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PHYTOCHEMICAL STUDY AND EVALUATION OF THE BIOLOGICAL ACTIVITY OF THE SPECIES JASMIM (*Plumeria rubra* L.)

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ABSTRACT

The plant species *Plumeria rubra* L. belongs to the family Apocynaceae, which originates from Central America and is widely distributed in the tropical climate regions of the world, including Brazil, as well as in Asia and East Timor. This plant is popularly known as jasmine, jasmine-mango, frangipani, pagoda tree, or "ai Santo Antonio. Studies reveal some critical biological activities of your polar extract, such as antimicrobial, anti-inflammatory, antioxidant, antitumor, and anticancer. The objective of this work was to identify the classes of secondary metabolites, antioxidant activity, and toxicity test of ethanol extracts from their flowers and leaves. The identification of the types of secondary metabolites, based on the appearance of staining and precipitation of extracts diluted in solution and specific reactive scans by phytochemical tests, the assay of antioxidant activity was performed with the method of DPPH free radical sequestration, with UV-vis spectrometer reading, using wavelength at 520 nm to obtain the IC_{50}. The toxicity test was performed in front of the *Artemia salina*, and the LD_{50} was calculated. The results of secondary metabolite tests revealed the presence of tannins, phenols, coumarins, flavonoids (absence in leaves), alkaloids, terpenoids, steroids, and saponins (absence in flowers). The results of spectrophotometric readings showed that the ethanolic extract of the flowers presented the IC_{50} of 495.17 ppm, and the ethanolic extract of the leaves gave the IC_{50} of 782.35 ppm. Where, the ethanolic extract of the flowers showed moderate antioxidant activity and the ethanolic extract of the leaves presented weak antioxidant activity, compared to the definite pattern of ascorbic acid (vitamin C). The results of toxicity tests against saline artemia showed a lethal dose (LD_{50}) 478.75 ppm of flower extract and lethal dose (LD_{50}) 818.28 ppm of leaf extract. Flower extracts showed moderate toxicity, and leaf extract showed low toxicity. The preliminary results obtained in this work the plant presents good oxidizing activity in flowers and larvicidal activity in leaf and flower extracts, however, further studies should be carried out in the search for biological activity, phytochemicals, isolation and other activities in the future for the development of new products in the pharmaceutical area, food among other applicability.

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INTRODUCTION

Nature produces most of the organic substances that are currently known. However, the plant kingdom has a significant contribution to the supply of materials that are delimited as effective active ingredients and that are now used in the treatment of diseases (SOUSA *et al*., 2011). The role of natural products as a source of medicines has been recognized since ancient times (PRIYASAMY *et al*., 2013). Plants have traditionally been used by populations of all constituents in the control of various diseases and pests, besides representing a valuable source of biologically active natural products, many

of which are models for the synthesis of many drugs. The fact that generates interest in the products found in nature is that these have enormous diversity in terms of structure and physicochemical and biological properties (LUPE, 2007). Traditional medicine is also considered as the use of medicinal plants in the accessible tract, especially in the context of preventive, promotional, and rehabilitation. The use of products of natural origin (plants, minerals, products of animal origin) to treat diseases is an integral part of the cultural heritage of all civilizations. It is still currently the most important medicinal resource of rural societies of developing countries (GOMES, 1990). One of the countries that presently still use medicinal plants as an alternative method to cure diseases is East Timor. In Timor-Leste, there are several mountainous elevations and mainly forest and savannah monsoon, although the study area also includes small pockets of green forest, rainforests, and deciduous rainforests. In the urban environment, there is a deficiency of vegetation that, in most populations, uses as medicinal plants (COLLINS *et al*., 2007; COSTA, 2010). One of the commonly used medicinal plants is jasmine (*Plumeria rubra* L.). Plant species are known as jasmine (*Plumeria rubra* L.) or vulgar name "ai Santo Antonio" in East Timor is a plant popularly cultivated as an ornamental plant in residences and cemetery decoration. Also, it is still commonly used in the treatment of diseases such as abdominal pain. For, in Timor-Leste, now still has traditional knowledge associated with the use of plants as a therapeutic activity as an ethnobotanical study associated with ethnopharmacological. This species is also endemic in Brazil, according to Costa (2010), which states that most of the plants existing in East Timor grow in Brazilian lands. This study showed the importance of sharing phytochemical knowledge of medicinal plants, their possible therapeutic use, and their contribution to multidisciplinary knowledge.

MATERIALS AND METHODS

Obtaining Botanical Material: The identification of the plant material was performed by the botany Profa. Dr. Maria Iracema Bezerra Loiola, in July 2018, at UFC, PICI campus, in Herbarium Prisco Bezerra, department of Biology. Exsiccate was deposited in the herbarium under the registration number EAC #59835.

Collection and obtaining Plant Extracts: Fresh jasmine leaves and flowers (*Plumeria rubra* L.) were collected in the municipality of Redemption, in the Region of the Baturité Massif, Ceará, Brazil, at coordinates 04º13'34"S and 38º43'52"W, between September and October 2016. After collection, fresh leaves and flowers were dried in a greenhouse at 50 ºC for seven days, and then manually crushed. 1 kg of the crushed leaves and 500 g of the crushed flowers were submitted to extraction with ethyl alcohol P.A. and left up to 7 days at room temperature. Soon after, the ethanol extract was filtered and evaporated under reduced pressure at 50 ºC temperature. The dry extracts obtained were submitted to phytochemical and biological tests.

