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Phytochemical study of shells *Schinus terebinthifolius* Raddi (Anacardiaceae)

Estudo fitoquímico das cascas de Schinus terebinthifolius Raddi (Anacardiaceae)

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Abstract

The objective of this work was to perform a phytochemical study and to analyze the secondary metabolites of the vegetal extract of the bark of *S. terebinthifolius*. As a methodology to obtain the extracts, the dried and crushed peels were placed in erlenmayer and kept in contact with one liter of ethanol for seven days. The material was then filtered and subjected to the simple distillation process until the syrup was obtained. Subsequently, the tests that determine the presence of secondary metabolites were performed. It was verified that the ethanolic extract of the bark presents classes of secondary metabolites of great interest in the pharmaceutical industry.

Keywords: Medicinal plants; *Schinus terebinthifolius*; Biological Trials

Resumo

O objetivo desse trabalho foi realizar um estudo fitoquímico e analisar os metabólitos secundários do extrato vegetal das cascas de *S. terebinthifolius*. Como metodologia para obtenção dos extratos, as cascas devidamente secas e trituradas foram colocadas em erlenmayer e mantidas em contato com um litro de etanol, por sete dias. Em seguida, o material foi filtrado e submetido ao processo de destilação simples até a obtenção do xarope. Posteriormente, realizaram-se os testes que determinam a presença de metabólitos secundários. Verificou-se que o extrato etanólico das cascas apresentam classes de metabólitos secundários de grande interesse na indústria farmacêutica.

Palavras-chave: Plantas medicinais; *Schinus terebinthifolius*; Estudo fitoquímico

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INTRODUCTION

Brazil stands out for being the country with the world's largest biodiversity possessing, according to 22% of all biological species in the world. As regards the plant diversity is estimated about 350 to 550 thousand species, of which only about 55 thousand have cataloged (MARTINS, 2012).

Within this unique range of biological wealth, the country also stands out in another aspect with regard to plants: Brazilian forests hold a significant number of species that have therapeutic and medicinal purposes. Thus, Brazil has a huge genetic potential to be explored and it is estimated that this plant assets represent about 16.5 billion genes (ALVES et al., 2008).

Phytotherapy is a therapeutic characterized by the use of medicinal plants in their different pharmaceutical forms. The use of medicinal plants in the art of healing is a form of treatment of very ancient origins, related to the beginnings of medicine and based on the accumulation of information by successive generations over the centuries, vegetable products constituted the basis for treatment of different diseases (BRASIL, 2006).

Currently, the medicinal plants are used for much of the world's population, as an alternative medical use for the treatment of various diseases, since in many communities, represent a more affordable remedy for allopathic medicines (CARNEIRO, 2014).

The Anacardiaceae family consists of about 76 genera and 600 species. His genres are divided into five tribes (Anacardieae, Dobineae, Rhoeae, Semecarpeae and Spondiadeae). About 25 % of genera of this family are known to be toxic and cause severe contact dermatitis. In general, the poisonous species of this family are restricted to Anacardieae tribes Rhoeae and Semecarpeae. Contact dermatitis caused by these plants is mainly ascribed to phenolic compounds and catecólicos or a mixture of these substances, known as phenolic lipids (CORREIA et al., 2006).

These substances may be present in different parts of the plant material, mainly occurring in the *Rhus* genus. In recent years, the source of lipids and phenolic derivatives has also been the subject of investigation; Furthermore, Anacardiaceae family species have been shown to be very promising in the search for bioactive substances. The studies of these species made it possible to verify the occurrence of flavonoids, terpenes, steroids, xanthones, and especially of phenolic lipids and derivatives. It is noteworthy that among the flavonoids, the bi flavonoids are the most common (RADI; TERRONES, 2007).

The *Schinus terebinthifolius* Raddi, popularly known as red aroeira, is a fruit native to South America, belongs to the division Tracheophyta, class Magnoliopsida, order Sapindales, family Anacardiaceae, genus *Schinus*, species of *terebinthifolius* and division of species Raddi (RIBAS et al., 2006).

