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Research Article

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Phytochemistry, Toxicology and Antioxidant Evaluation of the species *Rosmarinus officinalis* Linn (Rosemary)

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ABSTRACT

The research conducted with the Rosmarinus officinalis L. species known as rosemary was aimed at making the phytochemical study, antioxidant activity, and toxicological analysis of ethanol crude extract of the leaves. The phytochemical analysis of the species presented as the main secondary metabolites: alkaloids, phenols and tannins, and depsides depsidones, reducing sugars and coumarins. The antioxidant activity of the species was determined by linear regression of the inhibiting concentration 50% (IC50), which showed a value of 2.24 2.24 mg/mL^{-1}, strong correlation coefficient (R^2) of 0.9425, showing its potential therapeutic, and this is an important activity for inhibiting free radicals. The toxicological analysis with Artemia salina, which is considered the life and death of metanauplius, the crude extract of R. Officinalis L. did not show in any of its concentrations death of these marine organisms, which causes the extract of the species not present toxicity at the concentrations tested.

Keywords: *Rosmarinus officinalis* L., Medicinal Plants, Biological Activity, Secondary Metabolites

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INTRODUCTION

The existing biodiversity in Brazil is very rich, which is why it attracts a lot of research with medicinal plants due to the therapeutic use of these species [1]. The therapy performed with medicinal plants is a popular practice widely used. They are knowledgeable of several species with healing power, thus contributing to the studies to the pharmacology and phytotherapy use of these plants [1]. Phytochemical aims to analyze and record the components derived from plants as secondary metabolites by the isolation and elucidation of its molecular structures [2].

The species *Rosmarinus officinalis* Linn from Lamiaceae family, popularly known as Rosemary is a herb widely used for medicinal purposes and in cooking, this species has been studied for the identification of chemical compounds, physiological effects, and microbial activity [3]. This species is a perennial feature of plant, native to the Mediterranean and is one of 2800 species existing Lamiaceae family in the world.

The antioxidant activity of compounds present in plants such as phenolic compounds has raised great interest in investigating the effects and its benefits related to diseases linked to oxidative stress and other illnesses. And the teas and condiments these plants has a great interest also in relation to conservation due to the antioxidant and antimicrobial activity [4].

According to Asolini et al. (2006) [5] the oxidative stress process is an imbalance between prooxidant and antioxidant, in favor of pro-oxidative status, promoting potential damage. This process is related to chronic diseases that occur in large numbers, such as cardiovascular diseases and neurodegenerative diseases.
Thus, based on testing natural substances or chemicals obtained from plants, which are identified as reactive oxygen species obtainers, defending the human body of these effects, and inhibited the onset of many chronic diseases [4].
As there is great interest in the natural potential in herbal medicine of plants, it is necessary to have a great attention to its use. Even being natural, plants can bring great risks of intoxication, and one of the most economical and effective methods is the Animal toxicity test on *Artemia salina* Leach, a kind of micro-crustaceans of Anostraca order, used as a biomarker in laboratory tests and considered safe [6].
Thus, this toxicity test is a phase of great importance to the phytochemical study, it aims to evaluate the toxic effects on the biological system and seek the safe use of medicinal plants [7]. It is possible to evaluate the acute lethal tests, where it aims to assess the effects on selected marine organisms, summarizing the results evaluated by the LC50, which is the lethal concentration in a test that it is possible to present death in 50% of tested marine organisms [2].

Toxicity tests against *Artemia salina* L. bioassay are valid since the effects produced by a compound in laboratory animals are also applied to man. Thus, tests made based on the dose per unit body surface area, effects on humans are considered in the same boundaries that were observed in the laboratory [6]. This research aimed to make the phytochemical study in the pursuit of their major classes of secondary metabolites, to evaluate the antioxidant activity and toxicity with the front test of *Artemia salina* crude ethanol extract of the leaves of *Rosmarinus officinalis* L.

EXPERIMENTAL SECTION

Obtaining the crude extract

The plant species was collected on August 29, 2014, in the city of Macapá, Amapá, Brazil, sent to the laboratory of Pharmacognosy and Phytochemistry of the Federal University of Amapá, campus Zerão, to be clean and dry at room temperature. The dried leaves were ground in an electrical mill and extracted using ethanol as a liquid extractor maceration for a period of two days. After filtration and evaporation of the solvent under route and steam, there was obtained the crude ethanol extract.

Phytochemical analysis

To be performed the analysis has been adopted the methodology of Barbosa et al. (2001) [8], in which the extract obtained is analyzed by staining reactions and/or precipitation, for identification of the major classes of secondary metabolites.

Toxicity on *Artemia salina*

Preparation of artificial marine solution: The saline solution was prepared with 34.2 g of sodium chloride; 1.425 g of magnesium sulfate; 4.75 g of sodium bicarbonate and 951 mL of distilled water. Once homogenized and its pH adjusted to 9.0 using a solution of 2 $mol.L^{-1}$ sodium hydroxide.

