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Research Article

HPLC Isolation of Rutin, Hesperidin and Quercetin from *Ruta Chalepensis* Extract Growing in Iraq

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ABSTRACT

Background: Ethnopharmacological relevance: *Ruta chalepensis L* For years, various portions of this plant have been utilized in traditional medicine to treat a number of illnesses, including nervous disorders, rheumatism, menstrual issues, fever, convulsions, mental disorders, dropsy, and neuralgia. **Objection:** In this study, we aim to characterize three flavonoids extracted from *Ruta chalepensis*. **Material and methods:** In this study, various concentrations (80% and 100%) of methanol and ethanol in water were used as solvents in the extraction of flavonoids (rutin, hesperidin, and quercetin) from *Ruta chalepensis L*. by using conventional extraction methods (reflex and maceration). **Result:** HPLC results confirm Using maceration with 80% methanol was recommended for extracting flavonoids from *Ruta chalepensis L*. to obtain the highest yields, whereas reflex with 80% ethanol showed a slight increase in quercetin amount. The phytochemical screening of *Ruta chalepensis* L. exhibited the presence of flavonoids. **Conclusion:** The highest amount of the three flavonoids was found in maceration with 80% methanol.
Key words: Reflex, Maceration, Ethanol, Methanol, Extraction.

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INTRODUCTION

The biological activity and therapeutic efficacy of various plant parts are attributed to the bioactive phytochemical elements that are found in them. Secondary metabolites called flavonoids have a benzopyrone ring with phenolic or polyphenolic groups attached at various places. The most typical places where they can be found are in seeds, cereals, nuts, vegetables, flowers, herbs, stems, and fruits. Over 10,000 flavonoid chemicals have so far been isolated and figured out. The majority of flavonoids are commonly used as pharmaceuticals.[1] These are produced naturally using the phenylpropanoid pathway, and their bioactivity depends on how they are absorbed and how readily they are available to the body.[2] They are divided into different categories according to their level of unsaturation, degree of carbon ring oxidation, and chemical composition. The various subclasses of flavonoids include Isoflavonoids, chalchones, flavanonols, Anthaxanthin's (flavanone and flavanol), flavanones, flavans, and anthocyanidins All of these flavonoids can be found in large quantities in the natural world.[3] Numerous health advantages come with eating more foods abundant in flavonoids.[4] Since all these organic substances benefit human health, considerable effort is being undertaken to separate these substances from different plants. Citrus fruits, for example, are abundant in flavonoids. Grapes, Oranges, and lemons contain the flavonoids hesperidin and naringenin.[5] Mulberry contains quercetin and anthocyanins glycosides flavonoids.[1]

Flavonoids have potent antioxidant activities, also can inhibit platelet aggregation, antibacterial, anticancer actions, and anti-inflammatory. They also have great antioxidant capacity, reduce alpha-tocopherol radicals, can eliminate free radicals, and activate antioxidant enzymes.[6]

Fringed rue is the popular name for *Ruta chalepensis L. (family: Rutaceae)*, a perennial herb that is indigenous to North Africa and Eurasia and grows to a height of about 80 cm. This little shrub is now found throughout the world and was brought to America following Spanish colonization.[7] Various parts of this plant have been used for centuries in traditional medicine to treat a variety of conditions, including nervous disorders, rheumatism, menstrual issues, fever, convulsions, mental disorders, dropsy, and neuralgia.[8-10] Its anti-parasitic, antibacterial, anti-cancer, anthelmintic, anti-acetylcholinesterase, antioxidant, analgesic, and anti-inflammatory effects were determined by pharmacological analyses.[7]

Due to the chemical content of R. chalepensis, phytochemical investigations of its Roots, aerial parts, and fruits have revealed a prospective therapeutic use for this plant in neurological illnesses.[11,12] Since screening revealed the presence of triterpenes, saponins, coumarins, flavonoids, alkaloids, sterols, tannins, cardiac glycosides, and anthraquinones. the aerial parts of *R. chalepensis* appear to be particularly responsible for the significant effects of the plant.[11] Additionally, the presence of rutin, quercetin, scopoletin, and kaempferol in the medium polar fraction of an ethanol extract of the aerial portions of R. chalepensis results in substantial antioxidant activity.[13]

MATERIALS AND METHODS

Plant

Family: *Rutaceae*

Genus: *Ruta*

Species: *R. chalepensis*

Plant sampling

The arial part of *Ruta chalepensis L.* plant were cultivated in a private orchard at Al-FAW

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district, Basra, and authenticated by Asst. Prof. Dr. Ula AlMousawi, Pharmacognosy Department, Pharmacy College, Basra University. Fresh material of the plant sample was collected in August (2022), cleaned, shade dried for seven days at room temperature and crushed to medium-milled pieces and stored in airtight bottles.

