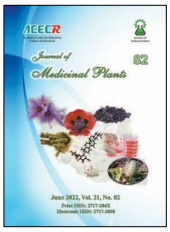
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Research Article

Assessment of the anti-oxidative potential of *Nepeta crispa* Willd. (Lamiaceae) and its effects on oxidative stability of virgin sunflower oil under accelerated storage conditions

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ABSTRACT

Background: *Nepeta crispa*, is an annual plant native to certain regions of Hamadan province in Iran. **Objective:** This study aims to investigate the antioxidant potential of *N. crispa*. **Methods:** Hydro-distillated essential oil and methanol extract from aerial parts of *N. crispa* were obtained. They were subjected to several assays (DPPH, FRAP, TBARS, peroxide value and *β*-carotene-linoleic acid assay) in order to evaluate their antioxidant properties and their effect on prevention of oxidation in sunflower oil. **Results:** The extract consistently exhibited more activity than the essential oil in each and every one of the assays. The activity of the essential oil was not significant compared to positive controls or even the extract. The results from DPPH and FRAP assays correlated well with one another but those obtained from *β*-carotene-linoleic acid assay were to some extents inconsistent with the others. The results from TBARS and peroxide value assays revealed that certain concentrations of the extract effectively prevent the oxidation process in sunflower oil. **Conclusion:** Our data suggest that the methanol extract of *N. crispa* may be considered as novel bio-resource of natural antioxidants for using in pharmaceutical and food industries**.** Hopefully as the future research progress, new lights will be shed on characterization of components in the extract and the mechanisms through which they provide antioxidant activity.

1. Introduction

Nowadays synthetic antioxidants have been suspected to cause numerous harmful or negative effects on health [1] and therefore more strict regulations are imposed to limit their usage. On the other hand, natural antioxidants have attracted much attention due to their fewer side effects.

The genus *Nepeta* is categorized under Lamiaceae family that consists of more than 250

Abbreviations: TBARS, Thiobarbituric Acid Reactive Substances; FRAP, Ferric Reducing Antioxidant Power; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl

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species, 69 of which have been found in Iran [2]. *Nepeta crispa* Willd. is one of those species that is endemic to Hamadan region and exclusively grows in the heights of Alvand mountain. It has a strong aroma and consequently its local name is "Moffarrah" which roughly translates to "what brightens and animates" as a result of its sweet and intense odor. This plant has had a large impact on Iranian traditional medicine especially in Hamadan province. It has been used for various purposes through the years in local homeopathy and herbal medicine. Anti-depressant, antipyretic, anti-asthmatic, expectorant and antiseptic uses are among the others [3, 4].

Recent studies have shown that *Nepeta* genus in general has a rather high antioxidant profile [5-9]. Considering that *N. crispa* mostly grows in Hamadan region, there is not much research regarding this particular specie. Therefore, despite the fact that It might have strong antioxidative properties, there are no studies yet to prove the hypothesis. Hence in this paper, we aimed to evaluate antioxidant activity of its extract and essential oil considering It would be favorable to be used as a substitute for synthetic antioxidants especially in oil products with the aim of reducing oxidation and oil decay.

Lipid oxidation is an elaborate process which is affected by light, temperature, air and presence of oxidation catalysts. Each protective property of antioxidants is carried out by different mechanisms which target a specific stage in oxidation process. Mainly there are two general types of antioxidants; primary which is either a free radical scavenger or a breaker of oxidation chain, and secondary; which its course of action is to deactivate metals, inhibit lipid breakdown or regenerate primary antioxidants.

Different methods are employed to determine the properties of antioxidants in plants. Each method has its own pros and cons. In our study, the essential oil and the methanol extract from *N. crispa* were analyzed by means of the following methods; DPPH, FRAP, β-carotene-linoleic acid, TBARS and peroxide value assay. The overall goal of this paper is to evaluate antioxidative profile of the essential oil and the methanol extract of *Nepeta crispa* Willd. along with their effects on prevention of lipid oxidation in sunflower oil through utilizing five different tests and remark whether or not this plant has the potential to be used in preservation of oils and other products.

2. Materials and Methods

2.1. Preparation of the Plant Material

Aerial parts of *N. crispa* Willd. (Herbarium code: 72) were collected in May of 2021 from the heights of Alvand Mountain, Hamadan province, Iran (at 3500 meters altitudes) and then dried and stored at 25°C until the day of the experiment.

2.2. Preparation of the extract

100 g of the dried parts of the plant was soaked in methanol for 3 days at room temperature. The solvent was evaporated using rotary vacuum evaporator. The remains were concentrated and isolated in a separate container. This procedure was repeated 7 times to attain the full extent of plant extract. The resulting solid masses were preserved in 4 °C in a dark ambient until the day of experiment.

2.3. Preparation of the essential oil

The essential oil of *N. crispa* Willd. was prepared by hydro-distillation of 100 g of the dried parts of the plant material, using a clevenger-type apparatus. The total of 1.9 ml extracted essential oil was then isolated and kept in sealed dark glass vials at 4 °C until use.

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8 to 0.625 mg/ml concentrations of essential oil in methanol were prepared to use in further assays.

2.4. In vitro antioxidant activity

2.4.1. DPPH assay

Free radical scavenging ability of the methanol extract and the essential oil from *N. crispa* Willd. was measured using the method described by Kamkar et al. with a few modifications [10]. Briefly, a 750 µl of various concentrations of plant extract and essential oil in methanol (4000 µg/ ml – 31.5 µg/ml) was added to 300 µl of 0.3 mM DPPH radicals in methanol at a ratio of 5:2, respectively. The mixture was allowed to stand at room conditions for 30 minutes and at It was gently shook from time to time. The absorbance of the resulting solution was measured against a blank at 518 nm. All measurements were performed in triplicate.

