concentrations (75 & 100 µg/ml). Inoculated plates were then incubated at 37ºC for overnight. The diameter of inhibition zone were carried out in triplicate.

Antioxidant activity

DPPH assay

Free radical scavenging ability of the lichen extract was assessed by DPPH assay with slight modification. Lichen extract was prepared of different concentrations (20, 40, 60, 80 & 100 µg/ml) in test tubes. About 1 ml. of 0.1 mM DPPH was prepared and added in each test tubes containing lichen extracts and the mixture was incubated at room temperature in the dark for 15 minutes. Phosphate buffer (pH 6.6) was used to make up the standard solution and sample. Absorbance was measured at 517 nm. Percentage inhibition was calculated (Saliha *et al.,* 2015) by the following formula.

$$\% \text{ Inhibition} = ((A_0 - A_1)/A_0)*100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Determination of ferric reducing antioxidant power

The reducing potential was determined by ferric reducing antioxidant power assay **(**FRAP**).** Lichen extract was prepared at different concentrations (20, 40, 60, 80&100 µg/ml) in test tubes. Phosphate buffer (200 mM, pH 6.6) was used to make up the standard solution. About 1.25 ml lichen extract and 2.5 ml of potassium ferricyanide (1%) were added in test tubes. The tubes were then placed in water bath for 20 minutes at 50˚ C, cooled and mixed with 1.25 ml of trichloroacetic acid (10%) and 0.5 ml of ferric chloride (0.1%). The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 595 nm after 10 minutes (Junaid *et al*., 2013).

RESULTS

Lichen sample was collected from Kolli Hills, Eastern Ghats, Tamil Nadu, India. The collected specimen was identified as *Ramalina fastigiata* based on chemical tests and morphological features. Cold extraction method was used to extract the bioactive compounds from identified lichens. Phytochemical investigation of the different solvent extracts of *Ramalina fastigiata* revealed the presence of phytochemical compounds like alkaloids, phenol, protein, saponins, terpenoids, glycosides, and flavonoids in most of the lichen extracts tested (Table 1). Most of the lichen compounds belong to phenolic constituents, which were confirmed by acetone extract hence acetone extract was used for further testing of biological activities. Benzene extract also showed the presence of alkaloids, saponins, tri terpenoids and flavonoids. (Rashmi *et al*.. 2014)

Table 1: Phytochemical analysis *Ramalina fastigiata*

Phytochemical tests	Benzene Extract	Chloroform Extract	Acetone Extract	Water Extract
Alkaloids	+	-	-	-
Flavonoid	+	-	+	-
Glycosides	-	-	+	+
Phenol	-	-	+	-
Protein	-	-	-	-
Saponins	+	+	-	+
Triterpenoid	+	-	-	-

+ Presence; – Absence

The antibacterial effect of acetone extract was tested against human pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia*. The antifungal effect of acetone extract was tested against *Candida albicans* and *Candida krusei*. *Ramalina fastigiata* showed maximum activity against *Klebsiella pneumonia* with the inhibition of 2.1±0.00 mm followed by *Proteus vulgaris* with 1.1±0.00 mm at the concentration of 100 µg/ml. No inhibition was observed with the concentration of 75 µg/ml against *Staphylococcus aureus, Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* (Table 2).

Table 2: Antibacterial activity of *Ramalina fastigiata*

Concentration (µg/ml)	Diameter of Inhibition zone (cm)		
	75	100	Control
Staphylococcs aureus	ND	ND	1.2±0.28
Escherichia coli	ND	ND	1.65±0.07
Proteus vulgaris	ND	1.1±0.00	1.4±0.00
Klebsiella pneumonia	ND	2.1±0.00	2.45±0.07

Values are in arithmetic mean ± SD; Control-Amoxycillin; ND- Not detected

Significant antifungal activity was exhibited by the acetone extract of *Ramalina fastigiata.* The zone of inhibition for acetone extract of *Ramalina fastigiata* was found to be 1.1±0.00 cm for *Candida albicans* and 1.3±0.00 cm for *Candida krusei* at the concentration of 100 µg/ml and 1.15±0.07 cm for 75 µg/ml concentration. No inhibition was observed with the concentration of 75 µg/ml against *Candida albicans* (Table 3).