Phytochemical prospecting: The extracts obtained were submitted to phytochemical tests to identify the classes of secondary metabolites based on the appearance of staining and precipitation of extracts diluted in specific solutions and reactive in each experiment. First, a stock solution of the ethanol extracts of the species was prepared, where 500 mg of sample was weighed and dissolved in a hydroalcoholic solution (80%). The methodology that was used for the detection of secondary metabolite groups was adapted by Silva and Lima (2016); Silva *et al*. (2015); Matos (2009); Patil *et al*., (2016); Vishwakarma *et al*., (2014). In the ethanolic extract of flowers and leaves, the following tests were performed: Alkaloids, Coumarins, Flavonoids, Tannins and phenols, Saponins, Terpenoids, and Steroids.

Alkaloid identification test: Initially, 2.0 mL of the first solution was added, 2.0 mL of HCl (10%), where it was heated for 10 minutes. It cooled, filtered, and the filtrate was divided into three test tubes, and a few drops of the Dragendorff and Mayer reactivates. Slight turbidity or precipitate (respectively purple to orange, white to cream, and brown) evidences the possible presence of them.

Test for identification of coumarins: In a test tube, 2.0 mL of the stock solution was placed, 2 mL of NaOH 10%, yellow color formation, which indicates the presence of coumarins.

Flavonoid identification test: A tube was placed, 2.0 mL of the stock solution, some fragments of Mg, and added, by the walls of the tube, a few drops of concentrated HCl. It was observed the possible change of color to red, red to purple, or pink, orange color in solution is indicative of the presence of Flavonoids.

Testing for the identification of Tannins and Phenols: It placed 2 mL of the stock solution in a test tube and added 4-5 drops of $FeCl_3$ 10%. The formation of green, blue, or black or between blue and red in solution is indicative of the presence of tannins and phenols. Or, the appearance of a dark precipitate of blue tint indicates the presence of water-soluble tannins and green the presence of condensed tannins.

Test for the identification of saponins: In this test, with 2.0 mL of the stock solution, 5.0 mL of distilled water was added and heated in a water bath for 10 minutes. After cooling, he stirred vigorously, leaving him at rest for 20 minutes. The presence of saponins is classified by persistent foaming.

Test for the identification of terpenoids: In a test tube, 2 mL of the stock solution was placed, and 2 mL of chloroform was added, and 4 to 5 drops of concentrated sulfuric acid, red-brown color formation, or the appearance of colorations ranging from blue to green indicates the presence of this metabolite.

Steroid identification test: In a test tube, 2 mL of the stock solution was added, and 2 mL of chloroform and 1 mL of concentrated sulfuric acid, red-brown color formation or the appearance of stains ranging from blue to green indicates the presence of this metabolite.

Antioxidant activity test: The preparation of the DPPH solution and evaluation test of the kidnap activity of the radical DPPH was used the methodology adapted by Rufino *et al*., (2007); Sousa *et al*., (2007); Alves *et al*., (2010); Boroski *et al*., (2015); Silva *et al*., (2012); Silva *et al*., (2013) and some modifications. In prepare of 60 μM DPPH solution, 2.4 mg of DPPH was weighed and dissolved in P.A. ethyl alcohol by completing the volume to 100 mL in a volumetric balloon. The samples were solubilized in ethanol and diluted up to the concentrations of 1000, 500, 250,125, 62.5, and 31.2 ppm. In the prepared concentrations, 2 mL of the ethanolic solution of

DPPH 60µM was added. Next, the absorbance of the samples and the white in spectrophotometric model T80 UV/Vis. Was read, using a wavelength of 520 nm. Ethanol was used to calibrate the spectrophotometer. The ethanolic solution of DPPH 60µM and ethanol were used as a negative control, and ascorbic acid was used as a positive control.

The percentage of inhibition of free radical elimination activity was calculated using the following formula:

$$\%\ InhibitionDPPH = \frac{(Abs_{DPPH} - Abs_{sample})}{Abs_{DPPH}} x\ 100$$

Where,

Abs_{DPPH} = absorbance of radical ethanolic solution DPPH;
Abs_{sample} = absorbance of the sample after 30 minutes of reaction with the solution of DPPH.

The value of IC_{50} (mg/mL) is the concentration necessary to inhibit 50% of the initial DPPH free radical, was calculated by linear regression and equation of the chart straight. All reactions were performed in triplicate, and the value was expressed as the mean ± standard deviation (r).

Toxicity test against *Artemia salina:* The toxicity test in the face of *Artemia salina* was performed according to Rosa *et al*.'s methodology (2016); Merino *et al*., (2015); Amarante *et al*., (2011); Meyer *et al*., (1982) and some modifications. Initially, a solution was prepared with sea salt at a concentration of 30 g/L. This solution was used for the hatching of *A. salina* eggs and in the preparation of the other dilutions of the samples. The eggs were placed to hatch in the saline solution for 48 hours with artificial lighting at room temperature 26ºC at 29ºC. Ten mg of ethanol extracts of flowers and leaves were used and dissolved in 10 mL of DMSO 1%, in preparation of the 1mg/mL concentration stock solution or 1000 ppm. From the stock solution, dilutions were prepared in saline water in five different concentrations (500, 250, 125, 62.5, 31.2 ppm) in a test tube. About ten larvae aged 48 hours were transferred to cells containing the samples tested. The larvae were incubated and under artificial lighting. After 24 hours, the dead larvae were counted. The test was accompanied by negative control (saline water only). The experiments were performed on duplicates.

According to WHO (2009), if the mortality in controls is between 5% and 20%, the results with the treated samples are corrected using Abbott's formula:

$$\%\ mortality = \frac{X - Y}{100 - Y} x\ 100$$

Where: X = percentage mortality in the treated sample and Y = percentage mortality in control.

The confidence intervals of the lethal dose (DL50) were determined from the 24-hour counts of dead larvae using the probit analysis method described by Finney (1952).

