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Review Article

Biologically-Active Compounds from Brazilian Lichens and their Affinity with Ether

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Abstract

It can be obtained from lichens biologically-active extracts and pure substances, many of them of phenolic nature. They are usually obtained by using organic solvents, such as diethyl ether. In this paper the usefulness of ether for the obtainment of crude extracts and the subsequent purification of pure substances from Brazilian lichen is reviewed, as well as alternatives to their production through cells or thallus immobilization in bioreactors and their entrapment in inert matrix.

ABBREVIATIONS

ASA: Acetylsalicylic Acid; ATR: Atranorin; BAR: Barbatic Acid; DIV: Divaricatic Acid; DYD: Dydimic Acid; FUM: Fumarprotocetraric Acid; LEC: Lecanoric Acid; NOR: Norstictic Acid; PRO: Protocetraric Acid; PUL: Pulvinic Acid; SAL: Salazinic Acid; STI: Stictic Acid; USN: Usnic Acid; VIC: Vicanicin; ZEO: Zeorin.

INTRODUCTION

An efficient extraction method and the adequate solvents are required to obtain bioactive compounds from plants and lichens. Organic solvents are indicated for extracting compounds with a different range of polarity, according to an eluotropic sequence, that indicates the more polar to apolar nature. In this context, lichens secondary metabolites are bioactive molecules, and due to their phenolic nature, their obtainment with organic solvents is feasible. Successive extractions in eluotropic series were achieved using *n*-hexane or petroleum ether, diethyl ether, acetone and methanol.

Lichens are symbiotic organisms formed by a fungus component, the mycobiont, and one or more green algae or cyanobacteria, the photobiont, forming a stable body, the thallus (Figure 1,2). The fungal partner comprises about 95% of the thallus and it is usually an ascomycete or less frequently a basidiomycete, whereas the photosynthetic partner is usually green algae or less frequently a cyanobacteria and comprises the remaining 5% of the thallus. They can be identified in various growth forms, being the most common: crustose, foliose, fruticose and dimorphic thalli (Figure 3-6). Despite of their slow growth and small sizes, some fruticose species are chosen for chemical and biotechnological assays [1-3].

Many lichen secondary metabolites originate from the fungal partners, but they are produced primarily in symbiotic association. When the mycobiont is isolated from its holobiont, phenolics are usually different from those produced in symbiosis. Mainly lichens produce unique secondary metabolites that are not produced by other fungi and plants, and that show some biological activities with interesting applications from a pharmacological point of view.

This way, there are many studies focusing on the biochemical and physiological activities associated with the lichen ether-extracts or with the purified components or derivatives. From these data, our paper focuses on the description of the relationship between lichen compounds and ether, as a useful solvent for extraction and purification of biologically-active compounds from lichens, with emphasis to Brazilian species.

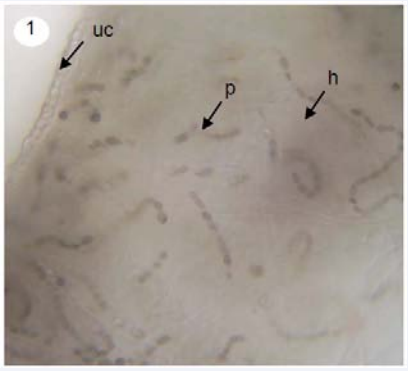

Figure 1 Thallus organization. A homoiomerous thallus (*Leptogium* sp.), showing upper cortex (uc), and photobiotic (p) and fungal hyphae (h) partners mixed internally.

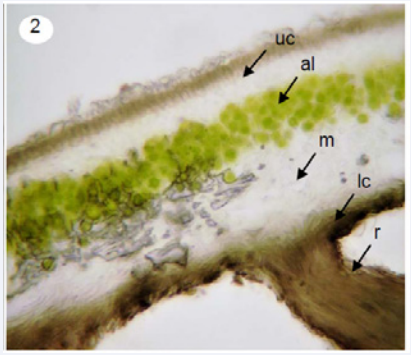

Figure 2 Thallus organization. A heteromerous thallus (*Physcia* sp.) with upper (uc) and lower (lc) cortices, and internally divided in algal layer (al), with its photobiont, and medulla (m), without photobiont. We can see a rhizine (r) from the inferior cortex for attachment to the substrate.

