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Lichens used in the Traditional Medicine by the Pankararu Indigenous Community, Pernambuco-Brazil

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Introduction- Traditional knowledge refers to the knowledge accumulated over the years and transmitted through generations over time(Martin, 2005). For some communities the only resource available for health disorders is the traditional phytotherapy (Forero, 2004).

Ethnobiology has grown increasingly the scientific knowledge about organisms popularly used as medicinal; it made the researchers be aware of the substances that were found in order to produce new drugs(Posey, 1992). In this regard, lichens have been extensively studied in temperate countries, in the Euro-Asiatic axis or on the USA, with particular emphasis on survey work conducted by Sylvia Sharnoff (Brodo et al., 2001; Sharnoff, 2015). In Neotropical countries, however, its study is scarce; there are just a few cases related to Brazil, used as dyes (Mors, 1966),or for tingling and sneezing when sniffed (Prance, 1972).It is known that Brazilian lichen flora is highly diverse (Cáceres, 2007; Eliasaro and Adler, 2000; Fleig and Grüninger, 2008; Marcelli, 2003), showing great pharmacological potential (Pereira, 2012), and ethnolichenological studies may assist in targeting and selecting species for future pharmacological research.

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I. Introduction

Traditional knowledge refers to the knowledge accumulated over the years and transmitted through generations over time(Martin, 2005). For some communities the only resource available for health disorders is the traditional phytotherapy (Forero, 2004).

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In Brazilian Northeast, in the west side, opposite to the Atlantic coast, it can be found the semi-arid region, that presents an exclusive biome – Caatinga, where endemic species of several taxa are reported (Leal et al., 2003), with an endemism level that varies from 4.3 % (birds) to 57 % (fishes)(Brasil, 2002).

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In this context, lichens are also found in this region, much of them are new report to Brazilian semi-arid northeast, or Country, and many species are new to the Science. Studies conducted by Cáceres (2007) refermainly crostose lichens, whereas Buril (2015) reports 22 new species and one new genus of foliose lichens, *Parmeliaceae* family, from semi-arid region of Pernambuco one of the States that makes part of Brazilian Northeast.

Even almost unknown the lichen biota of Brazilian semi-arid, the reported species have biologically-active substances in their chemical composition, that can be useful in the future in a sustainable way. By other hand, until this moment no report was found in traditional use of lichens in this region.

Among the traditional existent communities in Brazilian semi-arid, Lodoño-Castañeda (2010), selected Pankararu people for ethnobotanical studies, and observed that indigenous people use higher plants and also lichens for medicinal purposes.

This way, in this paper we show the use of foliose lichen species by indigenous Pankararu people in the semi-arid of Pernambuco State, Northeast of Brazil, and the biologically-active compounds found in these species.

II. Material and Methods

a) Site Description

The indigenous community Pankararu occupies an area of 8,100 ha with a population about 4,850inhabitants distributed in 13 villages (Socioambiental, 2009). The territory, inserted onto the Caatinga ecosystem, was homologated by the Brazil government, and named as “Pankararu land”. It is located in the “Sertão Pernambucano”, between the hills Serra Grande and Serra da Borborema, near the banks of São Francisco river, in the municipalities of Petrolândia, Tacaratu and Jatobá and the border of the states of Alagoas and Bahia – Brazil, whose Geographic coordinates are 09°07`16``S and 38°15`25``WGr North and 09°11`56``S and 38°13`52``WGr South (Fig. 1).

According to Köppen's classification, the climate is BSHs' (semi-arid of low latitudes), with mean annual temperature of 25°C, and mean annual pluviosity around 600 mm. The vegetation is dry tropical forest type, characterized by a predominance of xerophytic and deciduous species, endowed with a high floristic and physiognomic variation. Amongst the typical woody species there are found *Ziziphus joazeiro* Mart. (*Rhamnaceae*), *Schinopsis brasiliensis* Engler (*Anacardiaceae*), *Caesalpinia pyramidalis* Tul. (*Fabaceae*), *Bauhinia cheilanta*(Bong.) Steud. (*Fabaceae*), *Maprounea guianensis* Aubl. (*Euphorbiaceae*) (Araújo *et al*., 1995); succulent plants of *Cactaceae* and *Bromeliaceae* families are also typical, while lianas are scarce (Araújo and Martins, 1999).

