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# BIOPROSPECTING IMPORTANCE OF CERTAIN *RAMALINA SPECIES* AND PROMISING ROLE OF ITS BIOLOGICAL ACTIVE COMPOUNDS

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The aim of the study is to list out the economically important species of *Ramalina* genus based on the worldwide studies and data collected. From the early time of civilization, lichen species have been utilized by various ethnic groups. Certain ethno-botanically important *Ramalina* species are used as spices in flavouring vegetables and meat, prepared as cold dish in Yunnan marriages, as a natural dye on fabrics, in perfume industries, as an efficacious medicine for jaundice and hydrophobia, as an ingredient of medical decoction called sciba. *Ramalina* species possess important bioactive secondary metabolite compounds which show antitumor, antibacterial, antifungal, antioxidant, cytotoxic, antiviral and anti-inflammatory activities and ability to fight against *Mycobacterium tuberculosis*, life threatening *Staphylococcus aureus* infections. The sustainable use of lichens over other artificially prepared products could prevent us from side effects of certain chemical constituents. Even air pollution could be bio-monitored by this lichen genus. The world needs to have an attention over the immense and significant uses *of Ramalina* species.

**Keywords:** Bioactive Secondary Metabolites, Bio-monitored, Bio-prospecting, Ethno-botanically, *Ramalina.*

## Introduction

At the beginning of the nineteenth century, botanists were making their first attempts to classify the lichens and in order to systematize the immense variety of growth habits, they first divided the class on the basis of several major growth forms. The lichens of one family, even of one genus, may belong to the crustose, foliose, and fruticose growth forms[1]. Lichens being the nonvascular cryptogams and excellent example of symbiotic association have a composite thallus comprising of two components, mycobiont and photobiont. Lichens could also be defined as a symbiotic organism formed by fungi and algae and/or cyanobacteria[2]. Lichen is also defined as thalli that can be considered as self-contained mini-biosphere resulting from the close, symbiotic, morphological and physiological integration of a nutritionally specialized fungus (mycobiont) and at least a green alga and/or cyanobacteria (photobionts)[3].

With its share of just 2-4% of global land surface, India is a rich centre of lichen diversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world. Lichens produce a diverse range of secondary metabolites/chemical products, many of which have been found to have antimicrobial activity[4]. All of the secondary substances in lichens are of fungal origin. These substances are the crystals deposited on the surface of the hyphae, which are poorly soluble in water, and usually can be isolated from the lichens by organic solvents[5]. Specific conditions in which lichens live are the reason of production of many such metabolites as these metabolites are responsible for providing good protection against various negative physical and biological influences[6].

During the middle-ages, lichens figured prominently among the herbs used by medicinal practitioners. *Lobaria pulmonaria*, *Cetraria islandica*, and *Cladonia* species have been reported to be effective in the treatment of pulmonary tuberculosis and other lung diseases[7,8]. Lichens have been used in folk medicine in many countries. The utilization of lichen in medicine has been cited in different pharmacopoeias of the world[7]. Lichens have certain features which have resulted in their intensive use as indicators of air quality and as monitors of the atmospheric deposition of various elements. These salient features include the lack of roots or structures which have the absorptive function of roots and thus some lichens are dependent for their mineral nutrients to a large extent on material landing on the lichen thallus as the result of wet and dry deposition from the atmosphere[9].

