

RESEARCH ARTICLE

Antioxidant Activity of Chamomile Essential Oil and Main Components

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Abstract

The objective of this study was to characterize the antioxidant capacity of the chamomile (*Matricaria recutita* L.) essential oil (EO) as well as its major constituents. For this purpose, Pharmacopoeia grade *Matricariae aetheroleum* was used after the analytical evaluation, which was confirmed by GC/MS and GC/FID, respectively. The main components were identified as α -bisabolol oxide A and B, (*E*)- β -farnesene, α -bisabolone oxide A, chamazulene, and α -bisabolol according the European Pharmacopoeia, respectively. The major constituents α -bisabolol oxide A and chamazulene were purified using prep-TLC from the EO, and the identification and quantification of the constituents were confirmed by GC/MS and GC/FID analyses. Chamomile EO, α -bisabolol oxide A, (*E*)- β -farnesene, chamazulene, and α -bisabolol were evaluated comparatively for their *in vitro* antioxidant activities using the spectrophotometric 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging microdilution assay. The highest free radical inhibitory activity was observed for chamazulene followed by α -bisabolol oxide A, the chamomile EO and (*E*)- β -farnesene, respectively.

Keywords: Chamomile essential oil, *Matricaria recutita* L., DPPH antioxidant activity, chamazulene, sesquiterpene

Introduction

In general, antioxidant substances present in foods or in the biological system at low concentrations compared with oxidative substrates may delay or prevent the oxidation process. Assessments of antioxidant properties of natural compounds including essential oils (EOs) are important due to their uses in food, medicine, and cosmetics (Halliwell et al., 1995; Mishra et al., 2012).

It is well known that EOs have various properties, and the use of EOs as natural antioxidants is common practice in several cases. Essential oils are mainly composed of terpenes such as monoterpenes and sesquiterpenes (Amorati et al., 2013; Baser and Demirci, 2007). The sesquiterpenes are subject for a vast number of biological evaluations and activities including antioxidant potential (Bartikova et al., 2014).

Chamomile, *Matricaria recutita* L. of the Asteraceae family, is an annual herbaceous plant, growing in Germany, Hungary, France, Russia, Yugoslavia, India including Turkey, which is cultivated in several countries such as Egypt, Algeria, Hungary, Germany, among others (Das, 2015). Chamomile EO is highly demanded, which is obtained by steam distillation, and is also described in several pharmacopoeias for its specifications. Pharmaceutically it is commonly used for the treatment of various diseases associated to inflammation, infection, and spasms among others. The EO is characterised by the presence of sesquiterpenes such as chamazulene, farnesene and α -bisabolol and oxygenated sesquiterpenes were the most characteristic chamomile EO such as bisabolone oxide A and bisabolol oxide A and B. The sesquiterpenes are the major biologically active substances in the EO (Rhind, 2012; Schilcher, 2005; Singh et al., 2011; Srivastava et al.,

2010). Among other uses chamomile EO has been reported as a natural antioxidant (Abdoul-Latif et al., 2011; Ayoughi et al., 2011; Owlia et al., 2007; Stanojevic et al., 2016) and also is dark blue coloured oil that contains chamazulene, which is an important factor for the antioxidant power of chamomile EO (Buckle, 2015; Capuzzo et al., 2014; Ornano et al. 2013; Rekka et al., 1996).

The aim of this present study was to evaluate the biological activity of Pharmacopoeia grade chamomile EO. For this purpose, the EO was subjected to *in vitro* antioxidant activity by microdilution spectroscopy. In addition to the EO the major components α -bisabolol oxide A, (*E*)- β -farnesene, chamazulene, α -bisabolol; where the purified bisabolol oxide A and chamazulene were used and evaluated using the DPPH radical scavenging activity.

Materials and Methods

EO and chemicals

Pharmacopoeia (PhEur) grade chamomile EO (*Matricaria recutita* L.) was acquired from (Phatrade, Cairo in Egypt). α -Bisabolol and (*E*)- β -farnesene are purchased from Sigma-Aldrich. All chemicals used were of high purity and analytical grade which were supplied by Sigma Aldrich, Fluka and Merck and used without further purification unless otherwise stated. The chamomile EO composition was previously reported in detail (see Goger et al., 2018).

Gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC-MS) analyses

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 °C. In order to obtain the same elution order with GC-MS, simultaneous auto-injection was done by using same column and an appropriate operational conditions. The GC/MS analysis was carried out with an Agilent 5975 GC/MSD system as previously described in detail (Demirci et al, 2015; Goger et al., 2018).

Analytical and preparative thin layer chromatography (TLC, prep-TLC)

Chamomile EO was analysed by TLC. TLC analysis was performed on silica gel 60 G_F 254 (Merck) using chloroform-toluene (3:1, v/v) as a mobile phase by spraying with anisaldehyde/sulphuric acid and heating for 5 minutes at 110 °C. α -Bisabolol oxide A and chamazulene were isolated using successive prep-TLC techniques from the chamomile EO and the identification and quantification of the constituents were by GC/MS and GC/FID analysis.

The DPPH free radical scavenging activity

The DPPH free radical scavenging activity is based on the ability of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants (Kumarasamy et al., 2007). Briefly, stock solutions and DMSO (control) were prepared and added 80 µg/ml DPPH solution in MeOH. The mixtures were shaken vigorously and left to stand in the dark for 30 min at room temperature, then absorbance was read at 517 nm. Radical scavenging capacity was expressed as percentage inhibition (I %) and calculated using the following equation:

$$(I \%) = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \text{ (formulae)}$$

Ascorbic acid was used as a positive control. The DPPH IC₅₀ values (IC₅₀ value is the concentration of the sample required to inhibit 50 % of radical) of the samples were calculated and are listed in Table 2.

Results and Discussion

Chamomile EO composition

The chemical composition of the study material chamomile EO was recently reported where α -bisabolol oxide A (**1**) (47.7 %), (*E*)- β -farnesene (**2**) (21.5 %), α -bisabolol oxide B (**3**) (6.2 %), α -bisabolone oxide A (**4**) (5.7 %), chamazulene (**5**) (4.1 %) and α -bisabolol (**6**) (2.1 %) (Goger et al., 2018), were identified as the main components. The MS fragmentation of the main components are shown in Figure 1. which was detailed and listed in Table 1.

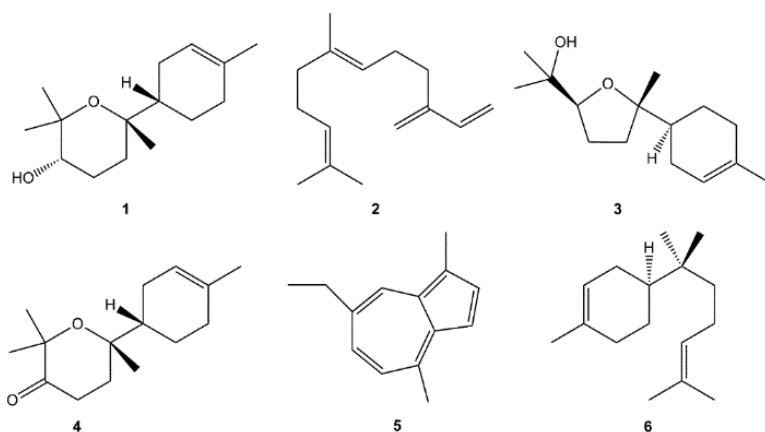
Stanojevic et al. (2016), identified 52 components consisting of β -farnesene (29.8 %), α -farnesene (9.3 %), α -bisabolol and its oxide (15.7 %), chamazulene (6.4 %), germacrene D (6.2 %) and spiroether (5.6 %) in chamomile EO. In another study, major compounds were identified as chamazulene (31.2 %), 1,8-cineole (15.2 %), β -pinene (10.1 %), α -pinene (8.14 %), α -bisabolol (7.5 %) and terpinen-4-ol (4.1 %) (Farhoudi, 2013). Also the chamomile EO consisted of α -bisabolol oxide A (48.2 %), α -bisabolol oxide B (23.31 %), α -bisabolol (12.1 %) and β -farnesene (5.2 %), chamazulene (2.4 %) sesquiterpenes as major constituents (Roby et al., 2013). Ayoughi et al. (2010), reported that (*E*)- β -farnesene (24.2 %), guaiazulene (10.6 %), α -bisabolol oxide A (10.2 %), α -farnesene (8.7 %) and α -bisabolol (7.3 %) were present in *M. recutita* EO.

