Chemical and Pharmacological Survey on Brazilian Medicinal Plants Using Ethnopharmacological Information as a Tool

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Abstract

The project encompasses plants from the following families: Palmae, Lamiaceae, Acanthaceae, Leguminosae and Gesneriaceae. Regarding the pharmacology, several models have been used like antinociceptive, anti-inflammatory, antioxidant, molluscicidal, anti-diabetes, anti-microbial and nitric oxide production inhibition. Results showed that utilizing ethnopharmacological information is a very important way to search for new bioactive molecules. It is noteworthy to mention the activity of Açaí fruit extracts in the inhibition of nitric oxide production. It was also possible to identify flavonoids responsible for the antidiabetic activity in plants belonging to the family Leguminosae. Acanthaceae extracts showed important antinociceptive and anti-inflammatory activities, as they are very rich in steroids and triterpenes. The same could be said about plants belonging to Lamiaceae that gave several examples of this kind of pharmacological property due to its steroid and triterpenoid compounds. One specie of Lamiaceae also produced a great amount of dihydroxylated triterpenoids with great molluscicidal potencial. Palmae species, rich in fatty acids and steroids led to enriched extracts responsible for the anti-BPH activities. Plants belonging to Gesneriaceae were antioxidant due to their flavonoid content. Polar extracts and isolated molecules, isolated from many species were able to donate hydrogen radical to DPPH.

INTRODUCTION

Brazil is one of the greatest centers of plant biodiversity. This research project was started to identify this diversity from chemical and pharmacological point of view. The scientific approach could be the only way to avoid losing this biological value.

Ethnopharmacology has been used for a long time as a tool for the study of medicinal plants (Holmstedt, 1991; Holmstedt and Bruhn, 1983). Thus, the chemistry of natural products isolated from medicinal plants is now facilitated with the previous knowledge from popular information of use of plant. By this way, we have studied

several plants belonging to different families trying to validate their chemical constituents as well as their uses by Brazilian people.

MATERIAL AND METHODS

Table 1 lists the plants/parts/families used in this study. All of these plants were previously reported to treat some disease or group of diseases. However, the plants were collected in different periods of time and an herbarium sample of each plant was deposited in the Universitary Botanical Center.

All collected plants were submitted to a successive extraction procedure using ethanol as solvent. Each ethanolic extract was dried under reduced pressure and after that, suspended in water. A liquid-liquid extraction procedure was done in order to obtain extracts of different polarities: hexane, dichloromethane, ethyl acetate and *n*-butanol. These extracts of different polarities were treated separately according to their physical-chemical properties. Crude extracts, isolated compounds and semi purified extracts were tested in pharmacological models.

Several pharmacological models were performed in order to validate the information given by people traditionally using the plant. In this sense, techniques of antioxidant measurement (as discoloration of a DPPH solution), techniques for the evaluation of anti-inflammatory activity (as induce paw edema with several inductors), techniques for the evaluation of the antinociceptive potential (as the count of writhings after intraperitoneal injection of acetic acid), techniques for the evaluation of hypoglicemiant activity (as the peroxidation of glucose) and toxicological tests were performed.

Tea made of species listed in Table 2 in 10% w/v was prepared and administered to mice. The glucose concentration of each mouse was measured at this time in order to achieve the basal blood glucose concentration (time 0h). After four hours of tea ingestion each group of mice that received each plant tea had their blood glucose measured again (time 4h).

Statistical analyses were done. Student's T test was used for a comparison between two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means complemented by the Tukey's test (Runyon and Haber, 1984). A difference was considered statistically when $p<0.05$.