Figure 3 Lichens growth forms. Crustose lichen (*Herpothallon rubrocinctum*).

METABOLIC PATHWAYS

The primary metabolites of lichens are intracellular, as proteins, amino acids, polyols, carotenoids, polysaccharides and vitamins, usually found in the protoplast, often water soluble [4,5]. Products from secondary metabolism are water insoluble, have low molecular weight and are accumulated in the cortex or in the medulla. All of them are found extracellularly and/or on the hyphae surface in a crystal form. They are correlated to different functions, as allelopathic [6,7], antimicrobial [8], mucolytic [9], antitumor [10], molluscicide [11,12], teratogenic [13], etc.

Those substances are phenolic derivatives, grouped in four well differentiated structural groups: depsides, depsidones, dibenzofurans (including usnic acid - USN) and depsones. They are named as lichen substances or lichen acids [14], and formed by two or three phenolics units, originated from poliketonic carboxylic acids, derived from acetic acid. However, species whose photobiont is a cyanobacterium do not produce phenolic derivatives [15].

Many molecular structures of lichen substances and biosynthetic pathways were described by Asahina and Shibata

Figure 4 Lichens growth forms. Foliose lichen (*Parmotrema praesorediosum*).

Figure 5 Lichens growth forms. Fruticose lichen (*Usnea* sp.).

Figure 6 Lichens growth forms. Dimorphic lichen (*Cladonia* sp.).

[16], who divided them in aliphatic (fatty acids, polyols, and triterpenes) and aromatic (derivatives of tetralonic and pulvinic acid - PUL, depsides, depsidones, dibenzofurans and diketopiperazin derivatives). Nowadays, the more used classification system is that proposed by Culberson and Elix [17], in which the substances are ordered according to their biosynthetic origin, those derived from acetate-polymalonate, from shikimic or from mevalonic acids pathways, according to Table 1 [18].

USEFULNESS OF ETHER AS AN EXTRACTOR OF LICHEN BIOLOGICALLY-ACTIVE COMPOUNDS

Biological assays with plants and lichens usually require a previous screening by using crude extracts, to verify the presence of any bioactive substance in those preparations. Several lichen secondary metabolites, with recognized pharmacological and biological potentials [12,19], such as USN, barbatic acid (BAR) and atranorin (ATR), are extracted by processes that include ether [10].

The selective extraction with organic solvents, considering the polarity of target molecules, is so remarkable in the process to obtain bioactive compounds.

In the case of lichens, one may find water soluble active substances, and/or lipophilic compounds with phenolic structure. The extraction with ether of thalli of several species allow to obtaining compounds with remarkable activity and/or with industrial interest, such as USN. The ether extract of some species, such as *Cladonia substellata*, precedes the purification of this acid, as well as the first step to obtain its water soluble salt (usnate), also bioactive [11]. In other cases, the ether extracts from *C. substellata, Cladonia crispatula*, and *Cladina dendroides*, are active against four lines of cancer cells, inhibiting their proliferation, with remarkable action of ether extract of *C. substellata* and *C. dendroides*, using adriamicin and taxol as reference products [20]. Testing *in vivo* ether extract of *C. verticillaris*, or a combined extraction with ether and ethyl acetate, against solid tumors (Ehrlich carcinoma and Sarcoma-180), they were able to inhibit tumor development. Extracts were active mainly from samples collected in the dry season, probably by the retention of the bioactive compounds, fumarprotocetraric (FUM) and protocetraric (PRO) acids, due to low rain occurrence [21].