Nuclear magnetic resonance spectroscopy (NMR): The nuclear magnetic resonance spectra of ^{1}H and ^{13}C, uni and two-dimensional, were obtained in Bruker spectrometer, DRX-300, and DPX-500 models, operating at a frequency of 300 and 500 MHz for proton and carbon 75 and 125 MHz, respectively. The deuterated solvents used in the dissolution of the samples and obtaining the spectra were: chloroform ($CDCl_3$), acetone [$(CD_3)_2CO$], methanol (CD_3OD), and pyridine (C_5D_5N). The multiplicities of the absorbers were indicated according to the convention: s (singlet), d (dub), dd (double dubbing), t (triplet), ddd (double doublet-doublet) hep (heptet) and m (multiplet). The carbon hydrogenation pattern in NMR ^{13}C was determined using the Distortionless Enhancement by Polarization Transfer (DEPT) technique, with a nutation angle of 135º, CH, and CH_3 with an amplitude as opposed to CH_2. Non-hydrogenated carbons were characterized by subtraction of bb (Broad Band) and DEPT and second convention signals, methyl, methyl, and methodical terminology were applied to CH_3, CH_2, and CH groups, respectively.

RESULTS AND DISCUSSION

Obtaining extracts : The dry vegetable material of the flower (500 g) and leaf (1 kg) of *Plumeria rubra* L. were macerated in ethanol for a given period and about 16.29 g (3.26% yield) of the dry extract of the flower and 18.32 g (1.83% yield) of the dry extract of the leaf. The results are in Table 1.

Table 1. Results of ethanolic flower extractions and jasmine leaf

Dry sample	Solvents	Mass obtained from extract (g)	Extract yield (%)
flowers(500 g)	Ethanol P. A.	16.29	3.26
leaves (1kg)	Ethanol P. A.	18.32	1.83

Through the ethanol extracts of the leaves and flowers of the species of *P. rubra* L. obtained, phytochemical tests, antioxidant tests, and toxicity tests were performed.

Phytochemical test: From a stock solution obtained, experiments were performed to react to the sample with a specific reagent solution described in the methodology for the identification of secondary metabolites present in the sample. Phytochemical analyses provide information on the presence of secondary metabolites in plants (ALMEIDA *et al*., 2015). The results obtained are described in Table 2, and, the results present or are not evidenced in Figures 5 and 6.

Table 2. Result of the identification of possible secondary metabolites found in the ethanolic extract of *P. rubra* L

Secondarymetabolites	Reaction	Ethanolextract	
		flowers	leaves
Alkaloids	Reagent Mayer	+	+
	Dragendroff Reagent	+	+
Coumarins	Sodium hydroxide 10%	+	+
Flavonoids	Shinoda	+	-
Tannins	Ferric chloride 10%	+	+
Phenols	Ferric chloride 10%	+	+
Saponins	Foaming	-	+
Terpenoids	Salkowski	+	+
Steroids	Salkowski	+	+

+ (positive) - (negative)

Phytochemical tests were performed from the extract of jasmine leaves and flowers (*Plumeria rubra* L.); these tests used to characterize secondary metabolites. According to the results obtained, it was noticed that some secondary metabolites were positive, and some were negative in the leaves and flowers of the species *Plumeria rubra* L. The secondary metabolites present in the ethanolic extract of the

leaves were alkaloids, coumarins, tannins, phenols, saponins, terpenoids and steroids, flavonoid was the only one that negative in the present test. And the secondary metabolites present in flower extract are alkaloids, coumarins, tannins, phenols, terpenoid flavonoids, and steroids, saponin was the only test that tested negative in the current analysis. From the positive tests for secondary metabolites, thus signals the potential for antioxidant activity (ZUANAZZI, 2001; BOROKSI *et al*., 2015), and other biological experiments. For the ethanolic extract of the leaves and flowers, it was possible to identify phenols. These compounds have antioxidant action as a result of their chemical structure and reducing properties, thus presenting characteristics such as antifungal, antiallergic, disinfectants, and in the manufacture of medicines (SOUZA *et al*., 2016). The identification of phenolics is based on its ability to suffer oxidation before oxidizing agents such as ferric chloride, where the complexation occurs with Fe^{3+} cations of the solution. The reaction is showing in Figure 7.

The results of phytochemical screening performed on the ethanolic extract of the species *Plumeria rubra* L. indicated the presence of flavonoids in the flowers and absence in the leaves. Flavonoid identification was performed through Shinoda's reaction. This reaction is based on the oxidation capacity that most flavonoids have in the presence of magnesium powder and hydrochloric acid. The color formed depends on the chemical structure of flavonoid, through yellow, violet, red in intense red (WOLF, 2008). The reaction is showing in Figure 8. The positive result of flavonoids may indicate the presence of the phenolic compound, thus signaling the potential for antioxidant activity. Flavonoids represent one of the most important and diversified phenolic groups among products of natural origin. This class of compounds is widely distributed in the plant realm. And flavonoids can be found in various structural forms (ZUANAZZI, 2001). The presence of flavonoids in vegetables is related to the protection of plants against the incidence of ultraviolet and visible rays, protection