Obtaining metanauplius A. salina: To obtain the metanauplius, cysts of *A. saline* were incubated in artificial sea solution (pH 9.0 and 28°C) under artificial illumination of a 40 W lamp for 24 hours. After hatching, the metanauplius migrates through a perforated plate to another compartment with a free incidence of light, due to its phototropism, thereby separating the waste larvae of cysts and cyst, not hatch.

Sample preparation and bioassay: The bioassay of *A. saline* was based on the technique described by Meyer et al. (1982) [9]. 10 mg the crude ethanolic extract was used, added 1 mL of Tween 80 to 5% to aid the solubilization. The solutions were mixed and the volume was made up to 5 mL with an artificial marine solution at pH = 9.0. Aliquots of these solutions were removed 2500, 1875.1250, 625, 250, and 125 µL and then transferred to 5 mL and the volume completed with the same solvent vials to give the concentrations of 1000, 750, 500, 250, 100 and 50 $mg.mL^{-1}$ for each extract.

Metanauplius were divided into seven groups, each containing ten individuals. The first group received the control solution (Tween 80 solution at 5%) and the following six solutions of the extracts in different concentrations. The samples were submitted to artificial light for 24 hours after this period were recorded live and dead larvae. Larvae that show no active movement in about twenty seconds of observation were considered dead. The experiment was performed in triplicate for each concentration.

Antioxidant activity

The evaluation of the antioxidant activity was based on the methodology proposed by Andrade et. al., (2012) [10] with the use of 2,2-diphenyl-1-picryl-hidrazila (DPPH), and some modifications.

A methanolic solution of DPPH at the concentration of 40 $\mu g.mL^{-1}$ was prepared. The extract was diluted in methanol at concentrations of 5, 1, 0.75, 0.50 and 0.25 $mg.mL^{-1}$. There were added to the test tube 2.7 mL of the stock solution of DPPH, and 0.3 mL of the crude extract solution at each concentration. After 30 minutes on a spectrophotometer (Biospectro SP-22), readings were performed at a wavelength of 517 nm. The antioxidant activity was calculated according to Andrade et al. (2012) [10].

$$\%AA = \{[100 - (Abs_{sample} - Abs_{white})] \times 100\}/Abs_{control}]$$

AA%: percentage of antioxidant activity;

Abs_{sample}: absorbance sample;

Abs_{white}: white absorbance;

$Abs_{control}$: control absorbance.

RESULTS AND DISCUSSIONS

In this study, were conducted thirteen phytochemicals tests, which have been identified in species only five secondary metabolites, which are: alkaloids, depsides and depsidones, tannins, reducing sugars and coumarins. Table 1 shows the classes of secondary metabolites search form.

Table 1: Classes secondary metabolites surveyed in this study of the species *Rosmarinus officinalis* L

	Secondary Metabolites	Positive	Negative
1	Saponins		X
2	Organic acids		X
3	Steroids and triterpenoids		X
4	Coumarins	X	
5	Purines		X
6	Alkaloids	X	
7	Tannins	X	
8	Depsides and Depsidonas	X	
9	Reducing sugars	X	
10	Flavonoids		X
11	Protein and Amino Acids		X
12	Anthraquinone		X
13	Polysaccharides		X

The alkaloids act by inhibiting acetylcholine in autonomic effectors innervated by cholinergic post-Ganglion nerves, as in smooth muscle, which is devoid of innervation cholinergic. The antimuscarinic actions, in general, have little effect on acetylcholine actions on nicotinic receptors, where the neuromuscular junction, the receptors are nicotinic. There are required high doses of the active ingredient to produce blocks [11].

The presence of depsides and depsidones secondary metabolites in the species comes from its originating biosynthesis orselínico acid, depsides are an example of polyketides, the biosynthetic reaction of formation of the acid is in orselínico acid synthase, where its mechanism involves dehydration reaction only in final step occurs when the cyclization of the chain to the formation of this acid. This has been characterized as an enzyme complex containing transacetylases activity, acyl carrier protein groups, condensing an enzyme and a hydrolase activity. The depsides and depsidones have been reported about the anti-inflammatory and antibiotic activity. Because of these activities, these metabolites have been synthesized for evaluation of their respective biological activities and the possibility of drug use [12].

The reducing sugars, substances belonging to the groups of carbohydrates are characterized by having a ketonic carbonyl-free group, able to oxidize in the presence of oxidizing agents in alkaline solutions. Monosaccharides, glucose, and fructose are examples of this class of compounds [13]. They are important in plants especially in drought situations, since they provide an increase in sucrose synthesis which contributes to the osmotic adjustment without inhibiting photosynthesis.

