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Estimation of flavonoids and phenolic constituents of *Matricaria Chamomilla L*. by HPLC.

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ABSTRACT

Chamomile (*Matricaria Chamomilla* L.) is a well-known medicinal plant species from the Asteraceae family often referred to as the "star among medicinal species". Phytochemical screening revealed the presence of various bioactive secondary metabolites as flavonoids, phenolics, saponins, glycosides, cardiac glycosides, tannins and alkaloids. Anthraquinones and sterols were not detected in the plant. The percentage of inorganic matter, organic matter, crude fibers content, total carbohydrates, total nitrogen, total proteins, total lipids, total tannins, total saponins, total alkaloids, total flavonoids, total phenolic of the plant were determined in this study. The flavonoid and phenolic contents of *Matricaria Chamomilla* L. were analyzed by the HPLC. The quantification of each compound was done according to the peak area measurements which were reported in calibration curves of the corresponding standards. The result revealed that *Matricaria chamomilla* L. consists of flavonoid of luteolin, apigenin and myricetin (23.1, 15.4 and 8.44 mg/ml) respectively, and phenolic of benzoic acid, Protocatechuic acid and gallic acid (12.5, 8.65 and 5.66 mg/ml) respectively.

Keywords: *Matricaria chamomilla*, phytochemical composition, HPLC.

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INTRODUCTION

The use of plants in the pharmacological treatment of disease began long ago. Centuries ago, Chinese, Japanese, and Indian used herbs in disease treatment as traditional medicine (Saito, 2000; Schulz et al., 20001). Plants have medicinal importance especially as antimicrobial activity and those are rich in their phytochemical contents whether alkaloids, flavonoids, etc. which encourages the researchers to investigate more uncommon plants for their medicinal importance (Suleiman 2020). Chamomile (*Matricaria Chamomilla* L.) is an annual herb belongs to the Asteraceae/Compositae family. Chamomile is a widely recognized herb in Western culture. Its medicinal usage dates back to antiquity where such notables as Hippocrates, Galen, and Asclepius made written reference to it. A common ingredient today in herbal teas because of its calming, carminative, and spasmolytic properties, it is also a popular ingredient in topical health and beauty products for its soothing and anti-inflammatory effects on skin. Chamomile has a sweet, grassy, and lightly fruity aroma. Its flowers are daisy-like, with yellow centers (approximately 1-1.5 cm in diameter) and white petals (between 12 – 20in number). It is from the plant's fresh and dried flower heads that infusions, liquid extracts, and essential oils are made (**Zadeh*et al*., 2014**).

MATERIALS AND METHOD

Plant material

Matricaria Chamomilla L. was bought from Agriculture Research Center (ARC), Dokki, Giza-Egypt.

Sample preparation

The powdered plant material (100 grams) from *Matricaria Chamomilla* L. was extracted with minimum amount of 70% ethanol, and purified according to standard procedures reported by **Mabry, et al. (1970)** and **Harborne, (1984)**. The slurry was allowed to stand for 24 h with occasional stirring, and then filtered off. The residue was repeatedly extracted with an excess volume of 70% ethanol. Combined filtrates were evaporated under reduced pressure using rotavapour apparatus until a minimum amount of solvent remained.

Phytochemical screening

The crude ethanolic 70% extract of *Matricaria Chamomilla* L. plant was subjected to preliminary qualitative phytochemical screening for the presence of bioactive constituents such as carbohydrates and/or glycosides, alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, and coumarins using standard phytochemical techniques as described by ***Clarke, 1975; Harborne, 1998***.

Determination of certain pharmacopeial constants of plant materials

Including inorganic (ash) and organic matter (**Brower and Zar, 1984**), acid-soluble and acid-insoluble ash, water-soluble and water- insoluble ash (**Askar and Treptow, 1993**) and crude fibers (**British pharmacopoeia, 1980**).

Investigation of metabolic products including determination of total carbohydrates, soluble and insoluble carbohydrates **(Chaplin & Kennedy, 1994)**, Total nitrogen and protein content in *Matricaria chamomilla* L. were determined using Kjeldahl method **(James, 1995).** Total lipids content, acid value ***(British Pharmacopoeia, 1993)***, ester value ***(Güenther, 1972)***, saponification value ***(Farag, 1995)***, iodine value ***(Mohamed and Amer, 1965)***.

Investigation of total active materials including estimation of total tannins using gravimetric method (Copperacetate method) according to **Ali, *et al.* (1991),** estimation of total saponins according to **Okwu and Ukanwa (2007),** estimation of total alkaloids (Gravimetric method) were carried according to the method described by **Woo, *et al.,* (1977)** and the amount of total phenolics and flavonoids were determined according to methods of ***Chun, et al. (2013); Malla, et al. (2013)***. **2.7. Qualitative and quantitative determination of phenolics and flavonoids** of *Matricaria Chamomilla* L. plant using HPLC was carried out according to the method described by **Mattila*et al*., (2000).** High performance liquid chromatography (HPLC) technique using HPLC Agilent 1100 series equipped with Quaternary pump, set at flow 1ml/min. Autosampler, degasser, column compartment set at 35°C and variable wavelength detector set at 330 nm for flavonoid compounds and 280 for

phenolic compounds. Column: Hypersil ODS 5µm, 250x4 mm was used. This work was carried out in the Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.