RESULTS AND DISCUSSION

Plants belonging to the family Lamiaceae were studied after the collection of popular information about their uses (Table 1). People from the Northeast Region in Brazil use plants of this family to produce syrup to treat cough and also in inflammatory processes, primarily skin inflammation. Several constituents were isolated from *Raphiodon echinus*, *Marsypianthes chamaedrys*, *Hyptis tetracephala*, *Hyptis heterodon* and *Hyptis fascidulata*. There is no doubt that the major constituents produced from these plants were triterpenes and steroids. Concerning the pharmacological properties it could be proven that some antinociceptice and anti-inflammatory activities probably due to the higher content of triterpene and steroids (sitosterol and stigmasterol) (Jie, 1995). It was also very interesting that the molluscicidal activity found for the hexanic extract of *M. chamaedrys* and also the bactericidal and antimicotic activity found for some extracts belonging to species of the genus *Hyptis* and the species *R. echinus* and *M. chamaedrys*. It is also noteworthy to mention the antioxidant activity found for the more polar extracts belonging to plants of the Lamiaceae family and also for rosmarinic acid isolated from *M. chamaedrys* and *Hyptis* species and for methylpiperitol isolated from *Hyptis fasciculata*.

Regarding Acanthaceae family, we have studied several extracts from the leaves and stems of *B. palisatii* and the flowers of *P. lutea* (Table 1). Among the extracts of *B. palisatii* it is noteworthy to mention the activity found for hexanic and dichloromethane extracts both for leaves and stems regarding inflammatory processes and also antinociception and specially for the stems (dichloromethane extract) it was shown the inhibition of nitric oxide production in a dose-dependant way achieving the maximum of

80% at a concentration of 200 µg/ml. For *P. lutea* it was possible to show a slightly hypoglycemiant activity probably due to its liver toxicity.

For both Gesneriaceae species studied it became clear the high amount of anthocyanins especially for *Nemathanthus* spp. leading to a great antioxidant activity evident by the discoloration of DPPH free radical.

Plants from Palmae family were studied beginning with *Euterpe oleracea* extraction and followed by the pharmacological evaluation (Table 1). For all the pharmacological assays, the extracts from fruits showed higher activities than the other plant materials. Regarding the chemistry, it was possible to isolate mixtures of fatty acids from both leaves and fruits, sitosterol and stigmasterol from fruits and sitosterol glucoside from leaves, in a great amount. From the more polar extracts of the fruits it was possible to quantify the amount of anthocyanis and for the less polar extracts it was possible to compare the amount of fatty acids of *E. oleracea* and those produced from *Sabal serrulata*, a plant often used for the prevention and treatment of benign prostrate hyperplasia. It was also possible to show the bactericidal activities for almost all the extracts produced by the fruit, which also had a very important antioxidant activity. Both very non-polar and very polar fruit extracts showed an important moluscicidal activity especially for those regions where this palm inhabits.

This study has investigated the AA of different extracts of Euterpe palm. The AA of the extracts in different concentrations (0,1; 1, 10 and 100 µg/mL) were analyzed by deoxyribose degradation assay, nitro blue tetrazolium (NBT) reduction test and inhibition of peroxidation. All hydrophilic extracts tested showed inhibition of deoxyribose degradation up to 10 µg/mL. Butanolic extract from fruits, ethyl acetate extract from leaves and spikes and ethanolic extract from flowers were able to inhibit around 75% of deoxyribose degradation The lipophilic extrats were able to inhibit deoxyribose degradation only at higher concentrations. However, in the NBT reduction test the dichloromethane extract from fruits was the most effective, showing 70% of O_2^- scavenger capacity. The other exracts tested showed around 25% O_2^- scavenger capacity in the higher concentration. As observed to NBT reduction test in the lipoperoxidation test the more effective extracts were the lipophilic extracts, hexane extract from leaves showed 50% of peroxidation inhibition at 100 µg/ml.

