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EVALUATING THE COMPOSITION OF *MATRICARIA RECUTITA* L. FLOWERS ESSENTIAL OIL IN HYDROPONIC CULTURE

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ABSTRACT

Chamomile plant (*Matricaria recutita* L.) is an herbaceous plant, which is belong to family of Compositae. Chamomile is an annual plant with 20 to 80 cm in height, which grows different environment such as farms, gardens, roadsides and arid lands as well as in the shades. Iranian chamomile's flowers usually contain 0.4% essential oils, in which bisabolol sesquiterpens, bisabolol oxide, bisabolone oxide, farnesene and chamazulene constitute the main portion of the essence. The aim of this study was to evaluate the composition of chamomile's flowers essential oils in hydroponic culture.

Chamomile plant was grown in hydroponic culture contains Hoagland's solution; The flowers were harvested at the end of growing season (when plants were three months old) and then dried. Essential oils were obtained by distillation with water steam using BP (British pharmacopeia) apparatus. Finally, analysis of the essential oils were accomplished by GC-MS and TLC methods.

Of the compounds identified in the *Matricaria recutita* L. flowers essential oil, bisabolol oxide A, with 59.53%, bisabolone oxide with 29.86%, bisabolol oxide B with 6.57%, chamazulene with 2.27%, spathulenol with 1.32% and farnesene with 1.23% are notable, respectively.

Key words: *Matricaria recutita* L, hydroponic, Essential oils, Bisabolol.

INTRODUCTION

Chamomile (*Matricaria recutita* L), is a perennial plant, 20 to 80 cm in height, which grows in pasture or mixed as weed in cultivated land. The plant has a branched stems, each lead to a flower head with a diameter of 1.5 to 2 cm. The leaves are long and thin with leaflet like notches. The flower heads are constructed by two types of white marginal ligulate florets and non-marginal yellow tubular florets[1,2].

Chamomile is one of the most widely used medicinal plants in the world and recorded in the pharmacopoeia of 26 countries. Chamomile has wide capability over a range of climate and soils and grown as aromatic plant in many countries of the world. The annual world consumption of chamomile flowers is more than 4000t. There are a number of species of chamomile spread over Europe, North Africa and the temperate region of Asia. Some of the most common species are Annual or German chamomile (*Matricaria*

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recutita L.) and perennial chamomile (*Chamaemelum nobile*). Unfortunately this species is at the verge of extinction due to improper and immature flower collection.

Chamomile's essential oil comprises 0.5% to 1.5% of the flower head. One hundred twenty chemical constituents have been identified in chamomile, including terpenoids, flavonoids and coumarins.

Iranian chamomile flowers contain at least 0.4 percent oil, which is mostly composed of the bulk bisabolol sesquiterpens, bisabolol oxide, farnesene, spathulenol and chamazulene. The European chamomile includes Bisabolol rather than Bisabolol oxides[2-4]. The amount of essential oil of chamomile flowers differs in various chamomile plants and can range between 0.3 to 1.5 percent or up to 3 percent in various types of crops. The minimum essential oil amount acceptable in other pharmacopoeia such as British Pharmacopoeia is 0.25 to 0.4 percent[5]. The specific blue color of Chamomile essential oil is due to the presence of a substance called chamazulene, that this compound is acquired during distillation of Matrices in the secretary ducts of flowers. Matrices is a pro-azulene, which degrades within water steam distillation process followed by unfolding of lactone ring converts to chamazulene charbonic acid. Afterwards, undergoing decarboxylation, the mentioned compound converts to chamazulene[1,6].

Inflammatory effect of azulenes (Chamazulene, Prochamazulene and Guaiazulene) occurs with affecting on pituitary and adrenal glands and stimulating the release of cortisone and inhibiting histamine release[7].

Chamomile flowers contain a group of lipophilic substances with prominent anti-inflammatory effect and also have some hydrophilic materials with a strong spasmolytic impact. Lipophilic substances include sesquiterpens (Chamazulene) and hydrophilic substances, including flavonoids (apigenin) and cumarines[3].

According to the result of different researches using hydroponic culture in an industrial way seems to be rational and cost saving due to optimization of production functions and consequently producing more crops. For example, results of different researches using hydroponic culture for different crop production show advantages of ability to control essential nutrient consumption as well as plant environment in *Brassica napus* L. and *Lactuca sativa*[8,9]. The results of these researches showed that controlling the consumption of nutrient of media is much easier and more yield harvested.

