

## The Antioxidant Activity of Chamomile (*Matricaria chamomilla* L.) Extract in Sunflower Oil

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**Abstract:** Antioxidants prevent the reaction of free radicals with biomolecules and can remind the nutritional values and physiological properties of foodstuffs. There are two kinds of antioxidant agents, synthetic and natural, nowadays there is an increasing trend among food technologists to replace the synthetic kind with the natural ones. Chamomile (*Matricaria chamomilla* L.) is a well known and valuable medicinal plant that is used widely in Iranian traditional medicine. The antioxidant activity of the essential oil derived from Chamomile had proofed and in this study we show the antioxidant activity of chamomile extract in fat-containing foods. The extract was obtained by the mixture of equal volume of water and ethyl alcohol (1:1). The antioxidant activity was investigated with three methods, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging system, determine of the peroxide and thiobarbituric acid (TBA) number. The antioxidant activity of extracted oil in five concentrations 0.2, 0.4, 0.6, 0.8 and 1 mg/ml were determined by measuring of peroxide and thiobarbituric acid (TBA) values in crud *Helianthus Annuus* (sunflower) oil. In both systems, the antioxidant activity of the extract was valuable and the antioxidant activities were raised by increasing of the extraction oil concentrations. Results show that chamomile extract could be used as a natural antioxidant in foodstuffs as a complementary material.

**Key words:** Chamomile • Peroxide number • TBA number • Sunflower oil • Antioxidant

### INTRODUCTION

Chamomile is sometimes known as "the plant doctor", because it is thought to help the growth and health of many other plants, especially ones that produce essential oils. It is thought to increase production of those oils, making certain herbs, like mints such as *Mentha spicata* (spearmint), *Salvia officinalis* (sage), (*Origanum vulgare*) oregano and *Ocimum basilicum* (basil) stronger in scent and flavor [1-3].

Chamomile tea is also thought to be useful to suppress fungal growth, for example, Mist using the extracts of which part may prevent damping off [4]. The major chemical components of the oil extracted from chamomile are  $\alpha$ -pinene,  $\beta$ -pinene, camphene, sabinene, myrcene, 1,8- cineole,  $\gamma$ -terpinene, caryophyllene, propyl angelate, butyl angelate, chamazulene,  $\alpha$ -bisabolol, bisabolol oxide A, bisabolol oxide B and bisabolone oxide A [5,6].

Also apigenin 7-O-glucoside and various acylated derivatives of apigenin 7-O-glucoside were identified in chamomile [7-10].

Oil oxidation is a free radical chain process leading to the deterioration of oil and lipid containing materials [11,12].

Antioxidant addition is one of the most effective means to retard oxidation. The action of antioxidant depends on its participation in a series of reactions involving radicals [13,14].

Recently, the interest in natural antioxidants has increased since the application of the most widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propyl gallate (PG) has been questioned because of possible toxic and carcinogenic components formed during their degradation [15,16]. Phenolic compounds are the main class of natural antioxidants [17-19].

Chamomile (*Matricaria chamomilla* L.) is one of the popular ingredients in herbal teas. This herb has been traditionally used for medicinal purposes such as selective COX-2 inhibitor with anti-inflammatory activity [20], antimicrobial property, antioxidant property, antiplatelet property and chemopreventive properties [21, 22].

Therefore, the research on active constituents in chamomile extract is widely being carried out [23-25].

The conjunction of chamomile with sedative drugs such as analgesics, benzodiazepines, or alcohol may be contraindicated [26-29].

## MATERIALS AND METHODS

**Materials:** The powder of Chamomile (*Matricaria chamomilla* L.) was obtained from Bajgah research garden, faculty of agriculture, Shiraz university, which is located in 30km north of shiraz, Iran. All samples were collected by hand in spring 2010 at 27°C and were dried in a dark room. A sample of crud sunflower oil without any additives was obtained from Ghoo oil company, Tehran.

**Preparation of Chamomile Extracts:** Extract from 10g dark dried powder of sample was obtained with 100ml of 1:1 ethyl alcohol and water the sample along with the solvent was shaken at room temperature for 1h and then heat the mixture at 80°C for 30 min. to give an initial extract, the infusion was cooled to room temperature, filtered (fraction I). The residues were extracted with the same process again (fraction II) the two fractions were combined and dried with evaporated under vacuum at 50°C and weighed to determine the yield [30, 31].

