

Qualitative and Quantitative Evaluation of Total flavonoids Extracted from *Mentha arvensis* Aerial Parts

التقييم النوعي والكمي للفلافونويدات الكلية في الجزء الورقي لنبات البطنج البري في العراق

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Abstract

Mentha arvensis is an important medicinal plant that contains many groups of secondary metabolites that have extensive biological activity. The flavonoids were extracted from the aerial parts of the mature plant by using reflex extraction, and the aglycan fraction was isolated with ethyl acetate. The flavonoids of the extract were detected qualitatively with TLC technique. The total amount of flavonoids derived from the plant was estimated using a colorimetric method and the total value was calculated using the straight line equation of the standard flavonoid curve (Rutin). The plant was found to be rich in flavonoids, especially rutin, quercetin, kaempferol, luteolin and low concentration of coumarin. Total flavonoids were about 0.78 mg for each gm. powdered aerial parts.

Key words: arvensis, areal parts, TLC, Rutin

المخلص

البطنج هو من النباتات الطبية الهامة التي تحتوي على مجموعة من نواتج الأيض الثانوي التي تمتلك الفعالية البيولوجية الحيوية واسعة النطاق. استخلصت الفلافونويدات من الجزء الورقي للنبات الناضج باستخدام الاستخلاص الانعكاسي، ومن ثم عزل الجزء غير السكري باستخدام خلاص الاثيل. كشفت عن الفلافونويدات من المستخلص باستخدام تقنية كروموتغرافيا الطبقة الرقيقة. قدرت كمية الفلافونويدات المستخلصة من النبات باستخدام طريقة لونية ومن تم احتساب القيمة الكلية بتطبيق معادلة الخط المستقيم للمنحنى القياسي للفلافونويد (روتين). وجد أن النبات غني بالفلافونويدات، وخاصة روتين، كوارسيتين، كامفيرول، اللوتولين وتراكيز قليلة من الكومارين. الفلافونويدات الكلية لكل غرام من مسحوق اوراق البطنج الجافة حوالي 0.78 ملغ.

الكلمات المفتاحية: نبات البطنج، الجزء الورقي، تقنية كروموتغرافيا الطبقة الرقيقة، روتين

Introduction

Mentha arvensis the perennial herbaceous plants of the mints genus belonging to the *lamiaesae* species have been found in different parts of Europe, Asia and North America [1]. *M. arvensis* is characterized by its special character. Plant height ranges from 10-60 cm. Green leaves grow around the leg in the form of reverse pairs. These leaves are serrated 2-6.5cm long. The leaves have a strong fragrance. Flowers are colored pale Purple and can see some colors in sub-species that grow as large groups. The color of the stem is differed from brown to green [2]. It grows in the spring and is an evergreen plant with random roots, as is the case in all family types of oral crawling freely under the soil [3] and spread in the fields and wetlands and on the edges of the canals and rivers, so called Iraq water mint but a lot of differences between the wild mint and *M. arvensis* in some features such a distinctive taste of the later that does not like mint, when eaten freshly or grounded [4].

M. arvensis flowers were used as anticonvulsant, gastric pain, carminative and disinfectant since the plant posses antimicrobial effect that causes bacterial cell damage [5-6]. Many of the chemical compounds, such as essential oil, alcohol, flavonoids, sugars, alkaloids, saponnines and others, which have protective effect against inflammation [7,8] are extracted from this plant. Many studies have been carried out on this plant in Brazil to evaluate the importance of phenolic compounds for industrial purposes, medicinal and aromatic preparations [9,10]. These compounds are not needed by the plant for its growth and reproduction but are believed to have a role in the survival of the plant in its environment such as preventing attacks of insects and for protection. The production of volatile oils in Human use for several aspects: pharmaceutical, food and industrial and other compounds of the secondary metabolism of plants (unlike the primary metabolites) are used in medicine, currently called alternative medicine

[11,12]. Human had been observed to cure with herbal medicine because of its ease of access, low expenditure and popular belief that it was safer than chemical drugs [13,14]

The current study aimed to identify the flavonoids of the plant quantitatively and qualitatively as one of the secondary metabolites in the plant and the biological effect is attributed to these major secondary metabolites in most recent studies.

Material and Method

First: Plant collection and Extraction

Aerial parts of plant were collected from the Abu Ghraib / Baghdad-Iraq area and were classified in the Faculty of Agriculture, Anbar University, then samples were taken to the laboratory after cleaning well from suspended soil. They were dried at room temperature 25°C and manually grinded, then packaged in sterile containers and kept away from direct light until use.