RESULTS AND DISCUSSION

Phytochemical evaluation was performed for qualitative and quantitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major biological response of the plant. In the present work, the preliminary phytochemical screening of *Matricaria chamomilla* L. plant proved the presence of various bioactive secondary metabolites as flavonoids, phenolics, saponins, glycosides, cardiac glycosides, tannins and alkaloids. Anthraquinones and sterols were not detected in the plant as represented in table (1).

Kayani*et al*., (2007) recorded that, these secondary metabolites are chemicals produced by means of secondary reactions resulting from primary carbohydrates, amino acids and lipids. These phytochemical constituents are of physiological importance and possess hypolipidemic, anti-tumor or stimulating properties which can reduce the risks of cardiovascular disease and cancer **(Kretovich, 2005).**

Certain pharmacopeial constants of *Matricaria chamomilla* L. including inorganic (ash) and organic matter, acid-soluble and acid-insoluble ash, water-soluble and water- insoluble ash and crude fibers are summarized in table (2). Higher ash content indicates that, the total inorganic mineral is high **(Oloyede, 2005)**. Also, **Smith, (2009)** stated that, the high content of ash is useful in assessing the minerals present in the sample.

It was observed from the obtained data that, the percentage of crude fibers in plant reached to15.50±0.17. Fibers used as prebiotic, where it has the ability to promote bacteria fermentation in colon. Dietary fibers play an important role in human health, which consists mainly of cellulose, hemicelluloses and lignin, which exert different physiological effects on human health **(Zia-ur-Rehman*et al*., 2003).** Food fiber promotes absorption of trace elements in the gut; reduce absorption of cholesterol and lower blood glucose in diabetic patients **(Aliyu*et al*., 2009)**.

The metabolic productsof *Matricariachamomilla* L. including determination of total carbohydrates, soluble and insoluble carbohydrates,Total nitrogen, protein content, total lipids content, acid value, ester value, saponification value and iodine valueare summarized in table (3).

The percentage of total lipids content in plant may be due to an increase in the metabolic rate, which leads to increase in carbohydrate concentrations that convert to lipid by oxidation reaction **(Stocker**, **1960**).

It was also, observed that, the percentage of total nitrogen and total protein reached to (1.57±0.081 and 9.80±0.20), respectively in the plant. Nitrogen is a universally occurring element in all living beings and major component of protein, therefore the concentration of protein is closely linked to the concentration of nitrogen in the plant.

The obtained results in table (4) declared that, total active materials in *Matricaria chamomilla* L. plant like flavonoids reached to 290±0.63mg/g rutin and total phenolics reached to 355.33±0.78 mg/g gallic acid, flavonoids and other plant phenolics are reported to have multiple biological activities in addition to their antioxidants or free radical terminators activity **(Bendini*et al*., 2006)**. Therefore, it is worth, while to determine their total amount in the plant chosen for the study.

Table (1): Phytochemical constituents in *Matricaria chamomilla* L. plant

Bioactive constituents	Observation
Flavonoids	+
Phenolics	+
Saponins	+
Glycosides and/or carbohydrates	+
Cardiac glycosides	+
Tannins	+

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Alkaloids	+
Sterols and/or terpenes	-
Anthraquinones	-

Table (2): Certain pharmacopeial constants of *Matricaria chamomilla* L. plant

Item %	Mean ± SE
Total ash	16.90±0.11
Organic matter	83.10±0.11
Acid soluble ash	10.47±0.22
Acid insoluble ash	7.03±0.15
Water soluble ash	10.90±0.20
Water insoluble ash	6.00±0.19
Crude fibers	15.50±0.17

Data are presented as mean ± SE for 3 replicates.

Table (3): The metabolic products of *Matricaria chamomilla* L.

Item %	Mean ± SE
Total carbohydrates	33.77±0.36
Soluble carbohydrates	18.27±0.22
Insolublecarbohydrates	15.50±0.31
Total nitrogen	1.57±0.081
Total protein content	9.80±0.20
Total lipids content	1.23±0.13
Acid value	15.17±0.15
Ester value	96.57±0.40
Saponification value	111.73±0.39
Iodine value	81.40±0.41

Data are presented as mean ± SE for 3 replicates.

Table (4): The total active materials in *Matricaria chamomilla* L. plant

Item	Mean ± SE
Total Flavonoids (mg/g rutin)	290±0.63
Total phenolics (mg/g gallic acid)	355.33±0.78
Total saponins (%)	1.30±0.15
Total tannins (%)	1.80±0.11
Total alkaloids (%)	1.13±0.17

Data are presented as mean ± SE for 3 replicates.

