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Research Article

ISOLATION AND STRUCTURE ELUCIDATION OF ERYTHRIN AND BIOPROSPECTION STUDIES OF *ROCCELLA MONTAGNEI* EXTRACT

JEYA PREETHI SELVAM[1], KALIDOSS RAJENDRAN[1], SHENBAGAM MUTHU[1], PONMURUGAN PONNUSAMY[1]*, ARUMUGAM P[2]

[1]Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India. [2]Armats Biotek Training and Research Institute, Chennai, Tamil Nadu, India. Email: drponmurugan@gmail.com

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ABSTRACT

Objectives: Lichen is a stable mutually beneficial organism. Composed of fungi, a Mycobiont is united with algae, a phycobiont or cyanobacteria. Since the ancient period, the extract derived from Lichens is utilized in the medication of numerous diseases. An attempt was made to isolate and structure elucidation of the erythrin, a biologically active compound from corticolous lichen *Roccella montagnei,* and analyze the antimicrobial and antioxidant activities of lichen's acetone extract under *in vitro* conditions.

Methods: An attempt was made to isolate and characterized the erythrin, a biologically active compound from corticolous lichen *R. montagnei* using ultraviolet, Fourier transform infrared, High-performance liquid chromatography, nuclear magnetic resonance, and gas chromatography–mass spectrometry techniques. The antimicrobial activities of lichen extract were evaluated against six pathogenic microorganisms using the standard disc diffusion technique. For *in vitro* antioxidant activity, the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging method, hydrogen peroxide scavenging method, and phosphomolybdenum assay were done.

Results: The outcomes show that the lichen extract has the maximum effective antibacterial activity compared to antifungal activity against pathogenic microorganisms. The maximum zone of inhibition was recorded in *Escherichia coli* (8.75±0.61 mm). Among fungal pathogens, the extract inhibited the growth of *Candida albicans* with a maximum inhibition zone of 7.50±0.52 mm. *In vitro* antioxidant activity, the DPPH scavenging method, IC_{50} value of lichen extract, and ascorbic acid were found to be 45.70 µg mL^{-1} and 39.74 µg mL^{-1}, respectively. In the hydrogen peroxide scavenging method, it was observed that the IC_{50} value of lichen extract, ascorbic acid, and rutin were found to be 39.39 µg mL^{-1}, 40.66 µg mL^{-1}, and 45.58 µg mL^{-1}, respectively. The maximum antioxidant content of 44.66 mg/g ascorbic acid equivalents was observed in the 100 µg mL^{-1} lichen extract in the phosphomolybdenum assay. Total phenolic content was higher in lichen extract with 214.84±14.84 mg gallic acid equivalent (GAE/g lichen extract).

Conclusion: The present study did the isolation and structure elucidation of erythrin obtained from *R. montagnei* from Anaikatty and reveals that the lichen extract has the potential to control the human pathogenic microorganisms in the future and the study also suggested that the lichen extract possesses active antioxidant substances to scavenge free radicals.

Keywords: Lichens, *Roccella montagnei*, Erythrin, Lichen extract, Antimicrobial, Antioxidant activity.

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INTRODUCTION

A unique kind of extracellular specialized metabolites from lichens, which are known as lichen metabolite. A wide variety of biological actions is done by lichen metabolites such as antibiotic, anti-inflammatory, antiviral, anti-pyretic, analgesic, anti-proliferative, and cytotoxic effects [1]. According to the Botanical Survey of India, in India, there are about 2900 species of lichens are recorded which is 14.8% of the total world's known species. In India, lichens are utilized in folklore as medicine and many Indian medicinal practitioners used lichen as a herb during the medieval period [2]. Several infectious bacteria have evolved into drug-resistant forms. This issue is being addressed through the development of new antibacterial drugs. Numerous lichens are screened for antibacterial activity. The major factor for many chronic and degenerative diseases such as diabetes and cancer is oxidative stress, which is due to the release of free radicles. Production of the free radicle is prevented by antioxidants. One of the natural sources of antioxidants is lichen, *Roccella montagnei* is a fruticose lichen that is often found in India's mangroves. In this study, *R. montagnei* was collected in a high altitude region, and as a result of the stressful physiological adaptations they evolved, they show a difference in phytochemical components than other typical lichens. In this work, we isolated and characterized erythrin, a bioactive compound, and did bioprospecting assays on lichen extract from *R. montagnei* collected in Anaikatty, the foothill of Nilgiri Biosphere Reserve, Tamil Nadu.

METHODS

Collection and identification of lichen material

R. montagnei thalli were collected in the month of July in Anaikatty, Coimbatore District, Tamil Nadu. It is located at an altitude of 2130 m above mean sea level, a latitude of 10°14'17.2" N, and a longitude of 77°29'21.06" E at the foothills of Nilgiri Biosphere Reserve, Tamil Nadu, the part of Western Ghats, India. The collected material is identified morphologically by the method described in Awasthi's lichen identification manual [3]. The obtained material was carefully cleaned with distilled water and air-dried. The dry material was weighed and ground into powder.

Extraction

Dried lichen material was ground into a powder and then extracted for 36 h in an extractor with acetone (2.5 *l*). To obtain the 7.5 g of acetone extract, the extract was evaporated in a rotatory evaporator and dried using a vacuum pump.