The fruits are the drupa type and have green color at first and then become red. The parts used to have medicinal properties are: shell, leaves and fruit. It is astringent, antidiarrheal, anti-inflammatory, purifying, diuretic and febrifuge. It is used as a component in cosmetology. Due to their essential oil composition is used in the treatment of respiratory disorders. In the shell of mastic takes action against fever, hemoptysis and uterine disorders in general. Shell extract an oil used against tumors and diseases of the cornea (DEGASPARI et al., 2004).

On the Amazon biodiversity numerous plants have been studied, but others are in process of study. Therefore, this study aimed to conduct a phytochemical study of the plant extract of the shell of *S. terebinthifolius*.

2. MATERIALS AND METHODS

Collection and identification of the plant

Shells *S. terebinthifolius* used to obtain the plant extract were collected in May during flowering time in a rural area of the municipality of Itapuã-the-West-RO (latitude 09º12'18 "south and a longitude 63º10'48" west). They collected four copies of the plant and led to the Herbarium Dr. Ary Pinheiro Penna Tupinambá of St. Luke School (HFSL) in Porto Velho, where he had the procedure in which the material was sandwiched between newspapers, cardboard, corrugated (aluminum) and press wooden. For each paper containing the specimen was identified with the number of collection, date, place and collector's name, staying in a three-day period in electric oven.

After dried, the material was taxonomically described with the aid of stereoscopic magnifying

glass and specialized and proven literature, or by comparison with material collection already identified. The material identified is recorded under number 00004285 of and incorporated into the herbarium's collection Dr. Ary Pinheiro Penna Tupinambá of St. Luke School (HFSL) in Porto Velho - RO.

Plant extract of preparation

The extraction was carried out from the dry and milled hulls were placed in conical flask containing one liter of ethanol for seven days. Then, the material was filtered and subjected to simple distillation process to obtain syrup. Phytochemicals tests were performed with ethanolic extract, based on precipitation and coloration of extracts diluted in solution and specific reagents for each test (RADI; TERRONES, 2007):

Alkaloids

To perform the assay we used 2.0 mL of the ethanolic solution is added 2.0 mL of hydrochloric acid (10 %), which mixture was heated at 100 °C for 10 minutes. After cooling, the extract was divided into three test tubes and placed in eight drops using Pasteur pipette, the reagents following recognition:

Tube 1 - Reactive Mayer: watching white precipitate formation or light white haze.

Tube 2 - Reactive Dragendorff: watching precipitate formation of orange color red.

Tube 3 - Reactive Wagner: observing orange color precipitate formation.

Glycosides cardiotonic

A 2.0 mL solution of the extract was added 3.0 mL of lead acetate solution 10 % and 2.0 mL of distilled water. Heated the mixture in a water bath for 10 minutes. Then the extract was filtered and stirred with 10.0 mL of chloroform, the chloroform phase separated in 4 test tubes. After evaporation of chloroform, the formation of residues in the tubes, which were added the following reagents:

Tube 1: the reaction was carried out for the determination of Salkowski steroidal nucleus. Going from yellow color to purple is a positive result.

Tube 2: 1.0 mL of Reactive Kedde. Pink or blue-violet to visible indicates cardenolide the bufadienólidos not react. The color is attenuated in a few minutes.

Tube 3: the reaction was carried out Keller-Kiliani (glacial acetic acid in a drop III ferric chloride to 5 % methanol and concentrated sulfuric acid). Intense staining is positive.

Tube 4: This was the Liebermann-Burchard reaction (1.0 mL of sample / few drops of acetic acid + 3.0 mL acetic anhydride / sulfuric acid (50: 1, v/v) Positive Result: Coloring green, blue-green, purple to blue.

Tube 5: This was the reaction Baljet (1.0 mL of sample / eight drops of acetic acid + 3.0 mL of chloroform). Positive: orange color, purple or red.