Antioxidant activity (%) was obtained using the following formula:

$$AA\% = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$$

Where $Abs_{Control}$ is the absorbance of Control reaction (which contained all reagents except for the test compound) and Abs_{Sample} is the absorbance of the test compound. Galic acid and ascorbic acid were used as standards. Extract and essential oil concentrations providing 50% inhibition (IC_{50}) were figured from the graph plotted of inhibition percentage against their concentration.

2.4.2. β-carotene-linoleic acid assay

In this paper *β*-carotene bleaching assay was performed according to Gursoy et al. (2006) with minor modifications [11].

A stock solution of *β*-carotene-linoleic acid was prepared as follows: 0.5 mg of *β*-carotene was dissolved in 1 ml of chloroform. Afterwards, 25 µl of linoleic acid and 200 mg of Tween 40 was added to the mixture. Chloroform was entirely evaporated using a rotary vacuum evaporator. Then 100 ml of deionized water was added and the mixture underwent a vigorous shaking in order to form an emulsion. 1.5 ml of this reaction mixture was placed in the test microtubes along with 250 µl of various concentrations (0.625 - 8 mg/ml) of the extract and the essential oil in methanol; the absorbance was immediately read at 490 nm at T zero. Then the system was incubated up to 2 hours at 50 °C and the absorbance was measured again. The same procedure was repeated for ascorbic acid and gallic acid as positive controls along with a blank sample. Antioxidative activities of the extracts were compared to those of ascorbic acid and gallic acid at 0.5 mg/ml and blank, which was consisted of only 250 µl of methanol. All measurements were performed in triplicate.

The antioxidant activity (%) were calculated as following:

$$AA\% = [1 - (Abs_{sample\ (2h)} - Abs_{sample\ (T\ zero)}) / (Abs_{control\ (2h)} - Abs_{control\ (T\ zero)})] \times 100$$

Where $Abs_{control(2h)}$ and $Abs_{sample(2h)}$ are the absorbance of control reaction (which contains all reagents except for the test compound) and test compound at 2 hours, and $Abs_{control(T\ zero)}$ and $Abs_{sample(T\ zero)}$ are their absorbance at the beginning of preparation.

2.4.3. Ferric Reducing Antioxidant Potential (FRAP) Assay

The ferric reducing power was evaluated using a modified version of the FRAP assay described by Szőllősi et al. [12]

The working FRAP reagent was freshly prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10mM/L TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40mM/L HCl and 2.5 ml of 20 mM $FeCl_3$. A standard curve was generated using various concentrations (100-

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0.625 mM) of $FeSO_4 \times 7H_2O$. All solutions were made on a daily basis and used the same day. 50 µl of various concentrations (0.062-8 mg/ml) of the extract and the essential oil were added to 1.5 ml of FRAP reagent. The reaction mixture was incubated for 30 minutes at 37 °C in a water bath. The absorbance was then read at 593 nm against a blank containing 50 µl of methanol. Ascorbic acid and gallic acid were employed as positive controls. Relative activities were derived from standard curve of $FeSO_4 \times 7H_2O$ standard solutions, then FRAP values were expressed as mmol Fe^{2+} per 1 gram of plant extract. All measurements were performed in triplicate.

2.5. Antioxidative activities of the extract in sunflower oil

2.5.1. Preparation of the oil samples

Various concentrations (250, 500, 1000 and 2000 ppm) of the plant extract as methanol solutions were added to each of the virgin sunflower oil samples, which were bought from downtown Hamadan two days prior to the experiment, in separate glass vials. Later on, the samples were stirred while exposed to airflow in a dark environment until the solvent was completely evaporated. Afterwards, the samples were capped and stored in an oven at 60 °C under dark conditions over a period of 7 days. Sampling was done every other day. At each sampling time, samples were randomly removed from the oven and were subjected to chemical analysis. Same procedure was repeated for BHT (200 ppm) as positive control and a control sample; which contained the oil sample without the plant extract.

2.5.2. TBARS assay

Thiobarbituric acid-reactive substances (TBARS) were determined parallel to PV evaluation, based on a method described by Borneo et al. with minor modifications [13]. In this assay, the pink color generated by the formation of malondialdehyde and thiobarbitoric acid complex is measured and the results are reported as mg of MDA in 1 kg of oil sample. Presence of malondialdehyde in oils is due to secondary stages of fatty acid oxidation and existence of secondary substances of oxidation. Thus, the thicker the color-formation, the less stability of the samples. In this regard, 50 µl of the oil samples (various concentrations of 250, 500, 1000 and 2000 ppm of the extract) were combined with 300 µl of the thiobarbituric acid reagent (0.2 % w/v of TBA powder in sulfuric acid 0.05 M) in separate test tubes and placed in a water bath at 90 °C for 30 minutes. Then the samples were cooled down for 10 minutes at room temperature and the absorbance was read at 532 nm. Concentrations of TBARS were calculated from the standard curve using 1, 1, 3, 3-tetraethoxypropane as standard. All measurements were carried out in triplicate (Fig. 1).

2.6. Peroxide value

Peroxide value of oil samples was measured using AOAC method number 965.33. for this purpose, a known weight of the oil sample (3 g) was added to 50 ml mixture of glacial acetic acid and chloroform at a ratio of 3:2, respectively. Swirling was continued until the oil was completely dissolved. Then 1 ml of saturated potassium iodide was added to the mixture with gentle shaking in a dark environment for 1 minute. Afterwards, the solution was diluted with 50 ml of distilled water. Mixture was titrated against 0.01 N sodium thiosulfate with vigorous shaking until the yellow color had almost disappeared. In order for all of iodide to be released from chloroform layer, 0.5 ml of starch

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solution was added to mixture and titration was continued with vigorous shaking until the blue color turned milky white. A blank titration was performed in the same manner parallel to treatment. Peroxide values were calculated using following equation:

Peroxide value (meq of oxygen/kg) = (V×N×1000)/W

Where V is the volume of sodium thiosulphate solution (blank corrected) in ml, N is normality of sodium thiosulphate solution (0.01 N) and W is the weight of oil sample in gram.

