

# Determination of total polyphenolic compounds and flavonoids in *Matricaria chamomilla* flowers

Muhammad Nasimullah Qureshi<sup>1,2\*</sup>, Guenther Stecher<sup>3,4</sup> and Guenther Karl Bonn<sup>3</sup>

<sup>1</sup>Department of Chemistry, University of Swabi, Swabi, Pakistan

<sup>2</sup>Department of Chemistry, Abdul Wali Khan University, Mardan, Pakistan

<sup>3</sup>Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University Innsbruck, Innsbruck, Austria

<sup>4</sup>Bionorica Research GmbH, Mitterweg, Innsbruck, Austria

**Abstract:** *Matricaria chamomilla* is of medicinal importance and has been used for its various pharmacological activities against different diseases due to the presence of polyphenolic compounds especially flavonoids and their glycosides. Flowers of the chamomile plant were studied for the quantification of total polyphenolic compounds and flavonoids aglycon. Total polyphenolic compounds obtained were 83.22mg as gallic acid equivalents/g of plant material; while for the determination of flavonoids aglycon the plant material was subjected to acid hydrolysis before quantification through HPLC-PDA. Results showed that luteolin, quercetin, apigenin, isorhamnetin and kaempferol were quantified. Apigenin was found in highest concentration (0.071mg/ml) while amounts of the rest of the flavonoids quantified are: luteolin (0.012mg/ml), quercetin (0.032mg/ml), kaempferol (0.001mg/ml) and isorhamnetin (0.023 mg/ml).

**Keywords:** *Matricaria chamomilla* flowers, Polyphenolic compounds, flavonoids, hydrolysis, HPLC-PDA

## INTRODUCTION

*Matricaria chamomilla* (Family: Asteraceae) is an annual herb, native to Europe and Western Asia; commonly known as the chamomile plant. It has been used for medicinal purposes since long ago (Srivastava *et al.*, 2010). Different pharmacological proven effects have been attributed to this specie like sedative, relaxation, anti-inflammatory, tenseness, aching muscles, indigestion, acidity, asthma, morning sickness, eczema, sore nipples and exhaustion etc (Gupta *et al.*, 2010; McKay and Blumberg, 2006; Srivastava *et al.*, 2010). The plant has been used in variety of ways such as cosmetics, teas, air fresheners, dyes etc (McKay and Blumberg, 2006). Flavonoids, coumarines and mucilages are important chemicals present in the flowers of chamomile plant, which contribute to its medicinal vitality (Gupta *et al.*, 2010; McKay and Blumberg, 2006). Besides its pharmacological activities, its side effects are also there. Those who are allergic to this family may suffer from some allergic reactions or some dermal complexities.

Flavonoids are the compounds which have a number of proven effects for its pharmacological activities (Gupta *et al.*, 2010; Qureshi *et al.*, 2014; Sultana *et al.*, 2008). They are present in plants in the form of various glycosides (Qureshi *et al.*, 2014). Their qualitative and quantitative profile is needed for the use of such type of raw materials for the preparation of pharmaceutical products. The present study deals with the quality control of flower part of *Matricaria chamomilla* focusing on: the determination of polyphenolic compounds and flavonoids by HPLC

using photodiode array detector (PDA).

## MATERIALS AND METHODS

### Chemicals and reagents

Methanol, ethanol absolute, THF, acetonitrile and formic acid were from Merck KGaA (Darmstadt, Germany). Folin-ciocalteu's phenol reagent 2 N, HCl (37%) and ortho-Phosphoric acid (85%) were purchased from Sigma Aldrich (Steinheim, Germany). Sodium carbonate and tetra-butylhydroxyanisole (*t*-BHA) were obtained from Fluka Biochemika (Buchs, Switzerland). Water purified by a Nano Pure-unit (Branstead, Boston, MA, USA) was used. All these chemicals and reagents used were of analytical grade. Standards gallic acid ( $\geq 97\%$ ), quercetin dihydrate (min. 98%) were purchased from Sigma-Aldrich. Apigenin ( $\geq 95\%$ ), kaempferol ( $\geq 96\%$ ), isorhamnetin (HPLC grade), luteolin (HPLC grade) were obtained from Extra synthese (Genay, France). *Matricaria chamomilla* flowers were provided by Bionorica AG a phytonengineering company GmbH (Neumarkt, Germany).

### Microwave assisted extraction

10ml of 50% methanol were added to one gram of powdered plant material and extraction was performed using the microwave digestion for 200 seconds (Hong *et al.*, 2001; Sultana *et al.*, 2008) employing high performance microwave digestion unit mls 1200 mega, with degasser EM-30 exhaust module, MLS GmbH (Germany). The extraction was performed at 150 watt power. Extracts were allowed to cool at room temperature and then centrifuged for 10 minutes at 14×1000 g using Eppendorf centrifuge. They were preserved at -20°C temperature for further work.