Second: Extract the total flavonoids from the plant

About 86 g of powdered plant samples were placed in a 1 liter glass flask and then added 600 ml distilled water with (10% v/v) HCl. Reflex extraction was performed for 8 hours continuously to ensure that the cleavage and broken of glycoside linkage of the flavonoids and the aglycon part was obtained. The plant extract was filtered and cooled. The aglycon portion that is the biologic active part of flavonoids is extracted by an organic solvent such as ethyl acetate by adding 50 mL per each 50 mL extract and repeated three times using a separating funnel. The acetate layer is collected in the separating funnel again and an equal amount of distilled water is added to remove HCl residues used in extraction. The acetate layer is then dried using rotary evaporator at 45°C. The output is saved to complete the rest of the analysis [15].

Third: Phytochemical tests were done according to [16]

For the purpose of the tests, 10 g of dried powdered plant macerated in 100 ml of 75% ethanol for overnight. Then filtered and the concentrated filtrate was identified for the plant chemical components.

A. Detection of tannins tests

Few drops of the 3% solution ferric chloride in distilled water were added extract of. A greenish blue color indicates the presence of tannins.

B. Detection of glycosides

1 mL of the plant extract was mixed with 2 ml of the benedict reagent, then the mixture placed in boiling bath for 5 minutes and left to cool. The red deposit indicated a presence of this group.

C. Detection of alkaloids (Draganoff test)

Weigh about 60mg of bismuth subnitrate and dissolved in 0.2ml HCL, this is solution. A Solution B contains 600mg potassium iodide in 1 ml distilled water

We mix the solution [A + B] and then add the plant extract; an orange to brown color will indicate the presence of alkaloids.

D. Detection of the saponines

The detection process will be proceeding by shaking the plant extract well. Formation of foam at the top will indicate presence of saponines.

E. Detection of flavonoids

i- (alkaline reagent test) by using Sodium hydroxide solution is mixed with few amount plant extract solution and left, a yellow color is obtained in presence of flavonoids.

ii- (Acidic test using a sulfuric acid): formation of orange-reddish color indicated the presence of flavonoids.

F. Detection of Polyphenolic compounds

Using 1% ferric chloride solution to the plant extract solution a brown deposition will formed.

Fourth: Determination of Total Flavonoids.

1. Qualitative assay

A stock solution from the extracted total flavonoids was prepared by dissolving 0.150 gm. Residue in 5 ml of 50% ethanol. A standard rutin, quercetin, and luteolin solutions were prepared in 50% ethanol also. Thin layer chromatography (TLC) was carried out using a silica coated silica 60 plate with a thickness of 0.1 mm which represents the stationary phase in the chromatography separation process. As for the mobile phase, different phases were used as shown in Table (1)

Table (1): shows the ratio the mobile phase used in the qualitative evaluation of flavonoids

Mobile Phase numbe	Ratio for Mobile Phase
1	Toluene: Ethyl acetate :Formic acid (36 : 12 : 5)
2	Chloroform : Glacial acetic acid : Formic acid (44 : 3.5 : 2.5)
3	n-Hexane : Ethyl acetate : Glacial acetic acid (30 : 20 : 1.5)
4	n-Hexane : Ethyl acetate : Glacial acetic acid (31 : 14 : 5)
5	n-Butanol : Glacial acetic acid : Distill water (20 : 5 : 25)

The TLC process is applied and the plate is placed in the mobile-saturated chamber as mentioned above. Flavonoids are left to be separated depending on their binding to the stationary-phase and solubility in the mobile phase. When the mobile phase reached about three-quarters of the plate or more, plates were removed and solvent front line was boarded. The type of flavonoids separated can be detected in corresponding to standard flavonoids spots in their distance that called RF value. This value is derived from dividing the distance travelled by each flavonoid in each model phase to the distance traveled by the solvent:

$$\text{Rf value} = \frac{\text{The distance traveled by each flavonoid}}{\text{Distance traveled by the mobile phase}}$$

Each Flavonoid can be detected separately by the exposure of the silica plate to the UV light as a colored spot. The silica plate is covered with Fluorescent material, which flashes when it binds to the active groups of flavonoids under UV at a wavelength of 254nm. The result is shown as bright spots under the UV light [17].