In this study nine villages were considered, mainly that one known as "Brejo dos Padres", located in a valley between the Serra Grande and Serra de Tacaratu, near to the left margin of São Francisco, one of the main river of Brazilian Northeast.

The villages are inserted in areas with several stages of ecological succession, where often can be found fruit trees as "murici" (*Byrsonima crassifolia* (L.) H.B.K., *Bignoniaceae*) and "umbu" (*Spondias tuberosa* Arr. Câm., *Anacardiaceae*), as well as woody and medicinal species.

Pankararu people perform subsistence agriculture practices, spanning in some cases informal marketing of food stuffs in local markets, as "macaxeira" (*Manihotesculenta* Crantz.), maize (*Zea mays* L.), and bean (*Phaseolus vulgaris* L.).

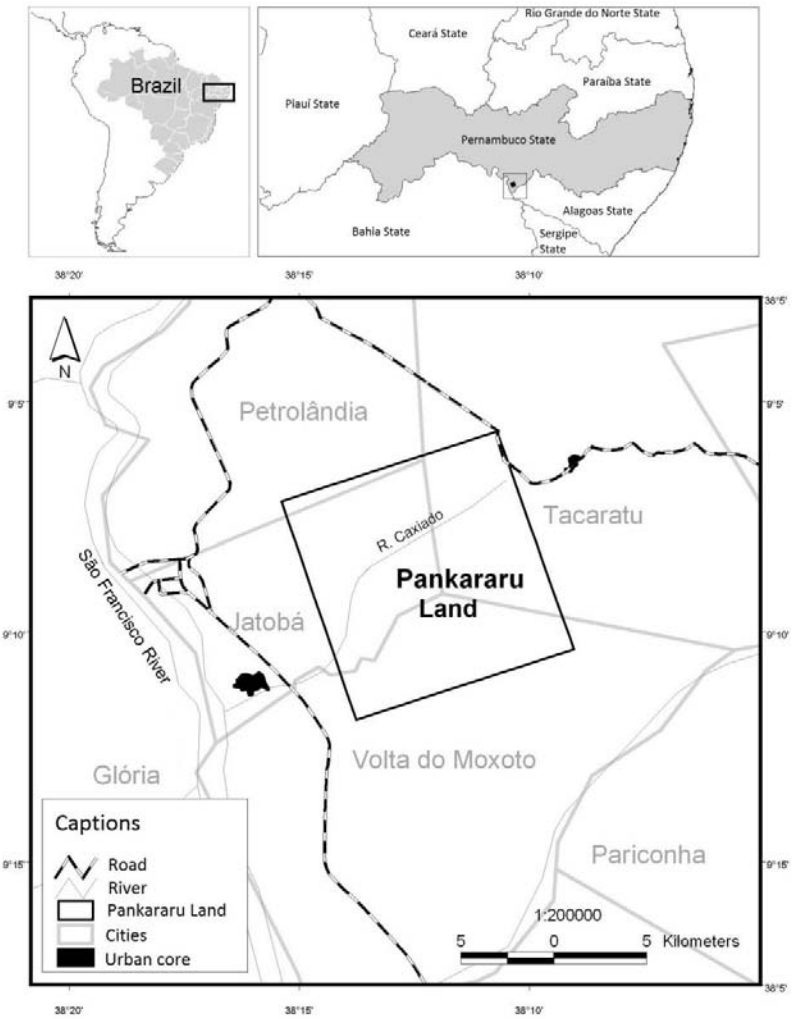

Fig. 1: Localization of Pankararu Land in Pernambuco State, Brazilian Northeast

Map designed by A.K.O. Silva (2014), adapted from FUNAI (National Indian Foundation) and IBGE (Brazilian Institute for Geography and Statistics) (2001).

b) Data Collection

The ethnobotanical information in the traditional medicine Pankararu were obtained within 60 days of field activities through semi-structured surveys, in which were employed standardized forms and recorded notes on the therapeutic indications of the local flora. The

survey was targeted to specialists of traditional medicine, using the "snowball" technique (Albuquerque et al., 2008).

