

Phenols, flavonoids and antioxidant activity of *Jatropha multifida* L. collected in Pindamonhangaba, Sao Paulo State, Brazil

Abstract

A number of herbs belonging to the genus *Jatropha* of Euphorbiaceae family are noted for their medicinal benefits. It has already been observed the antibacterial, anti-inflammatory, antifungal, antioxidant activity, anti-tumor and others. They are also employed as ornamental plants and energy crops. Qualitative and quantitative methods were used for the evaluation of total phenols and flavonoids in the latex and leaves extract of *Jatropha multifida* Linn and their antioxidant activity. This study was able to confirm the presence of phenols and flavonoids in the samples analyzed and its antioxidant activity corroborating the use of latex of the plant in the pharmaceutical industry.

Keywords: medicinal plant, *Jatropha multifida*, phytochemical composition, flavonoids, phenols, antioxidant activity

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Introduction

Traditional medicinal plants have been recognized as a rich source of candidate compounds for the development of pharmaceuticals.^{1,2} The genus *Jatropha* belongs to the family Euphorbiaceae and has a great variety of species, among them *J. multifida*, *J. curcas*, *J. molissima*, *J. gossypifolia* that are currently the source of studies for the production of biodiesel and also for the medicinal character that have.³

Jatropha multifida (Linn), commonly called coral bush, is characterized as a shrub up to 1.30m high, and can reach 7m in its habitat. It has green leaves up to 17cm long and 23cm wide, multifidus stipules up to 2cm long, red inflorescence up to 20cm long, fruit up to 3cm long and 2.5cm in diameter. The entire plant has whitish latex, so leaves, stem, bark and roots of the *Jatropha* plant have been used in ethno-medicines for a long time principally in India⁴ and in Africa.⁵ *J. multifida* is used in African folk medicine for the treatment of infection, pain, fever, various inflammatory conditions, tumor and tumor related diseases.⁶

Chemical investigation of the *J. multifida* has led to the isolation of nine diterpenoids, including a new jatromulone A, four podocarpane diterpenoids, two lathyrane-type diterpenoids and two dinorditerpenoids.⁷

Among the components already isolated from different parts of the plant and identified are diterpenes 15-*O*-acetyl japotagron, (4E)-jatrogrossidentadione acetate, (4E)-jatrogrossidentadione, multifidone, multidione, multifolone.⁸ Earlier examination of the latex of the plant afforded some cyclic peptides, phenolics and glucosides.⁹

The Genus *Jatropha* is a rich source of phytochemicals that can be utilized in nutritional, agricultural and pharmaceutical industries. The pharmacological properties presented by the plant are diverse, being the latex produced by it the substance that presented the highest therapeutic potential described in the literature to date.

The plant is known to possess different medicinal properties including antimicrobial activity,^{10,11} anti-inflammatory and antioxidant activities,¹² phytochemical and larvicidal properties,¹³ antioxidant, hemolytic and toxic.¹⁴ The stems of *J. multifida* could be regarded as an anti-influenza herbal medicine as well as a potential crude drug source for the development of anti-influenza compounds.¹⁵

According to Adesola and Adetunji,¹⁶ the alcohol extract obtained from leaves, stems or barks showed antifungal activity in a study against *Candida albicans*. The studies of Hirota et al.¹⁴ showed that from the methanolic extract of the leaves three di-C-glycosidic biflavones were identified and the extract presented significant analgesic and anti-inflammatory properties compared to Indomethacin, in addition to being hypotensive.

The leaves, the latex and the fruits of this plant are used in the treatment of infected wounds, skin infection and as a cicatrizing, ulcers, oral thrush, constipation and fever.¹⁷ Roots and stem are used as anticancer, antitumor, cytotoxic, atimalarial, antileishmanial, antimicrobial, insecticidal and molluscicidal because of the diterpenoid Lathyrane.¹⁸

Materials and methods

In this research were used spectrophotometric methods to determine the content of phenols and flavonoids of the hydroalcoholic

Jatropha multifida leaves extract and latex. The evaluation of the antioxidant activity was based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH), which is reduced in the presence of antioxidant substance.¹⁹

The leaves of *J. multifida* were collected from six adult specimens of the plant, in a residence of the municipality of Pindamonhangaba, Sao Paulo State, Brazil. Identification was made by Dr. Gokithi Akisue and sent an exsiccate to the Herbarium of the University of Sao Paulo-USP.