**Sample Preparation:** Inhibition effect of the oil substrate was achieved by adding of the methanolic solution of the antioxidant to a weighed oil sample. Samples contained 0.2, 0.4, 0.6, 0.8 and 1 mg/ml of the naturally obtained antioxidant from Chamomile in the crude sunflower oil (without antioxidant) were prepared. The samples and a control sample, crud sunflower oil (without antioxidant), were kept at 60°C for 1hr. The peroxide and Thiobarbituric acid number of samples were measured in 0, 8, 16, 24 and 32 days according to the below methods.

**Peroxide Method:** This measurement was accomplished according to the AOCS method (Ce 5b-89). In a container 250 ml, added to 3g of each samples to 30 ml of a solution

of acetic acid and chloroform (1:1) and mixed to prepared a suitable solution, then added 0.5ml saturated solution of KI and stirred it for one minute, 30ml stiller water was added and titrated with 0.01N sodium thiosulphate, the yellowish color was disappeared. Now added 0.5ml starch glue indicator to the solution, continued the titration until the blue color was disappeared. The peroxide number calculated by this equation:

$$PV = (S - S_0) \times N \times 1000 / W$$

Where:

*S* - The amount of consumption thiosulphate for samples;

*S<sub>0</sub>* - The amount of consumption thiosulphate for control sample;

*N* - The thiosulphate solution normalization;

*W* - The weight of samples (g).

**Thiobarbituric Acid Method (TBA):** The thiobarbituric acid number (TBA) is a complement test to determine of the antioxidant activity. The thiobarbituric acid number measures the Malon dialdehyde (MDA) per kilogram of oil. This was carried out using the method recommended by the AOCS (Ca 5c-87). In a container 250 ml, dissolved 1g of each samples to 10ml of carbon tetrachloride and added 10 ml thiobarbituric acid solution (aqueous solution of 0.67% thiobarbituric acid in the same volume of glacial acetic acid) then stirred it for 2 h and centrifuged for 5 min. with 1000 rot/min. after this step the aqueous layer was separated and heated in boiling water bath for 1h. Finally, the absorption values was measured at 532nm wavelength.

TBA was calculated as bellow equation:

$$E = e / (d \cdot a)$$

Where:

*e* - Measurement absorption;

*d* - Cell thickness;

*a* - is weight of sample (g).

**DPPH° Method:** This measurement was carried out according to the Brand -Williams method. In this method prepared seven samples from 1 to 7 mg/ml of the antioxidant and the free radical of DPPH° reacted with the antioxidant and produced yellowish color and reduced the absorption value in wavelength to 517nm. Then added 0.5 ml of various concentrations of extracted

oil to 2ml of  $6 \times 10^{-5}$  methanolic solution of the DPPH° free radical and stay in room temperature for 1h. The absorption of solution was read in 517nm. The control sample was a solution of 0.5ml methanol in 2ml solution of the DPPH°. This test was repeated for 3 times. Radical Scavenging Activity (RSA) was determined in below:

$$\%RSA = [1 - ((A_{control} - A_{sample}) / A_{control}) \times 100]$$

Where:

$A_{control}$  - The control absorption;

$A_{sample}$  - The sample absorption.

We used from  $EC_{50}$  to explain the anti-radically activities which is the percent of the extraction that be able to neutralized 50% of the initial DPPH° free radical.

**Statistical Analysis:** All determinations were carried out in three triplicate and data were subjected to analysis of variance. Statistical analyses were performed using MSTATC software. Differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

Figure 1 illustrate the rate of increase in the anti-radically activities and decrease in the reminded DPPH° free radical with arise of the chamomile extract concentration from 1 to 7 mg/ml.  $EC_{50}$  for chamomile extract is  $5.52 \pm 0.15$  mg/ml.

Figure 2 show the rate of decrease in the remaining DPPH° in 60 min. for the various extract condensations. Radical scavenging activity rise with increasing in extract condensations and was observed to be constant after 60 min. There is no descending in the control sample.

Table 1 shows the average of peroxide number (meq  $O_2$ /kg oil) of the various samples of Chamomile extracts and the control sample in 0, 8, 16, 24 and 32 days.

There are the differences among the control sample and the different concentration of extracts and it is clear that the peroxide numbers depended on the sample concentrations. Increase the concentration decreases the peroxide number.

These numbers illustrate that Chamomile extracts have antioxidant effects. For example Figure 3 illustrate the peroxide number in 32th day and show the punctual differences in the various condensations.

Results from Table 2 indicate that the average thiobarbituric acid number (meq/kg oil) of the various samples of Chamomile extracts and the control sample at 0, 8, 16, 24 and 32 days.

There are the differences among the control sample and the different concentration of extracts.

The TBA numbers are depend on the sample concentrations such as the peroxide numbers and increase the extract concentration makes decrease the TBA number and increase the antioxidant effects.