Among the reports, it was quoted the use of plants, as well as lichens, and the cited species were collected to identification. The lichen material was collected and kept in paper bags until laboratory tests. Vouchers were deposited in UFP Herbarium of Botany Department of Universidade Federal de Pernambuco (*Parmelinella salacinifera* (Hale) Marcelli & Benattin° 61069, *Heterodermia galactophylla* (Tuck.) W.L. Culb. n° 75448 and *Parmotrema wrightii* Ferraro & Elixn°61212).

To develop the field activities in indigenous PankararuLandit was required the approval of the Research Ethics Committee – CEP, the National Committee of Ethics in Research – CONEP, and the Board of the Genetic Heritage Management - CGEN, with subsequent approval of FUNAI (National Indian Foundation) (Proc. n° 1253/08).

c) Identification of lichen species

i. *Morphotaxonomic Analysis*

To identify the species of lichenized fungi, anatomical and morphological characters were studied. Structures as cilia, rizines, maculae, type, size and form of thallus, cortex and medulla, apothecia, ascospores and others were analyzed under stereo microscope (10-50X) and optical microscope (40-1000X).

d) Chemical Analysis

i. *Obtainment of extracts from the thallus in natura*

A chemical study of the species was performedto confirm the secondary metabolites.

The phenolic composition was analyzed from organic extracts obtained from each lichen species. Samples of lichen thalli (50 mg) were successively extracted by maceration with diethyl ether (5 mL), chloroform (5 mL) and acetone (5 mL), with infusion time of 15 minutes in each solvent and then filtered, reunited into one single extract for each lichen sample and stored until evaporation at room temperature (28 ± 3 °C).

ii. *Thin layer chromatography (TLC)*

For a general characterization of lichen phenols contained in the species, the organic extracts obtained from the thallus *in natura* were subjected to thin layer chromatography (TLC). The samples were applied on silica gel chromatoplates $F_{254\ +366}$, along with the standards of norstictic acid, salazinic acid, atranorin, and the ether extract of *Heterodermia leucomela*, containing as main compounds atranorin and zeorin. The samples and standards were previously dissolved in a concentration of 0.01 mg.$\mu L^{-1}$and then applied5 μL of each extract. It allows a more careful and accurate chromatographic analysis. TLC was developed in a solvent system A (toluene/dioxane/acetic acid 90:25:4, v/v/v),according to Culberson (1972), and spots formed were visualized under UV light and subsequently revealed by spraying 10% sulphuric acid (H_2SO_4) over the plates and subjecting them to heat.

For a more detailed evaluation additional TLC assays were performed with acetone extracts of the species using the following solvent systems: toluene: ethyl acetate: formic acid (139:83:8, v/v/v); toluene: ethyl acetate: acetic acid (6:4:1, v/v/v), using salazinic and norstictic acids, as well as atranorin.

In all tests, value of Rf spots were calculated and compared to the Rf of standard substances.

III. Results

Although the records of lichen species which therapeutic value are rare in ethnobotanical studies in Brazil and non-existent for the Northeast regionso far, three lichen species were recorded being used by Pankararu as medicine (Fig. 2):

Parmelinella salacinifera(Hale) Marcelli & Benatti (*Parmeliaceae*)
Heterodermia galactophylla (Tuck.) W.L. Culb. (*Physciaceae*)
Parmotrema wrightii L. I. Ferraro & Elix(*Parmeliaceae*)

These species are commonly called stone flower (flor-de-pedra in Portuguese) by the community and are used to treat digestive system problems such as diarrhoea and vomiting. The mix of three species is employed in an aqueous extract. They are also used for the treatment of epilepsy and cultural diseases through the smoker.