Some studies demonstrated that ether extract is as active as the pure compound, even more active in some cases. For example, Martins et al. [8], reported the antimicrobial activity of BAR from *Cladia aggregata* and its ether extract, but also suggested through biochromatography any interaction of this acid with some other compounds of the extract. This was also

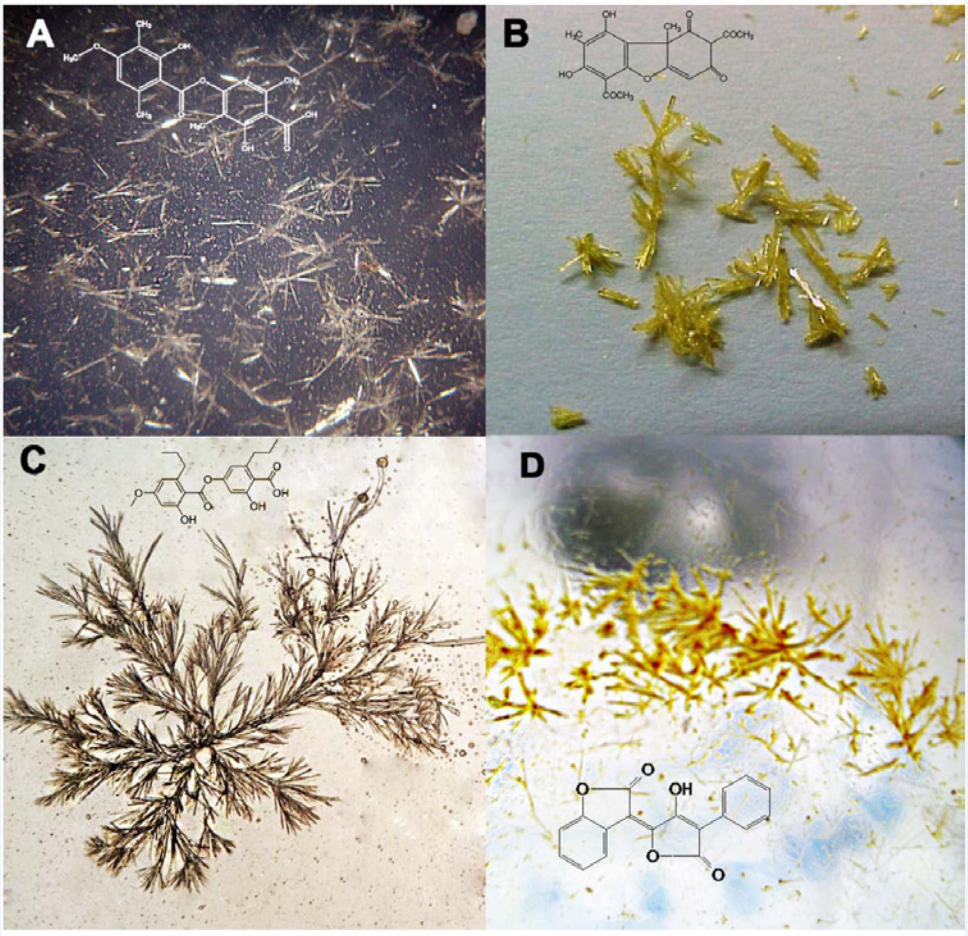

Figure 7 Crystal and chemical structures of some bioactive lichen substances isolated from Brazilian species. (A) BAR; (B) USN; (C) DIV; (D) Calicin.

detected for *Heterodermia leucomela*, an ATR rich lichen species, whose extract also contains zeorin (ZEO) and USN [22]. *Ramalina sorediosa*, whose ether extract contains salazinic acid (SAL) and USN, exhibited antibiotic action against *B. subtilis* and *S. aureus*, and a synergism was also reported [23]. Ribeiro et al. [24], also reported action of ether extract from *C. substellata* against human and plant pathogenic bacteria. The authors considered the extract more active against plant pathogens and supposed a synergic action of USN with norstictic acid (NOR). The same extract and substance were tested against *Staphylococcus spp*. obtained from skin and ears of dogs and cats with suspicion of pyoderma and otitis, being considered these products as effective for the treatment of these diseases [25].

In addition to antimicrobial action, antinociceptive activity of ether extract and ATR isolated from *Cladina dendroides* was achieved through induction of acetic acid and writhing test in mice. The presence of atranorin and FUM in the extract provided a remarkable action, higher than acetylsalicylic acid (ASA), low toxicity, and a probable synergism between both compounds [26]. On the other hand, ether extract from *Teloschistes flavicans*, rich in vicanicin (VIC) (60.26%), inhibited paw edema in mice at 120 and 180 min of treatment, and no granuloma or acute toxic symptoms were observed [27].