Many researches have been done in other countries related to the effect of different factors on Chamomile values, For example, the effect of Chamomile on reduction of cytochromes activity show that using chamomile tea for 4 weeks resulted in reduction of 39% activity of Cyp 1A2[10] but little is down using hydroponic culture.

Many researches around the world try directly and indirectly to increase valuable essential oil in Chamomile. However the variation of chemical composition of secondary metabolites of Chamomile in different location as well as in different varieties or genotypes makes essential oil analysis more valuable. For example, in a study by Gosztola et al.[11], on Chamomile plant in four geographic regions of Hungary (Sorokasari, Lutea, Goral, Bona), the results demonstrated the effect of geographical and environmental factors on the amount of produced oil and also metabolites in chamomile flowers. They reported that the most and least oil production rates were 0.93 and 0.61 g per 100 g of dried flowers, respectively and the production rate of Bisabolol oxide ranged between 30 to 41 percent, alpha - Bisabolol between 32 to 48 percent, and Chamazulene ranged between 21 to 25.5 percent. In a study conducted by Sashidhara et al.[12] on Himalayan chamomile alpha-Bisabolol with 16% can be mentioned which is regarded as a great valuable substance widely used in cosmetic industries.

Due to the more consumption of ingredients of chamomile and the verge of extinction of this plant, using new method for higher production of chamomile with high efficiency in a controlled condition such as hydroponic culture seems necessary. Unfortunately, there is no any report in using hydroponic media in order to harvest Chamomile in Iran. Therefore, due to lack of information about the composition of Iranian chamomile oil in hydroponic condition, this research was conducted to evaluate the amount and composition of essential oil of chamomile's flower in hydroponic culture.

EXPERIMENTAL

Hydroponic culture methods

Matricaria recutita seeds were obtained from Isfahan Agricultural Research Center. To produce seedlings, chamomile seeds were washed and disinfected by diluted solution of ethanol (70%) and water (with a ratio of 1 to 10) under laminar air flow. Collected seeds were separated precisely and transferred to sterile petri dishes due to the abundant mucilage. An autoclave with steam pressure of 105 Kilopascal and temperature of 121°C used to sterilize the dishes and other facilities. Seedlings were transferred to hydroponic culture contain Hoagland's solution[13] and kept in control condition (temperature 20 to 25°C, relative humidity 65%). Nutrient solution was replaced every two weeks.

Plant samples (flowers) were obtained when the white ligulate florets stood horizontally and 50% of yellow tubular florets bloom. At this time, flowers have the highest amount of essential oil[3]. It took 10 days for chamomile flowers to be harvested.

Isolation of the essential oil from the flower

Determining the amount of essential oil was performed by using steam distillation[14,5]. To do this, 20 g of dried flowers of the plant were poured into a 500 mL Erlenmeyer flask and 500 mL water was added and distillation was continued for 4 hours[14]. The obtained essential oil in the scaled tube was collected by adding 1 mL N- pentane as a solvent[5].

Identification of essential oil components

TLC Analysis

Extracted essential oil (21.0 g) were pured in 1 mL toluene, then 20 mL of mixture was planted on a silica gel GF 254 plate with approximately 2 cm distanced from the bottom edge. In a prepared chromatogram, standard bisabolol, azulene, dried flowers essential oil and dried extract of chamomile flowers were spotted on the plates. The solvent system used was toluene-ethyl acetate (93-7 respectively). After TLC developing, vanillin reagent (1% ethanolic vanillin) - Sulfuric acid (sulfuric acid and 10% ethanol) was used to visualize patches. The plates then were placed in oven on 110°C for 5 minutes[12].

GC/MS Analysis

The hydrodistilled flower oils of *Matricaria recutita* were analyzed by GC and GC/MS. Gas chromatography analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (39 m x 0.25 mm; film thickness 0.25 μm). The carrier gas was helium with a flow rate of 2 mL/min, the oven temperature for first 4 min was kept at 60°C and then increased at a rate of 4°C /min until reached to the temperature of 280°C, injector and detector temperatures were set at 280°C.

Confirmation of peak identity was effected by co-chromatography with standards and GC-MS. The mass spectra were recorded on a Hewlett Packard 6890 MS detector coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m x 0.25 mm; film thickness 0.25μm). The gas

chromatography condition was as mentioned previously. Mass spectrometer condition was as follow: ionized potential 70 eV, source temperature 200°C. Identification was based on retention data and computer matching with the WILEY 275.L library as well as by comparison of electron-impact-mass spectra (EI-MS) with those relevant reference samples and the literature.

