Evaluation of solvents extractors of rutin from Dimorphandra Gardneriana (leguminosae) and in vitro antioxidant tests

Avaliação de solventes extratores de rutina de Dimorphandra Gardneriana (leguminosae) e testes antioxidantes in vitro

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ABSTRACT

Dimorphandra gardneriana, popularly known as fava d’anta, is a typical tree from the Brazilian cerrado of large extractive use, due to its fruits being rich sources of the flavonoid rutin, which has pharmacological properties of commercial interest, especially vasodilator and antioxidant. In the present study, rutin was extracted by heating the extractor medium containing D. gardneriana fruits, where the use of different solvents, methanol, ethanol, ethanol/water and water were evaluated. The presence of rutin was analyzed by Thin Layer Chromatography and High Performance Liquid Chromatography. The antioxidant activity of rutin was evaluated at low concentrations using the following methods: DPPH free radical scavenging activity; reaction with thiobarbituric acid; Fe2+ chelation; degradation of deoxyribose; and iron reduction. Quercetin was used as a positive control for the antioxidant test. The yield of the extracts were: 19.13 % (Methanol), 18.23 % (Ethanol), 26.27 % (Ethanol/water) and 13.14 % (water). In the extraction processes, the rutin yield was equivalent for all extractions except for the one with water. Thus, it is concluded that methanol, the main solvent used in the extraction, can be replaced with ethanol or ethanol/water without compromising the rutin efficiency or purification degree. With the exception of the iron chelation test, in the other tests quercetin and rutin had a statistically significant relationship with the antioxidant potential. The efficiency of rutin and quercetin in low concentrations in in vitro antioxidant tests can justify the use of these substances in in vivo test or as positive control in in vitro test.

Keywords: Antioxidants, Dimorphandra gardneriana, extraction, quercetin, rutin

RESUMO

Dimorphandra gardneriana, popularmente conhecida como fava d’anta, é uma árvore típica do cerrado brasileiro de grande uso extrativista, por seus frutos serem fontes rutas da rutina flavonóide, que possui propriedades farmacológicas de interesse comercial, principalmente vasodilatador e antioxidante. No presente estudo, a rutina foi extraída por aquecimento do meio extrator contendo frutos de D. gardneriana, onde foram avaliados o uso de diferentes solventes, metanol, etanol, etanol / água e água. A presença de rutina foi analisada por cromatografia em camada fina e cromatografia líquida de alta eficiência. A atividade antioxidante da rutina foi avaliada em baixas concentrações usando os seguintes métodos: atividade de eliminação de radicais livres de DPPH; reação com ácido tiobarbitúrico; Quelação...
de Fe2 +; degradação de desoxirribose; e redução de ferro. A quercetina foi usada como controle positivo para o teste antioxidante. Os rendimentos dos extratos foram: 19,13% (metanol), 18,23% (etanol), 26,27% (etanol / água) e 13,14% (água). Nos processos de extração, o rendimento de rutina foi equivalente para todas as extrações, exceto a com água. Assim, conclui-se que o metanol, o principal solvente utilizado na extração, pode ser substituído por etanol ou etanol / água sem comprometer a eficiência da rutina ou o grau de purificação. Com exceção do teste de quebração de ferro, nos outros testes a quercetina e a rutina tiveram uma relação estatisticamente significativa com o potencial antioxidante. A eficiência da rutina e da quercetina em baixas concentrações nos testes antioxidantes in vitro pode justificar o uso dessas substâncias no teste in vivo ou como controle positivo no teste in vitro.

**Palavras-chave:** Antioxidantes, Dimorphandra gardneriana, extração, quercetina, rutina

1 INTRODUCTION

Rutin (Fig. 1), a substance belonging to the flavonol subclass, is the most common glucoside form of quercetin and is considered a prominent substance due to its various pharmacological activities, vasodilator and antioxidant activities being among them (Becho *et al.*, 2009; Pedriali, 2005). Polyphenols act in plants protecting them from damage caused by photosynthesis’ by-products and also protect against damage to its tissues and against attack by herbivores plants. Flavonoids, in particular defend against the action of free radicals (Sucupira *et al.*, 2012). Added to the human diet, polyphenols prevent various diseases derived from oxidative stress. It is believed that its performance is linked to the modulation of metabolic pathways through antioxidant activity (Sousa, 2013).