To obtain the hydroalcoholic extract were used 100g of a pool of fresh leaves of the plants. The leaves were washed thoroughly with normal tap water followed by sterile distilled water. Then the leaves were shade dried at room temperature and turned into a fine powder after well grounding using grinding machine.²⁰ Of the powdered leaves, crude extract was prepared by percolation; the solvents used were 50% ethyl alcohol 95% and 50% water. Starting with 100g of the leaves, 200mL of concentrated extract was obtained. The latex was obtained by dripping after cutting small branches of the plants. The hydroalcoholic extract and the latex were used for the research of flavonoids and phenols, and verification of antioxidant activity.

Preliminary phytochemical analysis

Test for flavonoid: 1mL of 2N NaOH was added to 1mL of leaves extract and latex, than the result of yellow color was taken as indicator for the presence of flavonoids.

Test for phenol: To 1mL of leaves extract and 1mL of latex, 1mL of sodium carbonate was added. To that 1mL of folin was added. Formation of blue or green color indicates the presence of Phenols.

Estimation of total flavonoid content in leaves extract and latex of *Jatropha multifida*

a. Construction of the standard curve

Metanolic solutions of quercetin at concentrations of 3.0, 4.0ng up to 10.0µg/ml were prepared. It was transferred to a 10mL volumetric flask containing approximately 5mL of methanol, an aliquot corresponding to each quercetin concentration and 200µL of the aluminum chloride solution. The final volume was set at 20°C. After the time of 30 minutes, the spectrophotometer - FEMTO Model Cirrus 80 - was read at 425nm. The equation of the standard curve was calculated by the least squares method.²¹

b. Flavonoid content in the hydroalcoholic extract and in the latex

A stock solution was made up with 100µL of the hydroalcoholic extract/latex in 10mL volumetric flask and completed with methanol until the meniscus. Then the procedures of item a were repeated. The procedure was done in triplicate.²¹

Estimation of total phenol content in leaves extract and latex of *Jatropha multifida*

a. Construction of the standard curve

A standard curve was constructed with gallic acid at concentrations of 3.0, 4.0µg up to 12.0µg/ml. It was transferred into a 10mL volumetric flask containing approximately 5mL of distilled water an aliquot corresponding to each phenol solution concentration and

800µL of the Folin-Ciocalteu reagent. Within about 4 minutes, 1.2mL of the 20% sodium carbonate-tartrate solution was added. It was allowed to react in a bath at 20°C for 2 hours and the final volume was adjusted, stirred for a few seconds. The absorbance was measured at 760nm in a UV-Visible Spectrophotometer, then the total phenolic content of the samples were estimated using mg Gallic acid equivalents (GAE)/g.^{20,21}

b. Total phenol content in the hydroalcoholic extract and latex

Stock solution was prepared with 1mL of hydroalcoholic extract/latex in a 100mL flask and the volume was quenched with water. Then the procedures of item a were repeated. The procedure was done in triplicate.²¹

Verification of antioxidant activity

For the verification of the antioxidant activity of the extract, the soluble solids content was first performed to know the exact concentration of the same. The latex was not necessary because it was used in its crude form.

For the determination of the soluble solids content, some adaptations of the method used were made. A 25mL beaker containing 5mL of the extract was taken to the oven at 60°C for two hours. After cooling in desiccator, the weighing was done; this procedure was repeated until constant weight. The soluble solids content was calculated according to the following formula:

$$\% \text{ soluble solids (m/v)} = \frac{m_2 - f}{V_a} \times 100$$

m_2 : final beaker mass with dry extract, f: initial mass of the beaker; V_a : volume of the aliquot

After the previous procedure two dilutions were made for the extract, one to 1% and another to 0.01% V/V; for the latex only 0.01%. In eleven tubes numbered from zero to ten, the volume of alcohol, extract/latex in each tube was added. Tube number zero was the control group. The volume of DPPH was added to the first tube and waited 1 minute. DPPH was added to the other tubes every 1 minute. The tubes were shaken. The spectrophotometer was read at 517nm, 30 minutes after the addition of DPPH in the 1st tube. The absorbance (%) versus concentration of extract/latex (µg/mL) was plotted. The EC50 was calculated by the least squares method.²¹ The experiment was carried out in triplicates.