Isolation of compound

The lichen substances were eluted using a column chromatographic procedure. The acetone extract was mixed with silica gel G (60–120

mesh) and applied in a silica gel G loaded column. The mobile phase solvent gradient containing n-hexane to ethyl acetate (10:0–10:20) was used. The eluent was condensed and lichen substance was identified using TLC and similar fractions were pooled and concentrated. The erythrin containing fraction was purified and subjected to spectroscopic analysis (ultraviolet [UV], Fourier transform infrared [FTIR], and ^{1}H and ^{13}C nuclear magnetic resonance [NMR]).

Evaluation of antimicrobial activity

The antimicrobial activity was determined using the disc diffusion technique, as described by [4].

Media preparation

Bacterial culture is performed on nutrient agar medium, while fungal culture is performed on potato dextrose agar media [5].

Source of microorganisms

The bacteria strains used in the biological tests were *Escherichia coli* (Microbial Type Culture Collection [MTCC] 732), *Staphylococcus aureus* (MTCC 3160), and *Vibrio cholera* (MTCC 3901), whereas the fungal strains were *Aspergillus niger* (MTCC 10180), *Penicillium citrinum* (MTCC 3234), and *Candida albicans* (MTCC 183), obtained from the Institute of Microbial Technology's MTCC in Chandigarh, India.

Antimicrobial activities

The antimicrobial activity of a sample was determined using the disc diffusion technique. 30 ml of nutrient agar (bacteria) medium and potato dextrose agar (Fungi) medium were poured into the Petri plates. The test microorganism was evenly inoculated on a Petri plate. The discs were loaded with 50 µl, 100 µl, and 150 µl of sample and 30 µl of Chloramphenicol (25 mg/ml distilled water), a standard solution for bacteria, and 30 µl of Fluconazole (25 mg/ml distilled water), a standard solution for fungus. Bacterial strains were incubated at 37°C for 24 h, whereas fungal strains were cultured at 37°C for 48 h. Each sample was tested 3 times.

***In vitro* antioxidant activity**

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity

The radical scavenging activity of DPPH was determined using the approach of [6], in summary, a 2 ml aliquot of DPPH methanol solution 25 g/ml was added to a 0.5 ml sample solution at various dosages. The mixture was quickly mixed and allowed to stand at room temperature for 30 min in the dark. A spectrophotometer was then used to measure the absorbance at 517 nm. The stronger the free radical scavenging activity, the lower the absorbance of the reaction mixture. By graphing concentration against percentage inhibition, the percentage inhibition of *R. montagnei* acetone extract was determined.

Hydrogen peroxide scavenging activity

Using the technique of, determining the hydrogen peroxide scavenging activity of lichen extract [7]. In triplicate, take 100 µl of plant extract and standards (BHT and Rutin). 600 µl of hydrogen peroxide solution should be added to the plant sample. Fill each test tube to a volume of 4 ml with phosphate buffer. As a negative control, an identical reaction mixture without the sample is used. Incubate all of the test tubes at room temperature for 10 min. Compare the absorbance of hydrogen peroxide at 230 nm to that of a blank (phosphate buffer). The following formula is used to determine the hydrogen peroxide scavenging activity.

Formula

Radical scavenging activity (%) = AC–AS/AC × 100

Where, The absorbance of the reaction mixture (in the presence of the sample) is denoted by AC = control and AS = sample.

Statistical analysis

Three separate experiments were carried out in triplicate. The quantity of sample required to inhibit free radical concentration by 50%, IC_{50}, was visually calculated using the Ms-Windows-based GraphPad Instat (version 3) tool. The results were shown graphically/mean±standard deviation.

Phosphomolybdenum assay

Evaluate the antioxidant activities of lichen extract using the phosphomolybdenum method [8]. In triplicate, add a standardized concentration of sample solution to test tubes. A reagent solution of 1 mL should be added. The standard will be a reaction mixture without a sample and the absorbance of the mixture at 765 nm will be measured against a blank. The findings were expressed in terms of ascorbic acid equivalents per gram of extract.

Total phenolic content determination

The plant's total phenolic content was determined using a significantly modified spectrophotometric method [9]. In a 25 ml volumetric flask containing 9 ml of distilled water, a diluted sample (1 ml) or gallic acid standard phenolic compound was added. The mixture was agitated after 1 ml of Folin-phenol Ciocalteu's reagent was added. After 5 min, 10 ml of 7% Na_2Co_3 solution was added to the test sample solution, which was then diluted to 25 ml of distilled water and properly mixed. The mixture was kept in the dark for 90 min at 23°C before the absorbance at 750 nm was measured. Total phenol content was calculated by extrapolating a calibration curve created by producing a gallic acid solution (20–80 mg/ml). The phenolic compound estimation was performed in triplicate. The total phenolic content was calculated as milligrams of gallic acid equivalents (GAE) per gram dry sample.

RESULTS

Collection and identification

The collected lichen sample is identified as *R. montagnei* Bel. em.D.D.Awasthi belongs to the family Roccellaceae. It is confirmed by Dr Sanjeeva Nayaka, Principal Scientist, National Botanical Research Institute, Lucknow and the lichen sample deposited in the lichen Herbarium of Bharathiar University.

