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Comparative Analysis of Rutin Content in Some Egyptian Plants: A Validated RP-HPLC-DAD Approach

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: To quantify the bioflavonoid rutin in three Egyptian plant families extracts in a comparative study and to prove the accuracy, precision, linearity and reproducibility of the used method.
Study Design: Development of RP-HPLC-DAD method. Rutin analysis in 29 plant extracts. Method validation using different parameters.
Place and Duration of Study: Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The study was performed in 11 months.
Methodology: A method was developed using reversed phased-high performance liquid chromatography coupled with diode array detector (RP-HPLC-DAD) and rutin as an analytical marker. Twenty-nine extracts from different Egyptian plants belonging to three families (Lythraceae, Lamiaceae and Asteraceae) were investigated for their rutin content. The method was then validated for accuracy, repeatability, precision, detection, quantification limits, linearity, and range parameters.
Results: The content of rutin (mg g^{-1} extract) was highest in *Punica granatum* bark (158.29), followed by *Melissa officinalis* leaves (133.09), *Lagerstroemia tomentosa* flowers (120.16) and leaves (118.17). The lowest rutin content (4.4 mg g^{-1} extract) was found in *Lagerstroemia speciosa*

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bark. The calibration regression equation was y = 7390x + 15.81 showing a correlation coefficient (r) of 0.9995, with best linearity in the range of 0.01-0.1 mg mL^{-1}. The detection and quantification limit values were 0.0031 and 0.0093 mg mL^{-1}, respectively, confirming the quantification method sensitivity. A recovery value of 100.17% indicates the best method accuracy.

Conclusion: The applied method was simple, precise, accurate, and proved successful for rutin determinations in different extracts for the first time in the selected plants, declaring regional variation in the phytoconstituents content of the Egyptian chemotypes and proved that Lythraceae plants were the richest in rutin, with *Punica granatum* bark extract showing the highest values. The method can be applied for the plants routine quality control analyses and the traces analysis of rutin in complex samples.

Keywords: RP-HPLC-DAD; rutin; Lythraceae; Lamiaceae; Asteraceae.

1. INTRODUCTION

Rutin is a citrus flavonoid glycoside. Its name comes from the plant *Ruta graveolens* (Rutaceae), which contains rutin. Chemically, it is a glycoside (Fig. 1) comprised of quercetin aglycone along with the disaccharide rutinose [1]. Rutin is a highly potent molecule due to its strong antioxidant properties [2], it protects the membranes from oxidative damage by interaction with polar heads of phospholipids, thus enhancing membrane rigidity [3]. It has shown a variety of pharmacological activities, including antiviral, antioxidant, cytotoxic, hepatoprotective, vasoprotective, anti-inflammatory, antiulcer, anticarcinogenic, neuroprotective, antihyperglycemic, anti-convulsant, and cardioprotective activities [4-7].

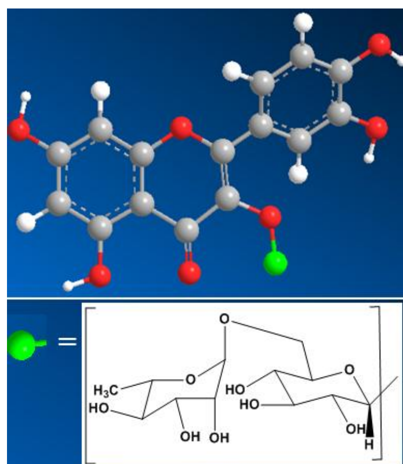

Fig. 1. Chemical structure of rutin

The family Lythraceae comprises about 31 genera and 620 species, which are widely distributed in the tropical and subtropical regions [8]. Recent phylogenetic analyses have included some genera, such as *Punica* L. (formerly in Punicaceae), in the family Lythraceae [9]. Preliminary phytochemical screening of different leaves extracts of some Lythraceae plants has revealed the presence of phenolics, alkaloids, carbohydrates flavonoids, coumarins, saponins, steroids and terpenoids [10]. Lythraceae plants are known for their medicinal importance, they have shown antioxidant, anti-inflammatory, antipyretic, antihyperglycemic, hepatoprotective, antihyperlipidemic and anticancer activities [10-12].