The species are the first report for Pernambuco state, being *H. galactophylla* the first report for Brazilian Northeast.

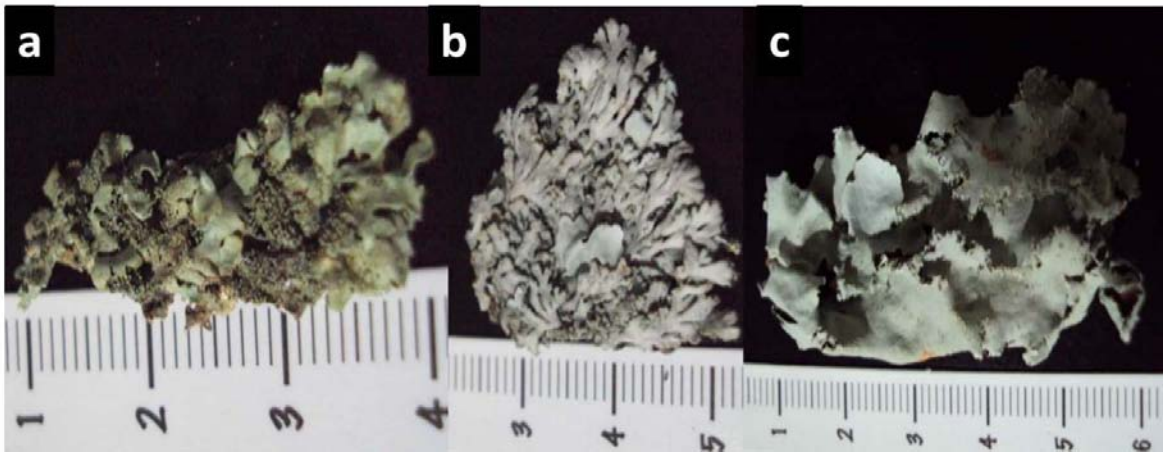

Fig. 2: Lichen species used as medicine by Pankararu people: a. *Parmelinella salacinifera* (Hale) Marcelli & Benatti, b. *Heterodermia galactophylla* (Tuck.) W. Culb., c. *Parmotrema wrightii* L. I. Ferraro & Elix

According to traditional knowledge, the different types of stone flower have contraindications of use: *P. wrightii* does not present any restrictions, while *H. galactophylla* is contraindicated for children and pregnant women, and *P. salacinifera* also presents restrictions of its use by pregnant women. The species are differentiated by the community by the colour and shape of the thallus. By other side, in the revised papers no mention about contraindications was found.

Through general TLC assays (Fig. 3) the presence of atranorin and zeorinin *H.galactophylla*, of atranorin and salazinic acid in *P. Salacinifera* and atranorin and norstictic acid in *P. Wrightii* was detected. It is quite likely that these substances are related to the therapeutic potential of these species as well as to their restrictive nature.