Tube 6: This was Raymond reaction (the extract was filtered and added 2 drops of ferric chloride solution of 10 % + two drops of lead acetate to 10 %). Positive result: color ranging from yellow to purple.

Coumarins

In a test tube was placed 2.0 mL the ethanolic solution was topped with filter paper soaked in 10 % solution of NaOH and brought to a water bath at 100 °C for some 10 minutes. It was removed and the filter paper was examined under ultraviolet light. The yellow or green fluorescence indicates the presence of coumarins.

Flavonoids

This study is based on the modification of the structure of the flavonoid in the presence of acid. Was placed in a tube, 2.0 mL ethanolic extract being added two drops of 10 % lead acetate. The presence of a colored precipitate indicates positive aspects of the reaction.

Tannins

The 2.0 mL of the ethanol extract was added 10 mL of distilled water. Were filtered and were added two drops using a Pasteur pipette, the 10 % ferric chloride solution. Blue color indicates possible presence of hydrolysable tannins and green staining tannins.

Saponins

In this assay, with 2.0 mL of ethanolic solution, was added 5.0 mL of boiling distilled water. After cooling, stirred vigorously, leaving at rest for 20 minutes. It is classified by the presence of saponins foaming.

Triterpenes

In this assay, with 2.0 mL of ethanolic solution, was added 5.0 mL of chloroform. After filtration, the extract was divided into two portions. In each tube there were the Liebermann-Burchard reactions and Salkowski. The stable color develop triterpenes and steroids develop color changing with time.

RESULTS AND DISCUSSION

For the secondary metabolites of shells *S. terebinthifolius*, positive results were to: alkaloids, glycosides cardiotonic using reagents Salkowisk and Baljet, coumarins, flavonoids, tannins and triterpenes. But negative results were to glycosides cardiotonic using Kedde reagents, Keller-Killiani, Liebermann-Burchard and Raymond and saponins, as shown in Table 1:

Table 1 - Identification results of secondary metabolites of ethanol extract of the shell of *S. terebinthifolius*

Metabolites secondary	Extract etanol	Coloration/Precipitacion
Alkaloids		
Reagent the Mayer	Positive	Orange
Reagent the Wagner	Positive	Purple
Reagent the Dragendorff	Positive	Orange
Glycosides Cardiotonic		
Reagent the Salkowiski	Positive	Green
Reagent the Kedde	Negative	Orange
Reagent the Keller-Killiani	Negative	Yellow
Reagent the Liebermann Burchard	Negative	Yellow
Reagent the Baljet	Positive	Orange
Reagent the Raymond	Negative	Red
Coumarins	Positive	Green fluorescence
Flavonoids	Positive	Orange
Tannins Condensed	Positive	Green
Saponins	Negative	Without foaming
Triterpenes		
Reagent the Liebermann-Buchard	Positive	Brown
Reagent the Salkowski	Positive	Red

The results of these studies are in agreement with those described in the literature mentioned by Matos (2002) *S. terebinthifolius* contain essential oils widely distributed in their plant parts such as leaves, fruits, stem, and in amounts varying compositions. In addition to the strong presence of these substances, phytochemical analyzes show the presence of high tannin content, biflavonoids and triterpenic acids in the shell of *S. terebinthifolius*. The essential oil of fruit and leaves are up to 5 % of mono and sesquiterpenes, components with high potential to provide protection from predators and weeds.

According to Silva et al. (2011) the essential oil of mastic is red-constitituint mainly monoterpenes (90.00 %) with a higher concentration of δ-3-carene (29.22 %), α-pinene (12.94 %), β-phellandrene (13.04 %) and ß-phellandrene (18.08 %), although it has alsδo been observed to occur sesquiterpenes, such as D-germacrene (3.09 %).