![Figure 1. Structural representation of rutin](image-url)
Dimorphandra gardneriana is an important source of rutin, popularly known as "fava d'anta" or "faveiro", a leguminous tree native of Brazil. Its occurrence is verified in the states of Pará, Maranhão, Piauí, Ceará, Pernambuco, Bahia, Minas Gerais, Goias and Mato Grosso (Landim and Costa, 2012).

The main form of extraction of rutin in D. gardneriana is by heating with use of methanol as a solvent (Santos, 2016) that, due to its polarity, dissolves various substances, being an important input in chemical research (Camargo, 2017). Methanol is a flammable and toxic substance that can cause serious damage to human health, has peculiar narcotic properties, and causes irritation of the mucous membranes. Its massive ingestion produces a state of coma that may persist for up to four days. Considered a cumulative poison, even short exposures to vapors which would not cause harmful effects, when constant, can cause health damage (Pereira and Andrade, 1998). In the atmosphere, the half-life of methanol is approximately nine days. This alcohol may react with the SO$_2$ and sulfuric acid present in the atmosphere, producing dimethyl sulfate, which has been shown in studies to be highly toxic and mutagenic, having been classified as a probable carcinogen for humans (Pereira and Andrade, 1998).

In activities that aim the extraction of natural substances, toxicity, the handling risks, availability and cost of solvent must be observed, as well as extraction efficiency (Falkenberg et al., 2000).

Previous studies made by means of in vitro tests demonstrated the efficiency of rutin and quercetin as antioxidants (Jiménez et al., 2011; Yang et al., 2008). The use of these substances as stabilizers of the antioxidant activity of other compounds as well as food additives has been the main focus of much scientific research (Hernández and Frutos, 2015; Cho et al., 2014).

Techniques of aqueous rutin extraction from D. gardneriana have been tested and improved in several scientific researches in order to minimize environmental pollution and protect human health with reduced costs and enhancement of the final product (Craveiro, 2012). The objective of the present study was to evaluate the rutin extraction yield from raw D. gardneriana pod extracts using different solvents, establishing a comparative analysis between them and establishing the minimum rutin concentrations for antioxidant activity efficiency.
2 MATERIALS AND METHODS

2.1 CHEMICALS AND REAGENTS

Acetonitrile, milli-q and distilled water, ethyl alcohol, methyl alcohol, Activated charcoal powder, celite, standard rutin (99.9 %), standard quercetin (99.9 %), Tris-HCl, thiobarbituric acid (TBA), 1´-1´-diphenyl-2´-pyrlylhydrazyl (DPPH), malonaldehyde bis-dimethyl acetal (MDA), trichloroacetic acid (TCA), methanol, acetone, phenanthroline, iron (II) sulphate, chloridric acid, 2,4,6-Tripyridy1-s-Triazine (TPTZ), Sodium acetate buffer, Iron(III) chloride were obtained from Sigma (St. Louis, MO, USA). All chemicals were of analytical grade. The solvents and reagents were obtained from the company Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

2.2 HPLC INSTRUMENTATION AND CONDITIONS

The Shimadzu Chromatograph, model LC-20 AT, with shim-pack CLC-ODS (250 x 4.6 mm) column and UV detector, absent furnace and quaternary pump were used. The mobile phase consisted of water (20 %), acetonitrile (40 %) and methanol (40 %). All samples were filtered through 0.20 μm membranes, and 20 μL were injected using a manual valve. Pumping occurred at a flow rate of 0.5 mL.min⁻¹. The detector was set to 275 nm and 320 nm wavelengths.

2.3 PLANT MATERIAL

Dried fava d'anta pods were acquired between June and July 2013, from the Horizonte district, in the city of Jardim, state of Ceará, Brazil. Species specimens were collected for botanical identification and exsiccates, registered under nº 10862, were deposited at the Caririense Dârdano de Andrade Lima Herbarium (HCDAL) at the Regional University of Cariri (URCA).

2.4 OBTAINING THE CRUDE EXTRACT

Fava d’anta fruits were milled and extracted using the decoction method, adapted from Paniwnyk et al. (2001), with different solvents. In the decoction process, 100 g of the ground pods and 1000 mL of the solvents were subjected to extraction, where after 2 h material separation was performed by common filtration and the material was concentrated. Following the cooling of the filtrate, precipitate formation occurred which was separated by common filtration.
The solvents used were: methanol, ethanol, ethanol/water (1:1) and water, all of which were subjected to the same procedure as described above. With the water extraction, material concentration was not necessary in view of the poor solubility of the desired substance at room temperature.