Extraction of flavonoids from *Ruta chalepensis L.*

Two methods (Cold extraction and hot extraction), solvent (80% Ethanol-80% Methanol-100% Ethanol-100% methanol).

Cold extraction (Maceration) Time: 24h hot extraction Reflux Time 2-3 hours heat: 50 C For each method of extraction, 10 g of medium-milled *Ruta chalepensis L.* was placed in a glass container with 200 ml of diethyl ether, which underwent defatting for two hours, filtration with filter paper, the collected plant was dried at room temperature for 24 hours. Reflex and maceration were used as methods of extraction with 250 ml of two different solvents (ethanol and methanol) and two different concentrations (100% and 80%, respectively)(solid-liquid ratio 1:25 W/V),[14,15] with modification.

RESULTS

Detection of flavonoids by phytochemical tests

Alkaline reagent test: A few drops of sodium hydroxide solution were added to the extracts. The presence of flavonoids is indicated by the formation of a bright yellow hue that becomes colorless when diluted acid is added.[16]

Shinoda test: A small piece of magnesium ribbon was added to the alcoholic solution of the crude extract, followed by the dropwise addition of concentrated HCL, which reveals the presence of flavonoids by the formation of a color ranging from orange to red within one to two minutes.[17]

Ferric chloride test: Two milliliters of 10% aqueous ferric chloride were added. The appearance of a bluish color indicated the presence of flavonoids in the extracts.[18,19]

Chromatographic analysis for the detection of flavonoids in crude extracts

High performance liquid Chromatographic analysis (HPLC) for the detection of flavonoids in crude extracts: HPLC analysis carried out using Sykam HPLC system S 600 series (Germany) connected to S 1130G quaternary gradient pump, S5300 sample injector, S3250 UV/VIS detector and S 4115 column oven. Before the final validation and real samples analysis, the sample preparation, mobile phase, and stationary phase conditions were optimized. Solutions for each of the rutin, hesperidin, and quercetin standards were prepared, 10µl of each standard solution was prepared, a mixture of the three standards was prepared, and a mixture of the rutin standard solution with crude extract was injected into the HPLC system. The detection was accomplished through elution with a mobile phase of acetonitrile and water (30%:70% v/v), a flow rate of 1.0 ml/min, and UV detection at 254 and 30 C. HPLC analysis resulted in the detection of rutin, hesperidin, and quercetin together in a crude extract by comparison of the retention time to the retention time of the standard solution.

DISSCUSION AND CONCLUSION

Figures 2-7 show reverse-phase HPLC chromatograms of each standard solution, a mixture of the three standard solutions, and a mixture of the rutin standard solution and crude extract. Flavonoids extracted from *Ruta chalepensis L.* were identified by comparison of their retention times with those of the reference standards. The mixture of rutin standard solution with crude extract showed a sharp peak at 3.313 min. that confirmed the rutin peak position as shown in figure 6.

To determine the content of the flavonoids, calibration curves were prepared in the range of 5–10,000 to 10000 µg/mL for each standard solution. increased linearly for all standards over the indicated concentration range (Table 3). The calibration curve is given in Figures 8 and 9. The injection volume for standard solutions and crude extracts was 50 µl for each run. The levels of flavonoids present in crude extracts and the results are shown in Table 4. The main compounds were determined.

The yield of extraction depends on the solvent with varying polarity, extraction method, temperature, and extraction time. In this work, *Ruta chalepensis L.* extracts were obtained by using different concentrations of methanol and ethanol (80% and 100%, respectively). The highest amount of the three flavonoids was found in maceration with 80% methanol. HPLC results confirm Using maceration with 80% methanol was recommended for extracting flavonoids from *Ruta chalepensis L.* to obtain the highest yields, whereas reflex with 80% ethanol showed a slight increase in quercetin amount.