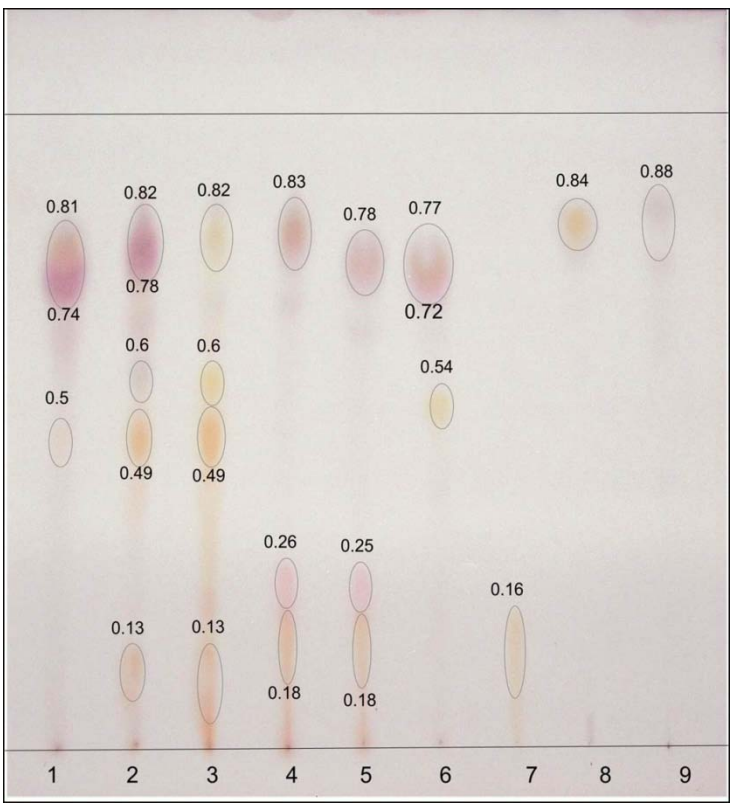

Fig. 3: Thin Layer Chromatography of 1, 2 – *Heterodemia galactophylla* extracts; 3 – *Parmotrema wrightii* extract; 4, 5 – *Parmelinella salacinifera* extracts; 6 –Norstictic acid (Rf0.54); 7 –Salazinic acid (Rf0.16); 8 - Atranorin (Rf0.84); 9: Ether extract of *Heterodermia leucomela* containing zeorin (Rf0.88)

IV. Discussion

Yavus (2012) investigating the pharmacopoeias written by Dioscorides, a physician who acted in the army of Roman legion in Italy, France, Greece and Turkey, had observed that in great part of species used as medicinal, *Parmelia* species were used among other species.

Most of *Parmeliaceae*, grows on different substrate as branches, barks, rocks etc. forming a total or partial rosette (Yavus, 2012), due to its radial growth form, resembling a flower, probably reason of the name "stone flower" given by Pankararu people.

Despite of the knowledge of lichen use in traditional medicine comes from ancient times (Agelet and Vallés, 2003), the relationship between their use with vascular plants is unbalanced. Soukand and Kalle (2013) studying plants used for tea with medicinal and/or recreational purposes in several places of Estonia, reported the use of 69 vascular plants and only one lichen species, *Cetraria islandica.* From 180 interviewed persons, 22 used this lichen as tea for medicinal purposes, as cough, cold, bronchitis, lung diseases, respiratory problems and fever. From these persons, only one used *C. islandica* tea for recreational purpose, and the predominance of its use as medicinal was justified due to be considered culturally unpleasant.

Singh et al. (2014) studied medicinal plants in sacred groves of Kumaon region of central Himalaya, and found 89 species, two of them were the lichens *Everniastrum cirrhatum* and *Parmotrema reticulatum* (*Parmeliaceae*), both used for cold.In the same region, in Nepal mountains Devokta et al. (2017) documented the use of lichens in nine different communities. The authors found ethnic and different value uses for lichens, since medicinal (most part) to spiritual and aesthetic. In addition, three species had been mentioned their use for cooking. Probably due the high availability of lichens in mountain regions, all kind of thallus were reported by using (fruticose, foliose and crustose).

Agelet and Vallès (2003) worked in Iberian Peninsula and mention 272 medicinal plants used by traditional communities, being five of them lichen species. They refer *Alectoria sarmentosa, Cetraria cucullata, C. islandica, Pseudevernia furfuracea* and *Ramalina capitata* as antiasthmatic, as well as an anticatarrhal and hypotensive activity for *P. Furfuracea* and hypotensive and antituberculosis action to *C. islandica.*