The crude extract was dried in an incubator with a temperature surrounding 60 °C. The thin layer chromatography (TLC) methodology on silica gel was used to confirm the presence rutin in the extracts obtained. Commercially acquired rutin with a purity percentage of 99.9 % was used as the standard. Methanol was used as the mobile phase. All study plates were developed with a spray containing a 2 g ferric chloride solution dissolved in 100 mL of methanol.

2.5 RUTIN PURIFICATION

The extract obtained with the use of different solvents passed through a purification process through crystallization to obtain rutin. A 1 g mass from each material obtained in each extraction was dissolved in 20 mL of methanol, then 50 mg of powdered active charcoal were added and the solution was filtered through a celite pad. The methanol evaporation ensued, followed by drying and subsequent yield calculation.

2.6 RUTIN QUANTIFICATION

The material was quantified by HPLC with acetonitrile (20 %), methanol (40 %) and doubly distilled water (40 %) as the mobile phase. For the construction of the calibration curve, a 0.2 mg.mL⁻¹ standard methanolic stock solution was prepared. From this solution, six dilutions with concentrations of: 0.12, 0.10, 0.08, 0.06, 0.04 and 0.02 mg.mL⁻¹ were obtained. A stock solution of purified rutin was prepared as described for standard rutin, this procedure was performed for all extractions and the 0.08 mg.mL⁻¹ dilution was used for analysis. All samples were injected in triplicates. The UV detector was set to a wavelength of 320 nm.

2.7 RUTIN HYDROLYSIS

To obtain quercetin, rutin hydrolysis was carried out in an aqueous sulfuric acid medium (5 %) using a reflux system for 6 h at 80 °C. After cooling, the reaction mixture underwent successive distilled water treatments to control a pH 6.
The quercetin obtained was purified with heated ethyl alcohol, treated with powdered active charcoal and filtered to form a precipitate. After complete drying, the material was monitored by TLC using commercial standards.

2.8 DPPH FREE RADICAL SCAVENGING ACTIVITY

The preparation of methanolic solution of rutin and quercetin was carried out, in concentrations ranging from 25 µg.mL\(^{-1}\) to 1.0 µg.mL\(^{-1}\). The methanolic solution of DPPH was prepared protected from light at a concentration of 24 µg.mL\(^{-1}\). For the analysis, 1 mL of the DPPH solution was added to 2.5 mL of each concentration of rutin and quercetin. A negative control was made using only methanol and DPPH solution. A blank test of the concentrations was also done, made in the absence of DPPH solution. After 30 min of reaction in the dark, at room temperature, the absorbance reading was carried out at UV-Visible spectrophotometer of Shimadzu Manufacturer, Model 10S Genesys, set to 518 nm. The consumption of DPPH was calculated in percentage according to the equation:

\[
\text{Antioxidant Activity (\%)} = \left( \frac{A_{\text{sam}} - A_{\text{bla}}}{A_{\text{con}}} \right) \times 100
\]

In which \(A_{\text{sam}}\) corresponds to the absorbance of each sample, \(A_{\text{bla}}\) corresponds to the absorbance of the blank test referring the sample and \(A_{\text{con}}\) is the absorbance of the negative test.

2.9 IRON CHELATION ASSAY

As described in Puntel et al. (2005) with minor modifications, phenanthroline ethanolic solution of 0.25 % aqueous solution of FeSO\(_4\) and 2 mM TRIS HCl 0.1M (pH 7.4) were prepared. Samples of rutin and quercetin were prepared with methanol yielding concentrations ranging from 25 µg.mL\(^{-1}\) to 1.0 µg.mL\(^{-1}\). For the analysis, solutions were prepared containing 100 µL of each concentration, 300 µL FeSO\(_4\) (2mM), 438 µL distilled water. Two negative controls were made without rutin and quercetin, one of them however also did not have FeSO\(_4\) solution (2 mM). All samples were incubated, protected from light for a period of 5 min, then proceeded with the addition of 26 µL phenanthroline 0.25 % in all samples except the blank. The absorbance was measured on a Spectrophotometer UV-Visible at 510 nm. The chelating ability was expressed as a percentage according to the equation:

\[
\text{Antioxidant Activity (\%)} = \left( \frac{A_{\text{sam}} - A_{\text{bla}}}{A_{\text{con}}} \right) \times 100
\]
In which $A_{sam}$ corresponds to the absorbance of each sample, $A_{bla}$ corresponds to the absorbance of the blank test referring the sample and $A_{con}$ is the difference between the absorbances of the two controls.