The family Lamiaceae contains 250 genera and 6700 species, which are distributed worldwide [13]. Members of this family are phytochemically characterized by the presence of essential oils, flavonoids, alkaloids, iridoids and terpenoids [14]. Both essential oils and plant extracts of this family have demonstrated a wide range of biological activities, such as antioxidant, antiseptic, anti-inflammatory, antimicrobial and anticancer activities [15-17].

The family Asteraceae (sunflower family), formerly Compositae, comprises the largest family of flowering plants, with 1700 genera and 24000 species, distributed throughout the world [18,19]. The main chemical constituents identified in the family are sesquiterpene lactones, polyacetylenes, terpenoids, flavonoids, seed oils, essential oils, alkaloids, cyanogenic glycosides and coumarins [20]. The plants and their secondary metabolites demonstrated various pharmacological activities, such as antioxidant, antimicrobial, antiprotozoal, anti-inflammatory, cytotoxic, antidiabetic, antispasmodic, cardioprotective and hepatoprotective effects [21].

Validation of analytical methods has become of great concern to guarantee that the used procedure gives exact, reliable and interpretable

information about the sample [22]. The main parameters for method validation are accuracy, precision, linearity, range, detection limit and quantification limit [23]. Linearity determines the capacity of the used method to give results directly proportional to the sample concentration within a certain interval [24]. The detection limit (LOD) is the concentration of sample which can give a significantly different signal from the background signal [25], while quantification limit (LOQ) is the smallest value of analyte that can be determined quantitatively, below which, measurements do not present sufficient confidence for quantification [26].

Thus, the goal of this research is to establish a rapid, precise and reproducible chromatographic technique to quantify rutin in Egyptian plants of different families other that the Rutaceae, in an attempt to find potential sources for that magnificent drug to serve as useful supplements in different disease prevention and treatment protocols.

2. MATERIALS AND METHODS

2.1 Plant Materials, Solvents and Chemicals

Plant materials were collected from El-Orman Botanical Garden, Giza, Egypt and from a local farm. All the specimens were authenticated by Mrs. Trease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture. Voucher specimens were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. All used solvents were of HPLC grade. The standard compound rutin was purchased from Sigma-Aldrich, USA.

2.2 Standard Solution Preparation

A stock solution was prepared by dissolving 1 mg of rutin in 10 mL of methanol. Aliquots of the stock solution (1, 3, 6 and 8 mL) were then diluted in a volumetric flask, each separately, with methanol up to 10 mL.

2.3 Samples Preparation

1 mg of the methanol extract of each plant sample was dissolved in 1 mL HPLC grade methanol and then filtered using a membrane filter.

2.4 Chromatographic Conditions and Procedure

High Performance Liquid Chromatography (Agilent 1200, Germany), with an autosampler, and DAD-Detector has been used for rutin analysis. The mobile phase used was first filtered through a millipore filter and then degassed by the use of sonication for 30 minutes. A C18 column (150 mm L × 4.6 mm I.D., 5 µm) and gradient elution were used, as described in Table 1 with a flow rate adjusted to 1.5 mL min^{-1}. The injection volume has been adjusted to 20 µL, and the UV-detection was made at 240 nm. Several trials have been made for selecting a proper mobile phase and a development method for obtaining a rapid assay with the reasonable runtime, and sharp peaks, this is because of the complex composition of the different plant materials.

Table 1. Timetable for the HPLC gradient elution process using water and acetonitrile as solvents

Time (min)	Solvent A (Water) (%)	Solvent B (Acetonitrile) (%)
0.10	93.0	7.0
2.00	50.0	50.0
4.00	40.0	60.0
6.00	40.0	60.0
6.10	0.0	100.0
12.00	0.0	100.0
12.10	93.0	7.0
15.00	93.0	7.0

2.5 Calibration Curve

The curve was constructed by injecting, in triplicates, five concentrations of stock solution (0.01, 0.03, 0.06, 0.08 and 0.1 mg mL^{-1}). The regression equation and the coefficient of the correlation (r^2) were then derived from the curve.