***Ramalina spp.***

*Ramalina* is a large genus containing several hundred species[10]. The genus *Ramalina* belongs to the family Ramalinaceae and is a widespread fruticose lichen genus[11]. The genus is characterized by usually having pale yellow-green flattened strap-like branches with small disk or cup-shaped apothecia. In New Zealand the genus is widespread and easily identified, being found on native and introduced trees in suburban gardens, forest edges and coastal localities[10]. The genus *Ramalina* was first described by Achariusin 1810, when he split a group of species from the genus *Parmelia.* The features used to differentiate the two included the possession of a cartilaginous thallus and several apothecial characters. A number of different genera and subgenera have been created from *Ramalina* specimens and revised in the two centuries since Acharius[12]. *Ramalina* has about 246 species distributed around the world, of which only 118 species were investigated for their chemical and biological studies[13]. Generally, *Ramalina* genus is described on the basis of morphological characteristics such as fruticose thallusattached by holdfast, erect or pendulous, variously branched; branches circular, narrow strap shaped or wide lobed; greenish-grey, yellowish-grey to yellowish brown; soralia and pseudocyphellae present or absent, based on the anatomically features like thallus heteromerous, corticated on all sides by a thin amorphous layer followed internally by a chondroid tissue, either continuous or in separate bands, uniform or split into bundles of hyphae (cracked); photobiont, a green alga (*Protococcoid*); medulla loose or arachnoid, solid or sometimes hollow. Apothecia laminal or terminal, sessile to peltate, lecanorine; disc yellow ochraceous, or buff coloured, often pruinose; hypothecium colourless; paraphyses simple; asci unitunicate, 8-spored; ascospores colourless, usually 2-celled, ellipsoid to fusiform, straight or slightly curved. Pycnidia species laminal or subterminal; pycnoconidia cylindrical, elongated. Out of about more than 200 species known from the world, 23 species, reported from India, Nepal and Sri Lanka[14].The members of *Ramalina* are distributed in diverse habitats such as lowlands, highlands, rainforests and alpines. The species occur on various substrates like rocks, peaty soil, wood, and bark. In fresh conditions, the thalli are greenish-gray to yellowish-gray in colour, the colour changes to yellowish-brown to dark-brown on drying. The species of *Ramalina* contain pseudocyphellae. Usnic acid is one of the major metabolites found in the members of *Ramalina*[15]. Atranorin is also present in cortex, but rarely[12].

**Bioprospecting role of genus *Ramalina***

Several *Ramalina* species are used traditionally as medicine, food and spice. Various bioactivities such as antimicrobial, antioxidant, enzyme inhibitory, insecticidal, cytotoxic, antihelmintic and immune-stimulatory activity are shown by some species of *Ramalina*[15]. Even some steroids extracted from the lichens show antitumor activities[16]. Certain *Ramalina* species are used as food in some Central and South East Asian countries. *R. farinacea* and *R. Conduplicans* are used as traditional food by the Rai and Limbu communities of East Nepal. *R. farinacea, R. conduplicans, R. sinensis,* and *R. subfarinacea*are cooked mixing with various foods[17]. *Ramalina* species were used for medicinal values in Nepal. Two respondents (more than 72 years) from Hangdewa and two (60 and 71 years) respondents from Phurumbu, Taplejung district used *Ramalina* species as antiseptic tincture to heal wounds[18]. Since the beginning of the 20th century, hair powder of *R. Calicaris* and *Ramalina* species was used in cosmetics in Europe[19], and India[17]. This lichen genus also has economic importance in production of a natural colouring dye. The secondary metabolites not only impart colour but also give unique aroma to the fibres. The lichen metabolites also have antimicrobial and insecticidal properties; hence lichen dyes have an inherent quality of insect resistance thus giving more life to the dyed fibres[20].

*Ramalina* species also act as biomonitors, which are mainly used for qualitative determination of contaminants. These can be classified as being sensitive or accumulative. Sensitive biomonitors may be of the optical type and are used as integrators of the stress caused by contaminants and as preventive alarm systems[21]. Lichens are perennial and this feature together with the other characteristics has led to the use of these plants as long term integrators of deposition from the atmosphere of elements originating from both natural and man-made sources[9]. To cope with the potential metabolic disturbances caused by intracellular Heavy Metal(HM) accumulation, lichen thalli have developed an armamentarium of biological responses. Indeed, there is vast diversity in heavy metal tolerance among lichens, ranging from moderately tolerant species, e.g., *Ramalina farinacea*, to sensitive ones. Based on all of these features, lichens can be viewed as extremely valuable biomonitors of environmental pollution. The physiological basis of such tolerance, and especially whether the mycobiont and photobiont differ in their sensitivity to heavy metals, is still poorly understood and somewhat controversial[22]. Algal member *Trebouxia* sp. Has been reported to be more sensitive to HMs and other pollutants than the corresponding mycobionts. Within chlorobiont lichens, those containing *Trebouxia* sp. as primary algae (*Parmelia caperata* or *R. farinacea*) were shown to be moderately Pb-tolerant, while the HM sensitivity of *Lobaria pulmonaria*, in which *Dictyochloropsis* is the primary phycobiont, is intermediate. The lichen *R. farinacea* has a world-wide distribution and is encountered in polluted and unpolluted areas indicating a moderate tolerance to Pb, as proved with the help of laboratory experiments[23]. Exposure to HMs in general, and to Pb in particular, increases the production of reactive oxygen species (ROS). At high levels, or following acute increases of metal pollutants, algal cells are injured because the resulting ROS levels exceed the capacities of cellular antioxidant protection systems[22].