These numbers illustrate that Chamomile extracts have antioxidant effects. Figure 4 illustrates the TBA number in 32th day and shows the significant differences in the various condensations.

Table 1: The peroxide number (meq  $O_2$ /kg) of samples in the five days. Data are the averages of the three repetitions  $\pm$  standard deviation

Sample	0 day	8 <sup>th</sup> day	16 <sup>th</sup> day	24 <sup>th</sup> day	32 <sup>th</sup> day
C-0.2	$0.43 \pm 0.12$	$14.56 \pm 0.03$	$35.65 \pm 0.25$	$45.76 \pm 0.30$	$60.37 \pm 0.67$
C- 0.4	$0.43 \pm 0.12$	$13.59 \pm 0.15$	$32.76 \pm 0.65$	$42.45 \pm 0.25$	$54.45 \pm 0.78$
C- 0.6	$0.43 \pm 0.12$	$13.66 \pm 0.35$	$32.45 \pm 0.40$	$40.86 \pm 0.72$	$52.40 \pm 0.12$
C- 0.8	$0.43 \pm 0.12$	$13.60 \pm 0.28$	$28.51 \pm 0.24$	$39.15 \pm 0.12$	$48.56 \pm 0.34$
C- 1.0	$0.43 \pm 0.12$	$12.43 \pm 0.05$	$26.33 \pm 0.06$	$36.14 \pm 0.27$	$44.20 \pm 0.24$
Control	$0.43 \pm 0.12$	$18.54 \pm 0.40$	$38.45 \pm 0.07$	$61.04 \pm 0.55$	$78.76 \pm 0.35$

Table 2: The TBA number (meq MDA/kg oil) of samples in the five days. Data are the averages of the three repetitions  $\pm$  standard deviation

Sample	0 day	8 <sup>th</sup> day	16 <sup>th</sup> day	24 <sup>th</sup> day	32 <sup>th</sup> day
C-0.2	0.000	$0.068 \pm 0.01$	$0.114 \pm 0.01$	$0.216 \pm 0.01$	$0.448 \pm 0.01$
C- 0.4	0.000	$0.059 \pm 0.00$	$0.107 \pm 0.01$	$0.133 \pm 0.01$	$0.432 \pm 0.02$
C- 0.6	0.000	$0.053 \pm 0.00$	$0.089 \pm 0.01$	$0.145 \pm 0.00$	$0.376 \pm 0.01$
C- 0.8	0.000	$0.050 \pm 0.01$	$0.085 \pm 0.00$	$0.110 \pm 0.02$	$0.269 \pm 0.01$
C- 1.0	0.000	$0.041 \pm 0.01$	$0.073 \pm 0.00$	$0.129 \pm 0.01$	$0.230 \pm 0.00$
Control	0.000	$0.093 \pm 0.02$	$0.119 \pm 0.02$	$0.243 \pm 0.03$	$0.586 \pm 0.02$

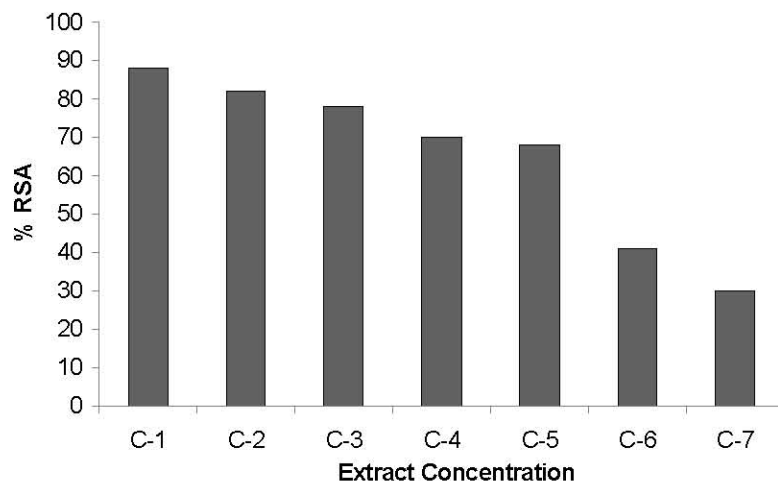


Fig. 1: Relationship between radical scavenging activity with Chamomile extract condensation. C-1 to C-7 are extract condensations from 1 to 7 mg/ml