2. Quantitative Assay

Several rutin standard flavonoid solutions were prepared with concentrations of (0.15625, 0.3125, 0.625, 1.25 and 2.5 mg / ml) in 50% ethanol solution. The following interaction is performed:

About 1 ml of plant total flavonoids extract is transferred to a glass tube, and 1 ml rutin standard solutions of each concentration is placed in separated glass tube. 1 ml of 5% sodium nitrite solution which dissolved in 50% ethanol was added to all tubes, then stirred and left in room temperature for 5 minutes, added to each tube 2 ml of aluminum chloride at a concentration of 10% dissolved in 50% ethanol. Then the tubes were mix and leave for another 5 minutes at room temperature. Finally, 5 mL of 1N NaOH solution was added stir the mixture and then read the resulting color with spectrophotometer at 510nm wavelength [19]. A standard curve is then performed between reading absorbance of each standard solution with their known concentration to get the straight line equation and then calculating the concentration of the total amount of flavonoids in extracted plant [18].

Results

First: Extract the total flavonoids from the plant

The results indicated that each 86g of the grounded aerial plant was produced about 1.3153 g residues resulting from the extraction and drying process of the rotary evaporator.

Second: Phytochemical investigation

Table (2): Phyto-chemical investigation of *Mentha arvensis* active compounds

Active compounds	Name of the detector	Result
Alkaloids	Dragandroff	+
Flavonoids	Base Alkaline test detection using NaOH	+
Phenols	Ferric chloride Detector 1%	+
The saponnines	Formation of foam	+
Glycosides and sugars	Benedict detector	+
Tannins	Ferric Chloride 3%	+

Positive results gave the symbol(+)

Third: Determination of total flavonoids

A. Qualitative assay

Flavonoids were extracted from the plan identified by thin layer chromatography (TLC). Spots were obtained by UV exposure when exposing the plate to a 254nm wavelength in comparison with standard flavonoids, Figure (1)

Table (3): flavonoids diagnosed in the Menthe plant and Rf values

Rutin	(Base line)
Quarecetin	0.43
Luteolin	0.30
Kaempfrol	0.49
Flavonoids <i>Mentha arvensis</i> extracted	All above rates with unknown flavonoids rates

Table (4) shows flavonoids and their Rf values of the standard flavonoids identified in plant extract.

Table (4): Diagnosed of rutin and quarstine in the *Mentha* plant

Number	Flavonoid Types	The R _f Values in Each Mobole Phase				
		Mobile Phase (1)	Mobile Phase (2)	Mobile Phase (3)	Mobile Phase (4)	Mobile Phase (5)
1	Rutin	(Baseline)	(Baseline)	0.33	0.36	0.71
2	Querecetin	0.43	0.14	0.92	0.91	0.86
3	The Extracted Flavonoids	All above flavonoids R _f values with another unknown flavonoid spots				

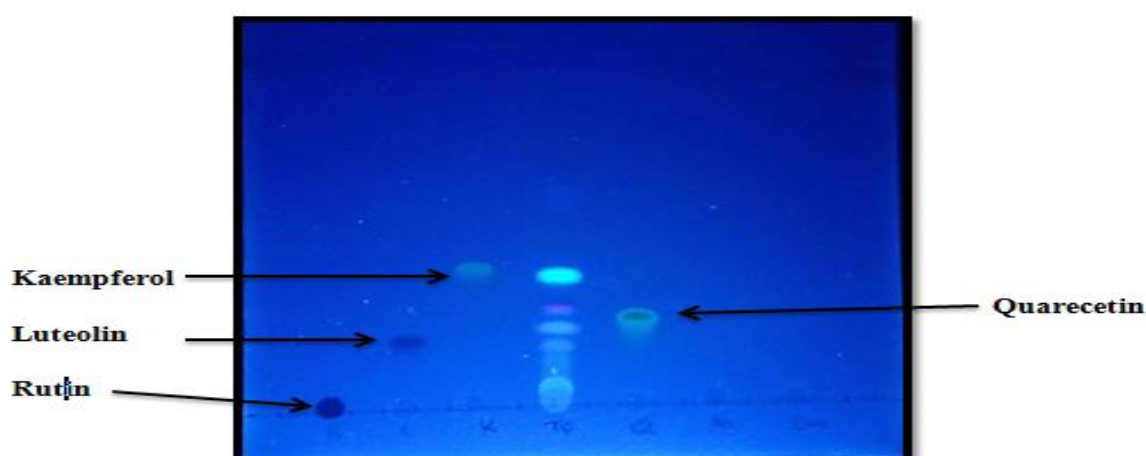


Fig. (1): TLC chromatogram used in the identification of the extracted flavonoids in corresponding to standard solutions ;including (T)_TTotal flavonoids extracted from *Mentha arvensis* , (K) kaempferol, (L) luteolin (R) rutin, and(Q) Quercetin