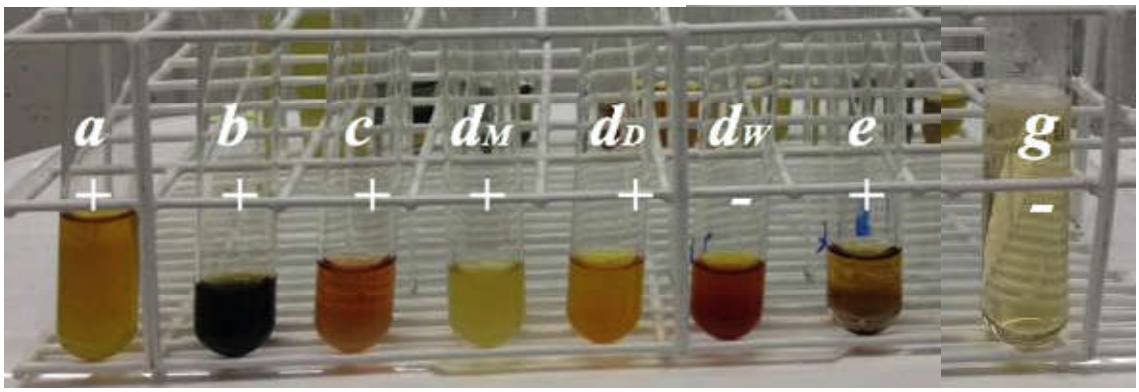


Figure 5. Phytochemical test of ethanolic extract of jasmine flowers.a) Coumarins; b) Tannins and Phenols; c) Flavonoids;d) Alkaloids (d_{Mayer}, $D_{ragendroff}$); e) Terpenoids; f) Steroids, and g) Saponins

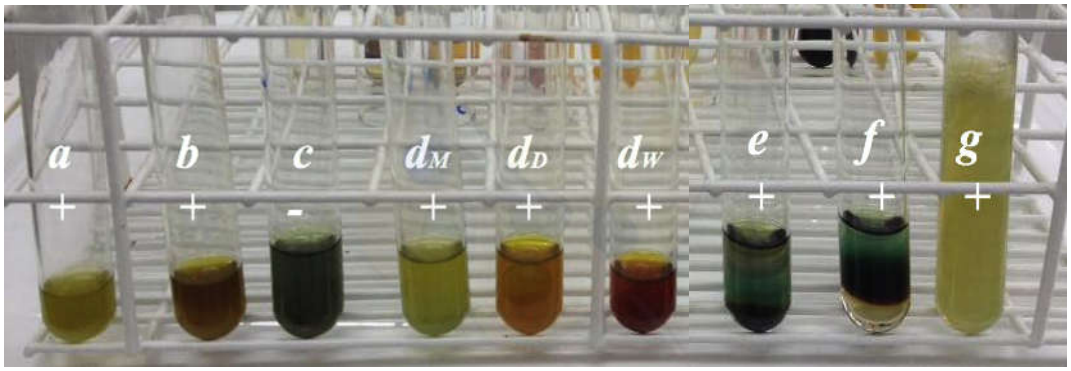


Figure 6. Phytochemical test of ethanolic extract of jasmine leaves.a) Coumarins; b) Tannins and Phenols; c) Flavonoids; d) Alkaloids (d_{Mayer}, $D_{ragendroff}$); e) Terpenoids; f) Steroids, and g) Saponins

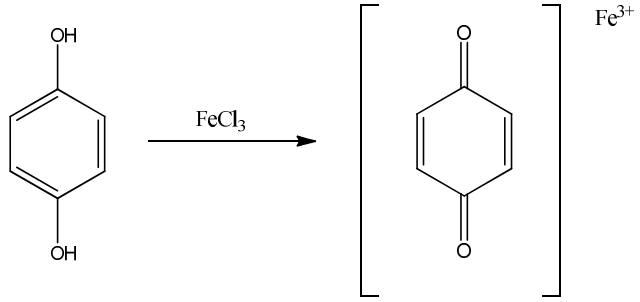


Figure 7. Reaction of identification of simple phenolics by ferric chloride

against insects, fungi, viruses and bacteria, attraction of animals for pollination purposes; antioxidants; control of action of plant hormones; allelopathic agents; and inhibition of enzymes (ZUANAZZI, 2001; BOROKSI *et al*., 2015). Flavonoids also present antibacterial, antispasmodic, antioxidant, anti-inflammatory activity, besides giving an immune response, however, in which there is the production of specific antibodies against a given aggressor agent (SOUZA *et al.,* 2016). The results obtained in this test, tannins were observed are present in *Plumeria rubra* L leaves and flowers. Tannins may be characterized by staining or precipitation reactions when reacting with ferric chloride. Hydrosoluble tannins produce, with a diluted solution of ferric chloride, an intense blue coloration, mainly in alkaline medium. Aqueous solutions of catechin derivatives, in turn, result in a green coloration, with weaker intensity than water-soluble tannins. In the mixture of both types of tannins, green coloration is not observed (WOLF, 2008). The reaction of tannins is showing in Figure 9.

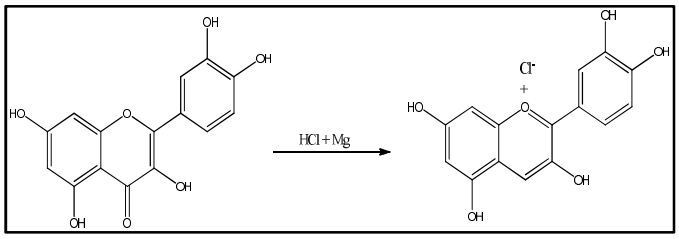


Figure 8. Shinoda reaction for flavonoid identification

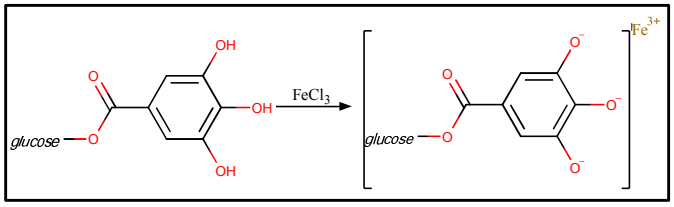


Figure 9. Reaction of identification of a galotanin (hydrolysible tannin)