Tannins are complexes of phenol and water-soluble nature. The action of tannins as free radical scavengers, which is a function of the active oxygen interception forming stable radical, helps prevent many degenerative diseases such as cancer, multiple sclerosis, atherosclerosis, and the aging process itself [14]. The bactericidal and fungicidal activities occur for three

general characteristics common to both tannins groups: complexation with metal ions; antioxidant activity and scavenging of free radicals; complexing ability with other molecules, especially proteins and polysaccharides [14].

The coumarins are derived from 5,6-benzothiazol-2-pyrone (*α*-chromone). Originate from the *trans*-cinnamic acid by oxidation, results in the - coumaric acid, whose phenolic hydroxyl condenses with a glucose unit. This compound isomerizes to its corresponding cis, which by coumarin cyclization form. This metabolite has pharmacological properties as the anticoagulant action, and some classes of coumarin have potent inhibitors of lipid peroxidation, eliminates the superoxide radical anion and quelarem iron ions. These properties make the substances of interest such as antioxidants, for possible application in the prevention of diseases caused by free radicals, and other pharmacological activities still under study [15].

The crude ethanol extract of rosemary not presented in any of his concentrations death of marine organisms, which causes the extract of the species does not show toxicity at the concentrations tested.

In the research of literature [6], it was observed that the toxicity tests done in essential oil from *R. Officinalis* L. has a non-toxic effect based on the rate of dead and living of metanauplius (cysts *A. salina*) similar to the results observed for the crude ethanol extract of *R. Officinalis* L. in this research.

Phytochemical analysis of the ethanolic crude extract of *R. Officinalis* showed compounds exhibiting antioxidant activity demonstrated in the literature, such as phenolic compounds, which are effective free radical scavengers.

According to Andrade et al., (2007) [16] the antioxidant activity of phenols is due to the redox properties, which allows acting as a reducing agent, hydrogen donor, and eliminators singlet oxygen. And its antioxidant effect of herbs is attributed to the presence of phenolic hydroxyl groups in their compounds.

The determination of activity was by linear regression of 50% inhibition concentration (IC50) which showed a value of 2.24 $mg.mL^{-1}$, strong correlation coefficient (R^2) of 0.9425. As can be seen in Figure 1

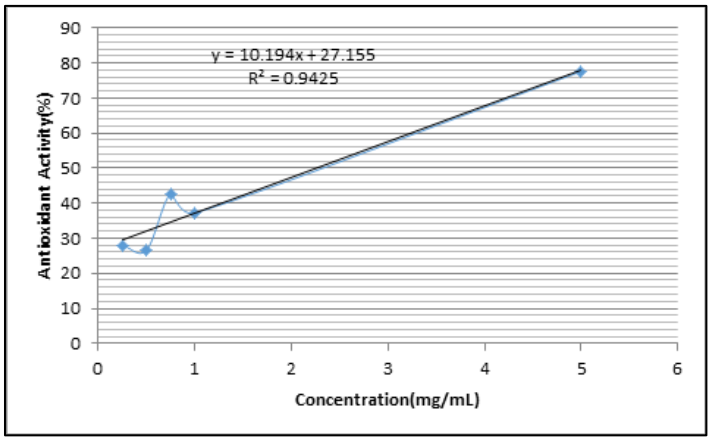

Figure 1: Percentage of inhibition of DPPH radical by the samples and the standards of the crude extract solution of *R. officinalis* L

In a study using various extracts of *R. officinalis* L., obtained by extraction in supercritical carbon dioxide, in IC50, 15.73 $\mu g.mL^{-1}$ was found for the ethanolic extract, and 9.23 $\mu g.mL^{-1}$ to hexane extract. The literature reports that the antioxidant activity depends on genetic conditions and plant growth, such as quality, origin and climatic conditions, storage and processing, in addition to the extraction process and its selected parameters [17].

CONCLUSION

Through studies of chemical and biological properties made it possible to verify and demonstrate the potential use of *Rosmarinus officinalis* L. species in therapy, where the crude extract of this species proved quite effective in the tests. Thus, the great variability of the chemical compounds found in this species due to various influences by various climatic and soil factors.

The chemical compounds found in the species are the main factors related to biological activities. Thus, tests performed as the phytochemical analysis, it was possible to find five major class of secondary metabolites: alkaloids, and depsides depsidones, tannins, reducing sugars and coumarins.

With the identification of such chemical compounds, emphasized that the phenolic compound, which is known as a compound for an antioxidant activity where it is able to inhibit the formation of, free radicals, which are reactive substances. It is the positive antioxidant activity test, and thus can be used as an herbal therapeutic way for chronic disease treatments.
The bioassays conducted in the laboratory to determine whether a plant has some toxic effect, highlight the toxicity test on *Artemia salina*, with negative results showing that the species under study is non-toxic at the concentrations tested.
Therefore, *Rosmarinus officinalis* species from the Lamiaceae family showed in their tests a very important potential for this to be used as herbal medicine, as the results described in this study encourage continued research to evaluate the potential of the biological activities of substances isolated species studied.

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