2.10 DEOXYRIBOSE DEGRADATION

The technique was adapted from Puntel et al. (2005). The 1mM iron sulfate solutions, deoxyribose 20 mM, potassium phosphate buffer (TFK) 0.5 M pH 7.5, 10 mM hydrogen peroxide, 2.8 % TCA and 0.6 % TBA were prepared. Methanolic solutions of rutin and quercetin were prepared at concentrations ranging from 25 µg/mL to 1.0 µg/mL. The test samples contained 100 µL of each concentration being added 80 µL of TFK 0.5 M, 120 µL of deoxyribose, 80 µL of H$_2$O$_2$, 80 µL of iron sulphate and 340 µL of distilled water. The blank samples did not contain deoxyribose, H$_2$O$_2$ and ferrous sulfate. A solution called induced was prepared without the substances rutin and quercetin.

All samples were incubated for 30 min at 37 °C. Elapsed this time, all samples received 800 µL of TCA, 400 µL of TBA and 500 µL of water, followed by further incubation for 20 min in boiling bath (100 °C). The standard curve followed the same testing procedure, without the phospholipid solution and the substance’s solutions, which is replaced with a MDA 320-500 µM. The reading of the absorbance was carried out in a UV-Visible Spectrophotometer at 532 nm. The results were expressed as a percentage, according to the quantity of present MDA, being induced solution equivalent to 100 %.

2.11 INHIBITION OF LIPID PEROXIDATION

The technique was adapted from Puntel et al. (2005). The solutions were prepared; egg phospholipid, TBA 0.6 % pH adjusted to 5.0 – 6.0 with NaOH. For acetic acid buffer solution 56.2 mL of 1 M acetic acid was added to 1 mL of 1 M sodium acetate supplementing the volume to 100 mL (pH 3.0), and iron sulfate 60 µM. Methanolic solutions of rutin and quercetin were prepared in concentrations between 25 µg.mL$^{-1}$ and 1.0 µg.mL$^{-1}$. In 500 µL of each test solution contained 50 µL of solution with specific concentration, 100 µL of phospholipid, 14 µL of FeSO$_4$ and 336 µL of distilled water. The blank samples did not contain phospholipid and FeSO$_4$. A solution called induced was prepared without the substances rutin and quercetin.

All samples were then pre-incubated for 1 h at 37 °C, passed the pre-incubation period the solutions received 500 µL of acetic acid buffer and 500 µL of 0.6 % TBA, and were again
incubated for 1 h at 100 °C. 1.5 mL of butanol were added and then centrifuged for 2 min at 2000 rpm. A 200 µL aliquot of the supernatant of each solution was collected and the reading was performed in a UV-Visible Spectrophotometer at 532 nm. The standard curve followed the same testing procedure, without the phospholipid solution and the substance’s solutions, which is replaced with a MDA 320-500 µM. The reading of the absorbance was carried out in a UV-Visible Spectrophotometer at 532 nm. The results were expressed as a percentage, according to the quantity of present MDA, being induced solution equivalent to 100 %.

2.12 FRAP (FERRIC-REDUCING ANTIOXIDANT POWER)

The preparation of the FRAP reagent followed the methodology described in Rufino et al. (2006). For analysis, aliquots of 10 µL of samples containing methanolic solutions of rutin and quercetin in concentrations ranging from 25 µg.mL⁻¹ to 1.0 µg.mL⁻¹ were transferred into microdilution plates, followed by addition of 200 µL of FRAP reagent. The negative control was made without the inclusion of samples, and blank for each concentration without FRAP reagent. All solutions for analysis were kept in a water bath at 37 °C for 30 min. The absorbance reading was then carried out at a Thermoplate ELISA reader, model TP-Reader with RS232 output, adjusted to 595 nm. The results were presented based on the registered absorbance.