Plants rich in tannins are used in traditional medicine as remedies for the treatment of diseases. For tannins help in the process of healing wounds, burns, and inflammations by forming a protective layer (complex tannin-protein and polysaccharide) on the skin or damaged mucosa (MELLO and SANTOS, 2001). According to the results obtained, alkaloids were found in the ethanolic extract of *Plumeria rubra* leaves and flowers. Alkaloids can be found in all parts of a vegetable; however, in one or more organs, there will be a preferential accumulation of these substances. It has been observed that animals or insects avoid many plants that produce alkaloids in their diet; this is undoubtedly due to their toxicity or the fact that most alkaloids taste bitter (HENRIQUES, KERBER, MORENO, 2001). According to Souza *et al*. (2016), this group has a variety of actions, such as burns, wounds, respiratory problems, antibacterial and antifungal activity, which can contribute to coronary artery dilation and cancer treatment. For the groups of saponins were proven through the duration of the existing foam for more than 30 minutes, being positive for leaf extract and negative for flower extract. Saponins are glycosylated, polar compounds, which are characterized by presenting surfactant property, that is, being able to form abundant and persistent foam after stirring their aqueous solutions. They can form complexes with proteins and phospholipids of the cell membrane, determining their biological actions (RODRIGUES *et al*., 2010). Its principal physical property is to reduce the surface tension of the water actively. Plants containing saponins also have anthelmintic, anti-inflammatory, larvicidal, molluscicide, anti-fungicidal, antibacterial, antiviral, mucolytic, diuretic and depurative action (SOUZA *et al*., 2016; SCHENKEL *et al*., 2001; RODRIGUES *et al*., 2010; SON *et al*., 2013). Steroids are formed from triterpenes by decarboxylations (ALMEIDA *et al*., 2015). The main biological and pharmacological functions of steroids involved are related to the development and control of the human reproductive system, functioning as cardiotonic, vitamin D precursors, oral contraceptives, agent anti-inflammatory, analgesic and anabolic agents (BESSA *et al*., 2013; RODRIGUES *et al*., 2010) and terpenoids, is recognized for the anti-inflammatory, analgesic, cardiovascular and antitumor effects (BESSA *et al*., 2013). The secondary metabolites of coumarins showed positive results in the leaves and flowers of ethanolic extract. Simple coumarins are not fluorescent, but in alkaline medium, cis-o-hydroxykinetic acid (WOLF, 2008) is formed. The coumarin reaction is showing in Figure 10.

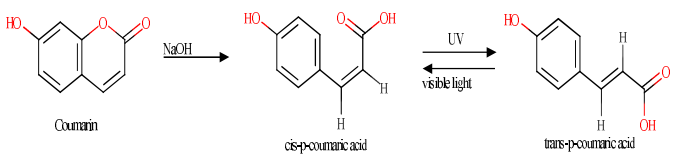


Figure 10. Coumarin identification reaction

Coumarin compounds also used to treat skin diseases such as psoriasis, dermatoses, vitiligo, and some compounds that have an anticoagulant effect. The main biological and pharmacological functions of coumarins are; antimicrobial, anti-inflammatory, antiviral, and antioxidant (BESSA *et al*., 2013; LUZ *et al*., 2014). With this promising potential, more studies should be conducted, such as fractionation, isolations for biological assays such as antibacterial, larvicide, antioxidant, and among others, to contribute to new drugs.

Analysis of antioxidant activity: The evaluation of the antioxidant activity used was the DPPH method, which is fast, simple, and sensitive. This method is based on the capture of DPPH free radicals (2,2-diphenyl-1-picrylhydrazyl) by antioxidants. This reaction produces a decrease in absorbance. The free radical reacts with hydrogen donors in the presence of antioxidant substances, and these donors receive H^+ and then reduced (SILVA *et al*., 2013). DPPH has a purple/violet coloration with maximum absorption in the region of 515 to 528 nm, becoming yellow after receiving the hydrogen atom from the antioxidant species, through an oxireducing reaction (BOROSKI *et al*., 2015). The staining of the DPPH solution in contact with the samples in tests goes from intense purple to yellow. And the intensity of color varies according to the concentration (VEDANA, 2008) (see in Figures 11-12). The higher the consumption of DPPH by a sample, the lower its CI_{50}, and the higher the antioxidant efficiency (SOUSA *et al*., 2007; BOROKSI *et al*., 2015).And the more upper the sample concentration and the lower the absorbance, the higher the consumption of DPPH (NASCIMENTO *et al*., 2011). From Table 3 (ethanolic extract of flowers), a curve of % AA values was constructed depending on the concentration of the extract. The value of IC_{50} is found by linear regression (Graph 1). Considering Graph 1, the values of IC_{50} can be obtained

through the equation of the straight: y = 0.0812x + 9.792, in which y corresponds to the axis referring to % AA and the x-axis to the concentration of the extract. As the IC_{50} represents the concentration of extract needed to inhibit 50% of the radical DPPH, the y = 50 axes were replaced in the straight equation. Thus, 50 = 0.0812x + 9.792. Therefore x = 495.17 ppm. Thus, the CI_{50} of Table 4 of the ethanol extract of the leaves was also calculated. The results of the IC_{50} of antioxidant activities are showing in Table 3 and 4. According to the research result, it was shown that the ethanolic extract of flowers has higher antioxidant potential than the ethanolic extract of leaves.

Because the ethanolic extract of flowers at the highest concentration presented 87.015% of inhibition percentage and foliage at the highest concentration presented 60.202% inhibition percentage. The results of CI_{50} (50% inhibition), in the flower extract, presented its CI_{50} (495.17 ppm) lower than the extract of the leaves that showed its CI_{50} (782.35 ppm). The value of the 50 represents the concentration of extract needed to inhibit 50% of the radical DPPH. The lower this value, the higher the antioxidant efficiency (BOROSKI *et al*., 2015). The standard for the DPPH assay was used ascorbic acid that has proven antioxidant activity.