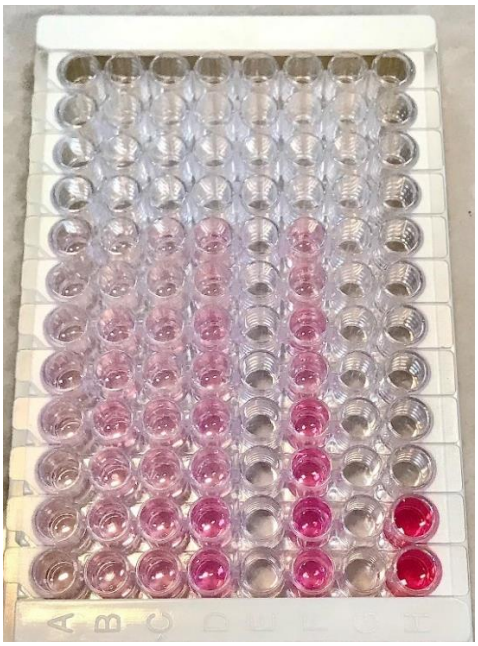


Fig. 1. TBARS assay

3. Results

3.1. Free radical-scavenging activity in DPPH assay

The concentration of antioxidant required to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity [14]. A lower IC_{50} value correlate with a higher antioxidant activity.

IC_{50} values for both extract and essential oil of *N. crispa* are demonstrated in Table 1. Our findings showed that increase in extract concentrations, resulted in increase in free radical-scavenging activity. Activity of the methanol extract was much higher than the essential oil ($P < 0.05$) but still, It wasn't comparable to gallic acid (IC_{50} = 2.18 µg/ml) and ascorbic acid (IC_{50} = 8.82 µg/ml) as positive controls.

Table 1. Scavenging effect of the essential oil and the methanol extract of *N. crispa* on DPPH free radical (IC_{50} µg/ml)

Sample	DPPH (IC_{50} µg/ml)*
Galic acid	2.18 ± 0.375
Ascorbic acid	8.83 ± 0.589
Methanol extract	93.2 ± 6.11
Essential oil	757 ± 29.48

*IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%.

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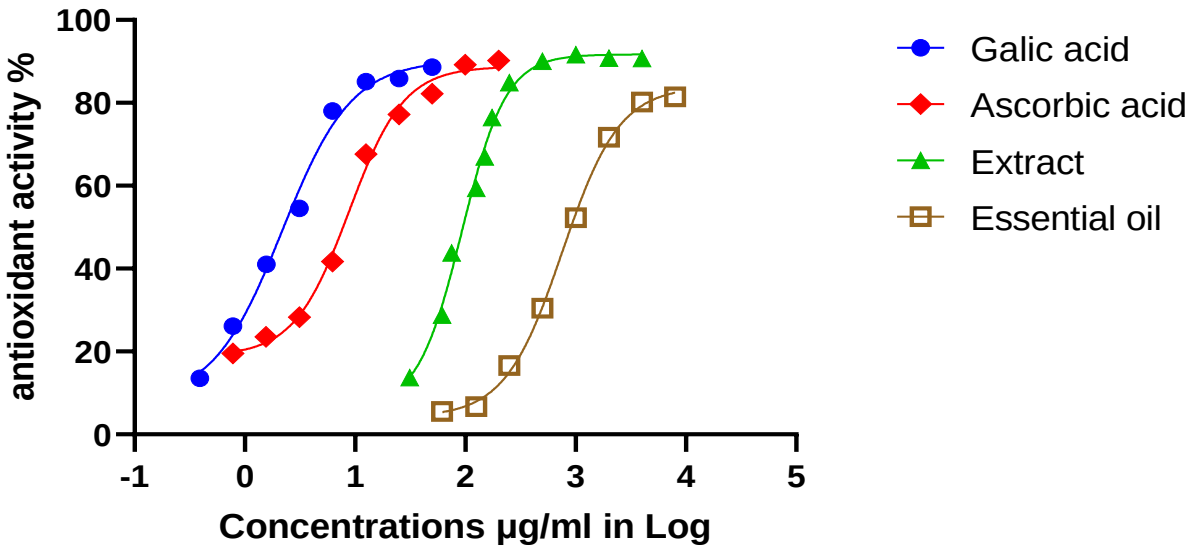


Fig. 1. Scavenging effect of the essential oil and the methanol extract of *N. crispa* on DPPH free radical

3.2. Inhibitory effect of essential oil and extracts on lipid peroxidation in β-carotene-linoleic acid assay

Inhibition on lipid peroxidation in response to the extract and the essential oil is illustrated in Fig. 2. The methanol extract effectively inhibited the oxidation of linoleic acid as much as 62.82 %, but the essential oil wasn't as effective and only provided 28.41 % of inhibition at the highest concentration. The methanol extract showed significantly more activity than the essential oil at all concentrations ($P < 0.05$). Notably, the inhibition of the extract at 8 mg/ml was comparable to Gallic acid at 0.5 mg/ml. At the same concentrations, neither extract nor essential oil were comparable to positive controls (Table 2).