2.13 STATISTICAL ANALYSIS

Programs used for statistical analysis were GraphPad Prism Software, Version 5.00, 2007 and Microsoft Excel 2010. The results were expressed as mean ± standard deviation (S.D.) and was considered significantly different when obtained $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 EVALUATION OF EXTRACTOR SOLVENTS

For the crude extracts obtained, the solvent which provided the most efficacy was the ethanol/water (1:1) combination with an average yield of 26.27 %. Purification of the extracted extract using different solvents obtaining rutin showed no difference in yield at the 5 % probability level, as shown in (Table 1).
Table 1. Rutin extraction yields from fava d’anta with different solvents before and after purification.

| Utilized Solvent | Extract Yield (%) | Rutin Yield (%) |
|------------------|-------------------|-----------------|
| MeOH             | 19.13± 1.82       | 67.00± 14.17    |
| EtOH             | 18.23± 0.74       | 79.66± 11.93    |
| EtOH /H₂O        | 26.27± 2.13       | 65.33± 8.50     |
| H₂O              | 13.14± 2.51       | 63.33± 6.02     |

The results are expressed as mean ± standard deviations (SD) of three determinations. Means followed by different letters in the same column differ by Tukey’s test with a 5% probability level.

By analysing the rutin percentage obtained from the crystallization process, the extract yield difference obtained with ethanol/water is compensated such that at the end of the process, there are no significant differences in rutin extraction between the extractions using methanol (12.81 %), ethanol (14.51 %) or ethanol/water (17.16 %). Water extraction compromises the final rutin yield, this being around 8.3 %, due to poor solubility of the substance in said solvent, even under high temperature.

TLC comparative analysis using standard commercial rutin allowed confirmation of the rutin extracted with the use of different solvents. The UV absorption calibration curve for rutin at 320 nm allowed the development of the linear equation y = 10927628.57x + 201940.96 (R² = 0.99), whereby the rutin purity degree was identified. Rutin extracted with methanol was 83.59 % pure, while rutin obtained with ethanol had 97.19% purity, and with ethanol/water 99.28 % purity, however it was not possible to determine the purity degree for the rutin obtained by water extraction.

3.2 ANTIOXIDANT TESTS

Quercetin, according to the results (Fig. 2), has a higher antioxidant activity to reduce the DPPH radical compared to rutin, even at low concentrations such as 1.0 and 2.5 µg/mL quercetin maintains levels of free radicals extremely low. The effective concentration able to reduce 50% of DPPH was 2.01 ± 0.12 µg.mL⁻¹ for rutin and 0.61 ± 1.05 µg.mL⁻¹ to quercetin. By donating an electron to the DPPH free radical quercetin and rutin become oxidized, but stabilized by resonance.
The results did not demonstrate efficient activity of quercetin and rutin as chelating agents at low concentrations, being the best percentage of chelation obtained with 25 µg.mL⁻¹ of rutin and quercetin (Fig. 2). Both quercetin and rutin were efficient in kidnapping the hydroxyl radical, and all concentrations tested for the two substances showed satisfactory results as shown in Fig. 2. The concentration to inhibit 50% of MDA formation to quercetin was 2.01 ± 4.27 µg.mL⁻¹ and rutin, 21.50 ± 0.31 µg.mL⁻¹. According to the results, quercetin has a great advantage in the antioxidant potential to prevent lipid peroxidation in relation to rutin, with a difference of extremely significant results. As seen in Fig. 3, quercetin has greater potential for iron reduction than rutin, and the in concentration of 25 µg.mL⁻¹ the difference between the two materials is highly significant with $p < 0.01$. 
swelling helps to expand the surface area for solute/solvent contact (Yang and Zhang, 2008). Thus justifying the ethanol/water combination efficiency which, in addition to minimizing toxic extraction effects since methanol use was not necessary, also reduced the costs of the process. For Machado et al. (2008), abiotic factors such as: solar radiation, drought or rainy periods, nutrients and season of the year, as well as artificial factors, such as pollutants, directly interfere in the mechanisms of metabolic production, which may justify the small yield variations in this study.

Among the several extractions with solvent variations, the ethanol/water extraction presented the best results confirming the efficiency of this solvent combination in rutin extraction from the fava d'anta. Although more sophisticated methods which reduce the time and solvent volume used exist, these require the use of expensive equipment and preparation to handle them, such as ultrasonic equipment (ultrasonic probe and ultrasonic bath), planetary mill and microwave ovens. The decoction method is easy to understand, requiring only basic equipment present in any laboratory.

