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# Isolation and Identification of the Major Chemical Components in the Capitula of *Matricaria chamomilla* Grown in Khuzestan Province of Iran

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*Matricaria chamomilla* is an important medicinal plant known for its antiinflammatory, wound healing, spasmolitic and antiseptic actions since long time. In this research, the major chemical components of the volatile oil obtained by hydrodistillation from *Matricaria chamomilla* plant grown in the surrounding fields of the city of Dezful, North West of Khuzestan province of Iran, were separated and identified. First the family, genus and species of the plant were identified then the capitula of the plant were hydrodistilled by using Clevenger hydrodistillation apparatus and the volatile oil was collected. Separation of different components was achieved by successive TLC and column chromatography techniques. Three major components were separated, purified and identified as chamazulene, bisabolonoxid and bisabololoxid A. The chemical structures of the components were determined by their IR, $^1$H NMR and MS spectra.

**Key Words: *Matricaria chamomilla*, German chamomile, *Matricaria recutita*, Wild Chamomile, Hungarian chamomile.**

## INTRODUCTION

German chamomile (*Matricaria recutita*), also spelt as Camomile, is an annual plant of the sunflower family Asteraceae. Synonyms are: *Chamomilla chamomilla*, *Chamomilla recutita, Matricaria chamomilla, Matricaria suaveolens*, Wild chamomile, Hungarian chamomile and Scented Mayweed. Scientific classification is: [Kingdom:Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Asterales; Family: Asteraceae; Genus: Matricaria; Species: *M. recutita*; Binomial name: *Matricaria recutita* L.] It usually grows near people all over Europe and temperate Asia. It is widely introduced in temperate North America and Australia. As the seeds need open soil to survive, it often grows near roads, around landfills and in cultivated fields as a weed. The branched stem is erect and smooth and grows to a height of 15-60 cm. The long and narrow leaves are bipinnate or tripinnate. The flowers are borne in paniculate capitula. The white ray florets are furnished

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with a ligule, while the disc florets are yellow. The hollow receptacle is swollen and lacks scales[1]. The flowers have a strong, aromatic smell and bloom in March and April in Iran. The flowers of chamomile provide 1-2 % volatile oils containing α-bisabolol, α-bisabolol oxides A and B and matricin (usually converted to chamazulene). Other active constituents include the bioflavonoids apigenin, luteolin and quercetin. These active ingredients contribute to chamomile's antiinflammatory, antispasmodic and smooth muscle-relaxing effects, particularly in the gastrointestinal tract[2].

Chamomile has been used for centuries as a medicinal plant, mostly for gastrointestinal complaints. It is used in various parts of the world as a table tea. It was used to regulate monthly periods. It is splendid for kidneys, spleen, colds, bronchitis, bladder troubles, to expel worms, for ague, dropsy and jaundice. The tea was believed to make an excellent wash for sore and weak eyes and also for other open sores and wounds[2]. German chamomile is used medicinally against sore stomach, irritable bowel syndrome and as a gentle sleep aid. It can be taken as an herbal tea, two teaspoons of dried flower per cup of tea. For a sore stomach, some recommend taking a cup every morning without food for 2-3 months. It is also used as a mouthwash against oral mucositis[3,4]. It has acaricidal properties against certain mites, such as *Psoroptes cuniculi*. The primary active ingredient of the essential oil from German chamomile is bisabolol. In homeopathy the remedy chamomilla is prepared from German chamomile and is used mainly to give relief to teething babies. Chamomile is also used cosmetically, primarily to make a rinse for blonde hair[1]. Chamomile also has calming and soothing properties. It is used for nervousness, headaches, anxiety and hysteria. It is also beneficial for colds and flu. Its antispasmodic properties benefit cramps and spasms, probably due to the easily assimilable form of calcium found in it. One tablespoon steeped in a covered cup of boiling water with two slices of fresh ginger is reported to be a very effective treatment for menstrual cramps and other pains and spasms. Chamomile is frequently used for digestive complaints and taken regularly will gently regulate the bowels. The same tea may be used for minor digestive problems such as acid indigestion and gas, weak stomach, stomach pains, lack of appetite and colic pain. It is also effective against intestinal parasites, like worms. Chamomile has tonic, diaphoretic (causes sweating) and analgesic (pain-relieving) properties. Thus, this plant is tonic, stomachic, anodyne, antispasmodic, laxative, diaphoretic, analgesic, carminative, antiinflammatory[5], sedative[6].

## EXPERIMENTAL

The flowers of the wild-growing *Matricaria chamomilla* were collected from the surrounding fields of the city of Dezful, North West of Khuzestan province of Iran. The plant was identified as *Matricaria chamomilla* by the Herbarium Department and Faculty of agriculture of Shahid Chamran University, Ahwaz, Iran. NMR spectra were recorded on Bruker FT-NMR Spectrophtometer 80 MHz ($^1$H) using TMS as

internal standard. Mass spectra were measured on Vannian Mat: BII-A (Germany) mass spectrometer. Infrared spectra were recorded using a JASCO, IR700 Infrared spectrophotometer. All the chemicals were purchased from Merck.