Potassium usnate and BAR from *C. substellata* and *C. aggregata*, respectively, exhibited molluscicide action against schistosomiases vector. Ether extract of this last lichen species also exhibited synergism, probably due to the occurrence of stictic acid (STI) in the mixture [11,12].

Complementary studies focusing on the action of lichen substances in the organisms have been developing, as teratogenic ability of those drugs on organogenesis. USN isolated from ether extract of *C. substellata* caused reduction of weight in pregnant rats, increase of resorption, decrease of viable fetuses, besides morphological changes as exposure of eyes, atrophy of limbs and histological injuries [13].

Assays with crude extracts, sometimes, reveal promissory results, as those found with pure compounds, and studies focusing on side effects, toxicity and other injuries caused by drugs, are important for completing the knowledge about natural products. In other hand, new biotechnological approaches have been optimized the action of these lichen substances by using nanotechnology for diminishing their toxicity by encapsulation using nanoparticles or microspheres [28], or through electrospun fibers, leading a controlled liberation of the substance into the organism [29].

PERSPECTIVE OF SUSTAINABLE USE OF LICHEN PRODUCTS

Since lichen compounds show several properties adequate for use in laboratory, at industrial or semi-industrial level, the eradication and extinction of species is a problem for observing and be caution, since lichen grow slowly, and their reposition to the environment can be impossible in some cases [30]. Procedures employing high biomass amounts, such as those used in the perfume industry, must be avoided. The responsible collection is mandatory, since several tons of lichens are extracted for cosmetic and natural medicine industries [31,32].

In this context, biotechnological methods are the alternative for a sustainable use of lichen biomass. Among some techniques, the immobilization of cell or thallus fragments in different kind of bioreactors, using inert matrices for entrapment, demonstrated to be a promissory technique to obtain active compounds [33,34]. Two kinds of matrices are commonly used. Devitalizing matrices, such as polyacrylamide, are preferently indicated to immobilize enzymes, such as orsellinate depside hydrolase [35] or depsidone ether hydrolase [36], whereas those that preserve cell vitality, such as polyhydroxyurethane, calcium alginate or kaolinite, are preferred to cell or tissues immobilization. Polyhidroxyurethane immobilizes cells by adhesion in such a way that immobilizates are instable [30]. Calcium alginate shows to be a good matrix for cell immobilization although precursors containing monovalent cations, such as Na^+ or K^+ must be avoided in order to impede calcium replacement and the disaggregation of the immobilizate [37]. For long periods of bioreactors activity, the use of some antibiotics is required to impede that phenolics produced by the immobilizates could be catabolized by epiphytic bacteria accompanying lichen fragments [38]. Incubation of immobilizates with the phenolic precursors will be in statics (without stirring) or shaking conditions, being this last the most efficient procedure since the limit later around the immobilizates supports a resistance to the access of the precursors to the lichen cells and increases their efficiency.

To produce lichen phenolics in bioreactors, immobilized cells or tissues were mixed with aqueous solutions of a precursor (sodium acetate). For extracting mid-polarity phenolic compounds from the cell washes, a mixture of diethyl ether and ethyl acetate (65:35, v/v) is required [39]. In these extracts, it was possible to detect USN [39,34], didymic acid (DYD) [40], BAR [41] PRO, FUM [42] and lecanoric acid (LEC) [43] acids as well as ATR [44], all of them bioactive compounds. Even immobilization techniques can be used to elucidate unknown enzymatic mechanisms of lichen phenolics production, such as cofactor requirements to produce FUM from its precursor, PRO [45].

CONCLUSION

From the properties and viability of ether use for obtainment of bioactive compounds from lichens, the indicative of this solvent for preceding biological assays, or for cosmetic preparations is feasible.

Besides the extraction and purification of compounds in laboratory or industrial/semi industrial scales, the techniques of immobilization of cell or thallus fragment can preserve the natural resources and the environment where lichen occur, indicating a sustainable use of these organisms. More promissory results can be found in the next studies with lichens and their ether preparations.

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