The action of the extracts obtained from the fruits and flowers on the nitric oxide production, a very important molecule with a lot of physiological roles such as vasodilatation, neurotransmission, tumoricidal and cytotoxic activity, was carried out. Cells RAW264.7 stimulated with bacterial lipopolysaccharide (LPS, 100 ng/ml) and interferon-alpha (IFN-alpha, 10 U/ml) produce large amounts of nitric oxide (35 µM) when compared with non-stimulated cells (3 µM). The hexane, dichloromethane, ethyl acetate and *n*-butanol extracts have shown high inhibition capacity, concentration-dependent in the cells activated with LPS and IFN-alpha, and the highest concentration promoted almost 100% of inhibition (Fig. 1). We also have tested if the inhibitory effect was due to a scavenger action using a NO donor, SNAP (S-nitrogen-n-acetyl DL-penicilamine) (Fig. 2 and 3). Only the ethyl acetate extract has shown significant scavenger action. At this moment an effort is underway to try to understand the possible mechanisms associated with the inhibition of these extracts.

The other Palmae, *O. speciosa*, *C. cerifera*, *S. oleracea* and *M. vinifera* had their studies started after the beginning of *E. oleracea* evaluation. These other plants are following the same pattern found for *E. oleracea*.

Regarding the Leguminosae, plants from the genus *Bauhinia* are commonly named pata-de-vaca in Brazil and are used for hypoglycemic purposes. This study has started in order to show if all these plants have the same properties and if it is possible to isolate the compound responsible for the mentioned activity. The first results indicate that the more prominent activities are found for those plants that have in their HPLC chromatogram a signal, probably a flavonoid glucoside, which should be responsible, at least in part, for the hypoglycemiant activity.

The study, performed with the population from the southeast region of Rio de

Janeiro, aimed at knowing the plants used by them in order to lower blood levels was fantastic because we could prove that one plant frequently used by those people had no significant activity while the others had shown some activities. Fig. 4 and 5 clearly illustrate that carambola (*Averrhoa bilinbi*) has no activity.

ACKNOWLEDGEMENTS

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Tables

Table 1. Plant species, family, and part used.

Plant	Plant Material	Family
Raphiodon echinus	Aerial Parts	Lamiaceae
Marsypianthes chamaedrys	Aerial Parts	Lamiaceae
Hyptis tetracephala	Aerial Parts	Lamiaceae
Hyptis heterodon	Aerial Parts	Lamiaceae
Hyptis fasciculata	Leaves and Stems	Lamiaceae
Brillantaisia palisatii	Leaves and Stems	Acanthaceae
Pachystachys lutea	Flowerings	Acanthaceae
Euterpe oleracea	Leaves, Fruits, Spikes and Stems	Palmae
Orbgynia speciosa	Leaves, Fruits and Stems	Palmae
Copernicia cerifera	Leaves, Fruits, Stems and Wax	Palmae
Syagrus oleracea	Leaves, Fruits and Stems	Palmae
Mauritia vinifera	Leaves and Fruits	Palmae
Nemathanthus sp.	Leaves and Stems	Gesneriaceae
Besleria sp.	Leaves and Stems	Gesneriaceae
Bauhinia monandra	Leaves	Leguminosae
Bauhinia angulosa	Leaves	Leguminosae
Bauhinia forficata	Leaves	Leguminosae
Bauhinia blakeana	Leaves	Leguminosae

Table 2. Plants mentioned by the population from the southeast region of Rio de Janeiro as possessing hypoglycemic activity.

Popular Name	Family	Species	Used Part
Insulina	Tiliaceae	*Cyssus sycioides*	Whole plant
Carqueja	Asteraceae	*Baccharis trimera* (Less.) DC	Leaves
Pau ferro	Leguminoseae	*Caesalpinae ferrea* Mart.ex.Tul.	Barks of stems
Graviola	Annonaceae	*Annona muricata* L.	Leaves
Jambo	Myrtaceae	*Syzygium malaccense* Merr.& Perry	Leaves
Caju	Anacardiaceae	*Anacardium ocidentalis* L.	Leaves
Jamelao	Myrtaceae	*Eugenia jambolana* Lam.	Leaves
Carambola	Oxalidaceae	*Averrhoa bilinbi* L.	Leaves
Pata de vaca	Leguminoseae	*Bauhinia fortificata* Link.	Leaves