Table 1. GC/MS analysis results of chamomile EO

Compounds	Rt (min)	<i>m/z</i> (Relative intensity, %)	%
(<i>E</i>)- β -Farnesene	33,66	M ⁺ 204 (4), 189 (3), 175 (1), 161 (18), 148 (4), 133 (33), 120 (23), 107 (11), 93 (67), 79 (26), 69 (100), 55 (16), 41 (52)	21.5
α -Bisabolol oxide B	46,38	M ⁺ 238 (2), 220 (5), 205 (3), 179 (27), 161 (55), 143 (100), 134 (39), 125 (34), 107 (24), 105 (74), 95 (27), 85 (58), 71 (35), 59 (47), 43 (55)	6.2
α -Bisabolone oxide A	47,45	M ⁺ 236 (2), 218 (3), 200 (5), 178 (11), 169 (6), 150 (28), 141 (96), 132 (16), 121 (54), 107 (38), 93 (100), 79 (34), 67 (46), 55 (20), 43 (47)	5.7
α -Bisabolol	48,06	M ⁺ 222, 204 (33), 189 (6), 161 (15), 147 (6), 134 (11), 119 (97), 109 (100), 93 (46), 79 (20), 69 (85), 55 (23), 43 (63)	2.1
Chamazulene	52,73	M ⁺ 184 (97), 169 (100), 153 (34), 141 (13), 128 (19), 115 (13), 89 (6), 77 (6), 63 (3), 45 (6)	4.1
α -Bisabolol oxide A	52,96	M ⁺ 238 (1), 220 (2), 180 (3), 159 (3), 143 (100), 134 (18), 125 (36), 107 (26), 93 (37), 81 (12), 71 (24), 59 (17), 43 (40)	47.7

Rt: Retention time; *m/z*: Mass-to-charge ratio; % calculated from FID data.

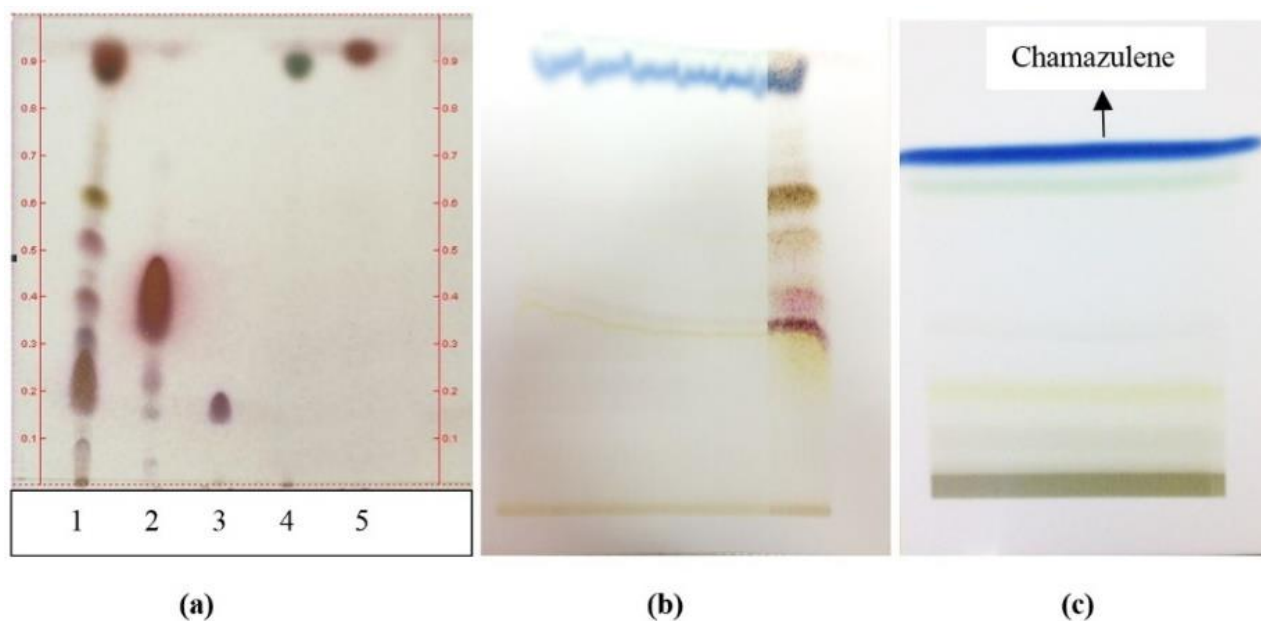
Figure 1. Chamomile EO major constituents