Characterization of bioactive compound

Analysis of the UV visible spectrum

Erythrin's UV visible spectrum examination revealed peaks at 210,270 and 310 nm (Fig. 1).

FTIR analysis of a sample

FTIR spectrum showed characteristic stretching vibrations for alcohol, phenol, aldehydes, saturated aliphatic alkenes, aliphatic amines, and aromatic groups present in erythrin. The peak, bonds, and related functional groups are shown (Fig. 2) (Table 1).

NMR spectrum

The examination of ^{1}H NMR and ^{13}C-NMR spectrum (400 MHz, DMSO-d6) data provided supporting evidence for the structure of the erythrin, and a comprehensive assignment is presented (Tables 2 and 3) (Figs. 3 and 4).

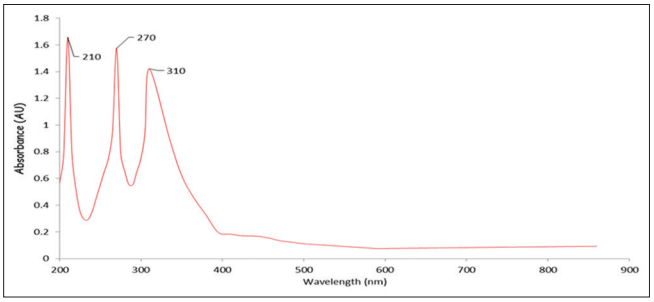


Fig. 1: Ultraviolet visible spectrum

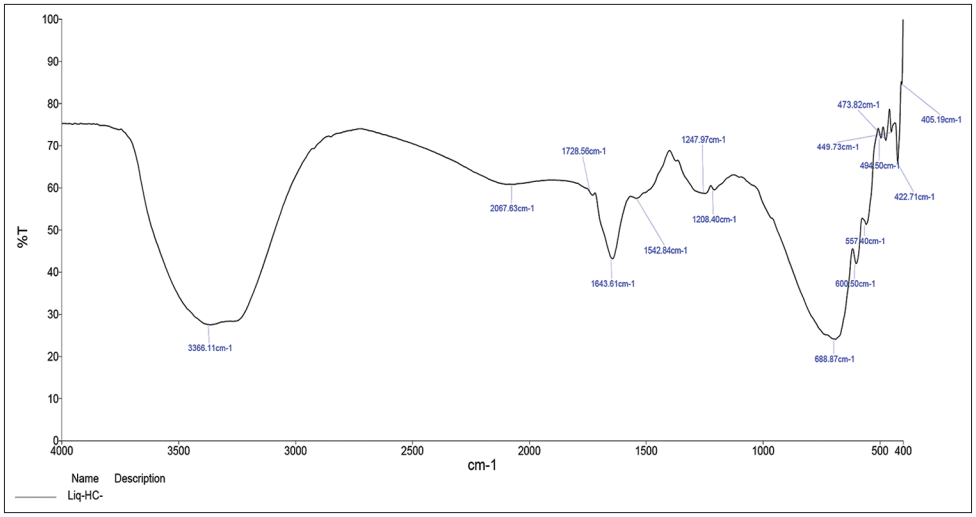


Fig. 2: Fourier transform infrared spectrum analysis of a sample

Table 1: Identification of functional group in the sample by FTIR spectrum

Peak	Bond	Functional groups
3366.11	O–H stretch, H–bonded	Alcohol and phenol
1728.56	C=O stretch	Aldehydes and saturated aliphatic
1643.61	-C=C- stretch	Alkenes
1247.97	C-N stretch	Aliphatic amines
1208.40	C-N stretch	Aliphatic amines
688.87	C–H "oop"	Aromatic

FTIR: Fourier transform infrared

Table 2: 1H-NMR spectral data of erythrin

Location of Proton	Sample	Literature (Sundholm and Huneck, 1980, 1981)
2	10.50	10.48 (OH)
3	6.22	6.22 (H)
4	10.47	10.47 (OH)
5	6.64	6.62 (H)
8	2.33	2.35 (CH_3)
2′	10.31	10.33 (OH)
3’	6.56	6.60 (H)
5’	6.60	6.61 (H)
7’	10.04	10.01 (COO)
8’	2.38	2.37 (CH_3)
2”	10.02	10.01 (OH)
3”	10.00	10.00 (OH)
4”	9.90	9.95 (OH)

NMR: Nuclear magnetic resonance

The spectra indicate 20 carbon signals, two of which are methyls and two of which are carboxylic acid carbons. Because of methylation at the C-8 and 8'-positions, they absorb at 21.16 and 21.40 ppm, respectively. Compound chemical shifts were compared to published values [10,11].

Finding compound

Based on this, the ^{13}C and ^{1}H-NMR data have been characterized as erythrin.

Structure: erythrin (molecular formula: C20H22O10).

The structure of the erythrin is confirmed by comparing it with the results of [12], in which erythrin containing fraction from column chromatography was purified and subjected to spectroscopic analysis and the molecule was characterized as C20H22O10, [3-hydroxy-5-methyl-4-((2,3,4-trihydroxybutoxy) carbonyl) phenyl2,4-dihydroxy-6-methyl benzoate] (Fig. 5).