Table 2. Antioxidant activity (%) of the essential oil and the methanol extract of *N. crispa* in *β*-carotene-linoleic acid assay

Sample	Concentration (µg/ml)							
	8000	**4000**	**2000**	**1000**	**500**	**250**	**125**	**62.5**
Gallic acid	-	-	-	-	66.52 ± 0.84	-	-	-
Ascorbic acid	-	-	-	-	76.92 ± 1.26	-	-	-
Methanol extract	62.82 ± 0.31[a]	56.41 ± 0.27	51.28 ± 0.34	47.43 ± 0.25	42.31 ± 0.14	40.87 ± 0.17	38.93 ± 0.21	37.17 ± 0.11
Essential oil	28.41 ± 0.35	21.35 ± 0.28	19.17 ± 0.13	17.54 ± 0.27	15.27 ± 0.24	12.72 ± 0.19	6.58 ± 0.09	6.32 ± 0.12

[a]Values are expressed as mean ± SD of three parallel measurements.

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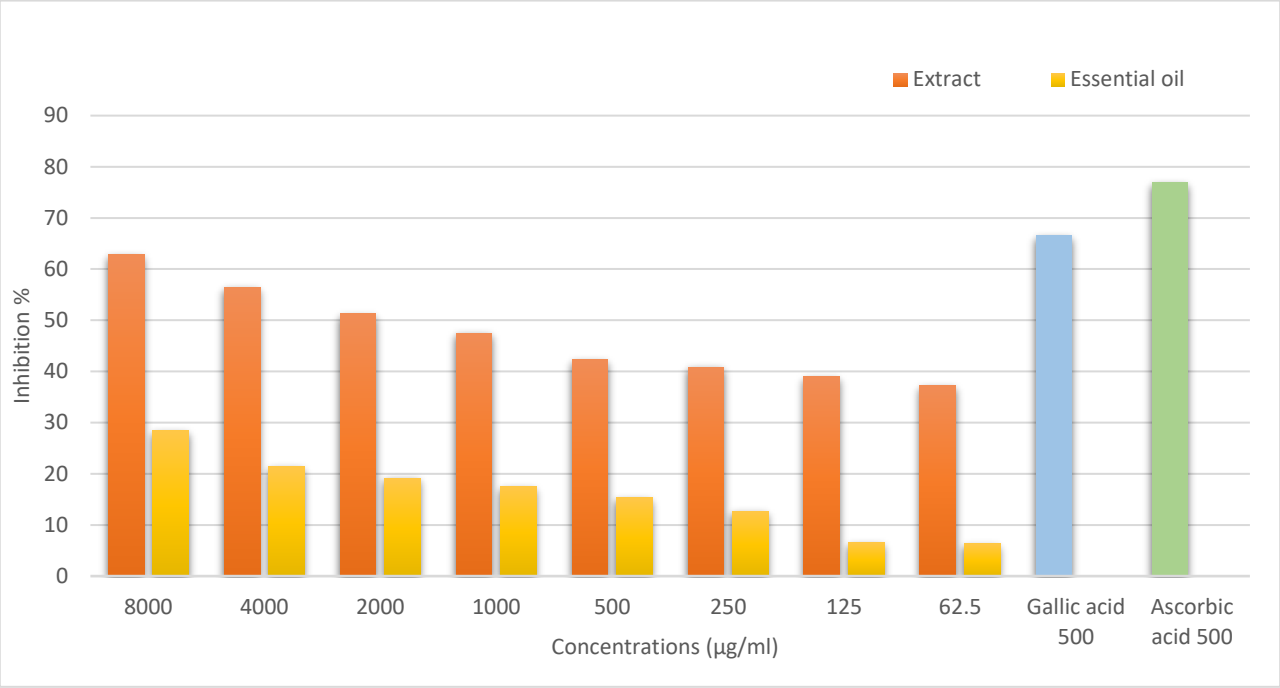


Fig. 2. Antioxidant activity of *N. crispa* extract and essential oil defined as inhibition percentage through *β*-carotene-linoleic acid assay.

3.3. Ferric Reducing Antioxidant Power Assay

Absorbance values for the essential oil and extract from *N. crispa* are shown in Fig. 3. The results of FRAP assay for the extract and the essential oil are presented in Table 3. The trend for the ferric ion reducing activity did not vary noticeably from DPPH assay. The essential oil was not considerably strong in this assay whereas the methanol extract was much stronger and demonstrated almost 5 times greater activity compared to the essential oil ($P < 0.05$). nonetheless the activity of extract was still modest when compared to ascorbic acid and gallic acid as positive controls. As it can be seen in Table 3, 1 g extract of *N. crispa* possessed an equivalent of 21.18 mmol Fe^{2+} ions. Since 100 g of dried powder of the plant aerial parts provided 14.136 g of extract, therefore there are an equivalent of 2.9935 mol Fe^{2+} (832.19 g $FeSO_4.7H_2O$) in 1 kilogram of plant material.

Table 3. Antioxidant activity of the essential oil and the methanol extract from aerial parts of *N. crispa* using FRAP assay

Sample*	**FRAP (mM Fe^{2+}/g)**
Galic acid	672.86 ± 21.25
Ascorbic acid	195.26 ± 4.69
Methanol extract	21.188 ± 1.604
Essential oil	4.934 ± 0.03

*Values expressed are means ± SD of three parallel measurements.