Isolation of the EO components

α -Bisabolol oxide A and chamazulene were purified by prep-TLC eluting with chloroform-toluene (3:1, v/v) from the chamomile EO (Figure 2), and the identification-quantification of the constituents were performed by GC/MS and GC/FID analyses.

Figure 2. (a) TLC of analytical standard, (1) chamomile EO, (2) α -bisabolol, (3) α -bisabolol oxide A, (4) chamazulene, (5) (*E*)- β -farnesene; (b) preparative TLC of chamomile EO; (c) isolation of high-purity chamazulene.



As previously reported (Ashnagar et al., 2009), three major components were separated, purified and identified namely as chamazulene ($R_f = 0.93$), bisabolonoxide ($R_f = 0.75$) and bisabololoxide A ($R_f = 0.3$) from *Matricaria recutita* EO. The chemical structures of the components were determined by their IR, ^1H NMR and MS spectra. These data also comply with our results.

Results of the DPPH free radical scavenging assay

In the DPPH assay the radical scavenging ability of the chamomile EO, and its four main components as listed in (Table 2), and also the positive control (ascorbic acid) was compared spectrophotometrically.

Table 2. Antioxidant activity of chamomile EO and its four main components on DPPH* assay

Samples	DPPH IC ₅₀ (mg/mL)
Chamomile EO	2.20
α -Bisabolol	43.88
α -Bisabolol oxide A	1.50
(<i>E</i>)- β -Farnesene	7.45
Chamazulene	0.27
Ascorbic acid (reference)	0.015

The results showed that the scavenging activity of the main substances were in the following order; chamazulene > α -bisabolol oxide A > chamomile EO > (*E*)- β -farnesene > α -bisabolol, respectively, accordingly to their IC₅₀ values compared to the positive control ascorbic acid, which exhibited high antioxidant activity with IC₅₀ value of 0.015 mg/mL in the same experimental system.

Previous antioxidant activity of chamomile EO showed the best antioxidant properties after 90 minutes of incubation with the EC₅₀ value of 2.07 mg/mL by DPPH free radical method (Stanojevic et al., 2017). In another antioxidant activity study by DPPH free radical scavenging and β -carotene/linoleic acid methods, the EO EC₅₀ value was determined as 5.63 \pm 0.20 mg/mL as reported by Ayoughi et al. (2010). In the β -carotene bleaching test, the EO gave the best inhibition result of 82.5 % after 120 minutes, supporting the antioxidant activity (Owlia et al., 2007).

Interestingly, Capuzzo et al. (2014), reported that chamazulene was unable to react with DPPH'. According to the literature, it is necessary to avoid DPPH assay to test chamazulene radical scavenging activity owing to its interference with the nitrogen-centred DPPH, colour factor and thus suggests the use of ABTS assay instead. Actually when chamazulene was evaluated using ABTS', a strong and significantly higher free radical scavenging activity was observed (IC₅₀ 3.7 \pm 0.7 μ g/mL).

Conclusion

The results showed that the *in vitro* scavenging activity of chamazulene was the highest after α -bisabolol oxide A, and the chamomile EO followed by the other chamomile major volatile constituents. To the best of our knowledge this is the first report on the antioxidant activity of α -bisabolol oxide A and (*E*)- β -farnesene constituents, which may responsible of the total activity of the EO.

ACKNOWLEDGEMENTS

This work was supported by the Anadolu University Scientific Research Projects (BAP-1401S008) fund, which is part of the PhD thesis of ZF. Part of this work was also presented in ISEO 2014, Istanbul.

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