Table 3: ^{13}C-NMR ^{1}H-NMR spectral data of erythrin

Location of Carbon	Sample	Literature (Sundholm and Huneck, 1980, 1981)
1	108.39	108.2
2	160.61	160.2
3	100.40	100.5
4	161.23	161.1
5	109.94	109.9
Location of Carbon	**Sample**	**Literature (Sundholm and Huneck, 1980, 1981)**
6	141.29	140.4
7	167.05	167.1
8	21.40	21.3
1’	116.60	116.4
2’	158.07	158.7
3’	107.47	107.4
4’	153.12	152.3
5’	114.76	114.8
6’	140.01	139.6
7’	170.90	170.7
8’	21.16	21.0
1”	101.23	
2”	63.97	
3”	60.22	
4”	50.81	

NMR: Nuclear magnetic resonance

High-performance liquid chromatography (HPLC) analysis

The HPLC analysis of erythrin showed a peak at 12.475 (Fig. 6) (Table 4).

Gas chromatography–mass spectrometry (GC-MS) analysis of the sample

GCMS analysis revealed a total of 20 bioactive lichen components in the thallus extract of *R. montagnei.* Supplementary Table 5 and Fig. 7 show the active principles together with their R. time, molecular formula, and molecular weight. The prevailing compounds in this acetone extract detected were 1,3-dioxolane-4,5 dimenthanol, propanoic acid, 2-hydroxy-2-methyl, 2,2-dimethyl-4-(2-propyl) aminobutanone etc., the biological activities of the active principles are presented (Table 6).