Figures

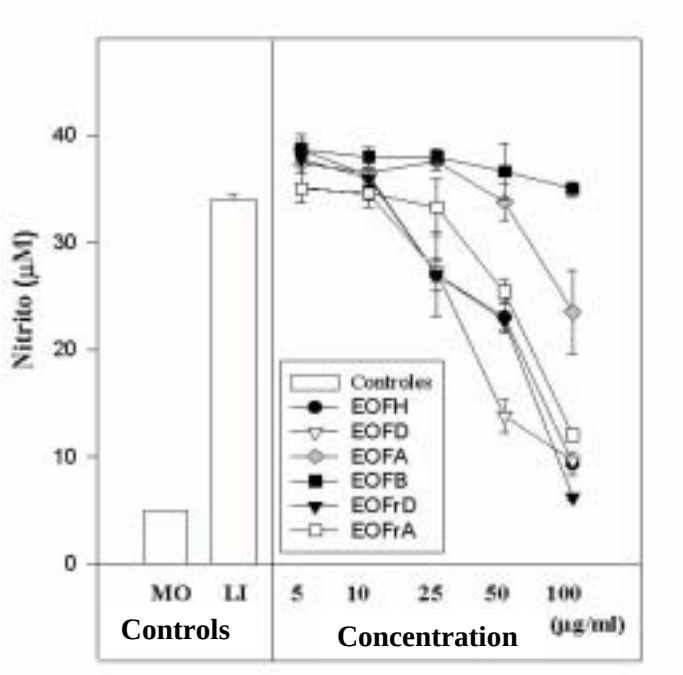

Fig. 1. Effect of *Euterpe oleracea* extracts in the nitric oxide production by macrophages.

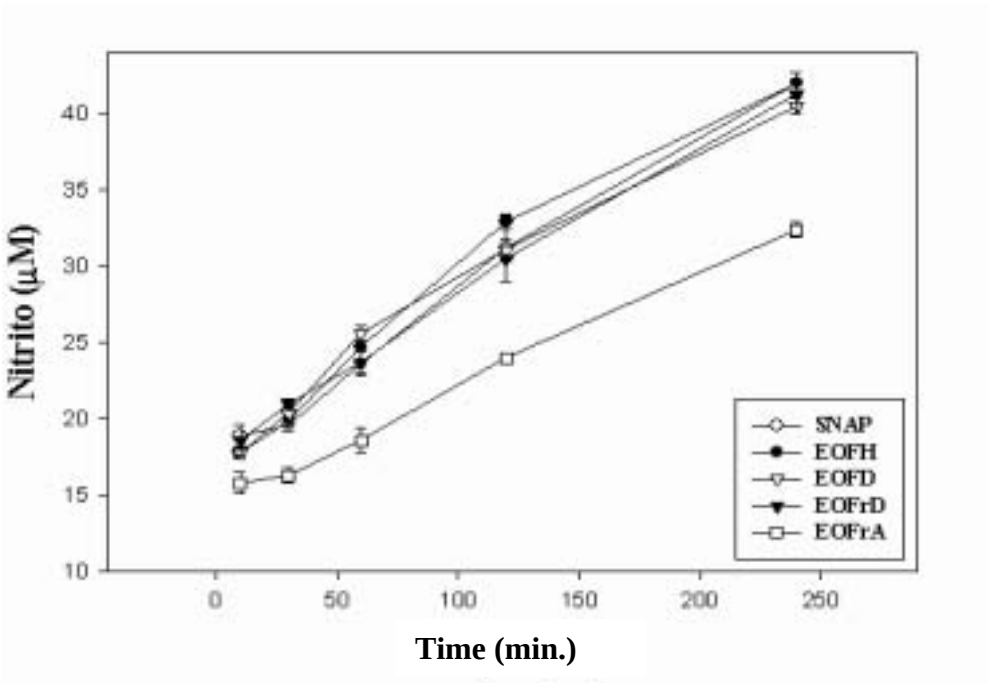

Fig. 2. Effect of *Euterpe oleracea* exctracts (50 µg/ml) on the liberation of NO after SNAP (1 mM).