Crawford (2015) summarizes studies made throughout the world, describing the use of 52 lichen genera as medicinal. The author consider *Usnea* the most common used genus, except in Australia, and so many others in Europe, USA, Canada, China, etc. For South America the data are scarce and many of them few informative. As example in Ecuador there are reported *Usnea* spp and *Dictyonema huaorani*, while in Argentina four species of *Usnea*are mentioned; the same genus is reported as useful in Uruguay, Venezuela and Chile. Marcelli (personal communication, 2015) mentions a saxicolous *Usnea* sp occurrant at Santa Catarina and Rio Grande do Sul coast (states of Brazilian South), used by local people for genitourinary diseases. The lichen thallus is mixed to "chimarrão" (typical drink of Brazilian South, made from infusion of "erva mate" – *Camelia sinensis*), and the users recognize the efficiency of the lichen thallus from its coloring; the more yellow it is, more effective its action. In Peru one *Roccella*sp is used by traditional communities. To Brazil the use of *Cladonia miniata*is reported, besides an inaccurate information about *Usneabarbata* with a local nomination with a Tupi Guarani term "membyrakú í ja", that means "hot daughter" (Ms Priscela Navarro, personal communication, 2015), and used for woman fertility. However, this species is more common in Brazilian South and neighbours Countries. The information to occur in Brazil is very much imprecise, due to size of this Country. In this context, the same author mention crostose white lichen in Peru used mixed to resins, as hallucinogen; a mix of five species (Chácobo) for several problems in Bolivia, and an unidentified species used for constipation in babies.

The mentioned papers did not mention if any species are used together, or due to their morphological similarity can be ethnosynonymous for these communities. This is the case of our study. Although *H. galactophylla*, *P. salacinifera* and *P. Wrightii* being ethnosynonyms for the Pankararu people being employed for the same purpose, they are differentiated by the degree of concentration of the therapeutic effect.

Considering the use and contraindications by popular and/or traditional use of lichens, neither Sõunkand and Kalle (2013), nor Agelet and Vallès (2003), Singh et al. (2014) and Crawford (2015) mentioned the active principles of the species, as well as reports of literature about effectiveness or toxicity of compounds contained in the studied species. By other hand, Agelet and Vallès (2003) reported studies performed by other colleagues about biological activities of related species or genus, nevertheless without refer any lichen compound.

It is known that substances like atranorin, zeorin, stictic and salazinic acids (Fig. 4) have antimicrobial and antibacterial activity (Tay et al., 2004; Yilmaz et al., 2004; Vicente et al., 2006; Marijana et al., 2010; Molnar and Farkas, 2010). To establish these relationships, more studies referring to their pharmacological uses are needed, due to the scarce information about toxicity at acute and both chronic and subchronic levels.