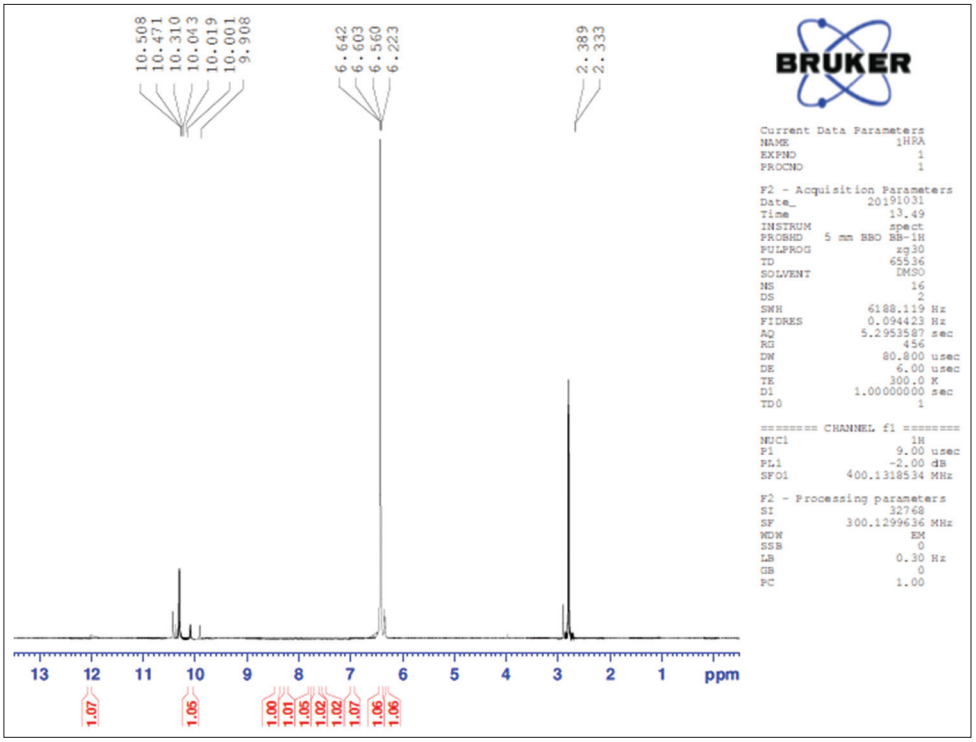


Fig. 3: ^{1}H-nuclear magnetic resonance spectral data of erythrin

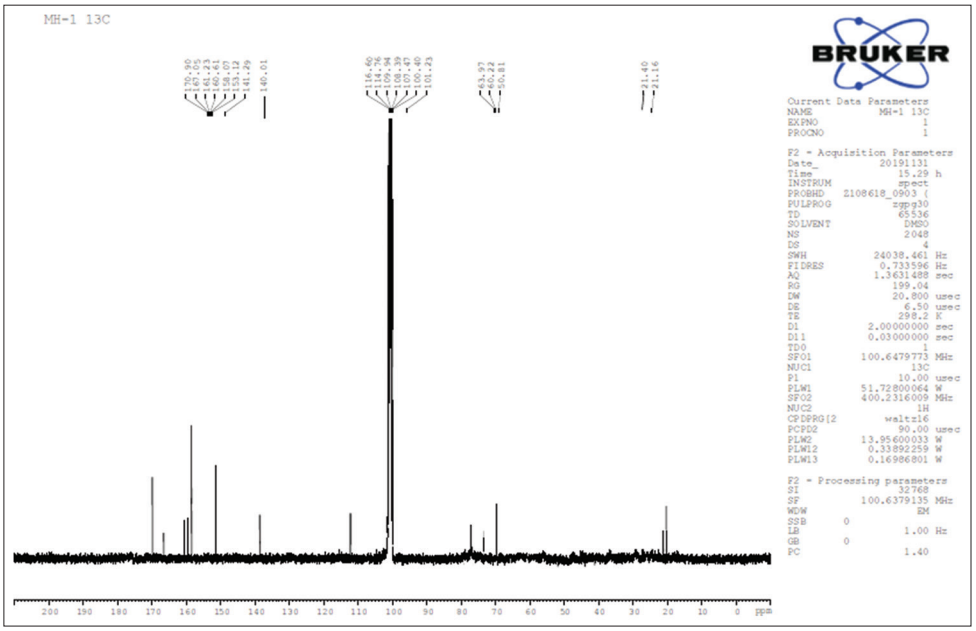


Fig. 4: ^{13}C-nuclear magnetic resonance (NMR) ^{1}H-NMR spectral data of erythrin

Bioprospecting assays

Antimicrobial activity

The antibacterial and antifungal activities of *R. montagnei* acetone extract were investigated. According to the findings, lichen extract has average antimicrobial activity against the test organisms (Table 7). At 150 µl of extract concentration, the inhibition zone of the extract against *E. coli* was found to be the highest (8.75±0.61 mm) and the lowest with *V. cholera* (7.90±0.55 mm). Furthermore, the inhibition zone against *S. aureus* was moderate (8.05±0.56 mm). The extract inhibited *C. albicans* growth with a maximum inhibition zone of 7.50±0.52 mm and a minimum inhibition zone of 7.20±0.50 mm against *A. niger*. *P. citrinum* is inhibited in a moderate zone with a diameter of 7.15±0.50 mm (Fig. 8).

Table 4: HPLC analysis. Detector AChl 254 nm–Peak table

Peak#	Ret. Time	Area	Height	Area%	Height%
1	12.475	5609417	114518	100.000	100.000
Total	12.475	5609417	114518	100.000	100.000

HPLC: High-performance liquid chromatography

In-vitro antioxidant activity

DPPH scavenging method

The DPPH scavenging method was used to evaluate the *in vitro* radical scavenging activity of *R. montagnei* acetone extract. The IC_{50} values of

Table 5: Identification of bioactive compounds in the sampleby GC-MS analysis

Peak#	R. Time	Area %	Molecular formula	Molecular weight	Name of the compounds
1	5.405	4.56	$C_7H_{14}O_4$	162	1,3-dioxolane-4,5 dimethaol, 2,2-d
2	5.526	4.46	$C_5H_{10}O_3$	118	Propanoic acid, 2-hydroxy-2-methyl-, methyl
3	6.404	0.85	$C_9H_{19}NO$	157	2,2-dimethyl-4-(2-propyl) aminobutanone
4	7.426	44.74	$C_7H_8O_2$	124	5-methyl-1,3-benzenediol
5	7.717	2.10	C_4H_6O	70	3-butyn-1-ol
6	7.783	1.59	CH_3BO	42	Borane, compd. With carbon monoxide (1:1)
7	7.842	0.53	$C_{22}H_{13}NO_4$	355	Ethyl 1-hexyl-4-hydroxy-2 (1h)-oxo-
8	8.560	0.49	$C_4H_4O_2$	84	But-3-ynoic acid
9	9.452	1.23	$C_8H_{16}O_2$	144	Octanoic acid
10	10.017	1.18	$C_{20}H_{26}O_4$	330	,1,2-benzoldicarbonsaeure
11	10.827	0.49	$C_{15}H_{32}O$	228	1-pentadecanol
12	11.078	0.50	$C_7H_6O_2$	122	Homo-p-quinone (bicyclo (4.1.0) hep
13	13.758	0.85	C_8H_{14}	110	Bicyclo[4.1.0]heptane, 2-methyl
14	13.994	3.53	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid
15	14.183	0.45	$C_{17}H_{26}O_2$	262	1,1'-bibicyclo (2.2.2) octyl-4-carboxylic acid
16	15.570	0.68	$C_{11}H_{22}O$	170	Trans-2-Undecen-1-Ol
17	15.683	0.60	$C_{15}H_{14}N_2O_2$	254	4-(methoxymethyl)-6-methyl-2-ph
18	15.807	18.70	$C_{22}H_{42}O_2$	338	13-docosenoic acid
19	16.479	12.02	$C_{22}H_{42}O_2$	338	13-docosenoic acid
20	19.311	0.46	$C_{23}H_{27}NO_3$	365	Didecyl 1,4-dihydro-2,6-dimethyl

GC-MS: Gas chromatography–mass spectrometry

Table 6: Biological activity of phytocomponents identified in the sample by GC-MS