**List of some important *Ramalina* species and their uses**

***Ramalina calicaris***

Ingredients in medicinal decoction called sciba[24].

***Ramalina capitate***

Taken as tea to relieve symptoms of asthma[24].

***Ramalina celastri***

An α-D-glucan polysaccharide isolated from *R. celastri* has shown cytotoxic effect against HeLa cells[25].α-D-glucan suggested to be a biological role response modifier (BRM), which acted as antitumor agent by its macrophage activity against Sarcoma-180 tumor cells[26]. Parietin isolated from *R. celastri* demonstrated antiviral activity against the arena viruses[27]. *Ramalina celastri* is one of the most common lichen species in the province of Córdoba

(Central Argentina) and its physiological response has been successfully used for biomonitoring the air quality in several transplantation studies. Elevated concentration of metals like zinc, arsenic and uranium could be detected by *Ramalina celastri*[28].

***Ramalina conduplicans***

Locally called as Jhavila by Baiga, Bhil, Bhilola, Gond, Korka and Muria tribal groups of India. In Central Indian region, this species is used as spice for flavoring meat and vegetables. Different tribal groups in Darjeeling and Sikkim use the species as effective medicine for hydrophobia and jaundice.

*R. conduplicans* is sold as a spice in Himachal Pradesh.Local names of *R. conduplicans*: shouxu, shikuacai, and shuhua (Jingu County, Yunnan) and shihuacai (Central Yunnan). Local name for *R. sinensis*: shuhua. In South-western Yunnan, the Yi, Dai, and Han people cook these two species of *Ramalina* to prepare a traditional cold dish served at marriage banquets. According to the local people of Jingu County, southern Yunnan, the dish has been used in this way since ancient times, and couples who eat it at their marriage will love each other more and never separate. In central Yunnan, however, the local people eat these *Ramalina* species at any time. In Kunming the dried lichen is readily available in public markets for about US$4 per kilogram. About 100–300 kg is sold per year in a shop in Lijiang. At Chuxiong, these two species are also used in a stir-fried pork dish. First the lichen is boiled in water with soda for 10–20 minutes and then soaked in water for 1–2 days. Sometimes the local people use the lichen with chili powder, salt, and other seasonings[24]. In the stocks of *Ramalina* sold in the local market in Jing Dong County, *R. conduplicans* and *R. sinensis* are always mixed together; usually with *R. conduplicans* in greater amount[29]. Methanolic extract of the lichens showed high inhibitory activity against eleven bacteria (*Bacillus cereus*, *B. subtilis*, *Enterobacter aerogens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pneumoniae*) and eight fungal strains (*Aspergillus flavus, A. nidulans, A. niger, A. sulphuricus, A. terrcues, Candida albicans, Cryptococcus albidus, Tricho phyton rubrum*)[25]. *Ramalina conduplicans* (secondary metabolites like Usnic acid, sekikaic acid aggregate and salazinic acid) gives cartridge buff color by Boiling Water Method (BWM), isabella color by Ammonia Fermentation Method (AFM), turtle green by Di-methyl sulphoxide Extraction Method (DEM)[20].

***Ramalina farinacea***

The acetone extract of the lichen *R. farinacea* and its usnic acid constituent showed antimicrobial activity against *Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis (Enterococcus faecalis), Yersinia enterocolitica, Candida albicans* and *C. glabrata (Torulopsis glabrata).* Norstictic acid was active against *Aeromonas hydrophila* as well as the above microorganisms except *Yersinia enterocolitica*. Protocetraric acid showed activity only against the tested yeasts *C. albicans* and *C. glabrata*[30]. In another study, water, ethanol, chloroform and n-hexane extract of *R. farinacea* exhibited antifungal, antibacterial and cytotoxic activities against several pathogens. Extracts from *R. farinacea* were evaluated against fifteen clinical isolates of *Staphylococcus aureus.* The results showed that two column chromatographic fractions, with minimum inhibitory concentrations against 90% of the isolates of 535.5 and 317µg/mL, respectively, generally performed better than tetracycline and ampicillin[31]. In an experiment, combination of methanolic extract of *R. farinacea* and ampicillin gave a desirable synergistic effect as an antibiotic against *Staphylococcus aureus* severe infection[32]. The methanol and water extract reduce HIV-I vector (lentiviral and adenoviral) infectivity in dose-dependent manner in a vector-based assay[33]. The aqueous extract of *R. farinacea* has a folklore value for treatment of mental disorders in Africa; and tinctures have also been used for treatment of ringworm (*Tinea*) in Nigeria[31].