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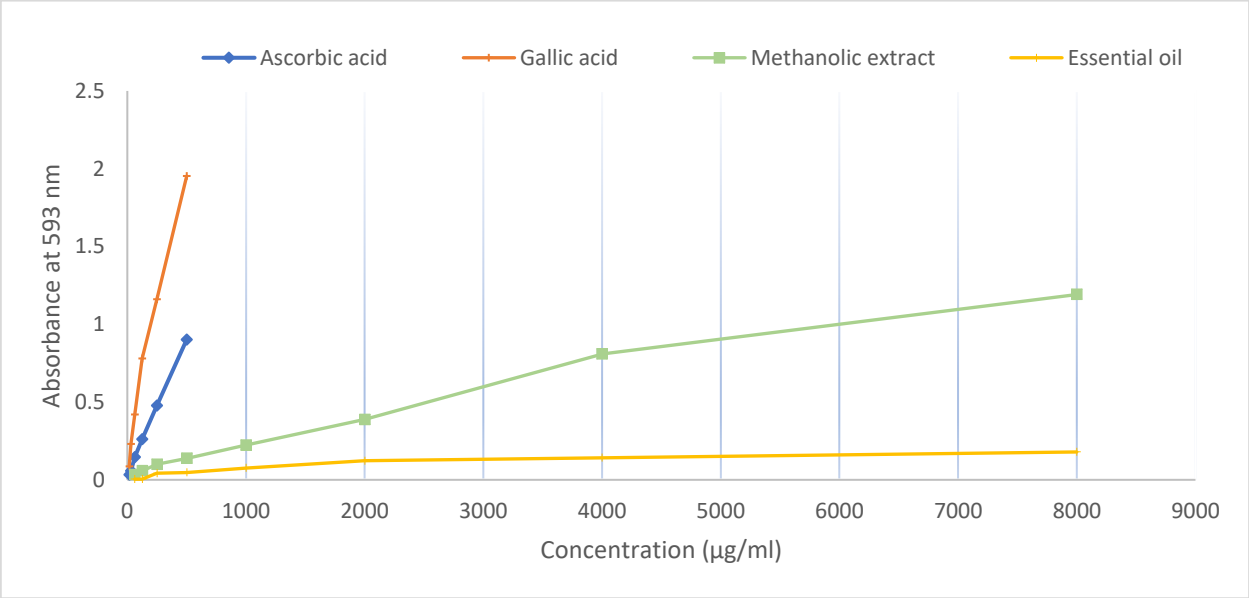


Fig. 3. Total reducing power of the essential oil and the methanol extract from *N. crispa*

3.4. Effects of the methanol extract on the oxidative parameters of sunflower oil

TBARS values are illustrated in Fig. 4. It shows that from day 3 of storage time onwards, TBARS values of the oil samples supplemented with BHT and *N. crispa* extract were remarkably lower than the control sample. TBARS values of all of the treated samples were significantly different from control sample ($P < 0.05$) from day 3 until the end of storage period. Even 250 ppm sample was effective enough to prevent TBARS value from rising compared to the control sample ($P < 0.05$). In general, higher concentrations of the extract made more decrease in both peroxide and TBARS values during the entire storage period but still, in terms of comparison with BHT, none were comparable ($P < 0.05$).

Fig. 5 shows the peroxide values of the sunflower oil samples in presence of various concentrations of the extract from *N. crispa* over a storage period of 7 days at 60 °C. We have demonstrated that peroxide values were reduced at different concentrations (250, 500, 1000, and 2000 ppm) of extract and incubation time points compared to the control (i.e. oil sample without the extract) (Fig. 5). Nonetheless, PVs in all samples increased from beginning of storage time until day 7 but in treated samples, the trend was slower compared to the control. For the control oil sample the gradual rise in peroxide values from day 3 to end of the storage period was significantly higher than the treated 2000 and 1000 ppm samples and BHT ($P < 0.05$). The decrease in peroxide values of oil samples supplemented with 250 and 500 ppm of the extract did not show much difference with the value of the control sample ($P > 0.05$) but the values of 1000 and 2000 ppm concentrations were significantly lower than control ($P < 0.05$), but still proved to be modest next to BHT as positive control.

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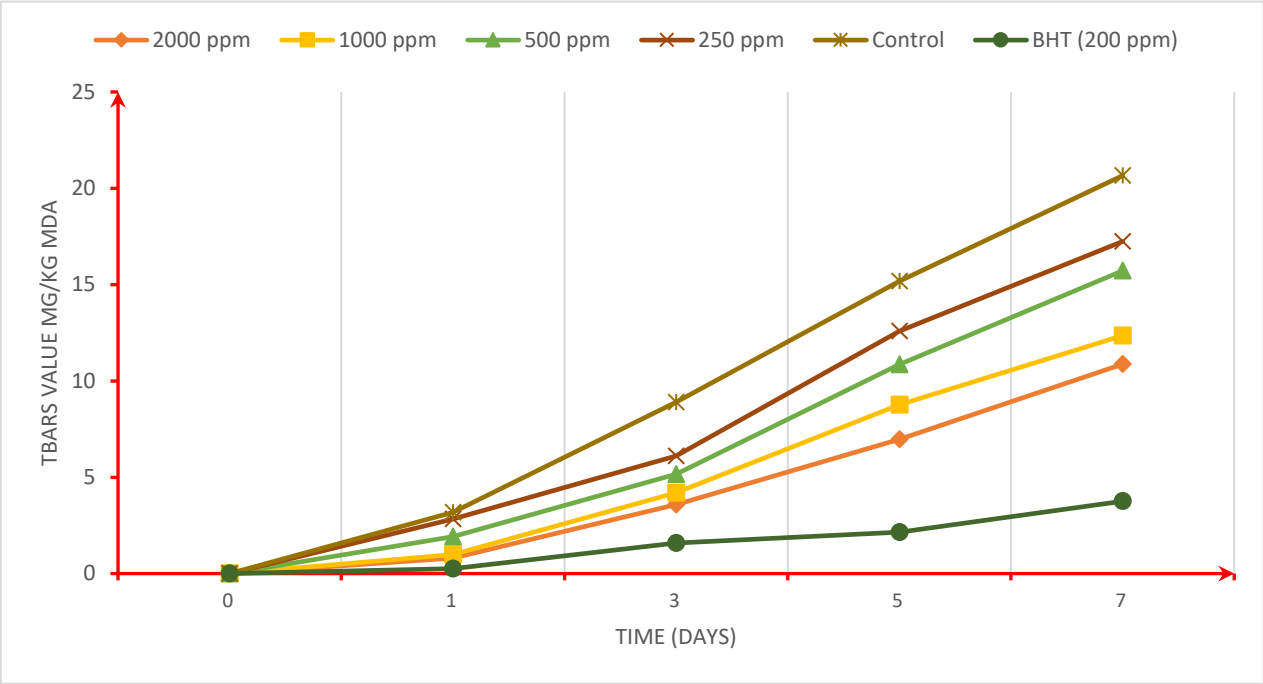