S. No.	Name of the Compounds	Biological activity**
1	1,3-Dioxolane-4,5 Dimetha OL, 2,2-D	Antimicrobial and anti-inflammatory activity
2	1,2-benzoldicarbonsaeure	Used as Softeners, used in the preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats, and boots. Used in textiles, as dyestuffs, cosmetics, and glassmaking
3	1-pentadecanol	Antiasthmatics and Bronchodilators
4	9-Octadecenoic acid	Antihypertensive, Increase HDL, and decrease LDL Cholesterol
5	Octanoic acid	Antimicrobial activity and Flavor
6	Trans-2-Undecen-1-Ol	Antimicrobial
7	4-(Methoxymethyl)-6-Methyl-2-Ph	Antimicrobial, Antioxidant, Anti-inflammatory, and Analgesic
8	13-docosenoic acid	Antipyretic, anti-inflammatory agents, Antibacterial, and viral agents

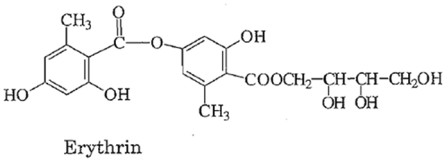
GC-MS: Gas chromatography–mass spectrometry

Table 7: Anti-microbial activity of *Roccella montagnei* in acetone extract

Strains	Concentration (µl)			Std. (30 µl)
	50	100	150	
Escherichia coli (mm)	3.15±0.22	5.40±0.37	8.75±0.61	11.65±0.81
Staphylococcus aureus (mm)	2.70±0.18	5.05±0.35	8.10±0.56	11.30±0.79
Vibrio cholera (mm)	2.30±0.16	4.75±0.33	7.90±0.55	11.15±0.78
Candida albicans (mm)	2.25±0.15	4.55±0.31	7.20±0.50	10.95±0.76
Aspergillus nige r (mm)	1.95±0.13	4.30±0.30	7.10±0.49	10.70±0.74
Penicillium citrinum (mm)	1.50±0.10	4.25±0.29	7.15±0.50	10.50±0.73

Values expressed as Mean±SD for triplicates, Standard: Chloramphenicol and Fluconazole, mm: Millime

lichen extract and ascorbic acid were determined to be 45.70 µg/ml and 39.74 µg/ml, respectively (Fig. 9) (Table 8). The extract inhibited DPPH activity by a significant percentage. The results showed that lichen extract has the same potential as standard L-ascorbic acid.



Erythrin

Fig. 5: The structure of the erythrin

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging method was used to evaluate the *in vitro* radical scavenging activity of *R. montagnei* acetone extract. The IC_{50} values of lichen extract, ascorbic acid, and rutin were determined to be 39.39 µg/ml, 40.66 µg/ml, and 45.58 µg/ml, respectively (Fig. 10). The extract inhibited hydrogen peroxide activity by a significant percentage. According to the findings (Table 9), lichen extract has the same potential as standard L-ascorbic acid and rutin.

Phosphomolybdenum assay

The *in vitro* antioxidant activity, phosphomolybdenum assay was done. The results are shown (Fig. 11). The maximum antioxidant content of 44.66 mg/g ascorbic acid equivalents was observed in the 100 µg/ml acetone extract of *R. montagnei.*

