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Original Article

Radical scavenging potential, antiinflammatory and antiarthritic activity of isolated isomer Methyl-γ-Orsellinate and roccellatol from *Roccella montagnei* Bel

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ABSTRACT

The burden of multiple drug resistance towards chronic diseases is rapidly increasing globally at an alarm rate. The present study aims to isolate active principles via bioassay guided fractionation of traditionally used lichen *Roccella montagnei* for *in vitro* radical scavenging activity with potential to combat arthritis and inflammation. The results of proximate composition and mineral analysis revealed the presence of essential amount of micro and macro nutrients which are required for secondary metabolite production. Fractions of Roccella montagnei and pure compounds were assessed for its *in vitro* antioxidant (DPPH, FRAP, OH radical, SOD, CAT, GPX), antiarthritic and antiinflammatory activity. The results indicates that lichen sample and fractions exhibited potent radical scavenger demonstrated *in vitro* antiarthritic and antiinflammatory activity in dose dependent manner. Bioassay fractionation lead to isolation of two pure compounds ie., Compound I and Compound II which were characterized using spectral data such as FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT NMR spectroscopy, COSY & HMQC NMR spectroscopy and LC-MS analysis and was identified as Methyl-γ-Orsellinate (C9H10O4) a novel isomer from fractions E2d5a with a molecular weight of 182.2 and Roccellatol ($C_{12}H_{16}O_7$) from fractions D3d4c with a molecular weight of 272.3. The present findings suggest that the secondary metabolites present in the lichens have direct relation, with biological activities revealing different properties and can be a future novel antioxidant and a potent anti-arthritic agent.

1. Introduction

Free radicals produced in the human system triggers oxidative damage by release of reactive oxygen species (ROS), reactive nitrogen species (RNS) from activated neutrophil and macrophages leads to various diseases like heart disease [1] autism, cancer, diabetes, arthritis, Alzheimer's dementia, Parkinson's disease, cataracts and aging [2]. Reactive oxygen species induces inflammation by stimulating the release of the cytokines such as IL- I, TNF-α, and INF-γ, which in turn stimulates recruitment of additional neutrophil and macrophages. These free radicals are chief mediators that induce inflammatory processes.

Rheumatoid arthritis an autoimmune disease with joint inflammation, synovial proliferation and destruction of articular cartilage [3] and the majority is seen in female. Prolonged treatment with allopathic drugs causes adverse side effect and hence alternative natural measure is in search. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins [4] and the effect can be reduced by inhibiting protein denaturation and regulating the production of auto antigen. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure to analyze *in vitro* anti-arthritic activity. The possible mechanism to decrease the risk of chronic diseases and constrain disease progression by inducing natural antioxidant defenses or by supplementing with dietary antioxidants [5].

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Lichens are universally distributed organisms occurring in the most adverse and varied climatical conditions. Lichens are symbiotic organisms with unique interdependence of two organisms (algal and fungal properties) that aids lichens to produce variety of secondary metabolites, some of which are common in plants or in higher fungi but about 80% metabolites are specifically produced by lichens [6]. Lichens have been widely used in food and in folk medicine in several countries over a considerable period of time.

More than 1000 lichen metabolites like dibenzofurans, quinones and pulvinic acid derivatives, depsones, depsidones, lactones, including phenolic compounds have been identified [7]. Various scientific reports suggest that the lichens have antioxidant, antiinflammatory, antitumor, analgesic and antipyretic, antiproliferative, antimicrobial, antiviral, and antiprotozoal potentials [8,9]. There are primary works carried out with different lichens but detailed bioassay isolation and biological activity of the isolated compounds are lacking, hence the present study focuses on bioassay guided isolation of active principles from *Roccella montagnei* and to validate its usage in traditional system of medicine experimentally using *in vitro* model.

2. Materials and methods

2.1. Collection, identification and authentication of lichens

Roccella montagnei Bel *(Rm)* was collected from Marine National Park, Pathapir Island, Jamnagar district, Gujrat, India and Pichavaram mangrove forest, Chidambaram, Tamil Nadu. Identified, authenticated and the specimen samples (Voucher Specimen No. LWG 13-027427) are kept in the Herbarium of Indian Lichenological Society, CSIR-National Botanical Research Institute (Govt. of India), Lucknow, U.P.

2.2. Chemicals and solvents

All the chemicals and solvents were of analytical grade and obtained from S.D. Fine Chemicals, Mumbai and Fischer Inorganic and Aromatic Limited, Chennai, India. TLC plate (pre coated plate 0.2 mm thick) from E. Merck, Germany.