Fig. 4. Effect of *N. crispa* methanol extract and BHT on TBARS values of sunflower oil over a 7-day incubation at 60 °C. Values were expressed as mean ± SD.

Table 4. Effect of methanol extract of *N. crispa* on the formation of thiobarbituric acid-reactive substances (TBARS) in sunflower oil during storage (60 °C)[a]

Sample[c]	Thiobarbituric acid-reactive substances (TBARS) (mg/kg. mean. n = 3)[b]				
	Storage time (days)				
	0	1	3	5	7
SO	0.001	3.182	8.891	15.171	20.657
SO + BHT 200 ppm	0.001	0.249	1.583	2.147	3.756
SO + ME 250 ppm	0.001	2.838	6.106	12.584	17.239
SO + ME 500 ppm	0.001	1.914	5.151	10.863	15.718
SO + ME 1000 ppm	0.001	0.984	4.189	8.769	12.353
SO + ME 2000 ppm	0.001	0.812	3.583	6.972	10.869

[a] TBARS values are expressed as mg of malonaldehyde per kg of sunflower oil.
[b] Standard deviation values ranged from 0.07 to 0.89; for the sake of clarity SD values were not included within the table.
[c] SO = sunflower oil; BHT = butylated hydroxytoluene; ME = methanol extract solids from *N. crispa*

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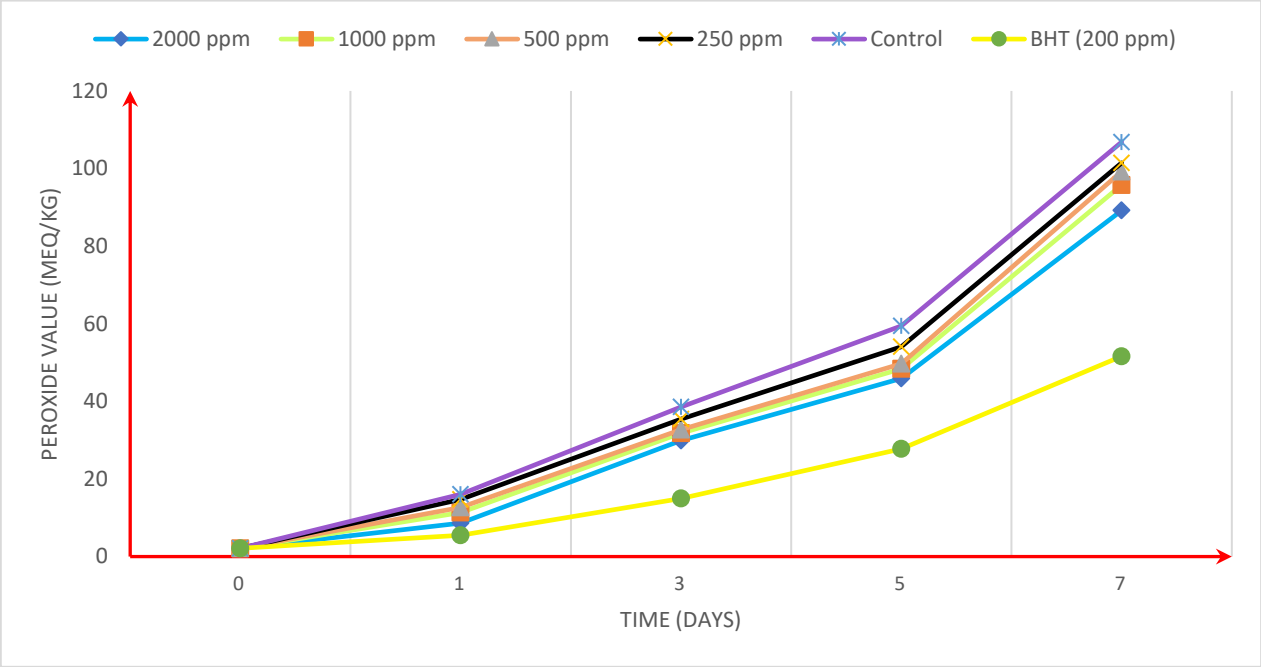


Fig. 5. Effect of *N. crispa* methanol extract and BHT on peroxide values of sunflower oil over a 7-day incubation at 60 °C.