\*Corresponding author: e-mail: mnasimuq@yahoo.com

**Determination of polyphenolic compounds****Preparation of standards**

Stock solution of gallic acid was prepared with a concentration of 0.25mg/ml in double distilled water. Further dilutions were made for the standard calibration curve diluting stock solution with double distilled water to the desired concentrations (0.01mg/ml-0.2mg/ml). Six calibration standards were made; each of which was measured thrice a time.

**Folin-Ciocalteu method**

Polyphenolic compounds were determined by Folin-Ciocalteu method (Qureshi *et al.*, 2014) using gallic acid as the reference standard. 5ml of diluted FC reagent (1:10 FC reagent to water) were added to one milliliter of each of standards, extract and blank (water) in test tubes. These were mixed thoroughly through vortex mixing. 4ml of Na<sub>2</sub>CO<sub>3</sub> solution (7.5%) were added to each mixture after 8 minutes and mixed thoroughly through vortex mixing. These test tubes were covered and stored for 2 hours at room temperature and away from direct light. Absorbance of these test solutions were read against the prepared blank at 740nm using UV-visible spectrophotometer (UV 2000, Hitachi).

**Quantification of flavonoids****Preparation of calibration standards**

Stock solutions of the five standards apigenin, isorhamnetin, kaempferol, luteolin, and quercetin were prepared in methanol/water (50:50, v/v) in the concentration range of 0.255mg/ml-2.02mg/ml and stored at -20°C until use. Working standard solutions for calibration curve were made by diluting stock solutions with methanol/water (50:50, v/v) to the desired concentrations (apigenin: 0.053mg/ml-0.315mg/ml; isorhamnetin: 0.01mg/ml-0.061mg/ml; kaempferol: 0.05 mg/ml-0.302 mg/ml; luteolin: 0.026 mg/ml-0.154mg/ml and quercetin: 0.01mg/ml-0.505mg/ml). Six calibration standards were made; each of which was measured thrice a time.

**Extraction and hydrolysis**

Quantification of flavonoids aglycon in plant extracts was performed after subjected them to simultaneous extraction and hydrolysis. 20ml of 62.5% of aqueous methanol containing 2g/L of tert-butyl hydroxyanisole (t-BHA) was added to about one gram of powdered plant material in a round bottom flask. 5ml of 6M HCl was added and mixed carefully. The mixture was refluxed at 90°C with

continuous stirring using carousel reaction station. Extract was left to cool at room temperature and centrifuged for 10 minutes at room temperature.

**Instrumentation**

Shimadzu HPLC was used for the quantification of flavonoids. The HPLC system comprised of an online degasser unit (DGu-14A), two solvent delivery pumps (LC-10Advp), an auto injector (SIL-10ADvp), a column oven (CTO-10Avp) and a system controller (SCL-10Avp). Detection of the analytes was performed using a photo diode array detector PDA (SPD-M10 Avp). LCMS-Solution, version 3 and LCMS-Post run, version 3-H2 software was used to control the system and to analyze the data respectively.

**Chromatographic parameters**

The chromatographic separation was performed on a reverse stationary phase column (Hypersil BDS 125×4 mm; particle size: 3µm and pore size: 130 Å). Elution was carried out in gradient mode using the mobile phase A: water (900ml) + methanol (100ml) + phosphoric acid (10ml) and B: water (600ml) + tetrahydrofuran (THF; 300 ml)+methanol (100ml)+phosphoric acid (10ml). Column temperature was 50°C and a flow rate of 0.5ml per minute was used. The gradient was started at 10% B and raised to 60% B in 10 minutes. The concentration of the mobile phase was hold at 60% B for 35 minutes and then increased up to 100% B till 53 minutes. The concentration of the mobile phase was kept constant for 3 minutes at 100% B and then changed to the zero time conditions in 4 minutes. 50% methanol was used as washing solution for the auto injector and the sample injection loop. The whole analysis took 70 minutes. Detection was performed in the wave length range of 200 to 600nm.

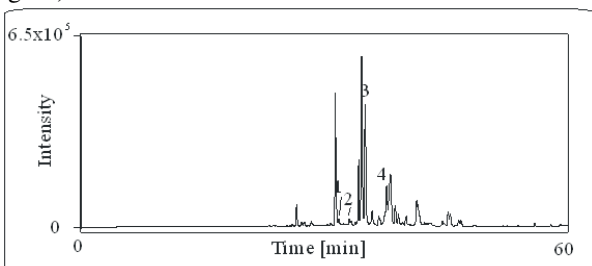
**RESULTS**

Analyses were performed on the powdered flowers of *Matricaria chamomilla* focusing on the quantification of total polyphenolic compounds and quantification of flavonoids by HPLC-PDA. Total polyphenolic compounds obtained using the micro wave assisted extraction, were 83.22mg as gallic acid equivalents/g of plant material. figs. 1 and 2 shows the HPLC-PDA chromatograms of non-hydrolysed and hydrolysed plant extract with 6M HCl obtained at 370nm. Different glycosides of the identified flavonoids (fig. 1) were also detected and confirmed through their masses. Results