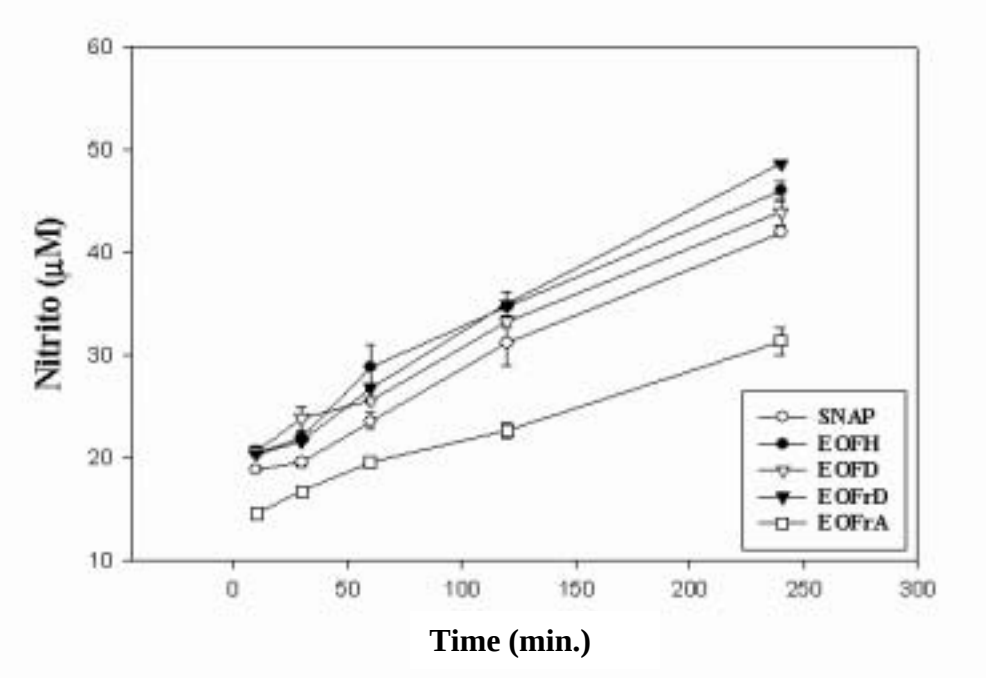

Fig. 3. Effect of *Euterpe oleracea* extracts (100 µg/ml) on the liberation of NO after SNAP (1 mM). (EOFH (hexane, leaves), EOFD (dichloromethane, leaves), EOFA (ethyl acetate, leaves), EOFB (*n*-butanol, leaves), EOFrD (dichloromethane, fruits), EOFrA (ethyl acetate, fruits), MO (macrophages), LI (macrophages activated with LPS and INF), SNAP (S-nitroso-n-acetyl DL-penicilamine).