Table 5. Effect of methanol extract *from N. crispa* on peroxide values of sunflower oil during storage (60 °C)

Sample[b]	Peroxide value (milliequivalents O_2 /kg)				
	Storage time (days)				
	0	**1**	**3**	**5**	**7**
SO	2.133	16.05	38.591	59.44	106.83
SO + BHT 200 ppm	2.133	5.48	14.93	27.74	51.61
SO + ME 250 ppm	2.133	14.69	35.41	54.04	101.46
SO + ME 500 ppm	2.133	12.66	32.67	49.74	99.24
SO + ME 1000 ppm	2.133	11.35	31.83	48.35	95.76
SO + ME 2000 ppm	2.133	8.636	29.88	45.91	89.18

[a] Peroxide values are expressed as milliequivalents O_2 per kg of sunflower oils.
[b] SO = sunflower oil; BHT = butylated hydroxytoluene; ME = methanol extract solids from *N. crispa*

4. Discussion

DPPH assay is frequently used in antioxidant studies as It provides a fast technique to evaluate radical-scavenging activity of pure compounds as well as crude plant extracts. The data were in agreement with those measured from the reducing power assay, which is to be expected. The activity of *N. crispa* extract was superior to *N. cataria* investigated by Adiguzel et al. [15]. IC_{50} of *cataria* specie was reported to be 171.98 ± 3.6 µg/ml as opposed to *N. crispa* which has an IC_{50} of 93.2 ± 6.11 µg/ml. However

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ÖZBEK et al. demonstrated that the activity of extracts from *Nepeta italica*, *Nepeta cilicia* and *Nepeta caesarea* are superior to *N. crispa* which was investigated in this study [16]. The IC_{50} values of the discussed species were reported to be 25.5 ± 0.55, 33.4 ± 1.25 and 39.1± 0.76 µg/ml, respectively. These differences in the antioxidative characteristics of *Nepeta* subspecies could be due to their constituting ingredients.

In this study and through employing *β*-carotene-linoleic acid assay, ascorbic acid showed the most activity (76.92 %). By evaluating diminishment rates of *β*-carotene, activities of the methanol extract of *N. crispa* ranged from 37.17 to 62.82 at the highest concentration. Similar to other assays, the essential oil was weaker in terms of antioxidant activity. Major components of the essential oil from *N. crispa* consist of 1,8 cineol and nepetalactones, according to Sonboli et al. [17]. Notably, 1,8 cineol is known for its Antimicrobial effects[18], but regarding antioxidant activity, there are not promising results. The same goes for nepetalactones, as they possess a good antimicrobial and antifungal profile [19], but they are not considered antioxidant agents.

To our surprise, Gallic acid, which was found to be superior to ascorbic acid in DPPH and FRAP assays, demonstrated lower activity in this test. This could be due to chemical structure differences between reagents in these assays and the way each test interprets the antioxidative activity. However further research on structural properties of the substances involved in these tests could be fruitful in order to specifically determine the cause of this issue.

As Seladji et al. reported in their work, other species of *Nepeta* (*N. nepetella*) presented similar to higher activity than gallic acid which is in accord with our results [20]. Although at the same concentrations, the extract was weaker, but at the peak concentration (8 mg/ml), same range of activity with gallic acid 0.5 mg/ml was reported.

Since *N. crispa* is endemic to Hamadan region, evaluation of its total phenols has not yet been determined. Therefore It would be beneficial that future research focus on this matter and examine the correlatability between FRAP values and amount of the polyphenols in this particular specie.

Our findings in this assay were in agreement with the data from DPPH test. The methanol extract possessed almost 5 times greater activity (i.e. equivalent amount of Fe^{2+}) than the essential oil. The activity of the extract was still modest compared to Gallic acid as gold standard. However, the FRAP value of the extract was closer to that of ascorbic acid which makes It impressive as It only contained crude methanol extract of the plant (Table. 2). In any case, the results from these two assays did not completely mirror the data from *β*-carotene test, in which, although the extract was still more powerful compared to the essential oil, ascorbic acid expressed more antioxidant activity as opposed to gallic acid.

Inhibition of TBARS formation has been approved as another approach to evaluate the antioxidant activity of crude plant extracts and chemical substances. Fig. 4 demonstrates TBARS content of the sunflower oil submitted to accelerated oxidation process and supplemented with reference standard (BHT) and methanol extract from *N. crispa*. As it can be viewed from Fig. 4, from day 3 onwards, all samples were statistically different ($P < 0.05$) from the control (i.e. sunflower oil alone). This shows that the extract was effective in inhibition of TBARS formation during the storage period. Notably,

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BHT expressed the most inhibitory activity on TBARS formation at any time during the storage period since the lowest concentrations of TBARS were observed in samples treated with BHT. The extract was effective on delaying the increase of TBARS content of the sample comparing to the control, but It was not at any day of the storage as strong as BHT. Nonetheless, the extract at 2000 ppm, reduced the amount of TBARS formed in the sample by almost half comparing to the control (Table 4). Taking into account that It was only crude methanol extract of the plant, the results are still considered impressive whereas the isolated components of the extract could show more activity.

These characteristics of the methanol extract from *N. crispa* can be credited to its phenolics, flavonoids and terpenoids constituents. In this regard, Luximun-Ramma et al. demonstrated a linear correlation between antioxidant properties and phenolic contents of the plant extracts, fruits and beverages [21]. As for the flavonoids, Sugihara et al. and Spencer et al. suggested that flavonoids too, may have the ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals [22, 23]. Terpenoids were studied for their powerful antioxidative activities by Joshi et al. [24].

As mentioned earlier, due to novelty of *N. crispa* to academical research and its rarity, there are still many analyses to be done concerning this specie. For example phytochemical investigation could shed light on its inhibitory profile on TBARS which could then be explain by the specific contents, such as flavonoids, terpenes etc., present in its extract.

Storage variables such as temperature and humidity are thought to have a significant impact on virgin oils. Such changes could be remarked in terms of acidity and rancidity. The unsaturated double bonds in lipids are the main participants in autoxidation. Thus oils with high level of unsaturation are more prone to autoxidation. Presence of peroxides is the first indication of rancidity in unsaturated fats and oils. Among the other available methods, determination of peroxide value is the most commonly employed, as It is one of the best assays for evaluation of autoxidation (i.e. oxidative rancidity). It determines the extent to which an oil sample has been subjected to primary oxidation. Peroxides take part as intermediates in the autoxidation process. Autoxidation is a free radical reaction, involving oxygen, which drives lipids and oils to deteriorate. This process leads to alteration in taste and smell of the oil products. Therefore, screening the concentration levels of peroxides is valuable to assess the extent of spoilage [25].