B. Quantitative Assay

The amount of flavonoids found in the abdominal plant was estimated using the optical spectrometer at 510 nm depending on the concentration of the standard routine solution Table (3) where the straight line equation is obtained as shown in Figure (2)

Table (5): Absorption values of standard flavonoids Rutin at different concentrations and total flavonoids of tubal plant

Absorption at (510 nm)	Rutin standard solution(mg/ml)
0.135	0.15625
0.341	0.3125
0.602	0.625
1.003	1.25
2.750	2.5
2.0012	Total Mentha flavonoids(double diluted)

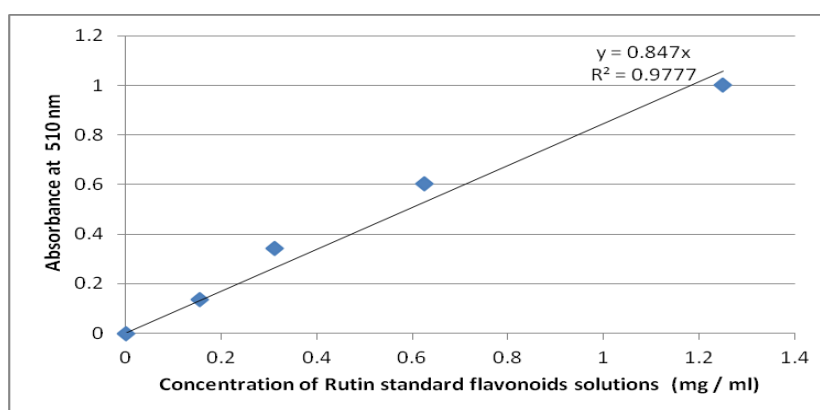


Fig. (2): Rutin standard curve

From the equation of the straight line of the standard flavonoid curve to the Rutin with different concentrations, the concentration of the total flavonoids for the extract of the *M. arvensis* is as follows:

$$Y = 1.0369X_1$$

$$2.0012 = 1.0369 X_1$$

$$X_1 = 1.94 \text{ mg/ml}$$

Since the extract is diluted twice and dissolved in 5 ml in an alcoholic solvent 50% alcohol, the total concentration of flavonoids per 86 g of the *Mentha arvensis* plant powder is approximately 170 mg.

Therefore, the total concentration of flavonoids per 1 g of *Mentha arvensis* powder will be:

$$170\text{mg} / (86\text{g}) = 2 \text{ mg (approximation)}$$

Where: Y = absorption; X = concentration mg / ml.

Discussion

In current study, the quantitative and qualitative value of flavonoids were estimated taking amount of plant aerial parts powdered from Wild Iraqi (*Mentha arvensis*), this study has shown that the plant is rich in flavonoids, where it has been shown that for every 100 g of powder aerial parts of the plant contain 200 mg. Flavonoids are major secondary metabolites consist of one or more hydroxyl phenolic groups with sugar moiety. *Mentha arvensis* plant is a good source of phenolic compounds such as glycosides and flavone, which have a protective effect against inflammation. One study done by (Olli and co 2011) showed that there is a significant effect in preventing or curing the injury due to the presence of Phenolic compounds in the extract of *Mentha arvensis* [19].

Total flavonoids of *Mentha arvensis* in present research were extracted and identified by the TLC technique to qualify and separate this extracted flavonoid in corresponding to standard solution including (Quercetin, kaempferol, luteolin and rutin). All these compounds play a major role in biological effectiveness. Flavonoids are the most important classes of active compounds found in the plant and the most abundant is rutin and Quercetin. Both have multiple bio-efficacy in alternative medicine may be

used as a treatment in many conditions and as free radical scavenger. In a study carried out on the infusion of a variety of herbs used in Brazil, including botanic plant for the verification of phenolic compounds, including flavonoids as antioxidants and free radicals, showed that the *Mentha spp.* contains about 3 mg / g of phenolic compounds using vegetable parts .It was also observed that this batch was statistically significant as an antioxidant [20]. In another study conducted in India on Indian herbs used in Indian foods, it was found that the total flavonoids content in this plant was 1.57 mg / gm dried leaves [21], while the total flavonoids in the aerial parts of the Iraqi *Mentha arvensis* was about 2mg / g, which is very abundant and very rich in flavonoids. Our study also showed that after the chemical tests, the plant contains a percentage other bioactive component such as: volatile oils glycosides, saponins, alkaloids, tannins and Polyphenolic compounds including flavonoids.

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