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Research Article

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HPLC based estimation and extraction of rutin, quercetin and gallic acid in *Moringa oleifera* plants grown in Saudi Arabia

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Abstract
Moringa oleifera L. *(Moringaceae) is a valuable medicinal plant with a high nutritional and industrial value. To analyse the major polyphenolic compounds rutin, quercetin and gallic acid present in the M. oleifera leaves, HPLC methods have been used. In the range of 10-30 µg for rutin, quercetin and gallic acid with determination coefficient (R^2) good linearity was observed and linear regression $y = 1232.9x + 106.05$, $y = 133.53x + 202$ and $y = 619.4x + 3442.5$ were achieved in 0.98, 0.99 and 1.04 min with the retention time respectively. The LOQs for rutin, quercetin and gallic acid; 3.4, 10.6 and 4.4 µg/ml and LODs ≤4.0 µg/ml for all standards were achieved. The rutin (555.6±1.79 µg/g), quercetin (37.54±1.07µg/g) and gallic acid (48.5±1.34 µg/g) were recorded in M. olefera. Rutin was the most abundant flavonoid analysed in M. oleifera.*

Keywords: *Moringa oleifera*, HPLC, polyphenolics, rutin, quercetin, gallic acid.

INTRODUCTION

Moringa oleifera L. (Moringaceae) is a valuable medicinal plant with a high nutritional and industrial value cultivated in Sudan, Latin America, Philippines, South Africa, India, and Pacific islands. It is native to the sub-Himalayan region [1]. *Moringa oleifera* is rich source of micronutrients, antioxidant, vitamins, protein and flavonoids [2-3].

Flavonoids are a group of polyphenolic compounds, distributed in plant kingdom. With 300 known varieties generaly used in medicine. Rutin, (5,7,3', 4',tetrahydroxy flavonol -3- rhamnoglucoside) and quercetin (5,7,3', 4',-tetrahydroxy flavonol) used in anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antidiabetic and antiviral as well as cardiovascular mortality [4-6]. Gallic acid used in anti-fungal and anti-viral properties, antioxidant and helps to protect our cells against oxidative damage [7].

Polyphenolics estimation and screening are one of the tools for the quality assessment used for these compounds, which include metabolites screening, profiling etc., using modern analytical techniques. In the past few decades, many methods have been used like thin layer chromatography, gas chromatography, NIR etc., for the extraction and quantification of the rutin, gallic acid and quercetin from the medicinal plants [4,8-9]. Some methods are non-specific and did not check the exact quantity of the metabolites present in the plants. Either they are more expensive

and time consuming. Keeping in view we, have optimized the HPLC based method to estimate the rutin, gallic acid and quercetin content in the methanol extract of *M. oleifera* leaves.

The objective of this study is mainly to focus on estimation of rutin, gallic acid and quercetin level variation influenced by either environment or regional distribution through High Performance Liquid Chromatography (HPLC).

EXPERIMENTAL SECTION

2.1. Plant material, seed germination and cultivation of *M. oleifera*

The experiment was conducted at Sara Alghonaim Research Chair, College of Science and Humanities, Prince Sattam bin Abdulaziz University (PSAU), Alkharj (24° 15′ N, 47° 30′ E), Saudi Arabia. The seeds were germinated at Sara Alghonaim Research Chair, in peat moss and sand (1:3). The seeds were germinated after 4 days of sowing in soil mixture at room temperature. 30 days old seedlings were thereafter planted in Prince Sattam bin Abdulaziz University (PSAU), campus for further study.

2.2. Extraction of phenolic compounds

The leaves were collected after six months of *Moringa* plants cultivated at PSAU's campus and leaves were sorted, destalked individually and washed under running tap water for 30 min at room temperature for estimation of rutin, quercetin and gallic acid analysis.

One gram of a fine leaf powder of *M. oleifera* was extracted in 20 ml methanol for overnight at room temperature. The supernatant was filtered with 0.22 µm filter (Milipore, USA) and vaccumed drying for maximum biomass recovery and dissolved in 2 ml of methanol which was screened for HPLC analysis.

2.3. HPLC analysis for rutin, quercetin and gallic acid

HPLC rapid analysis was carried out in a multi-solvent Agilent1260-Infinity Quaternary LC system with quaternary pump (G131B) tagged autosampler (G1367E) and coupled with thermostat Diode Array Detector (DAD) assembled with high accurate computer system (HP Intel i3 processor). The noise levels of <± 0.6 µAU revolutionary 6 cm flow cell set up to 10 times higher sensitivity than other method. Agilent Open LAB ChemStation version C.01.05 (Agilent, USA) was used for data processing for the rutin, quercetin and gallic acid on the basis of area and retention time of the chromatogram.

A RP C18 column (*Agilent eclipse Plus,* 4.6mm X 100 mm, with a pore diameter of 95 Å, 1.8 µm particle size) has been used for rutin, quercetin and gallic acid. A gradient of methanol (50%) in acetonitrile (50%) in the ratio with a flow-rate (1.0 ml/min) for 5 min elution at room temperature was used. The injection volume 25 µl of the sample was injected into the HPLC for the test run. A calibration curve was prepared on the basis of external standard using final concentrations of 10, 20 and 30 µg rutin, quercetin and gallic acid in triplicate. Quantitative analyses were performed according to molecular coefficient absorbance for rutin, quercetin and gallic acid acquired at the specific wavelength with consonant with other findings reported earlier. All constituents were calculated with the support of standard curve arranged by HPLC. Rutin, quercetin and gallic acid contents were expressed in microgramg^{-1} DW basis of *M. oleifera* leaves samples.