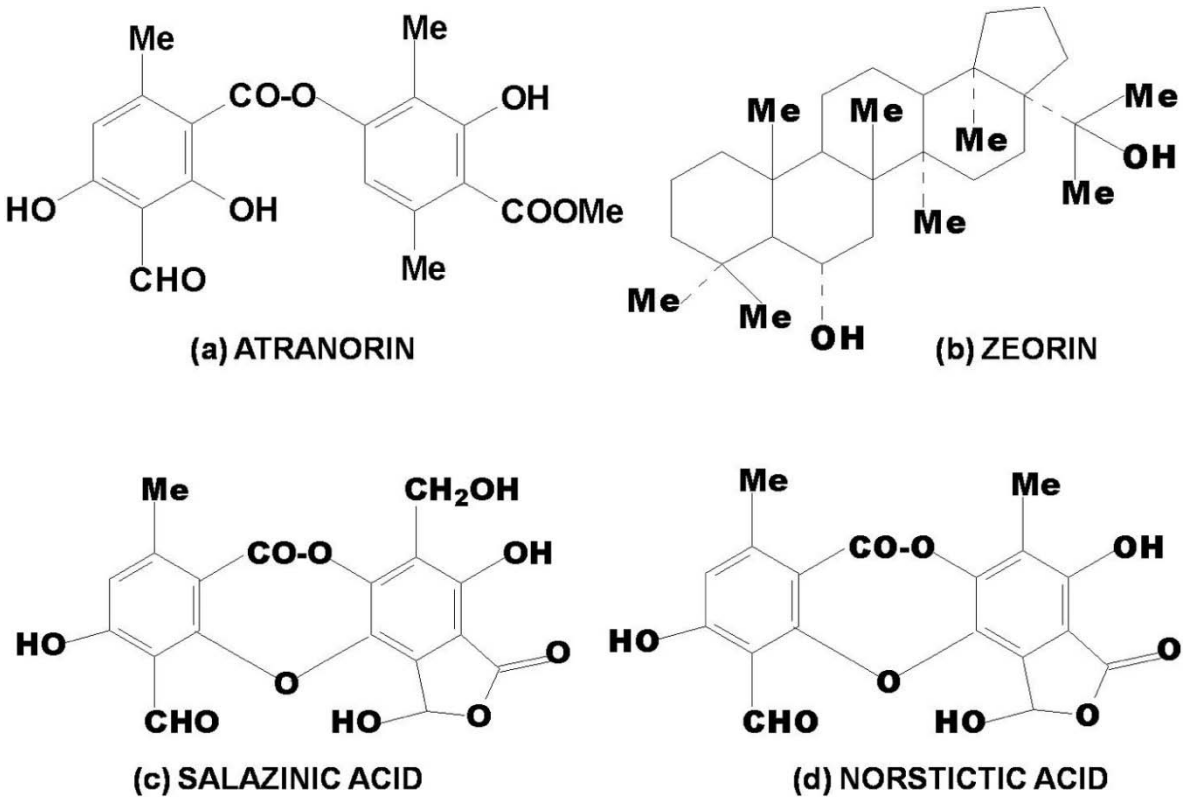

Fig. 4: Structural formulae of atranorin (a), zeorin (b), salazinic (c) and norstictic (d) acids

Graphical Abstract

Regarding to toxicity of substances found in studied species, it is known the low toxicity of atranorin. Melo et al. (2011) in anti-inflammatory assays with this substance (100 mg/kg and 200 mg/kg) obtained from *Cladina kalbii* a meaningful activity, but no significant toxicity at acute and subchronic levels was detected, as well as cytotoxity. These data are coincident to ones described to Maia et al. (2002), when tested the antinociceptive action of atranorin and crude extracts from *Cladina dendroides.*

Asakawa et al. (2013) describes cytotoxic activity of α-zeorin, isolated from several liverworts, against P-388 cells, whose IC50 was 1.1 μg mL^{-1}. Data is almost nonexistent for such compounds, and no information was found about salazinic acid.

By other hand, it is possible to attribute a more remarkable action, depending on the chemical group the lichen compound is placed. In this context, Correche et al. (2002) mention that the depsidones, in general, exhibit a stronger cytoxity than the depsides, attributing this bioactivity to the structural characteristics of their chemical group, where the aldehyde function is always linked to a C3, with an OH to the adjacent C4. This way, both salazinic and norstictic acids have these characteristics, while atranorin, being a depside, exhibit a lower toxicity. Regarding to zeorin, this compound is a terpenoid. Harrewijn et al. (2001) mention that several terpenoids have minimal toxicity to vertebrates, besides their usefulness in the cosmetics and pharmaceutics, due to be biologically actives. By other hand, some of them have evidenced their toxicity, suggesting a more accurate discussion about toxic effects of this chemical group, mainly concerning the terpenes obtained from lichens and lower plants.

V. Conclusions

Our results show that Pankararu people use lichens with active principles for several treatments, andknow the right dose, side effects and restrictions of each species.

Acknowledgements

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Highlights

- Traditional indigenous community in semi-arid Brazil use lichens as medicine.
- Lichens used have their properties and contraindications recognized by this people.
- This is the first report of use of lichens as medicament in Brazilian semi-arid.
- Active compounds were found in lichens used by Pankararu people.

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