Table 3: Antifungal activity of *Ramalina fastigiata*

Concentration (µg/ml)	Diameter of Inhibition zone (cm)		
	75	100	Control
Candida albicans	ND	1.1±0.00	2.65±0.21
Candida krusei	1.15±0.07	1.3±0.00	2.6±0.00

Values are in arithmetic mean ± SD; Control-Nystatin; ND- Not detected

The acetone extract of lichen *Ramalina fastigiata* exhibited significant radical scavenging activity in terms of % inhibition in the range of 41.84 % to 61.93 % (Table 4). Maximum inhibition was observed at the concentration of 100 µg/ml with IC_{50} of 2.052 µg/ml.

Table 4: DPPH radical scavenging activity of *Ramalina fastigiata*

Lichen sample	% Inhibition Concentration (µg/ml)					IC_{50} (µg/ml)
	20	**40**	**60**	**80**	**100**	
Ramalina fastigiata	41.84	54.25	55.08	57.44	61.93	2.052

In ferric reducing assay, the optical density values were observed to be increased with increasing concentration of lichen extract (Table 3) in the concentration range of 20 to 100 µg/ml. This shows the dose dependent activity of the extract (Ananthi *et al.,* 2015)

Table 5: Ferric Reducing power of *Ramalina fastigiata*

Lichen Sample	OD (nm)	Concentration (µg/ml)				
		20	**40**	**60**	**80**	**100**
STD	595	0.078	0.086	0.087	0.094	0.104
Ramalina fastigiata		0.11	0.142	0.152	0.17	0.19

Standard (STD) – Ascorbic acid; OD –Optical density

DISCUSSION

As a result of secondary metabolic process, lichens produce so many compounds which are naturally bioactive. These lichen secondary metabolites are awesome compounds exhibiting excellent bioactivities in terms of antimicrobial, antioxidant, anti-inflammatory and antidiabetic activities etc. The phenolic constituents are responsible for antioxidant property of lichens (Behra et al., 2005; Choi *et al*., 2007; Jayaprakasha and Rao, 2000). Hence the presence of phenolic constituents of acetone extract of *Ramalina fastigiata* from the phytochemical study confirmed that the lichens are very good antioxidant agents. Lichens belong to *Ramalina* species were reported with antidiabetic activity via glucosidase assay and α-amylase inhibitory assay (Vinayaka *et al*., 2013). It was evident that acetone extracts of *Ramalina fastigiata* (pers) was found to have resistance against *Escherichia coli* and it showed no activity against *Klebsiella pneumonia*, further the study revealed that the bioactivity of *Ramalina fastigiata* was due to the presence of usnic acid and evernic acid etc., (Rafika and Monia, 2018; Saliha et al., 2015). Lichens belong to *Parmotrema* genus are reported as the potential antimicrobial sources (Poornima *et al*., 2017). *Parmotrema austrosinense* was reported to have antioxidant activity (Subash *et al*., 2012). Solvent extracts of *Parmotrema reticulatum* was already proved with radical scavenging activity (Sharma *et al*., 2012). Ramalina species are the good antifungal agents (Ankith *et al*., 2017).

CONCLUSION

The study revealed the biological potential of *Ramalina fastigiata.* Lichens are of great interest now-a-days because of their unique secondary metabolites. The biological activities of the lichen is due to the presence of secondary compounds produced by the lichen mycobiont. Hence isolation and mass culturing of lichen mycobionts will lead to the extraction of bioactive compounds of interest which in turn helps to exploit the commercial value of the lichens in the field of pharmaceutical sciences.

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Conflict of interest

The authors declare that there is no conflict of interest in publishing the article.

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