In the course of the present study, some effective flavonoid and phenolic compounds in *Matricaria chamomilla* L. were identified by HPLC. The result revealed that *Matricaria chamomilla* L. consists of flavonoid of luteolin, apigenin and myricetin (23.1, 15.4 and 8.44 mg/ml), respectively (Table 5& Fig. 1) and phenolic of benzoic acid, Protocatechuic acid and gallic acid (12.5, 8.65 and 5.66 mg/ml), respectively (Table 6& Fig. 2).

Table (5): The flavonoid contents of *Matricaria Chamomilla* L.by HPLC.

Peak No.	Compound	Concentration (mg/ml)
1	Apegenin	15.4
2	Myrecetin	8.44
3	Luteolin	23.1

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Table (6): The phenolic contents of *Matricaria chamomilla* L.by HPLC.

Peak No.	Compound	Concentration (mg/ml)
1	Gallic acid	5.66
2	Protocatechaulic acid	8.65
3	Benzoic acid	12.5

Fig (1): Flavonoid contents of *Matricaria Chamomilla* L.by HPLC.

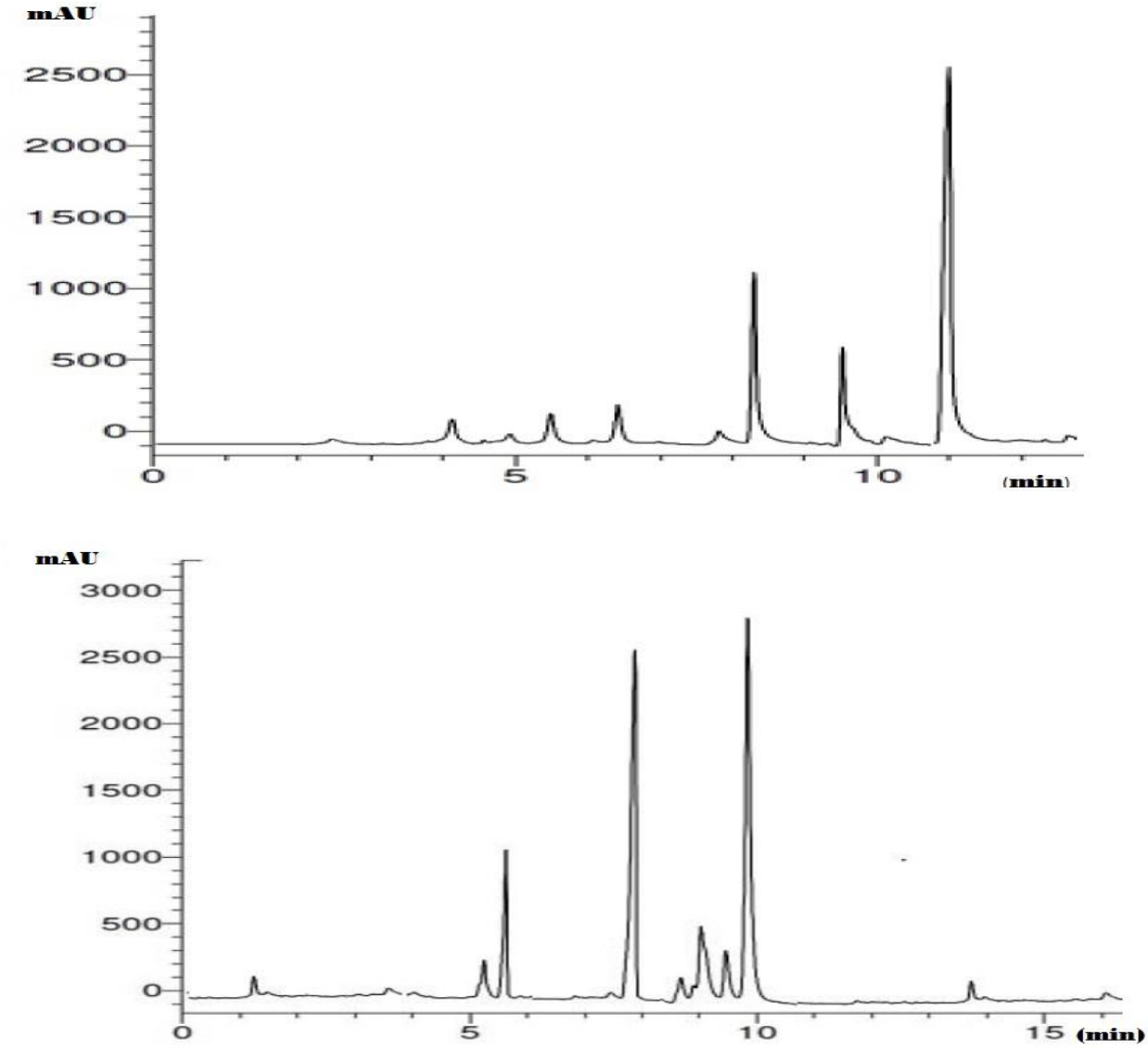

Fig (2): Phenolic contents of *Matricaria chamomilla* L. by HPLC.

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