In a study, it is clearly indicated that the ability of the lichen *R. farinacea* to moderately tolerate polluted environmental conditions in general, and Pb exposure in particular on the basis of physiological tolerance of its two phycobionts, TR1 and TR9. *Trebouxia* TR1 andTR9 were isolated in our laboratories from a population of the lichen *Ramalina farinacea*. In order to further characterize the defensive response of TR1 and TR9 microalgae, we studied the activities of several key antioxidant enzymes in response to increased levels of the HM. When external conditions surpass certain thresholds and extracellular barriers are no longer effective, the intracellular defense mechanisms of TR9 phycobionts can be rapidly induced. *R. farinacea* was proposed as a good example of biomonitoring lichen[22]. Ingredients in medicinal decoction called sciba along with *Ramalina calicaris, Usnea plicata, Pseudevernia furfuraceae*[24]. Many species of algal genus, *Trebouxia* were reported to be more sensitive to HMs and other pollutants than the corresponding mycobionts. Within chlorobiont lichens, those containing *Trebouxia* spp. as primary algae (*Parmelia caperata* or *R. farinacea*) were shown to be moderately Pb-tolerant, while the HM sensitivity of *Lobaria pulmonaria*, in which *Dictyo chloropsis* is the primary phycobiont, is intermediate[23]. Exposure to HMs in general, and to Pb in particular, increases the production of reactive oxygen species (ROS). At high levels, or following acute increases of metal pollutants, algal cells are injured because the resulting ROS levels exceed the capacities of cellular antioxidant protection systems[22]. The lichen *R. farinacea* has a world-wide distribution and is encountered in polluted and unpolluted areas indicating a moderate tolerance to Pb, as has been earlier reported in many studies[23].

***Ramalina hossei***

*Ramalinahossei* (secondary metabolites are Usnic acid and sekikaic acid) gives natural dye colors like pale pinkish buff color by Boiling Water Method (BWM), olive yellow color by Ammonia Fermentation Method (AFM), white color by Di-methyl sulphoxide Extraction Method (DEM)[20].

***Ramalina menziesii***

This lichen is used as baby diaper[24].

***Ramalina nervulosa***

The extract of this lichen is well known for insecticidal, larvicidal and antihelminithic activities[34].

***Ramalina obtusata***

Due to the sensitivity of *R. obtusata* to pollution and proximity to the oil and gas industry at the Izhma River site could be considered a monitoring site for future industrial impacts in the area. The first record of *Ramalina obtusata* in the Komi Republic was made on the bank of the Izhma River, 10 km from Ukhta, Russia[35].

***Ramalina pacifica***

It is known from Karnataka and Kerala. The methanol extract of cultured tissue of this lichen exhibited inhibition of tyrosine activity[34]. In Shimoga district, located in southern part of Karnataka, it is used as spices, curry powder and also for flavoring meat and vegetables[37].

***Ramalina pollinaria***

Methanol extract of the lichen showed antibacterial activity[38]. *R. pollinaria* collected from Turkey and its usnic acid extracted with acetone exhibited antimicrobial activities against *Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Staphylococcus aureus, Bacillus subtilis* and *B. megaterium*[39]. In a study, it was revealed that *Ramalina pollinaria* is sensitive to genotoxic stresses. This supported the view that RAPD analysis is a highly sensitive method for the detection of DNA damage induced by environmental pollutants like toxic chemicals[40].