Results and discussion

Phenol and flavonoid contents

Table 1 shows the values of total of flavonoid and phenols found in the latex and hydroalcoholic extract of *J. multifida* L.

Table 1 Total phenols and flavonoids in leaves extract and latex of *J. multifida* L.

	Latex	Leaves extract
Phenols (a)	4,808	0,130
Flavonoids (b)	0,000	2,322

a: Determined as gallic acid (mg GAE/g)

b: Determined as quercetin with $AlCl_3$ (mg/g)

The latex presented 4.808% of total phenols, while the value of flavonoids was null. In the extract, however, there is an inversion in these values, presenting less than 0.5% of phenols and a higher value of flavonoids 2,322%. The results obtained for the extract may have been influenced by the solvent used.²⁰ Rampadarath et al.¹³ also showed the effect of solvent on phytochemical extraction.

The qualitative and quantitative results of phenol and flavonoid compounds in latex and leaves extract of *J. multifida* are in concordance with that obtained by Rampadarath et al.¹³

Soluble solids

The soluble solids content found in the hydroalcoholic extract of *J. multifida* was 45.61%.

Antioxidant activity

The results were given in EC₅₀ (µg/mL), which is equivalent to the amount of extract required to reduce the DPPH radical by 50%.¹⁹

As showed in Table 2, the latex showed antioxidant activity with EC₅₀ value of 3.44 µg/mL while leaves extract 0.01% and leaves extract 1% showed 0.0µg/mL and 1,530.75µg/mL, respectively.

Table 2 Antioxidant activity of leaves extract and latex of *J. multifida* L. expressed in µg/mL of DPPH

	Latex 0.01%	Leaves extract 0.01%	Leaves extract 1%*
Antioxidant activity	3.44	0	1,530.75

*The leaves extract was tested at 1.0% because in its lower concentration did not present activity

Compared to the Green Propolis extract - EC₅₀ 15.71µg/mL,²² and to *Ginkgo biloba* L extract, that has EC₅₀ 38.91µg/mL and is considered by Mensor et al.²³ as an extract of high antioxidant activity, it is clear that the latex of *J. multifida* appears to be an excellent antioxidant. It should be noted that the lower the value of DPPH, the higher the antioxidant activity.¹⁹

The antioxidant activity is characterized by the inhibition or retardation of the oxidation of substances susceptible to this type of reaction, which consists in the elimination or capture of free radicals. Because synthetic antioxidants are toxic and expensive, the search for natural antioxidants has been increasing in studies and research. This activity in natural compounds has been related to the presence of phenolic compounds found in medicinal plants.²⁴

Jatropha plants are described to have several chemical constituents as alkaloids, cyclic peptides, terpenes (a monoterpene, sesquiterpenes, diterpenes and triterpenes), flavonoids, lignans, coumarins, coumarino-lignoids, a non-cyanogenic glucoside, phloroglucinols, ester ferulates, phenolics, deoxypreussomerins and fatty acids¹⁷ which makes them of high interest for the agricultural nutritional and pharmaceutical industries.³

Factors as climatic, seasonal and geographical conditions, age of plant, humidity of the harvested plant material, extraction technique, and the existence of chemo type may result in discrepancy in the composition of extracts of plants,²⁵ which necessitates further studies with samples of specimens from different areas.

Toxic effects have been reported after ingestion of *J. multifida* seeds, which contains a toxalbumin capable of causing agglutination and hemolysis of red blood cells and is also detrimental to other cells. This indicates that the seeds probably should not be used in the production of medicines.²⁶

Conclusion

There is variation in the chemical composition of phenols and flavonoids between leaf extract and latex of *J. multifida*, but this difference can be related to the solvent used in the extraction, and more studies are necessary to determine it with certainty. In relation to the antioxidant activity, *J. multifida* latex presents very expressive and promising activity, but it needs more research on it. Also the presence of phenolic compounds acts as a factor for the antioxidant activity.

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Conflict of interest

The author declares that there is no conflict of interest.

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