The total number of hydroponic container was 25, containing four plants in each container. Out of 100 plants, 75 plants were harvestd. Each Chamomile plant had 2 to 5 flowers, consequently an average of 3.84 flowers per plant were obtained. Drying of wet flowers led to have dried flowers, containing 85% water. Each dried flowers weighted 0.12 g in average, and the total weight of dried flowers was evaluated as much as 34.6 g. In fact, for each plant 0.46 g dried flower was obtained.

Extraction of 20 g of dried chamomile flowers resulted to have 0.21 g essential oil, thus 1.051 percent oil was obtained. According to previous researches conducted on the amount of essential oil of various chamomile flowers can differ between 0.3 to 1.5 percent, which has been reported up to 3 percent in cultivated species[15]. The percentage of essential oil had been estimated as 0.4 percent in Iran[16], which in our case is 2.5 times higher compared to Iranian estimated average.

According to chromatograph of the chamomile essential oil of chamomile flowers eight chemical compounds including: Bisabolol oxides, Farnesene, Alpha Bisabolol, En-yn-dicycloether, Bisabolone oxides, Farnesene and Chamazulene are identified[17]. In this study, of compounds identified in the essential oil of *Matricaria recutita* L. using GC/MS were Bisabolol oxide A (53.59%), Bisabolone oxide (29.86%), Bisabolol oxide B (29.86%), Chamazulene (2.27%), Spathulenol (1.32%) and Farnesene (1.23%). A complete list of essential oil compounds of chamomile flowers are tabulated in Table 1.

Table 1: Profile of major compounds identified in the essential oils of *Matricaria recutita* flowers: RT: Retention time

Name of compound	RT	Percentage
Beta – Farnesene	18.52	1.23
Spathulenol	21.97	1.32
Bisabolol oxide B	24.16	6.57
Bisabolol oxide A	26.64	53.59
Bisabolone oxide	26.24	29.86
Chamazulene	26.10	2.27

The result of a study conducted with the aim of determining the constituents of chamomile essential oil obtained from cultivation plants in natural areas of central Iran, the percentage of Bisabolol oxide A, Bisabolol oxide B and Chamazulene were 53%, 8% and 5/3%, respectively[18]. Comparing the results of hydroponic culture and planting chamomile in nature the difference was not statistically.

RESULTS AND DISCUSSION

Matricaria recutita plant is frequently cultivated in different parts of the world, especially in Europe, and its oil is used for anti-inflammatory, anti-spasmodic, carminative and tonic digestive properties[18]. Sesquiterpene bisabolol, which is present in cosmetic products, is widely applied in sunscreen creams and lotions and topical anti-inflammatory creams. The mechanism of the anti-inflammatory effect of bisabolol is inhibition of Lipo-oxygenase and Cyclooxygenase production[3].

In this study, hydroponic media was used .to produce Chamomile flowers because many characteristics and properties of the essential oil of chamomile flowers, such as morphological characteristics and also the amount of flower essence, are influenced by genetic factors such as sex, species as well as environmental factors. In hydroponic culture many factors such as the geographical climate, planting date, plant density, salinity and moisture of soil can be controlled. So the hydroponics method may be a good way for higher production of chamomile's flower with higher essential oil.

Applying hydroponic in many research was based on agricultural and industrial perspective rather than pharmacology and herbal medicine development, therefore, it is impossible to compare the results proficiently[19,20].

Most existing researches on various stresses, contain the effect of reducing and increasing the mineral composition in nutrient solutions, using different substrates and the integration of hydroponic cultivation and biotechnology of herbal drugs[21,22].

With regard to the acquired results, no significant difference was observed in type and the percentage of active ingredients in Chamomiles, while planted naturally or hydroponically. Also in this study, it was found out that the highest percentage of produced chamomile flowers essential oil composition belong to bisabolol oxide A, bisabolone oxide and bisabolol oxide B.

It is recommended for further researches to cultivate the refined or imported seeds hydroponically, in order to identify the effect of seed's type and source on the amount of produced secondary metabolites in *Matricaria recutita*. L. It is also suggested to provide the possibility of industrial cell culture of Chamomile with the aim of producing compounds such as Farnesene in callus derived from cell culture of Chamomile.

CONCLUSION

The composition of essential oils of chamomile's flower under hydroponic condition was almost similar to wild collected plant as well as cultivated crops.

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