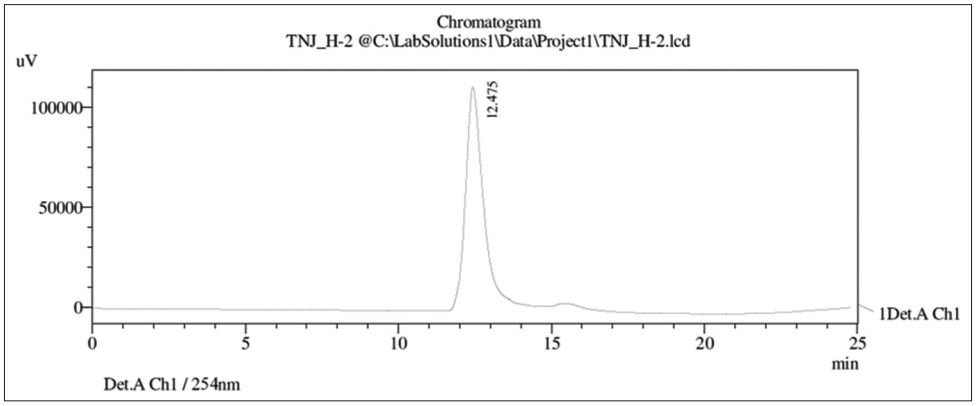


Fig. 6: High-performance liquid chromatography analysis

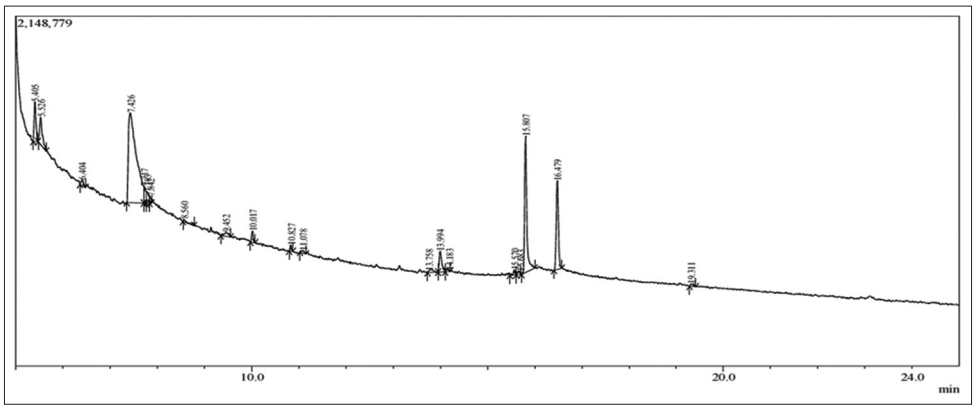


Fig. 7: Gas chromatogram and mass spectrum of sample

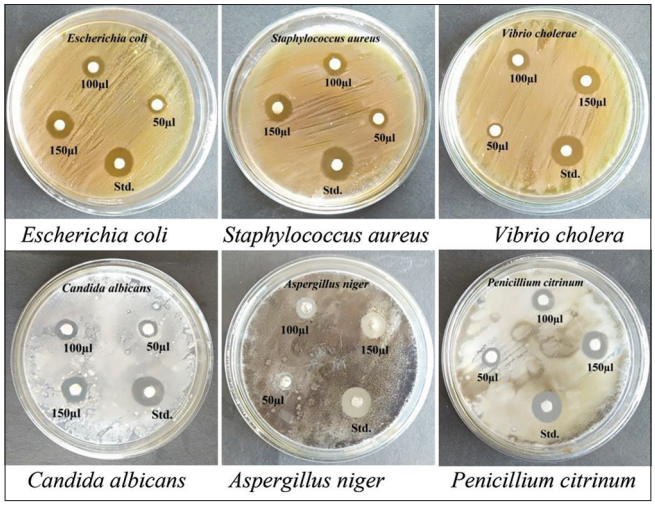


Fig. 8: Anti-microbial activity of the sample

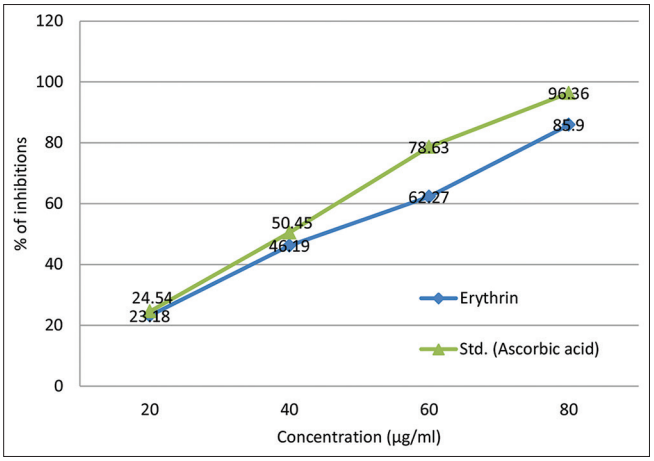


Fig. 9: DPPH radical scavenging activity of the sample

Quantitative analysis of total phenol content of the sample

Total phenolic content was higher in acetone extracts of *R. montagnei* with 214.84±14.84 mg (GAE/g lichen extracts). The results are shown (Table 10 and Fig. 12).

DISCUSSION

R. montagnei, which yields erythrin, a biologically active compound and characteristic analyzing of erythrin, through UV spectrum analysis, FTIR, NMR, HPLC, and a total number of bioactive lichen compounds were identified in thallus extract of *R. montagnei* by GCMS analysis.

Acetone aids in the quicker extraction of lichen components from lichens, hence, it was employed for erythrin extraction in the present investigation. For column chromatography, the extract was eluted in a silica gel column. Erythrin was revealed to be a significant component in the natural thallus of *R. montagnei* [13]. The present study's UV spectrum analysis results are consistent with [14] research, which stated that the UV spectrum analysis of erythrin revealed peaks at 212, 272, and 304 nm. The FTIR spectrum results are comparable to [15] study of the FTIR spectrum of *R. montagnei*, which displays the distinctive band at 3409 cm^{-1}, which we attribute to the OH stretching frequency. The carbonyl stretching vibration is responsible for the strong band at 1252 cm^{-1}.

Table 8: DPPH radical scavenging activity of the sample

Samples	% of inhibitions				IC_{50} value (µg/ml)
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	
Acetone extract	23.18±1.62	46.19±3.23	62.27±4.35	85.90±6.01	45.70
Std. (Ascorbic acid)	24.54±1.71	50.45±3.53	78.63±5.50	96.36±6.74	39.74

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, Values expressed as Mean±SD for triplicate

Table 9: H_2O_2 scavenging activity of the sample

Samples	% of inhibitions					IC_{50}value (µg/ml)
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)	
Extract	11.89±4.62	31.76±1.97	37.76±2.69	47.44±2.36	56.58±3.07	39.39429
Std (Ascorbic acid)	16	23.2	32.8	37.9	56.8	40.66667
Rutin	9.6	31.2	40.8	64	71.2	45.58974

Values expressed as Mean±SD for triplicate

Table 10: Total phenol content

Name of sample	Total phenol (Milligrams of gallic acid (GAE) equivalents per gram)
Lichen Extract	214.84±14.84