2.6 Validation of the Method

Method validation was done according to ICH guidelines [27], to ensure that it accurately describes the relationship between the estimated response (y) and the standard concentration (x). The linearity plot was also evaluated, to verify the values obtained for quantification limit. An analytical curve was constructed from the rutin standard solutions at concentrations which were close to the expected quantification limit.

2.6.1 Accuracy of the method

The average recovery percentage of different standard concentrations was used to estimate the method accuracy and was determined by applying peak area values to the calibration graph regression equations. Each concentration was calculated in triplicate.

2.6.2 Precision of the method (Repeatability)

The method precision was determined by the analysis (n=15) of different concentrations of the standard solution (0.01, 0.03, 0.06, 0.08 and 0.1 mg mL^{-1}) and the results were presented as % RSD (relative standard deviation).

2.6.3 Reproducibility (Intermediate precision)

Both the inter-day and intra-day precision of the developed method were determined through the analysis of different standard solution concentrations (0.01, 0.03, 0.06, 0.08 and 0.1 mg mL^{-1}) three times on the same day and another triplicate on a different day. The results were presented as % RSD.

2.6.4 Detection (LOD) and quantification (LOQ) limits

By the analysis of different rutin concentrations, in triplicate, the LOD was calculated on the basis of the equation, LOD = (3.3 * SD of response/slope), while the LOQ was calculated as LOQ = (10 * SD of response/slope).

2.7 Statistical Analyses

All experiments were made in triplicates and reported as mean value ± % RSD. All statistical analyses were made by the use of GraphPad InStat 3 Software, Inc. La Jolla, CA, USA. The graphs were sketched by GraphPad Prism version 5.01 software, Inc. La Jolla, CA, USA.

3. RESULTS AND DISCUSSION

3.1 Rutin Content

Twenty-nine Egyptian plant extracts were investigated for their content of rutin, as shown in Table 2. The results indicated that quantitative estimation of rutin in the tested samples ranged from 4.4 to 158 mg g^{-1} extract (Fig. 2). A comparative study was made among plant extracts of the same family. The Lythraceae plants have shown the highest concentrations of rutin as compared to the other families (Fig. 3), with *Punica granatum* bark representing the highest rutin content (158.29 mg g^{-1} extract), and *Lagerstroemia speciosa* bark represents the lowest one (4.4 mg g^{-1} extract).

Differences among the means of rutin content of different plant species were found to be significant at P = .05, using One-Way ANOVA results.

Tested plant extracts of family Lamiaceae show moderate concentrations of rutin compared to the other families, with exception of *Melissa officinalis* leaves extract that showed a relatively high rutin content (133.09 mg g^{-1} extract), while *Lavendula officinalis* flowers extract represents the least Lamiaceae plants tested extract (7.29 mg g^{-1} extract). Among the tested Asteraceae plants, the highest content of rutin was observed in *Calendula officinalis* leaves (71.73 mg g^{-1} extract), while the lowest rutin content in *Chrysanthemum frutescens* leaves (4.44 mg g^{-1} extract).