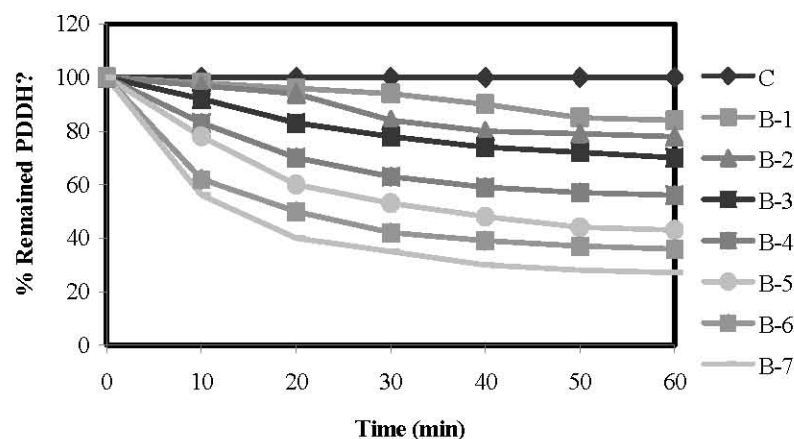


Fig. 2: Trend of the reduce percentage of remained PDDH° in 60 min with Chamomile extract condensation. B-1 to B-7 are extract condensations from 1 to 7 mg/ml.

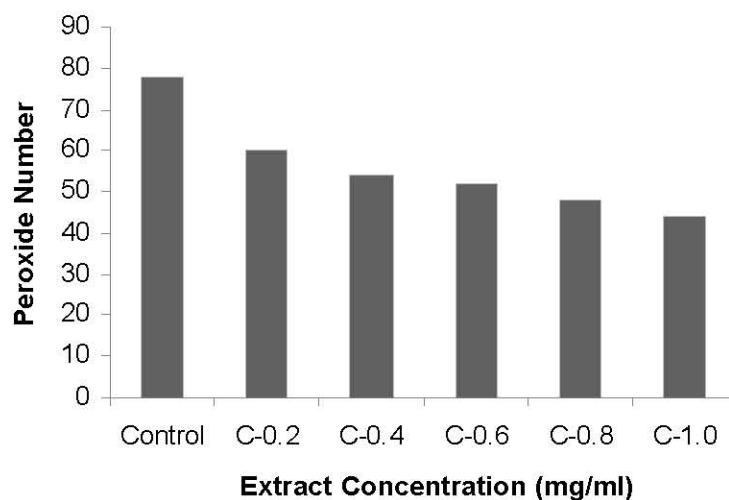


Fig. 3: Relationship between the peroxide number with the Chamomile extract condensation

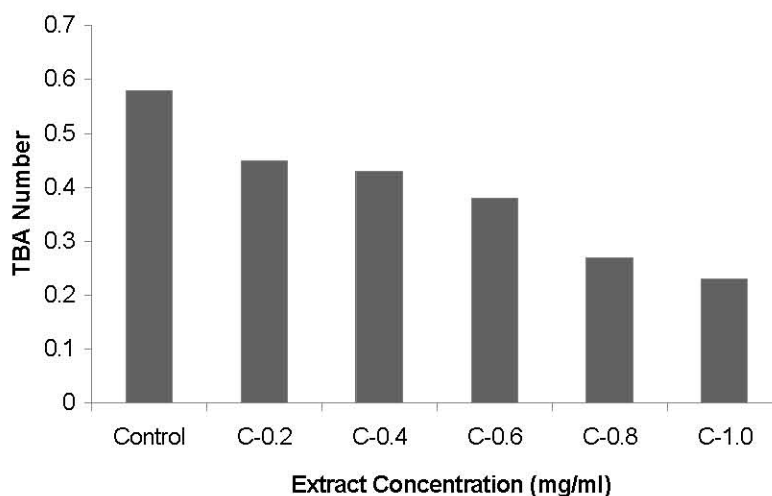


Fig. 4: Relationship between the TBA number with the Chamomile extract condensation