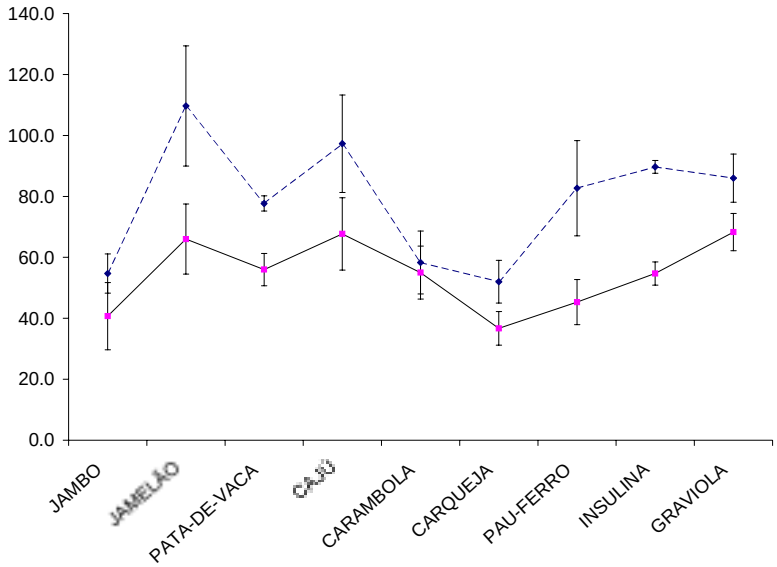

Fig. 4. Hypoglycemic effects of the tested teas at 10% showing the glycemia both at time 0h (dotted line) and 4h (solid line). Each point represents mean of n=10 ± SD. $p \leq 0.05$.

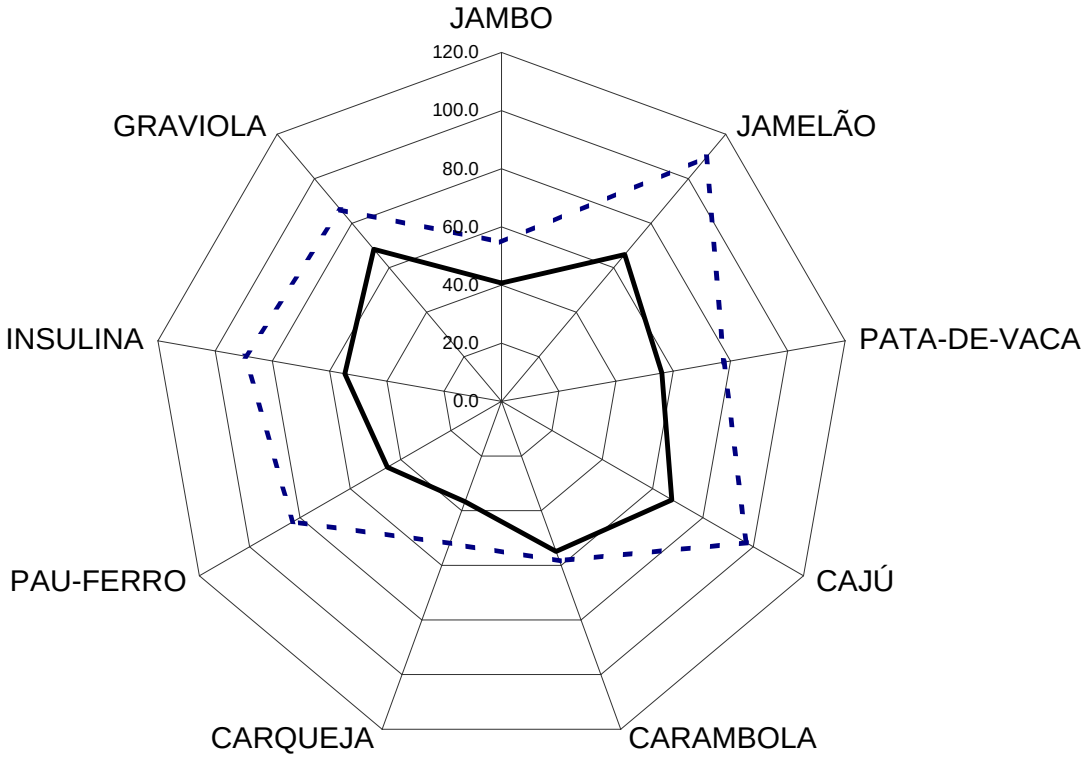

Fig. 5. Hypoglycemic effects of the tested teas at 10% showing the glycemia both at time 0h (dotted line) and 4h (solid line). Each point represents mean of n=10.