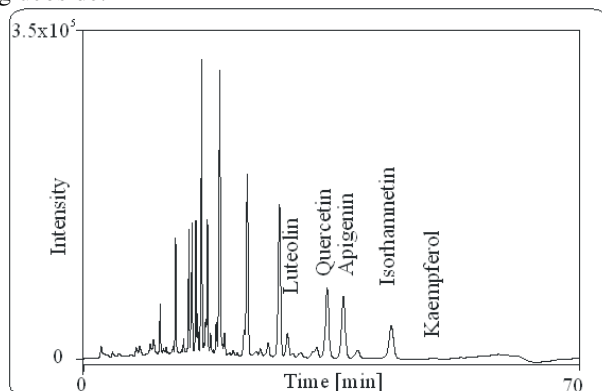
**Table 1:** Results of the quantification of flavonoids in *Matricaria chamomilla* flowers

| Parameters    | Luteolin              | Quercetin             | Apigenin              | Isorhamnetin          | Kaempferol            |
|---------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Conc. (mg/ml) | 0.012                 | 0.032                 | 0.071                 | 0.023                 | 0.001                 |
| Std. dev.     | $2.45 \times 10^{-4}$ | $4.91 \times 10^{-4}$ | $1.18 \times 10^{-3}$ | $1.78 \times 10^{-4}$ | $8.34 \times 10^{-6}$ |
| LOD (mg/ml)   | $7.34 \times 10^{-4}$ | $1.47 \times 10^{-3}$ | $3.54 \times 10^{-3}$ | $5.35 \times 10^{-4}$ | $2.5 \times 10^{-5}$  |
| LOQ (mg/ml)   | $2.45 \times 10^{-3}$ | $4.91 \times 10^{-3}$ | $1.18 \times 10^{-2}$ | $1.78 \times 10^{-3}$ | $8.34 \times 10^{-5}$ |

(table 1) showed that luteolin, quercetin, apigenin, isorhamnetin and kaempferol were quantified. Apigenin was found in highest concentration (0.071mg/ml) while amounts of the rest of the flavonoids quantified are: luteolin (0.012mg/ml), quercetin (0.032mg/ml), kaempferol (0.001mg/ml) and isorhamnetin (0.023 mg/ml).



**Fig. 1:** HPLC-PDA chromatogram of non hydrolyzed extract of *Matricaria chamomilla*. 1. Quercetin glucoside, 2. Luteolin glucoside rhamnoside or Kaempferol glycoside, 3. Isorhamnetin glucoside, 4. Apigenin glucoside.



**Fig. 2:** HPLC-PDA chromatograms for quantification of flavonoids in hydrolysed plant of *Matricaria chamomilla*.

## DISCUSSION

Flowers extracts of *Matricaria chamomilla* prepared under microwave digestion were subjected to the quantification of total polyphenolic compounds and flavonoids aglycons present in quantifiable amounts. Total polyphenolic compounds were determined by measuring the absorbance of the blue coloured product obtained as a result of the reaction of Folin-Ciocalteu reagent with polyphenolic compounds. The total polyphenolic compounds obtained were presented as equivalent of gallic acid used as reference standard for establishing the calibration curve.

As flavonoids are present in glycosidic forms in *M. chamomilla* flowers as depicted in fig. 1. To make the flavonoid aglycons free from glycosidic moiety, the extract was subjected to acidic hydrolysis using HCl through the optimized procedure previously performed by the author (Qureshi et al., 2014). After the hydrolysis, free flavonoids were quantitatively analyzed through reverse

phase HPLC coupled to PDA detector. Chromatographic parameters used were as conditioned by Qureshi et al., 2014 (Qureshi et al., 2014). Quantification of the three flavonoids quercetin, isorhamnetin and kaempferol were made by measuring their absorbance in plant extracts at 370 nm, for luteolin at 350 nm and for apigenin at 340 nm. Six points calibration curve for each standard were made and the  $R^2$  values obtained were: 0.9946, 0.9995, 0.9981, 0.9894, 0.9981 for luteolin, quercetin, apigenin, isorhamnetin and kaempferol respectively. Limit of detection and limit of quantification for each flavonoid quantified (table 1) were calculated using the Microsoft Office 2003 Excel program. These calculations were based on the standard deviation of the three replicate analyses taking signal to noise ratio as 3 for LOD and 10 for LOQ.

## CONCLUSION

The hydrolysis of flavonoid glycosides and the chromatographic conditions employed for the separation of flavonoids is reliable. These results, showing the presence of important antioxidants in the plant, can be used for developing the quality control profile.

## ACKNOWLEDGMENTS

Higher Education Commission (HEC) of Pakistan is acknowledged for providing scholarship during the course of work.

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