## REFERENCES

- Omid Beigi, R., 2000. Produce of the drug plants, 1<sup>st</sup> edn, Nashr Press, 2: 424-30.
- Zargari, A., 1991. Medical plants, 5<sup>th</sup> edn. Tehran University Press, 2: 942.
- Kulisic, T., A. Radonic and V. Katalinic, 2004. Use of different methods for testing antioxidative of oregano essential oil, Food Chemistry, 85: 633-40.
- Haji Sharifi, A., 2004. Secret of medical plants, Hafez Press, 2: 568.
- Szoke, E.G., A.L. Verzar-Petry and A.M. Smirnov, 1979. Difference in the component composition of the essential oil isolated roots, root callus tissue and cell suspensions of *Matricaria chamomilla* L., Izu. Akad. Nauk SSSR, Ser. Biol., 6: 943-9.
- Ogata, I., T. Kawanai, E. Hashimoto and Y. Nishimura, 2010. Bisabololoxide A, one of the main constituents in German chamomile extract, induces apoptosis in rat thymocytes, Arch Toxicol., 84: 45-52.
- Hoelzi, J. and G. Demuth, 1975. Influence of ecological factors on the composition of the essential oil and the flavones in *Matricaria chamomilla* of different origin, Plant Medical, 77: 46-52.
- Omid Beigi, R., 1999. Produce of the drug plants, 1<sup>st</sup> edn, Astan Ghods Press, 1: 283-95.
- Jaymand, K. and M.R. Rezaee, 2002. Identification of the essential oil compounds in The *Matricaria chamomilla* L. in Tehran and Hamedan, Research in Iranian Medical Plants, 13: 11-23.
- Zargari, A., 1992. Medical plants, 5<sup>th</sup> edn. Tehran University Press, 3: 890.
- Sashidhara, K.V., R.S. Verma and P. Ram, 2006. Essential oil composition of *Matricaria recutita* L. from the lower region of the Himalaya, Flower and Fragrance J., 21: 274-76.
- Sacchaetti, G., M. Muzzoli and M. Maietti, 2005. comparative evaluation of 11 essential oils of Different origin as functional antioxidants, antiradicals and antimicrobials in foods, Food Chemistry, 91: 621-32.
- Madhavi, D.L., S.S. Deshpande and D.K. Salunkhe, 1996. Food Antioxidants, New York, Marcel Dekker Inc., pp: 378.
- Koch, C., J. Reichling, J. Schneelee, *et al.*, 2008. Inhibitory effect of essential oils against herpes simplex virus type 2, Phytomedicine 15: 71-78.
- Kobayashi, Y., Y. Nakano, K. Inayama, A. Sakai and T. Kamiya, 2003. Dietary intake of the Xower extracts of German chamomile (*Matricaria recutita* L.) inhibited compound 48/80- induced itch-scratch responses in mice, Phytomedicine, 10: 657-64.
- Matos, F.J.A., M.I.L. Machado, J.W. Alencar and A.A. Craveiro, 1993. Constituents of Brazilian chamomile oil, J. Essent. Oil Res., 5: 337-39.
- Atoui, A.K., A. Mansouri, G. Boskou and P. Kefalas, 2005. Tea and herbal infusions: Their antioxidant activity and phenolic profile, Food Chemistry, 89: 27-36.
- Emad, S., 2006. Antioxidant effect of extracts from red grape seed and peel on lipid oxidation in oil of sunflower, LWT, pp: 883-92.
- Kamal-Eldin, A. and L.A. Appleqvist, 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols, Lipids, 31: 671-701.

20. Srivastava, J.K., M. Pandey and S. Gupta, 2009. Chamomile, a novel and selective COX inhibitor with anti-inflammatory activity, Life Sci., 85: 19-20, 663-669.
21. McKay, D.L. and J.B. Blumberg, 2006. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.), Phytother Res., 20: 519-530.
22. Duke, J.A., 2001. Handbook of medical herbs, 2<sup>nd</sup> edn., CRC Press LLC., pp: 398.
23. McKay, D.L. and J.B. Blumberg, 2008. An experimental study of the effects of Matricaria chamomilla extract on cutaneous burn wound healing in albino rats, Nat. Prod. Res., 22(5): 423-8.
24. Maeura, Weisburger and Williams, 2007. Wound healing activity of *Matricaria recutita* L. Extract, J. Wound Care, 16(7): 298-302.
25. Andres, C., W.C. Chen, M. Ollert, *et al.*, 2009. Anaphylactic reaction to chamomile tea, Allergol. Int., 58: 135-136.
26. O'Hara, M., D. Kiefer, K. Farrell and K. Kemper, 1998. A review of 12 commonly used medicinal herbs, Arch. Fam. Med., 7: 523-36.
27. Abebe, W., 2002. Herbal medicine: potential for adverse interactions with analgesic drugs, J. Clin. Pharm. Ther., 27: 391-401.
28. Larzelere, M.M. and P. Wiseman, 2002. Anxiety, depression and insomnia, Prim Care, 29: 339-60.
31. Honcariv, R. and M. Repcak, 1979. Chemotype of *Matricaria chamomilla* L., Herbal Pol., 25: 46-52.
29. Sahari, M.A., M. Barzegar and F. Aoughi, 2009. A study on antiradical properties and chemical composition of essential oil of Matricaria chamomilla, 18<sup>th</sup> National Congress on Food Technology, Iran.
30. Zandi, P. and M.H. Godron, 1999. Antioxidant activity of extracts from old tea leaves, Food Chemistry, 64: 285-88.