RESULTS AND DISCUSSION

The analysis of the major compound rutin, quercetin and gallic acid present in the *M. oleifera* leaves after six months of cultivation were estimated through HPLC methods described in the experimental section. The optimal HPLC view obtained after assay with the mobile phases (methanol and acetonitrile) with a C18 column in rapid HPLC system. Preliminary estimation of the standard and *M. oleifera* leaves extract were carried out with a mobile phase consisting methanol and acetonitrile with 50% (v/v) using the gradient mode of elution. Flow-rate of 1.0 ml/min for a maximum elution time of 5 min was setup at room temperature and thus the resolution was observed good.

The detection of rutin, quercetin and gallic acid were performed using the 3D mode operation, with UV spectra of the constituents from 200 to 500 nm with steps of 4 nm. This could be checked the purity of the peak in a real sense. Estimation of rutin, quercetin and gallic acid were carried out with observed wavelength near the UV absorbance and rutin, quercetin and gallic acid standards were quantified with fluorescence response at 265 nm (Table. 1). To obtain higher detection efficacy of rutin, quercetin and gallic acid, fluorescence mode was used that provide the retention time, resolved wavelength, quantification range and limit of detection for all analytes (Table 1). There is

no any peak was observed after 5 min hence we kept the maximum elution time 5 min for further study. This study, hence proved that this method is fast and reliable.

Calibration curve was constructed with purity against the peak of the rutin, quercetin and gallic acid. The rutin, quercetin and gallic acid concentration were estimated and calibration curve was made. The R^2 for the rutin, quercetin and gallic acid analytes were 0.999, 0.998 and 0.991 with the slope R.S.D., values lower than 1.5%. The injected range of rutin, quercetin and gallic acid concentrations was between 10-30 µg/ mL. The calibration curves were made through zero and the contents of rutin, quercetin and gallic acid were carried out using the regression equation. Good linearity was achieved in the range of 10-30 µg for rutin, quercetin and gallic acid with determination coefficient (R^2) and linear regression y = 1232.9x + 106.05, y = 133.53x + 202 and y = 619.4x + 3442.5 respectively (Table 1). On the basis of rutin, quercetin and gallic acid retention time, we have calculated and calibrated the yield percent recovery of the compounds present in *M. oleifera* extract (Table. 2). The LOQs for rutin, quercetin and gallic acid were achieved 3.4, 10.6 and 4.4 µg/ml. The LODs for all standards were ≤4.0 µg/ml. This study was also similar to other investigators' reports [4,9,10].

Analysis of rutin, quercetin and gallic acid in *M. oleifera* leaves

The flavonoid (rutin, quercetin and gallic acid) of dried powder of *M. oleifera* were extracted in methanol from collected leaves from the full bloom stage of plant after planting and drying. The quantifications of phenolics (µg/g dry matter) were accomplished by comparing retention times and peak areas between the standards and the samples. The results show that their chromatographic separations were different, however, the peak sizes were different, which determines the amount and proportion of each compound. The most abundant phenolics rutin (555.6±1.79 µg/g), quercetin (37.54±1.07µg/g) and gallic acid (48.5±1.34 µg/g) were recorded in *M. oleifera* (Table 2). The results showed that rutin is the most abundant flavonoid in all analysed samples in *M. oleifera*. This report is also consonant with Valdez-Solana et al. [9].

The developed HPLC method was appropriate and liable for the simultaneous analysis of rutin, quercetin and gallic acid in the leaf extract of *M. oleifera*. This method can also be applied for other valuable plants for the standardization and estimation of flavonoid concentrations.

Table 1: Parameters of calibration of rutin, gallic acid, quercetin standards for HPLC

Metabolite	Wavelength	Retention time	Linear regression	R^2	Limit of detection	LOQ	Peak
Rutin	265	0.98	y = 1232.9x + 106.05	0.9993	3.8	3.6	DAD1 A, Sig=265,4 Ref=263,100 (004-0401.D)
Gallic acid	265	0.99	y = 619.4x + 3442.5	0.99	1.36	4.4	DAD1 A, Sig=265,4 Ref=263,100 (007-0701.D)
Quercetin	265	1.04	y = 133.53x + 202	0.998	3.92	10.6	DAD1 A, Sig=265,4 Ref=263,100 (002-0202.D)

Table 2: Estimation of polyphenolic compounds (µg/g dry weight) separated by HPLC-DAD in *Moringa oleifera* leaves

Compounds	**µg/g dry weight**
Gallic acid	48.5± 1.34
Quercetin	37.54±1.07
Rutin	555.6±1.79

CONCLUSION

The most commonly used separation technique in analytical science is HPLC estimated more than 65% recovery of the compound separated worldwide in plant as well as other system. The method of extraction by soaking the plant material in solvent was selected due to its simplicity. As mentioned earlier, the abundance and diversity of flavonoids present in *Moringa oleifera* may be responsible for their therapeutic effectiveness against various diseases [11]. Rutin is present in substantial amounts in our *M. oleifera* leaves and some investigations showed that this compound has a broad range of physiological activities [12].

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