2.3. Extraction

75gms of powdered *Rm* samples were taken separately, mixed with 500 ml of methanol and then magnetically stirred in a separate containers overnight at room temperature. The residue was removed by filtration, concentrated under reduced pressure in a rotary evaporator at 60 ± 10 °C and the resultant extract was used for further studies [10].

2.4. Bioassay guided fractionation of crude methanol extract

The methanol crude extract (75 gms) was fractionated by column chromatography using silica gel (60–120 mesh), column (85 cm × 5.7 cm) following bioassay guided fractionation protocol with slight modification [11]. Elution was performed with different proportions of Hexane: Methanol (8:2. 6:4, 3:7, 0:1, 500 ml each fraction) to yield initial fractions A, B, C, D and E, monitored using TLC plates and the fractions with similar Rf values in TLC pattern were pooled together, concentrated and subjected to *in vitro* assay.

Fraction D and E exhibited significant activity were rechromatographed separately and fractioned with Hexane: Methanol (9:1, 7:3, 1:1, 0:1) yielding D (D1, D2, D3, D4, D5); E (E1, E2, E3, E4, E5, E6) and subjected for *in vitro* assays. D3 and E2 alone were chromatographed using silica gel (230-400mesh), elution with Hexane: Ethyl acetate (9:1, 8:2, 7:3, 0:1) yielding D3–D3a, D3b, D3c, D3d; from E2–E2a, E2b, E2c, E2d & E2e and tested for *in vitro* assays. Among the collected sub fractions, fraction D3d and E2d represented potent activity, hence again eluted using Hexane: Isopropyl alcohol in 9.5:0.5, 9:1, 8:2, and 0:1, the purity of the sample was continuously monitored using TLC plates. Fraction D3d4 and E2b5 demonstrated potent activity and were subjected for further purification and eluted with Hexane: Isopropyl alcohol in 9.5:0.5, 9:1, 8:2, and 0:1, using silica gel (234 mesh), column (85 cm × 2.4 cm) yielding fraction D3D4c from D fraction and E2b5a from E fraction. TLC pattern of these two compounds represented single peak further confirmed using HPLC chromatogram, tested for its biological activity, subjected for characterization and the possible structure was elucidated using the spectral data's.

2.5. Proximal composition and mineral analysis

The proximal composition and mineral contents was determined using atomic absorption spectrophotometer as per the method suggested by the Association of Official Analytical Chemists [12].

2.6. Characterization of isolated compounds

The structure of the isolated compounds were determined on the basis of FT-IR spectra (Shimadzu IR affinity instrument). HPLC (Agilent 1200 series, Phenomex C18 column with 200 × 3.9 mm id, particle size 5 µm). The ^{1}H, ^{13}C NMR, COSY and HMBC (Avance Bruker 400 MHz, using DMSO-d_6 with tetramethylsilane as internal standard). The mass spectra (Agilent 1200 MS series 2000 liquid chromatography-mass spectrometry).

2.7. In vitro assays

In vitro biological activity of *Rm* was carried out following the methods with slight modification. DPPH radicals scavenging assay [13], Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP) [14], Hydroxyl radical scavenging assay [15], Superoxide dismutase (SOD) [16], Catalase (CAT) [17], Glutathione peroxidase [18]. *In vitro* anti-inflammatory activity (albumin denaturation inhibition) [19], *in vitro* anti-arthritic activity-Inhibition (protein denaturation method) [20].

2.8. Statistical analysis

All the results were expressed as Mean ± S.D. and all the grouped data were statistically evaluated with SPSS\16.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test.

3. Results

3.1. Proximal composition and mineral analysis of lichen

Lichen thalli of *Rm* was taken to identify the mineral composition (Table 1). Ash content was found to be 94.3%, with higher amount of macronutrients like sodium, potassium, phosphorous and calcium. The results revealed that samples has lower quantity of microelements like nickel, arsenic, chromium and aluminum as compared to sulphur,

Table 1
Analysis of Micro and macro nutrients in *Roccella montagnei* sample.

Ash%	P mg/g	K mg/g	Mn mg/g	Zn mg/kg	Ca%	S%	Mg%	Cu ppm	Na ppm	As ppm	Ni ppm	Cr ppm	Al ppm
94.35	4.32	29.63	0.89	92.63	2.8	0.96	1.26	0.86	16.53	–	0.06	0.04	0.83

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