Essential oils can be related to different roles in plant interaction with the environment, as a defense against herbivores and pathogens, competition between plants and attracting beneficial organisms, such as pollinators, seed dispersers and symbiotic microorganisms. Furthermore, these compounds also have a significant protective action in relation to abiotic stress, such as those associated with temperature changes, light, exposure to ultraviolet radiation, nutrient availability and geographical distribution (RAVEN et al., 2001).

Based on the results obtained, the qualitative phytochemical analysis indicated the presence of large number of classes of secondary metabolites present in the shell of *S. terebinthifolius*. Of secondary metabolites analyzed the substances that are found present were alkaloids, coumarins, triterpenes, flavonoids, tannins and glycosides cardiotonic using any specific reagents.

The absence of other compounds may be associated with the degree of maturity at harvest, by genetic differences between cultivars, among other factors (GOBBO-NETO; LOPES, 2007). Also according to Bobbio; Bobbio (2003) the degradation of some compounds can occur during the extraction of vegetable, processing and storage of food, influenced by extrinsic and intrinsic factors (CORDEIRO, 2008).

Temporal and spatial variations in total content as well as the relative proportions of secondary metabolites in plants occur at different levels (seasonal and daily; intraplanta, inter- and intraspecific), and despite the existence of a genetic control, the expression may undergo modifications resulting from interaction of biochemical, physiological, ecological and evolutionary processes. They represent a chemical interface between the plant and the surrounding environment, so its synthesis is often affected by environmental conditions (COUTINHO, 2013).

In general, of shell in the *S. terebinthifolius* shell is the part of the plant material most used to isolate bioactive substances, although studies reveal that in addition to this, leaves and fruits are sources of chemical compounds that end up attracting great interest in scientific research (KWEKA et al., 2011; PAWULOWSKI et al., 2012; CARLINI et al., 2013).

Varela-Barca et al. (2007) found amentoflavona, dihydro-amine and tetrahydro-amine. These substances had already been identified in fruits by Skopp and Schwenker (1986). Lima (2009) when performing the phytochemical study of the essential oil of the leaves of S. terebinthifolius verified an oil yield that was 0,8%, 37 chemical constituents were identified, and the main components were germacreno D (25 %), (E)-β-cariofileno (17,5 %) e δ-elemeno (10,5 %), noting that this plant has a wealth of natural products that need to be checked for biological activity.

As shown in research Santana (2012), the main phytochemical products of red aroeira are: fatty acids, terpenoids and other acid derivatives such as 3 α-masticadienoico (schinol) and masticadienoico. However, it is known that the chemical composition of S. terebinthifolius is much more complex due to the compounds and chemical bonds to which they are involved. Therefore, it is necessary to investigate the chemosystematics of this plant in order to know which of these compounds can be used in the chemical industry (CARVALHO et al., 2013).

Galvão (2014) reports in his study addressing aspects, phytochemical, ethnobotanical and pharmacological of red aroeira that the traditional, pharmacological and clinical use of the extract of the shells of red aroeira can be used in the topical treatment of injuries of the skin, mucosa in general and in the and cervicitis and cervicovaginitis, and this activity may be useful for chemical-structural and pharmacological studies, as well as for the synthesis of new anti-inflammatory drugs as an alternative to those that cause gastric irritation.

Therefore, Ethnobotany, as the main source of popular knowledge among the generations, needs

to be further investigated from a medical, ecological and biological point of view, so that a strongly built and solid argument can be built, thus favoring recognition for the contribution among civilizations that use folk medicine.

CONCLUSION

It follows that the shell *S. terebinthifolius* exhibited qualitative study of secondary metabolites (alkaloids, coumarins, flavonoids, triterpenes, glycosides cardiotonic using reagents Salkowisk and Baljet and tannins) of great medical interest which can provide a high biological activity, by their herbicidal activity, insecticidal and fungicidal and / or drug.

However, it is necessary that this species is subject to phytochemicals biomonitorados studies, in order to isolate and identify the active compounds and establish a relationship with the biological activities observed in popular usage.

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