The results show that, in the proposed concentrations, the studied methods point efficient antioxidant activity for both rutin and for quercetin, and in the latter, lower concentrations already have antioxidant activity with a significant difference from rutin, as can be seen in Fig. 2. This difference can be explained by structural factors such as the number and position of hydroxyl groups, type and position of glycosylation and degree of sterile
impediment in the hydrogen abstraction site, which influences the antioxidant activity of these compounds, leading to different antioxidant potentials in several polyphenolic compounds (Sousa, 2013).

The DPPH is characterized as a stable free radical due to the presence of alternating double bonds in the benzene ring, giving the molecule one resonance effect that allows the delocalization of the unpaired electron throughout its length, aiding in its stability, only strong reducing reagents are able to react with this radical (Borges et al., 2011; Alves et al., 2010).

Iron is scavenging in vivo by proteins, hindering their participation in oxidation reactions mediated by free radicals. Therefore the tested substance’s ability to reduce the iron ions, reflects its ability to reduce the formation of reactive oxygen species, which gives them antioxidant characteristics (Sousa, 2013). Some flavonoids exhibit strong antioxidant activity on free radicals but demonstrate pro-oxidant activity in the presence of transition metals (Alves et al., 2010). Some studies mentioned by Behling et al. (2004) found that the pro-oxidant activity of flavonoids is directly linked to the total number of hydroxyl groups, suggesting that factors related to antioxidant activity are also somehow related to pro-oxidant activity, which requires more detailed and clarifying studies.

The hydroxyl radical (HO•) can be readily kidnapped in vitro and is very efficient in tests with antioxidants. However, the same does not occur in vivo, requiring high amounts of antioxidants. This is due to the short half-life of this radical (Alves et al., 2010; Vignoli et al., 2012; Lima and Adballa, 2001). Substances with antioxidant properties can compete with deoxyribose for the hydroxyl radical, preventing lipid peroxidation and the formation of MDA, with consequent decrease of absorbance. As the concentrations are low, in vivo tests were needed to confirm the effectiveness of the antioxidant potential of these substances against hydroxyl radical, which presents different behavior in biological organisms.

TBARS is an assay used to estimate the peroxidation of lipids in biological membranes and systems (Costa et al., 2012). Lipid peroxidation was assessed by the presence of substances reactive to thiobarbituric acid (Nasser et al., 2011). The homogenized egg yolk was used as substrate rich in lipids. In the oxidation process occurs the donation of a hydrogen atom from a chain of phospholipid unsaturated fatty acids generating lipid peroxides, spreading in a chain reaction, having as product MDA that reacts with TBA denouncing the occurrence of the lipid peroxidation process (Costa et al., 2012; Sreelatha and Padma, 2009).

The ferric complex tripiridil-triazine has light blue color, in the presence of an antioxidant under acidic conditions it undergoes reduction, forming the ferrous complex
tripiridil-triazine that has an intense blue color and a maximum absorption at 595 nm, being the antioxidant power evaluated by the increase in absorbance. The degree of hydroxylation and the extent of conjugations relate to iron reducing capability. Low absorbance values can be positive for antioxidants as the Fe$^{2+}$ is more reactive than the Fe$^{3+}$ to form hydrogen peroxide, which may have a pro-oxidant effect (Sousa, 2013).

The advantage of quercetin as an antioxidant substance regarding the rutin is evident in all in vitro tests carried out in this study. One issue to be addressed in further research is how the absorbs rutin and quercetin. While there are studies showing that the glycosylated Quercetin is absorbed more than its aglucone form, there are controversies, since there are also studies showing that rutin was less absorbed than quercetin (Behling et al., 2004).

4 CONCLUSION

Based on the results obtained, methanol, the main solvent used in rutin extraction, may be replaced with ethanol, or an ethanol/water (1:1) mixture without compromising the yield and purification degree of the substance. Only the use of water as a solvent extractor was not as efficient as the others. Preventative measures for diseases caused by excessive methanol exposure may be taken by using less toxic solvents, which in addition to preserving human health contributes to the balance of the ecosystem.

The results of the tests for antioxidant activity suggest that the substances rutin and quercetin may demonstrate antioxidant efficiency, as low concentrations already presented satisfactory results in most in vitro tests performed in this study, which enables its use in in vivo testing as well as the production of products based on rutin and quercetin, aimed at promoting a better use of their therapeutic properties, reducing potential damage to biological systems.

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