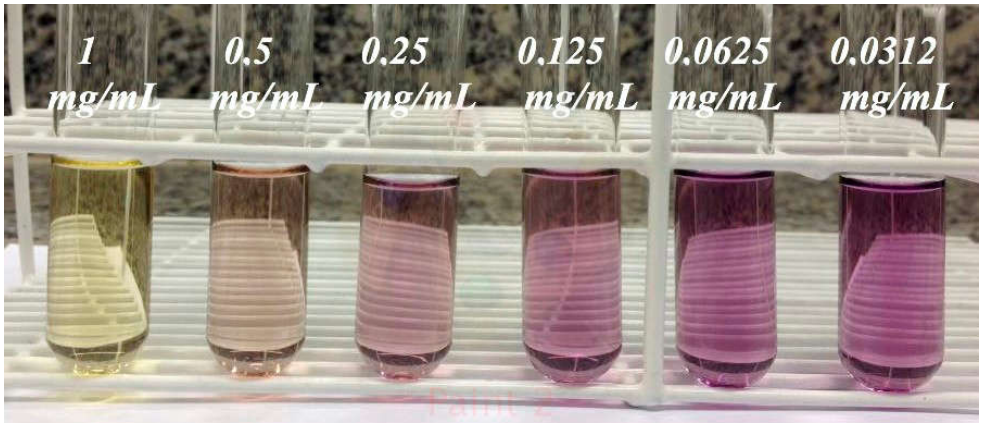


Figure 11. Antioxidant activity of ethanolic extract of flowers of the species *Plumeria rubra* L. evaluated by the DPPH method

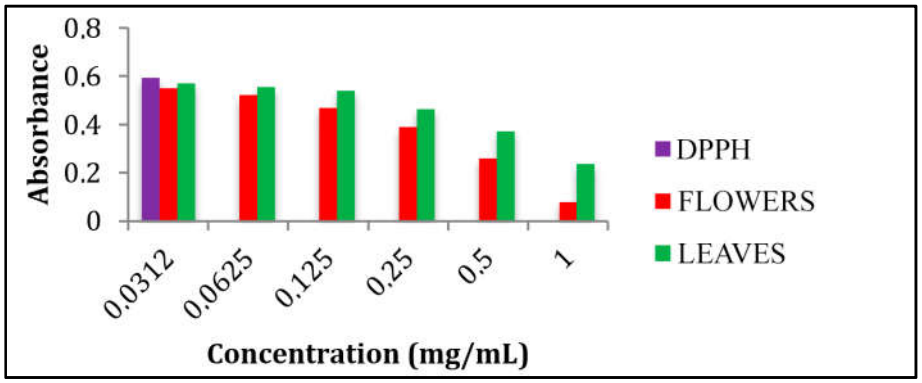
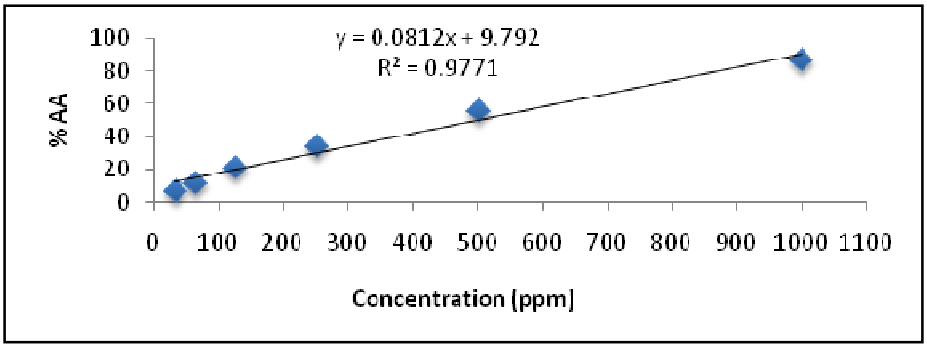


Figure 12. Relationship between the concentration and absorbance of the samples in the consumption of DPPH

Table 3. Antioxidant activity of ethanolic extract of flowers

Concentration(ppm)	Abs ($\lambda = 520\ nm$)	%AA	r	IC_{50} (ppm)
1000	0.08	87.02	± 0.01	495.17
500	0.26	56.38	± 0.02	
250	0.39	34.51	± 0.04	
125	0.47	21.30	± 0.01	
62.5	0.52	12.03	± 0.02	
31.2	0.55	7.31	± 0.02	
DPPH	0.59	0.0	± 0.00	

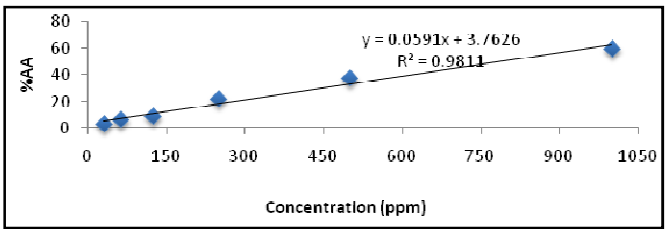


Graph 1. Percentage of antioxidant activity about the sample concentrations of the ethanolic extract of flowers

Thus, it can be used as a parameter for comparing the activities found for the fractions analyzed (WEILER *et al*., 2010).