***Ramalina sinensis***

No lichen like substance is present in this species of lichen[14]. It is sold as a spice in Uttarakhand, Uttar Pradesh and Himachal Pradesh. *R. sinensis* gives olive yellow natural dye color by Ammonia Fermentation Method (AFM)[41]. Yunnan in China, the Yi, Dai, and Han ethnic people cook *R. sinensis* to prepare a traditional cold dish served at marriage banquets[8]. Biosynthesis by reducing the iron chloride solution with *Ramalina sinensis* extract containing sulfate polysaccharide works as a major, reducing, stabilizing, and effective agent and showed that the synthesis of nanoparticles with plant extracts seems to be effective and can be used to remove toxic metals such as lead and cadmium. The ability to remove lead and cadmium by magnetic nanoparticles of iron oxide was respectively 82% and 77% for initial concentration of 50 mg/l and pH in the range of 4–5[42].

***Ramalina subcomplanata***

Used as spice and flavoring agent for meat and vegetables by Bhaiga, Bhil, Bhilala, Gond, Korka and Muria tribes of Madhya Pradesh.

***Ramalina subfarinacea***

*Ramalina subfarinacea* and *R. farinacea* are very close in morphology, the latter being more polymorphic, usually corticolous and with protocetraric acid in medulla[14]. It contains usnic acid, its acetone extract showed significant antibacterial activity against *Bacillus cereus, B. megaterium, Staphylococcus aureus* and *Klebsiella pneumoniae*[43].

**Table 1. Activity of various secondary metabolites found in *Ramalina sp.***

| **Secondary metabolites** | **Biological activity shown by these metabolites** | ***Ramalina* species in which these metabolites are found** |
|---|---|---|
| Norstictic acid | Antimicrobial, antioxidant, anticancer [30,44,45]. | *Ramalina arabum, Ramalina subfarinacea*[14]. |
| Evernic acid | Antifungal, antibacterial, antioxidant, anticancer[46,47]. | *Ramalina baltica, Ramalina himalayensis, Ramalina obtusata, Ramalina pollinaria*[14], *Ramalina yasudae*[48]. |
| Usnic acid | Antiviral, antitumour, antioxidant, antibacterial, antifungal, antipyretic, analgetic, anti-inflammatory, hepatotoxic, antiviral[49,30,50,45,51,52,53,54,55,56,57]. | *Ramalina celastri, Ramalina conduplicans, Ramalina flabelliformis, Ramalina nervulosa, Ramalina hossei, Ramalina pacifica, Ramalin apollinaria, RamaIina roesleri*[14], *Ramalina crassa*[58] and *R. reticulata*[17]. |
| Salazinic acid | Antitumour, antibacterial, antifungal, antioxidant[59,56,60]. | *Ramalina conduplicans, Ramalina pacifica, Ramalina subpusilla*[14], *Ramalinacrassa*[58]. |
| Sekikaic acid | Antioxidant, antibacterial [61,62]. | *Ramalina conduplicans, Ramalina hossei, Ramalina inflata, Ramalina intermedia, Ramalina leiodea, Ramalina nervulosa, RamaIina roesleri, Ramalina taitensis, Ramalina usnea*[14]. |
| Protocetraric acid | Antibacterial, antifungal, antioxidant, anticancer[31,50,44,60]. | *Ramalina farinacea*[14], *Ramalina yasudae*[48]. |
| Divaricatic acid | Antioxidant, antimicrobial[63,64]. | *Ramalina inflate*[14]. |
| Atranorin | Antimicrobial, antioxidant, anti-inflammatory, anticancer[65,66,67,51,45,68]. | *Ramalina siliquosa*[69,70]. |

## Conclusion:

Moost of the antibiotics are costly and their use is often associated with certain adverse effects on the health of the individuals, now a days, the scientific community is focusing more on the search for alternatives for disease therapy. Natural products such as plants, microbes and lichens have been investigated and are found to be promising alternatives[15]. Lichens have major environmental importance in maintaining ecology by playing role as biological weathering agents in the development of soils. However, it was formerly considered in a geological context only, but recent researches have shown that these organisms are capable of biodeteriorating stone substrates within a relatively short timescale. Information is now available to demonstrate that lichens can often contribute substantial biomass and support a high biodiversity of micro- and macro-organisms; creating complex food webs and adding significantly to energy flow and mineral cycling[71]. Since the lichens are slow growing organisms, unable to produce large amount of biomass, therefore, there is a need to develop proper harvesting techniques for obtaining good yield of lichens which have various bioprospects and at the same time for conserving lichen biodiversity[72]. Research should be conducted to enhance lichen biomass under short time period.

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