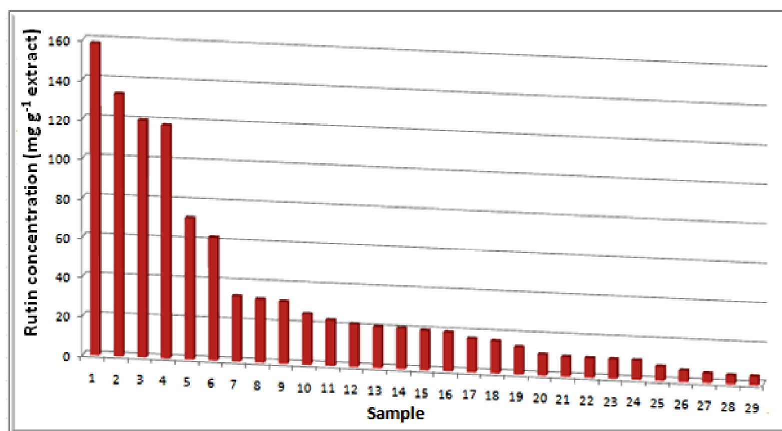

Fig. 2. Concentration of rutin in the tested samples

Table 2. Rutin concentration (mg g^{-1} extract) in Egyptian medicinal plants

Sample[a]	Scientific plant name	Plant family	Common name	Part used	Rutin content[b,c]
1.	*Punica granatum*	Lythraceae	Pomegranate	Bark	158.29
2.	*Melissa officinalis*	Lamiaceae	Lemon balm	Leaves	133.09
3.	*Lagerstroemia tomentosa*	Lythraceae	White crape myrtle	Flowers	120.16
4.	*Lagerstroemia tomentosa*	Lythraceae	White crape myrtle	Leaves	118.17
5.	*Calendula officinalis*	Asteraceae	Pot marigold	Leaves	71.73
6.	*Tagetes erecta*	Asteraceae	African marigold	Leaves	62.31
7.	*Thymus vulgaris*	Lamiaceae	Thyme	Herb	33.01
8.	*Lagerstroemia indica*	Lythraceae	Crepe myrtle	Leaves	32.03
9.	*Chrysanthemum frutescens*	Asteraceae	Marguerite Daisy, Paris Daisy	Flower	31.43
10.	*Ocimum basilicum*	Lamiaceae	Basil	Leaves	25.74
11.	*Rosmarinus officinalis*	Lamiaceae	Rosemary	Leaves	23.16
12.	*Lagerstroemia speciosa*	Lythraceae	Pride of India, Queen's crape myrtle	Leaves	21.62
13.	*Origanum majorana*	Lamiaceae	Sweet marjoram	Herb	20.72
14.	*Salvia officinalis*	Lamiaceae	Sage	Leaves	20.63
15.	*Bellis parennis*	Asteraceae	Common daisy, English daisy.	Flowers	19.99
16.	*Helianthus annuus*	Asteraceae	Common sunflower	Flowers	19.67
17.	*Tagetes patula*	Asteraceae	French marigold	Leaves	16.99
18.	*Hyssopus officinalis*	Lamiaceae	Hyssop	Herb	16.30
19.	*Lagerstroemia indica*	Lythraceae	Crape myrtle	Bark	13.75
20.	*Tagetes erecta*	Asteraceae	African marigold	Flowers	10.63
21.	*Mentha piperita*	Lamiaceae	Peppermint	Leaves	9.97
22.	*Helianthus annuus*	Asteraceae	Common sunflower	Leaves	9.88
23.	*Punica granatum*	Lythraceae	Pomegranate	Leaves	9.82
24.	*Gazania rigens*	Asteraceae	Treasure flower	Flowers	9.82
25.	*Lavendula officinalis*	Lamiaceae	Lavender	Flowers	7.29
26.	*Tagetes patula*	Asteraceae	French marigold	Flowers	5.69
27.	*Lagerstroemia tomentosa*	Lythraceae	White crape myrtle	Bark	4.83
28.	*Chrysanthemum frutescens*	Asteraceae	Marguerite Daisy, Paris Daisy	Leaves	4.44
29.	*Lagerstroemia speciosa*	Lythraceae	Pride of India, Queen's crape myrtle	Bark	4.40

[a]Samples are arranged descendingly according to rutin content, [b]Data expressed as mean value of three measurements, [c]Rutin concentration is measured as mg g^{-1} extract

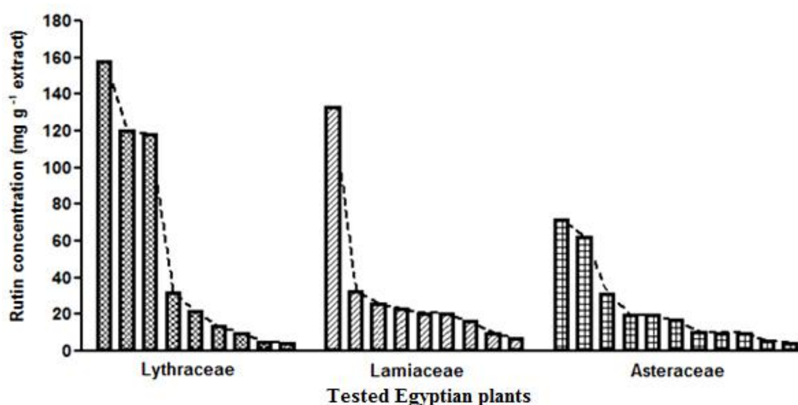

Fig. 3. Comparative levels of rutin among different families

3.2 Development of HPLC Method

The rutin peak retention time was found to be the same, while being injected several times, giving a symmetric and well-resolved peak. The runtime was 15 minutes for the whole chromatogram, the retention time of rutin was 2.88 minutes, while rutin appeared on the chromatograms of plant extracts at 2.83-2.92 minutes as shown in Fig. 4. This indicates that the developed method is convenient and rapid.