The most important factor in determining the success of an extraction procedure, especially for liquid-solid extraction, is the solvent utilized. According to the "like dissolves like" concept, a solvent having a polarity value close to the polarity of the target substance is more likely to dissolve it, and vice versa. This theory explains why rutin, hesperidin,

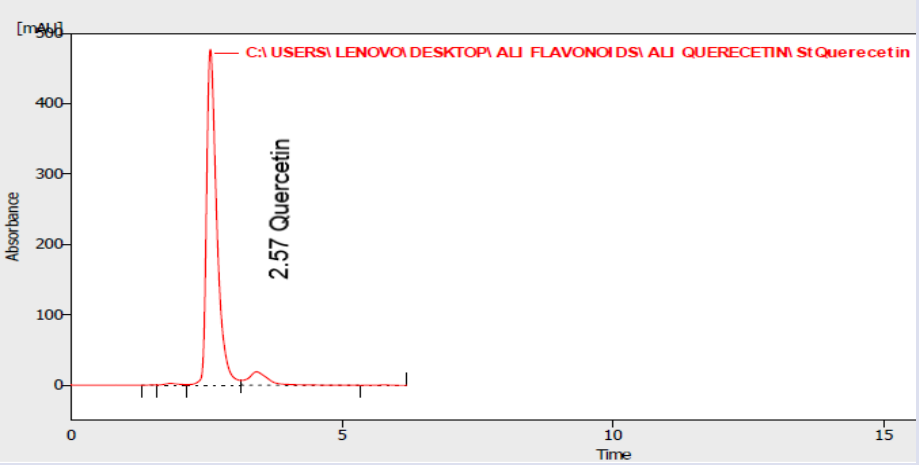

Figure 1: HPLC chromatogram of Quercetin stander