Table 4. Antioxidant activity of ethanolic extract of leaves

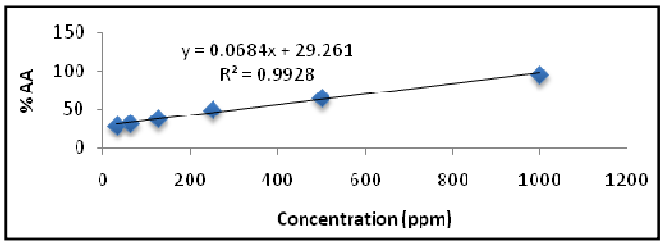
Concentration (ppm)	Abs ($\lambda = 520\ nm$)	%AA	r	IC_{50} (ppm)
1000	0.24	60.20	± 0.04	782.35
500	0.37	37.61	± 0.01	
250	0.46	21.92	± 0.00	
125	0.54	9.05	± 0.00	
62,5	0.56	6.41	± 0.01	
31,2	0.57	3.65	± 0.01	
DPPH	0.59	0	± 0.00	



Graph 2. Percentage of antioxidant activity in relation to the sample concentrations of ethanolic extract of leaves

Table 5. Antioxidant activity of the ascorbic acid pattern

Concentration (ppm)	Abs ($\lambda = 520\ nm$)	%AA	r	IC_{50} (ppm)
1000	0.0200	96.01	± 0.00	303.20
500	0.2100	65.43	± 0.01	
250	0.3000	49.52	± 0.00	
125	0.3700	37.66	± 0.01	
62.5	0.4000	32.49	± 0.02	
31.2	0.4200	29.06	± 0.02	
DPPH	0.5900	0.00	± 0.00	



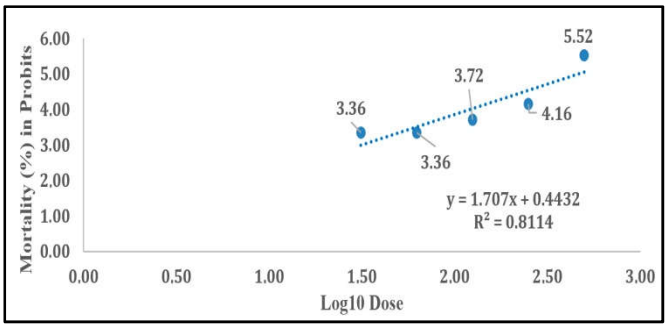
Graph 3. Percentage of antioxidant activity in relation to ascorbic acid concentrations

Ascorbic acid (Table 5 and Graph 3) was used as a standard and presented the CI_{50} equal to 303.20 ppm. In comparing with the pattern, the flower extract shows approximately IC_{50} to the model, and the leaf extract exhibits slightly larger than the ascorbic acid pattern. It means that ethanolic extract from jasmine flowers probably presents moderate antioxidant activity, and ethanolic extract of leaves perhaps presents weak antioxidant activity. The antioxidant activity found in the extracts may be due to the presence of phenolic compounds, flavonoids, tannins, coumarins, and others. Based on the results obtained, it can be concluded that the *Plumeria rubra* L. flower and leaf has potential as a source of natural antioxidants.

Toxicity test against *Artemia salina*: The results of toxicity tests of ethanol extracts of flowers and leaves in front of the *Artemia salina* are showing in Table 5-6 and on Graph 3-4.

Table 6. Result of the toxicity test of ethanol extract of flowers in front of *A. salina*

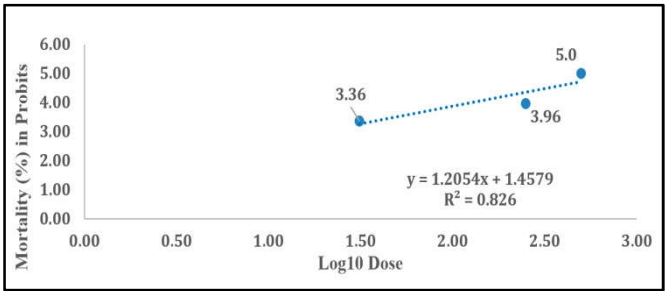
Concentration (ppm)	Total larvae	Deadlarvae	% Mortality	LD_{50} (ppm)
500	20	14	70	478.75
250	20	4	20	
125	20	2	10	
62.5	20	1	5	
31.2	20	1	5	
Control	20	0	0	



Graph 4. Percentage of mortality of larvae *A. salina* in concentrations of samples of ethanolic extract of flowers analyzing by the Probit program

Table 7. Result of the toxicity test of ethanol extract of leaves in front of *A. salina*

Concentration (ppm)	Total larvae	Deadlarvae	% Mortality	LD_{50} (ppm)
500	20	10	50	818.28
250	20	3	15	
125	20	0	0	
62.5	20	0	0	
31.2	20	1	5	
Control	20	0	0	



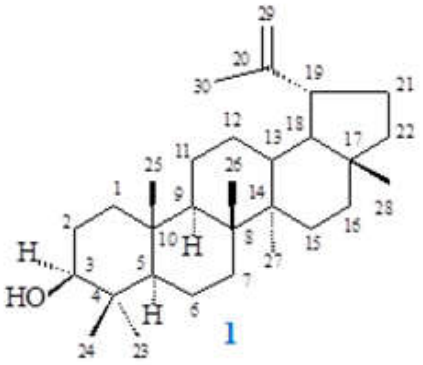
Graph 5. Percentage of mortality of larvae *A. salina* in concentrations of samples of ethanol extract of leaves analyzing by the Probit program

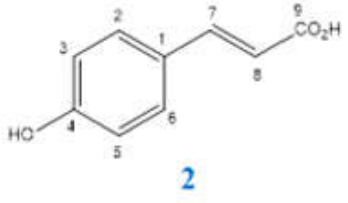
According to the results obtained in the toxicities tests of ethanolic extracts of flowers and leaves of jasmine against *A. salina*, the significant result was obtained. Where LD_{50} 478.75 ppm of flower extract and LD_{50} 818.28 ppm of leaf extract. According to the scale of the toxicity of Amarante et al., (2011), in his study on the toxicity assessment of extracts performed through the bioassay with *A. salina*, low toxicity was considered when the lethal dose 50% (LD_{50}) was higher than 500 ppm; moderate to LD_{50} between 100 and 500 ppm and very toxic when the LD_{50} was less than 100 ppm. According to the results obtained from jasmine extracts, considered quite toxic against larvae, where the ethanolic extract of the flowers presented moderately toxic with the value of LD_{50} = 478.75 ppm, and the ethanolic extract of the leaves exhibited mild toxicity with the value LD_{50} = 818.28 ppm. The median lethal dose (LD_{50}) is the necessary dose to

cause the death of 50% of micro crustaceans in the samples under study. According to Johnson and Johnson (2006), in their study of the classes of toxic plants, the species of *Plumeria rubra* L. considered as the poisonous plant with low toxicity class. With the results obtained, it was concluded that the ethanol extracts of the leaves and flowers of the species *Plumeria rubra* L. presented a possible toxic action, which makes it essential to continue the studies of this plant, with more analysis which could be used in the manufacture of drugs, ornamental sorters and medicinal products.