**Collecting and drying flowers of *Matricaria chamomilla*:** The flowers of the wild-growing *Matricaria chamomilla* were collected from the surrounding fields of the city of Dezful, North West of Khuzestan province of Iran at the early days of March while the plant was flowering. The picking up of the flowers was done from early morning till the mid days over several consecutive days by hand and scissors. After cleaning the flowers, they were spread over a table cloth in a room and left for three days to dry.

**Hydrodistillation of the dried flowers:** 50 g of *Matricaria chamomilla* dried flowers were crushed by using a mortar and pestle then placed in a 1000 mL round bottomed flask. 500 mL of 1 % saline was added then hydrodistillation was carried out. A blue coloured distillate was obtained. This process was repeated several times to collect sufficient amount of the essential (volatile) oil (the yield was 0.4-0.6 % w/v).

**Solvent extraction of the dried flowers:** A mixture of 20 g of *Matricaria chamomilla* flower powder and 60 mL of dichloromethane was placed in a 250 mL Erlenmeyer flask and stirred with a mechanical stirrer for 8 h at room temperature. After filtration of the mixture and removal of the solvent, a yellow-brown gelly like material was obtained which was kept in refrigerator.

**Chromatography analysis of the essential (volatile) oil:** Thin layer chromatography on silica gel with chloroform:toluene (3:1 v/v) as mobile phase was carried out. The spots were developed first by spraying about 8 mL of 5 % solution of sulphuric acid in ethanol then immediately by spraying 1 % solution of vanilla in ethanol. The results showed six coloured spots (Table-1). However, three major spots with $R_f$ = 0.92, 0.55 and 0.2 were the major ones and decided to separate them by preparative and column chromatography.

TABLE-1
THIN LAYER CHROMATOGRAPHY RESULTS OF THE ESSENTIAL (VOLATILE) OIL OBTAINED FROM THE FLOWERS OF *Matricaria chamomilla* PLANT GROWN IN THE CITY OF DEZFUL

| Spot No. | $R_f$ | Appearance |
|---|---|---|
| I | 0.97 | Dark violet spot |
| II | 0.92 | A large size red-violet spot |
| III | 0.74 | A medium size dark brown spot |
| IV | 0.55 | A very large size blue-violet spot |
| V | 0.35 | A brown-violet spot |
| VI | 0.20 | A very large size yellow-orange spot |

**Chromatography analysis of the extracted material:** Thin layer chromatography on silica gel with chloroform:toluene (3:1 v/v) as mobile phase was carried out. The spots were developed first by spraying about 8 mL of 5 % solution of

sulphuric acid in ethanol then immediately by spraying 1 % solution of vanilla in ethanol. The results showed five coloured spots (Table-2). However, one spot with $R_f$ = 0.53 was the major one. No further work was done on this fraction.

TABLE-2
THIN LAYER CHROMATOGRAPHY RESULTS OF THE EXTRACT OBTAINED FROM THE FLOWERS OF *Matricaria chamomilla* PLANT GROWN IN THE CITY OF DEZFUL

| Spot No. | $R_f$ | Appearance |
|---|---|---|
| I | 0.99 | A violet spot |
| II | 0.75 | A brown spot |
| III | 0.53 | A very large size blue-violet spot |
| IV | 0.35 | A yellow-brown spot |
| V | 0.30 | A coloured band |

**Preparative thin layer chromatography of the essential (volatile) oil:** Preparative TLC on silica gel with dichloromethane as mobile phase resulted in the separation of three major bands with $R_f$ = 0.93, 0.75 and 0.3. This process was repeated several times. Finally, further purification of each of the components separated from these bands was carried out by TLC with ether as the mobile phase. IR, $^1$H NMR and MS spectra of each component were taken.

**Column chromatography of the essential (volatile) oil:** Column chromatography of the essential oil on silica gel with dichloromethane as the mobile phase resulted in the separation of the fractions with $R_f$ = 0.97, 0.93, 0.85 and 0.75. Finally, further purification of each of the components separated from these bands was carried out by preparative TLC with ether as the mobile phase. Components with $R_f$ = 0.93 and 0.75 were the major components obtained.

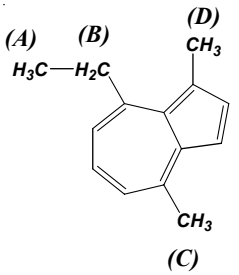
**Characterization of the component with $R_f$ = 0.93 (Chamazulene):** IR (neat liquid) $\nu_{max}$ ($cm^{-1}$): 3094, 3062 (C=C-H, aromatic ring, alkene), 1593, 1553, 1524 (C=C, aromatic ring), 1450 ($CH_2$), 1378 ($CH_3$), 812, 772, 711 (C=C-H, aromatic ring); $^1$H NMR ($CCl_4$, 80 MHz) δ( ppm): 1.33 (t, 3H, protons of A), 2.64 (s, 3H, protons of D), 2.80 (s, 3H, protons C), 2.82 (q, 2H, protons of B), 6.67-8.15 (m, 5H, protons of aromatic rings); its MS (EI) showed m/z: 184 [$M^+$, 90 %], 169 {[M-$CH_3$]$^+$, 100 %}, 155 {[M-$C_2H_5$]$^+$, 37 %}, 154 {[M-2 $CH_3$]$^+$, 28 %}. On the basis of these results, this component was identified as chamazulene.