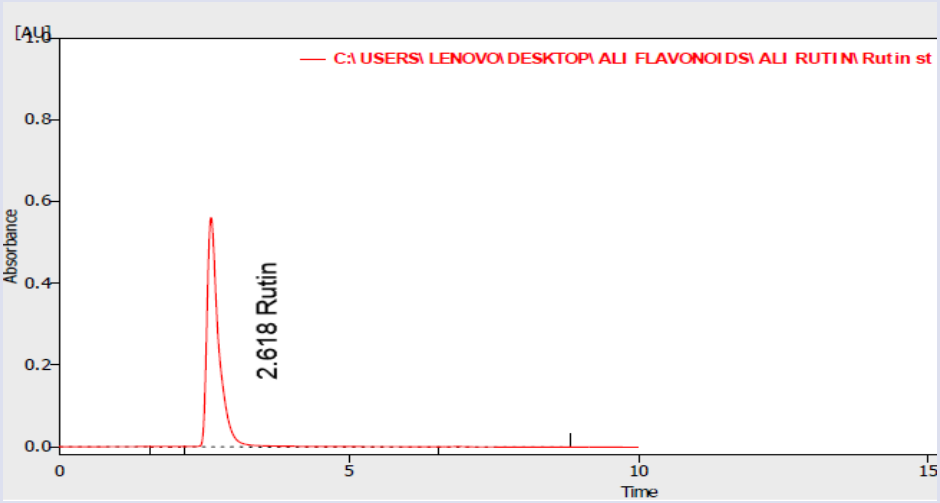

Figure 2: HPLC chromatogram of Rutin stander

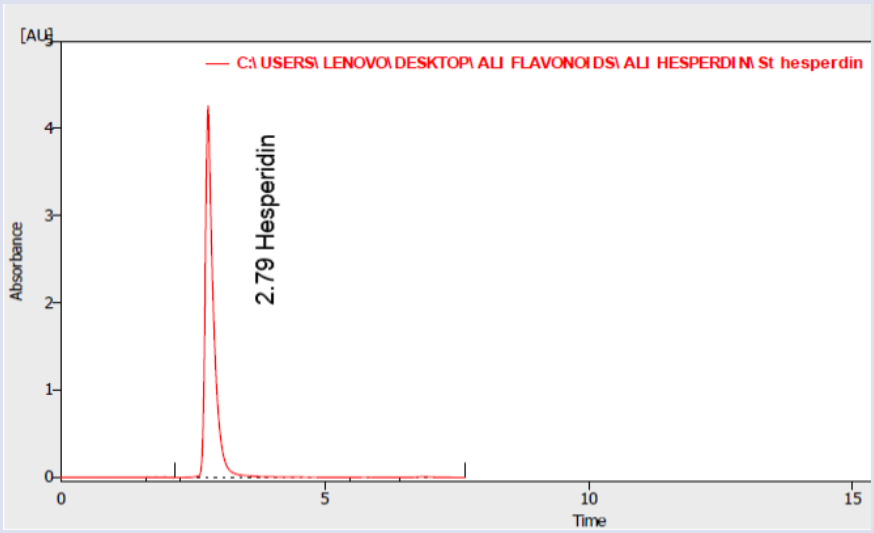

Figure 3: HPLC chromatogram of Hesperidin stander

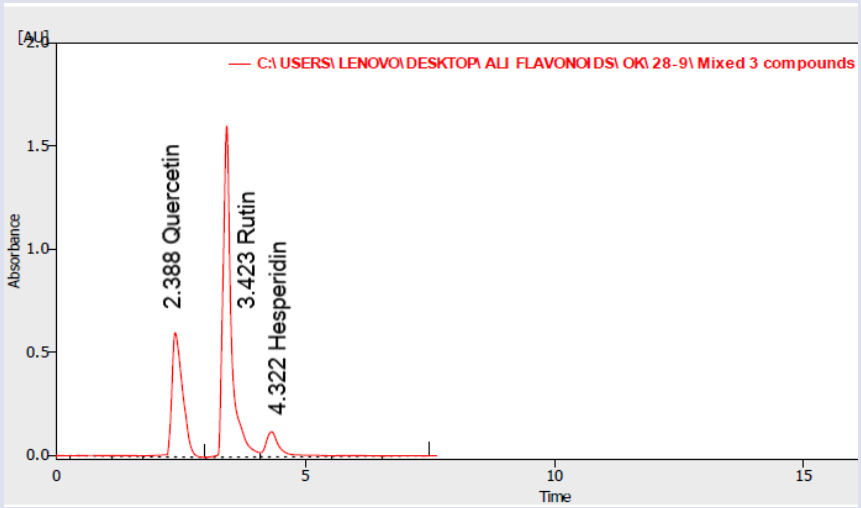

Figure 4: HPLC chromatogram of mixture of the three standers

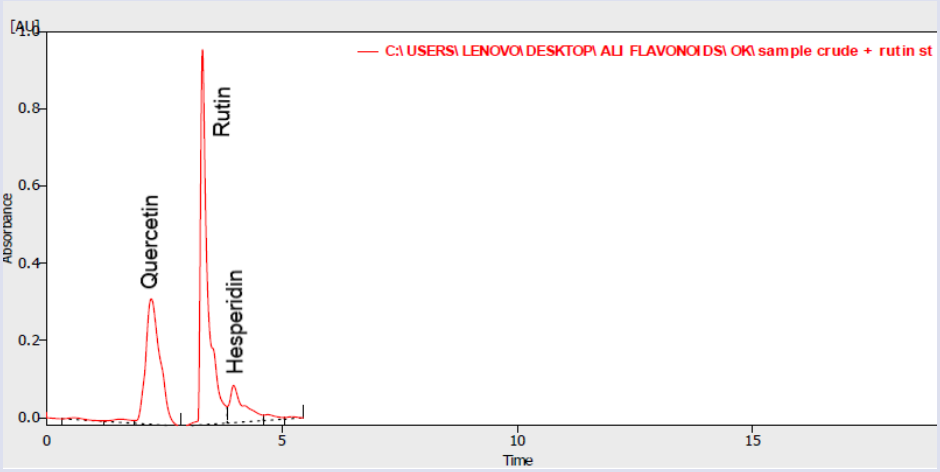

Figure 5: HPLC chromatogram of mixture of Rutin stander solution with crude extract