Characterization and identification of organic compounds of *P. rubra*

Structural determination of the isolated compound (1) and (2): The compound (**1**) was obtained as a white powder with the molecular formula $C_{30}H_{50}O$, which portrays the presence of six degrees of unsaturation, one of them by an oilefinic function. The terpenoid nature of this substance was confirmed by the number of methyl, methylene and methine signals observed in the NMR spectra of ^{1}H and ^{13}C, and approved by the 2D spectra of homonuclear (COSY) and heteronuclear correlation (^{1}H-^{13}C-HMQC and ^{1}H-^{13}C-HMQC), which allowed to characterize the structures of these pentacyclic triterpene as shown in Table 1, it is a pentacyclic triterpene, probably lupeol, i.e., (1R,3aR,5aR,5bR,7aR,9S,11aR,11bR)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-il)icosahydro-1H-ciclopenta[a]crisen-9-ol. The structure was compared with the literature data and is being unprecedented in this species studied (MAHATO; KUNDU, 1994; ALANDER; ANDERSON, 2005; IMAN *et al*., 2007). The compound (2) was obtained as a transparent film aspect with the molecular formula $C_9H_8O_3$, which portrays the presence of five degrees of unsaturation, one of them by an oiling function and the





Lupeol (1)			4-Hydroxicinamic acid (2)	
	δ_C	δ_H	δ_C	δ_H
C				
1			127.65	
4	38.8		161.05	
8	40.8			
10	37.1			
14	42.8			
17	41.9			
20	150.8			
28				
CH				
2			131.03	7.43 (*d*., 9.0)
3	78.9	3.45 (*dd*., 10.15; 3.28)	116.90	6.80 (*d*., 8.4)
5	55.1	1.35 (*dd*., 10.09; 2.08)	116.90	6.80 (*d*., 8.4)
6			131.03	7.43 (*d*., 9.0)
7			145.84	7.55 (*d*., 15.6)
8		1.04	117.05	6.29 (*d*., 15.6)
9	50.4	1.44 (*dd*., 9.33; 1.52)	171.99	
13	38.0	1.54 (*dddd*., 9.66; 9,57; 9.72)		
18	48.1	1.43 (*dd*., 9.33; 1.52)		
19	47.7	2.18(*dd*., 9.33; 1.52)		
CH_2				
1	38.7	1.56; 1.31(m)		
2	27.4	1.72; 1.47 (m)		
6	18.1	1.52; 1.27 (m)		
7	34.2	1.56; 1.31 (m)		
11	20.9	1.52; 1.27 (m)		
12	25.1	1.52; 1.27 (m)		
15	27.4	1.56; 1.31 (m)		
16	35.5	1.56; 1.31 (m)		
21	29.8	1.64; 1.39 (m)		
22	40.0	1.55; 1.30 (m)		
29	109.1	5.11; 4.92 (m)		
CH_3				
23	28.0	0.99 (s)		
24	15.4	0.99 (s)		
25	16.1	1.04 (s)		
26	15.9	1.04 (s)		
27	14.5	1.04 (s)		
28	18.0	1.04 (s)		
30	19.1	1.82 (s)		

other an aromatic. The kinetic structure of this substance as indicated by the number of methylene and methine signals observed in the ^{1}H and ^{13}C NMR spectra, allowing to identify and characterize, as shown in Table 1, a hydroxycinnamic acid, also based on comparison with literature data and being unpublished in this species (VINHOLES; WHITE; SILVA, 1994).

Conclusion

The jasmine plant species (*Plumeria rubra* L.) showed satisfactory results during the research. Where, the plant revealed the presence of some of the secondary metabolite classes, antioxidant activity action, and toxicity activity. Preliminary phytochemical screening revealed the presence of alkaloids, steroid flavonoid coumarins, terpenoids and tannins in the extract of *P. rubra* L. and alkaloid flowers, coumarins, steroids, terpenoids, tannins and saponins in the extract of leaves of *P. rubra* L. However, only the isolation of a triterpene (lupeol) and a cinnamic acid (4-hydroxymynomic acid). The presence of active phytochemicals, the plant can be used medicinally in the future. A more detailed study to arouse the interest of isolation from active principles. The abduction assay of DPPH-free radicals from the samples obtained by ethanolic extracts of flowers and leaves of *P. rubra* L. species showed significant antioxidant activity, compared with the ascorbic acid pattern. Where ethanolic flower extract probably presented moderately antioxidant activity and ethanolic leaf extract perhaps presented its weak antioxidant activity. The toxicity of extracts from flowers and leaves of *P. rubra* L. presented biological activity in front of crustacean *Artemia salina*, in which flower extracts gave moderately toxic, and leaf extracts showed slightly toxic. According to the research results, the species *P. rubra* L. demonstrated the presence of compounds of therapeutic interest, which makes it essential to continue phytochemical and biological studies in the future for the development of new products in the area pharmaceutical, food, cosmetics and among others.

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