**Characterization of the component with $R_f$ = 0.75 (Bisabolonoxid):** IR (neat liquid) $\nu_{max}$ ($cm^{-1}$): 1721 (C=O, ketone, ester), 1620 (C=C, alkene), 1450 ($CH_2$), 1372 ($CH_3$), 1020 (C-O, ether, ester); $^1$H NMR ($CDCl_3$, 80 MHz) δ (ppm): 1.14 (t, 3H, protons of A), 1.30 (s, 6H, protons of D+E), 1.63 (s, 3H, protons B), 1.8-2.58 (m, 11H, protons of rings), 5.35 (m, 1H, protons of C); MS (EI) showed m/z: 236 [$M^+$, 3.1 %], 178 {[M-$C_2H_6O$]$^+$, 3.1 %}, 150 {[M-($C_2H_6CO$+CO), 12.9 %]$^+$, 37 %}, 141 {[M-$C_7H_{11}$]$^+$, 42.5 %}, 121 {[M-($CH_3$ + $C_6H_{10}O_2$]$^+$, 32.3 %}, 95 {[M- $C_8H_{13}O_2$]$^+$, 47.1 %}, 93 {[M-($C_7H_{11}$ + 2H)]$^+$, 100 %}, 82{[M-($C_5H_8$ + $C_2H_6O$ + CO)]$^+$, 18.8 %}, 68 {[M-$C_{10}H_{16}O_2$]$^+$, 79.4 %}, 67 {[M-($C_5H_8$ + $C_4H_6O_2$ + $CH_3$)]$^+$, 91.3 %}. On the basis of these results, this component was identified as bisabolonoxid.

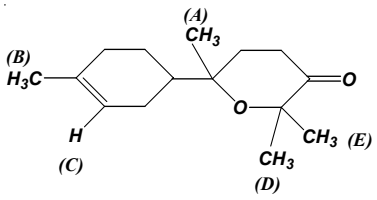
**Characterization of the component with $R_f$ = 0.3 (Bisabololoxid A):** IR (neat liquid) $\nu_{max}$ ($cm^{-1}$): 3600-3200 (O-H, alcohol), 1714 (C=O, ketone) 1450 ($CH_2$), 1350 ($CH_3$); $^1H$ NMR ($CDCl_3$, 80 MHz) $\delta$ (ppm): 1.11 (s, 3H), 1.17 (s, 3H), 1.26 (s, 6H), 1.63 (s, 3H), 1.76-1.93 (m), 2.3 (t, 1H), 3.41 (m, 1H), 5.35 (s, 1H); MS (EI) showed m/z: 219 {[M-($H_2O$ + H)]$^+$, 1.4 %}, 208 (0.5 %), 207 (10.2 %), 148 (0.9 %), 147 (2.8 %), 121 (0.9 %), 95 (20 %), 93 (5.1 %), 82 (39.1 %), 68 (44.7 %) and 67 (54.4 %). On the basis of these results, this component was identified as bisabololoxid A.

## RESULTS AND DISCUSSION

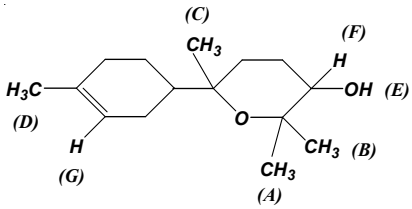
The main objectives of this research are: (i) Isolation of the main chemical components found in the essential oil obtained from the flowers of the wild-growing *Matricaria chamomilla* by hydrodistillation (ii) Identification and determination of the chemical structures of the above mentioned isolated components by using various spectroscopic techniques. After collecting the plant from the surroundings of the city of Dezful, it was identified as *Matricaria chamomilla*, then air dried and finally the flowers were crushed. Hydrodistillation of the crushed flowers of chamomilla plant by using Clevenger hydrodistillation apparatus gave a clear blue coloured liquid. Successive TLC and column chromatography on silica gel with methylene chloride then diethyl ether as the mobile phase resulted in the separation of three fractions with $R_f$ = 0.30, 0.75 and 0.93. $^1H$ NMR, IR and MS spectra of these fractions were taken. On the basis of their spectra, the fraction with $R_f$ = 0.93 was identified as chamazulene (**I**), the fraction with $R_f$ = 0.75 was identified as bisabolonoxid(**II**) and the fraction with $R_f$ = 0.30 as bisabololoxid A (**III**). The structures of some of the isolated components are given in Fig. 1.




(**I**) Chamazulene




(**II**) Bisabolonoxid




(**III**) Bisabololoxid A

Fig. 1. Chemical structures of the isolated components

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