3.3 Method Validation

The developed method was linear in the range of 0.01-0.1 mg mL^{-1}. The regression equation was found as $y = 7390x + 15.81$ with r^2 of 0.999, showing best linearity, as shown in Table 2. Calibration linearity is important for the quantification limit because all the concentrations are estimated from the regression line. The best chromatographic method is that based on statistically reliable parameters which are obtained from the calibration curve [24].

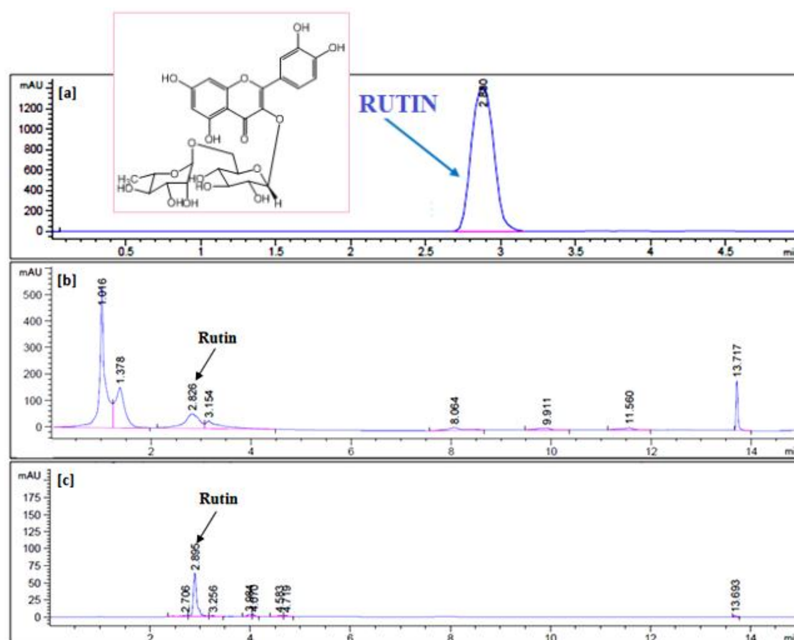

Fig. 4. HPLC chromatograms of 1 mg mL^{-1} of [a] standard rutin, [b] *Punica granatum* bark extract, [c] *Melissa officinalis* leaves extract

Where Y axis represents peak area (mAU) and X axis represents runtime (min)

Table 3. The validation parameters of the developed method

Parameter	Result
Regression equation	$y = 7390\ x + 15.81$
Accuracy (mean value ± %RSD)	100.17 ± 1.46
Precision	
Intermediate precision (%RSD)	
Intra-day (n=15)	1.12
Inter-day (n=15)	1.95
Repeatability (%RSD)	1.46
Linearity	
Correlation co-efficient (r)	0.9995
Slope	7390
Intercept	15.81
Range of linearity (mg mL^{-1})	0.01-1
LOD (mg mL^{-1})	0.0031
LOQ (mg mL^{-1})	0.0093

The quantification limit value was 0.0093 mg mL^{-1}, which is the lowest analyte concentration on the calibration curve, which shows linearity within the defined interval [24]. This result confirmed the quantification method sensitivity for the compound. A recovery value of 100.17%, as shown in Table 2, indicates that the developed method showed the best accuracy.

4. CONCLUSION

The used HPLC method was simple, precise, sensitive and accurate. Moreover, the method was successfully applied for the determination of rutin in twenty-nine different extracts, can be used for the routine quality control analysis for those plants and may also provide a potential application in the analysis of traces of rutin in complex samples.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

The author has declared that no competing interests exist.

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