Values are expressed as Mean±SD for triplicates

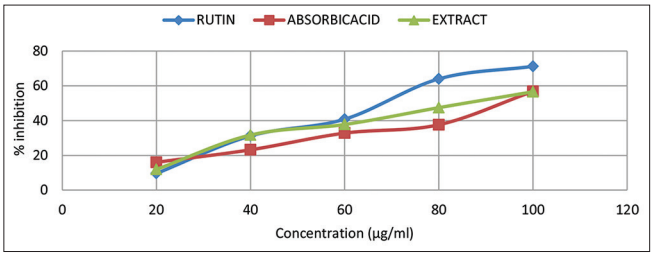


Fig. 10: Hydrogen peroxide scavenging activity of the sample

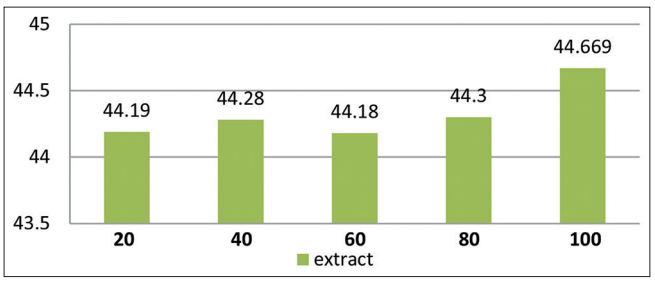


Fig. 11: Phosphomolybdenum assay

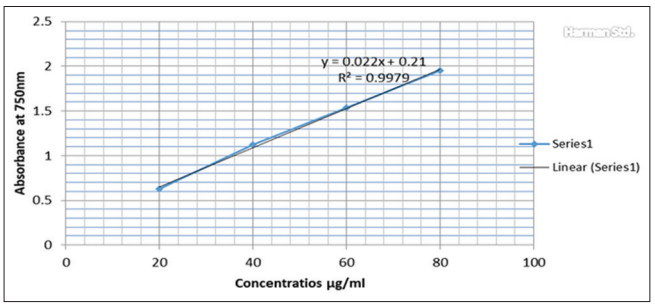


Fig. 12: Standard curve for total phenol using gallic acid

Lichen's secondary metabolites play a significant role in biological activities – antimicrobial, antioxidant, antitumor, anti-insecticidal, etc. In the present study, *in-vitro* antimicrobial and antioxidant activities of acetone extract from *R. montagnei*. In this experiment, the tested acetone lichen extract shows a similar anti-microbial activity potential to the standard. The strength of the antimicrobial action varies according to the lichen species, concentration, and bacteria examined. This antimicrobial study results that the highest 8.75±0.61 mm zone of inhibition occurs in *E. coli* and the lowest 7.20±0.50 mm zone of inhibition occurs in *A. niger*. The acetone extract of *R. montagnei* shows strong antibacterial than antifungal activity, this result accords with antimicrobial activity studies [16,17] which explain that fungi are less sensitive than bacteria in antimicrobial studies because of their cell wall composition and permeability differences. The Gram-positive bacteria's cell wall is made up of peptidoglycans, lipoprotein, and lipopolysaccharides [7,18] and the fungi cell wall consists of polysaccharides (Lichen and glucan) and the cell wall is poorly permeable. Our results are in agreement with the work done by [19,20] that the acetone extract of *R. montagnei* is stronger against bacterial pathogens especially Gram-positive bacteria than the fungi.

Acetone extract of *R. montagnei* was subjected to antioxidant activity using the DPPH scavenging method, hydrogen peroxide scavenging method, and phosphomolybdenum assays. In *in vitro*, the acetone extract of lichen has similar potential standards against different types of oxidative systems. The extract shows good radical scavenging activity because of the presence of phenolic content in lichen. Based on the study's results revealed that the antioxidative property of the extract is due to the presence of phenolic compounds in the lichen. Phenolic components stabilized the free radicals by donating hydrogen [21] and they are the potential antioxidant free radical terminators [22,23]. The correlation between phenolic content and antioxidative activities is found in numerous researches [16,24,25]. Even though some lichen extract shows no correlation between phenolic content and antioxidative activities and the antioxidant activity of various lichens may depend on other non-phenol components. One example of that is the aqueous extract of *Cetraria ilandica* has high antioxidant activity. According to [26] the acetone, extract of *R. montagnei* showed higher radical scavenging activity in all the antioxidant assays. Hence, in future, after proper clinical investigation, *R. montagnei* will be used as an effective antioxidant source.

CONCLUSION

This study concluded that erythrin, a biologically active compound is isolated from the *R. montagnei* is characteristic analyzing, was done through UV spectrum analysis, FTIR, NMR, HPLC, and a total number of bioactive lichen compounds were identified in thallus extract of *R. montagnei* by GCMS analysis. In further studies, erythrin is tested for medicinal properties. The tested lichen extract has an effective *in vitro* antimicrobial and antioxidant activity. Based on the results, this lichen has excellent and secure natural antimicrobial and antioxidant agents.

In the future, this lichen will have a significant role in human, animal, and plant diseases.

AUTHORS' CONTRIBUTION

The concept plan and experiment support were done by Corresponding Author Dr. P. Ponmuruganand Mr. P. Arumugam. The lichen collection, antimicrobial activities, and antioxidant activities were done by the first author Ms. S. Jeya Preethi and the manuscript editing and modification were done by Dr. R. Kalidoss and Ms. M. Shenbagam.

CONFLICT OF INTEREST

None.

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