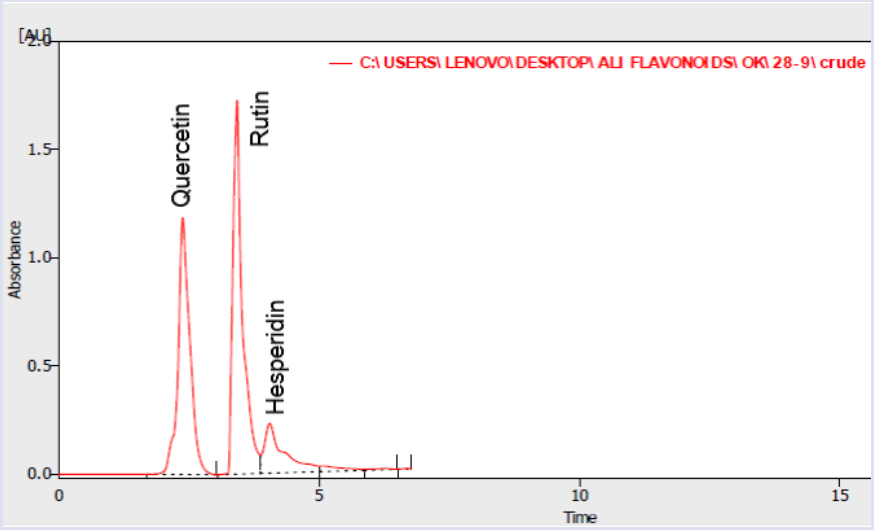

Figure 6: HPLC chromatogram of crude extract

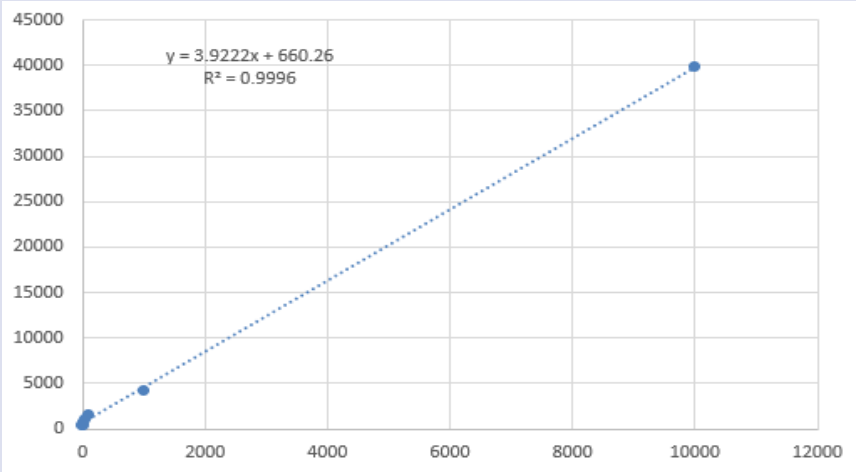

Figure 7: Calibration curve of the Quercetin standard

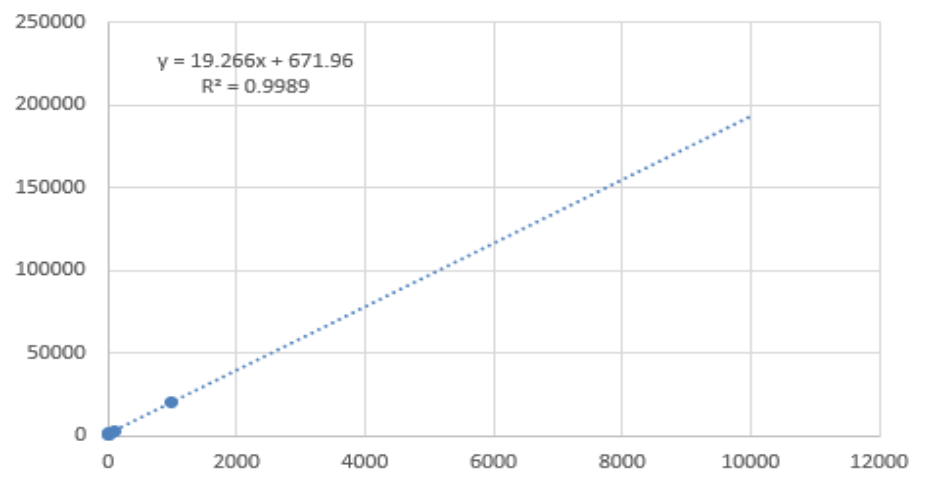

Figure 8: Calibration curve of the Rutin standard

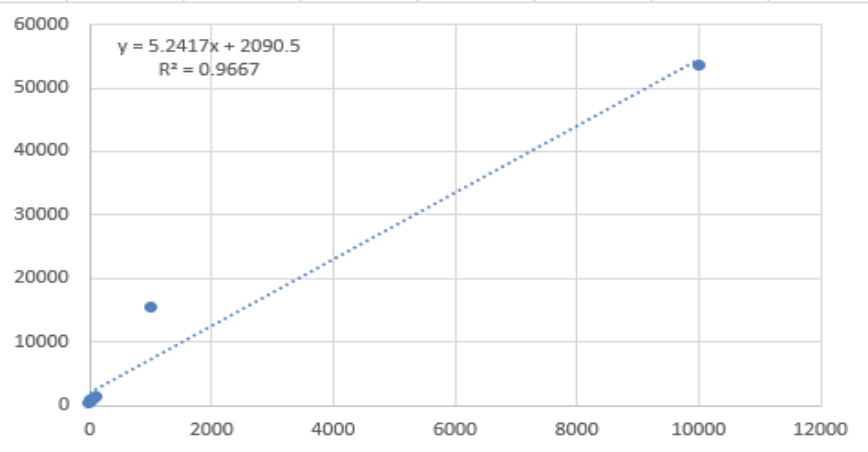

Figure 9: Calibration curve of the Hesperidin standard

Table 1: Qualitative phytochemicals tests of flavonoids in *Ruta chalepensis* L.

Extracts	Alkaline reagent test	Shinoda test	Ferric chloride test
Reflex Ethanol 80%	+	+	+
Maceration Ethanol 80%	+	+	+
Reflex methanol 80%	+	+	+
Maceration Methanol 80%	+	+	+
Reflex Ethanol 100%	+	+	+
Maceration Ethanol 100%	+	+	+
Reflex Methanol 100%	+	+	+
Maceration Methanol 100%	+	+	+

Key**:** (+) represents present; (-) represents absence of a particular phytochemical.

Table 2: Equations of calibration curves obtained for flavonoid standards.

Standard	Equation	r^2
Quercetin	Y=3.9222x+660.26	0.9996
Rutin	Y=19.266x+671.96	0.9989
Hesperidin	Y=5.2417x+2090.5	0.9667

Table 3: Flavonoids percentage detected in crude extracts (mg/g).

Crude extracts	Quercetin percentage	Rutin percentage	Hesperidin percentage
Reflex 80% ethanol	0.0777%	0.0069%	0.0025%
Maceration 80% ethanol	0.0566%	0.0095%	0.0026%
Reflex 80% methanol	0.0215%	0.0089%	0.0060%
Maceration 80% methanol	0.0674%	0.0165%	0.0062%
Reflex absolute ethanol	0.0047%	0.0057%	0.0001%
Maceration absolute ethanol	0.0110%	0.0020%	0.0001%
Reflex absolute methanol	0.0503%	0.0068%	0.0041%
Maceration absolute methanol	0.0423%	0.0040%	0.0054%

and quercetin are commonly extracted using a polar alcoholic solvent such as ethanol or methanol. Ethanol is a more suitable solvent because it is non-toxic and inexpensive. Despite the fact that organic solvent is the preferred solvent for rutin extraction, a modest amount of water would optimize extraction efficiency. Water has the potential to promote the diffusion of extractable polyphenols through plant tissues. Water's swelling impact on plant tissues increases the surface area of interaction between solute and solvent. The variance in extraction yield was caused by the solvent system's effect on the interaction between rutin and buckwheat starch as well as the solubility of rutin, hesperidin, and quercetin in the solvent system. Other parameters that must be considered during heat reflux extraction include extraction temperature and time. The mass transfer process is generally accelerated by high extraction temperatures. An extraction temperature that is too high may breakdown rutin, especially in an aqueous extraction technique. The majority of flavonoids are heat-sensitive chemicals. Longer extraction durations should result in a larger extraction yield. However, the concentration of rutin decreases proportionally to the length of the extraction time, especially when performed at high temperatures. This explains why the response with 80% ethanol revealed a modest rise in the quantity of quercetin. The results of this study are in agreement with the extraction yields of rutin.[20]

CONFLICTS OF INTEREST

None.

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