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Research Paper

Plant species used in dental diseases: Ethnopharmacology aspects and antimicrobial activity evaluation

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ABSTRACT

Ethnopharmacological relevance: Ethnopharmacological surveys show that several plant species are used empirically by the population, in oral diseases. However, it is necessary to check the properties of these plant species.

Aim of the study: To evaluate in vitro antimicrobial activity against Streptococcus mutans from plant species selected in a previous ethnopharmacology study.

Materials and methods: An ethnopharmacological survey was conducted with users of a dental clinic school services, located in Sao Luis, Maranhão, Brazil, aiming to identify plant species used in oral diseases treatment. From the ethnopharmacological survey, species were selected for in vitro antimicrobial activity evaluation against Streptococcus mutans, by agar diffusion method and determination of Minimum Inhibitory Concentration (MIC).

Results: Two hundred and seventy one people participated in the research: 55.7% reported the use of plants for medicinal purposes, 29.5% of which have knowledge and/or use plants for some type of oral disease. Thirty four species belonging to 24 (twenty four) botanical families were reported, being Aloe vera L., *Anacardium occidentale* L., *Schinus terebinthifolius Raddi*, *Chenopodium ambrosioides* L. and Punica granatum L. the most cited. The most commonly reported indications were healing after tooth extraction, followed by toothache, inflammation and bleeding gums., The determination of Minimum Inhibitory Concentration (MIC) demonstrated that Punica granatum L., *Psidium guajava* L. and *Schinus terebinthifolius Raddi* showed similar activity to 0.12% chlorhexidine, used as positive control.

Conclusion: That result is important to follow up the study of these species in the search for new anticariogenic agents originated by plants.

1. Introduction

In the process of developing new pharmacologically active compounds, natural resources of vegetable origin, represent an important source of drugs (Macedo and Oliveira, 2006). However, it is worth emphasizing the importance of defining criteria in the selection of the material for scientific research. Concerning those criteria, it has been found that ethnopharmacological approaches have provided important subsidies in establishing criteria for inclusion and/or exclusion of species for development of validation studies, allowing to evaluate efficacy and safety of plant resources employed therapeutically by the population (Elisabetsky, 2004; Gurib-Fakim, 2006).

In recent years, some studies have been developed in Brazil, aiming at assessing the popular use of plants in dentistry, making it possible to identify plant species with potential biological activity (Alviano et al., 2008; Alves et al., 2009; Santos et al., 2009; Albuquerque et al., 2010), in the search for new drugs to prevent and treat diseases.

Dental plaque is a complex biofilm that accumulates on the surface of the teeth, composed by more than 500 bacterial species, in which there is adhesion of initial colonizing bacteria in the salivary film of the enamel, followed by secondary colonization through the interbacterial adhesion. There is a variety of adhesins and molecular interactions in the base for the adhesive interactions, contributing to the development of caries and periodontal infection (Rosan and Lamont, 2000).

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Dental caries is a common chronic disease, being the leading cause of tooth loss in children and young adults. Initially, it presents itself as a white stain on the enamel (Balakrishnan et al., 2000). Its mechanism involves bacterial adhesion to dental surfaces, dental plaque formation and dental enamel demineralization by acids of bacterial origin. *Streptococcus mutans* has played a central role in the pathogenesis of the disease, by producing glycosyltransferase and synthesizing insoluble glucan from sucrose, mediating bacterial adhesion and accumulation on the surface of the tooth. After the formation of the plaque, the acids produced by bacteria demineralize the tooth surface (Hajishengallis and Michalek, 1999; Fejerskov and Kidd, 2007).

The chlorhexidine gluconate is the most used anti-plaque agent in the control of the dental biofilm (Figueiredo et al., 2004). It has high retentiveness, showing efficiency in the reduction of oral microorganisms, principally *Streptococcus* of the group *mutans*, for a long period, inhibiting the action of the enzyme glycosyltransferase, responsible for the accumulation of the bacterium on the dental surface (Ribeiro et al., 2007). However, the adverse effects of the chlorhexidine include dental pigmentation, stains on the restorations, burning sensation in the mouth and alteration in the taste (Torres et al., 2000).

The bacterial resistance to the antimicrobial traditional agents, besides its adverse effects, stimulates the development of researches on natural products with antimicrobial activity, aiming at new therapeutic alternatives in order to prevent caries (Normark and Normark, 2001; Sheldon, 2003; Hebbar et al., 2004).

In this sense, the present research was carried out to identify plant species used in the treatment of oral diseases and to investigate the antimicrobial activity *in vitro* of plant species more frequently referred to as plants of popular use in dentistry in the study population. The investigation took place in an odontology-teaching clinic, located in the city of *São Luís*, *Maranhão*, Brazil.

2. Materials and methods

The study was approved by the Committee of Ethics in Research of the Federal University of *Maranhão*, with consubstantiated legal opinion n° 23116-010660/2009-42. Before the collection of data, the participants were asked to sign the Voluntary and Informed Consent, authorizing their participation and dissemination of the collected data.

2.1. Ethnopharmacological study

2.1.1. Study area

The first stage of the research, characterized as an observational, cross-analytical study, had people over 18 years old as population of study. They were being cared at the odontology clinics of a higher education public institution, located in *São Luís* (south of Ecuador, in geographical coordinates latitude S 2°31′, longitude W 44°16), geographical micro region of the urban agglomeration, *Maranhão* State, Brazil; with basic dental care and specialized service to the population in general, besides services of prevention.

Maranhão is one of the 27 federal units of Brazil, located at the west end of the Northeast. Its area is 935.507 km², being the eighth largest state in Brazil. It has a population of 6,794,298 inhabitants. In terms of gross domestic product, it is the 16th richest state of Brazil. The main economic activities are industry (the work of transforming aluminum and alumina, food, timber), services, vegetable extraction (babassu), agriculture and livestock. *Tocantins* covers an area of 277.720 km². The economy is based on trade, agriculture (rice, corn, beans, soybeans, watermelon), and livestock (Fig. 1).

2.1.2. Sample

The data were collected from a sample of convenience, based on an oral diseases ethnopharmacological survey (Lima Júnior and Dimenstein, 2006), being considered the predominance of 40% for the use of plants with medicinal purposes, 5% error and a 90% confidence interval, when adopting a sample of 271 (two hundred and seventy one) people.

2.1.3. Collection of ethnopharmacological and socio-demographic data

To collect the ethnopharmacological data, structured and semi structured interviews were used from April to June of 2010, focusing on the approach of plant species used in dental diseases. The interviewed ones that mentioned the use of plants were questioned about the origin of the piece of information, the form of preparation, and what part of the plant is used. Socio-economic data were also collected to identify the average family income in accordance with criteria adopted by ANEP[1] (2009).

2.1.4. Collection and botanical identification

Identification of the plant material by botanist and sampling were collected from interviewees. The species that were referred in the ethnopharmacological survey were collected in the occurrence areas mentioned by the interviewees, including urban area (garden, square, backyard, domestic vegetable garden, medicinal herb garden) and native area; exsiccatae were prepared and sent to the *Ático Seabra Herbarium* of the Federal University of *Maranhão* to be botanically identified. Voucher specimens of the samples were kept in a herbarium.

2.2. Evaluation of the antimicrobial activity

2.2.1. Selection of vegetable species

From the ethnopharmacological survey, *Punica granatum* L., *Psidium guajava* L., *Schinus terebinthifolius Raddi* and *Chenopodium ambrosioides L.* were selected for investigation of the antimicrobial activity *in vitro* against *Streptococcus mutans*. The inclusion criterion was the plant species mentioned in the treatment of oral diseases with the highest frequencies of citations in the present study, native or exotic and of wide occurrence in the pre Amazon region. Toxic plant species were excluded, with study of validation of antimicrobial activity against oral pathogens, endangered ones and/or species that are not cultivated in the region.

2.2.2. Preparation of the extracts

Barks of *Schinus terebinthifolius* were obtained in *Araguaína*, *Tocantins*, Brazil and leaves of *Punica granatum*, *Psidium guajava* and *Chenopodium ambrosioides* were collected in the "Berta Langes de Morretes" Medicinal Herb Garden of the Federal University of *Maranhão*, *São Luís*, *Maranhão*, Brazil.

The plant material was dried separately in a greenhouse with air circulation, at the temperature of 38 °C for 48 h, followed by trituration in a cutting mill. The remaining water was evaporated by freeze-drying. The dry and crushed material of *Psidium guajava* (220 g), *Punica granatum* (248 g), *Chenopodium ambrosioides* (200 g) and *Schinus terebinthifolius* (200 g) was extracted through maceration with ethanol at 70%, for 24 h, in room temperature. That process was repeated 04 (four) times, with output of 12.5%, 14.6%, 11.8% and 26.6% (p/p) for the extracts of *Psidium guajava*, *Punica granatum*, *Chenopodium ambrosioides* and *Schinus terebinthifolius*, respectively.

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