Similar to TBARS assay, peroxide values were proven to be influenced by the addition of *N. crispa* extract. While the peroxide value of the control sample (sunflower oil without any supplements) increased from 16.08 meq/kg to 106.83 meq/kg after 7 days of accelerated storage, the peroxide value of oil samples treated with 2000 and 1000 ppm of extract did not rise at the same rate. (Table 5) at the end of storage period, the peroxide values of oil samples with 2000 and 1000 ppm of the extract were 89.18 and 95.76 meq/kg, respectively. Although their activities were humble when compared to BHT, but they were still effective in delaying the rise of peroxide values.

In other words, the application of *N. crispa* extract to sunflower oil would lessen the rate of rancidity within the course of time and provide good storing conditions. Overall in the peroxide value assay, the extract demonstrated less antioxidant activity than TBARS. Only concentrations of 1000 and 2000 ppm were significantly different from control sample ($P < 0.05$). BHT was still the most powerful in

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terms of antioxidant activity but in this test, the difference between BHT and the extract was more drastic comparing to the ones from TBARS test.

5. Conclusion

In this study, It was discovered that the methanol extract of *Nepeta crispa* Willd. possesses radical-scavenging and antioxidant activities, as determined by reducing power on ferrous ions, β-carotene-linoleic acid model system, scavenging effect on the DPPH• free radical, inhibitory activity on generation of TBARS and measuring peroxide value tests, but to different degrees depending on each test. In this investigation, the results from DPPH and FRAP assays found to be in correlation with one another but not with those achieved from β-carotene-linoleic acid assay. In this work, the extract from aerial parts of *N. crispa* was discovered to be fairly active and its potency, although lowers than positive controls, was remarkable given that It was merely crude methanol extract from the plant. Therefore, it is concluded that the extract from *N. crispa* might be considered as a good candidate for preservation purposes in sunflower oil. As for the prevention against oxidation in oils, the findings were not as impressive, suggesting that It might be more efficient to examine various solvent fractions of the extract in effort to achieve higher activity.

The essential oil from *N. crispa* was not effective in neither one of the tests. Considering its constituents, our data would be justified as it possesses rather antimicrobial and antifungal potential than antioxidant. Thus, extraction and isolation of specific contents of extract, such as increasing the concentration of phenolic compounds, means that more antioxidant activity could be expressed. Future studies could fruitfully explore this issue further by specification of substances present in the extract, measuring total phenolic and flavonoid content and determining the antioxidant activities of separate solvent fractions of the crude extract.

Author contributions

SM designed the experiments and supervised the work. MM carried out the experimental bench work and analyzed and interpreted the data. MM wrote the article. All authors approved the final version of the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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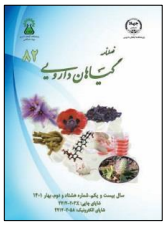
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مقاله تحقیقاتی

ارزیابی پتانسیل آنتی‌اکسیدانی مفراح (نعناعیان) و تاثیرات آن بر پایداری اکسیداتیو روغن بکر آفتابگردان تحت شرایط نگه‌داری تسریع شده

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اطلاعات مقاله

گل‌واژگان:
پونه‌سای مواج
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اسید چرب

چکیده

مقدمه: گیاه مفراح یا پونه‌سای مواج، گیاهی یکساله و بومی مناطق خاصی از استان همدان در ایران می‌باشد. **هدف:** این مطالعه به بررسی پروفایل آنتی‌اکسیدانی گیاه مفراح می‌پردازد. **روش بررسی:** اسانس گیاه با روش تقطیر با آب و عصاره‌ی آن از راه خیساندن در متانول تهیه شد. سپس آزمون‌های متعددی شامل سنجش DPPH، FRAP، TBARS، اندیس پراکسید و بتاکاروتن- لینولئیک اسید، به منظور مشخص کردن خواص آنتی‌اکسیدانی و اثرات جلوگیری‌کننده بر روی اکسیداسیون اسیدهای چرب بر روی آنها انجام گرفتند. **نتایج:** عصاره‌ی گیاه در تمامی آزمون‌ها دارای فعالیت آنتی‌اکسیدانی بیشتری نسبت به اسانس آن بود. اسانس گیاه در مقایسه با ترکیبات استاندارد و حتی عصاره نیز فعالیت آنتی‌اکسیدانی قابل توجهی از خود نشان نداد. تست‌های DPPH و FRAP تا حدود زیادی با یکدیگر همبستگی داشتند اما داده‌های حاصله از آزمون بتاکاروتن- لینولئیک اسید تا حدودی با سایر داده‌ها ناهماهنگ بودند. نتایج تست‌های TBARS و اندیس پراکسید نیز نشان دادند که غلظت‌های خاصی از عصاره به طور مؤثری از فرآیند اکسیداسیون در روغن آفتابگردان جلوگیری به عمل می‌آورند. **نتیجه‌گیری:** نتایج این تحقیق نشان دادند که عصاره‌ی متانولی گیاه مفراح می‌تواند به عنوان یک کاندید مناسب از منابع آنتی‌اکسیدانی طبیعی برای استفاده در صنایع دارویی و غذایی در نظر گرفته شود.

مخففها: TBARS، ساختارهای واکنش‌دهنده با تیوباربیتوریک اسید؛ FRAP، آزمون قدرت کاهشی آنتی‌اکسیدانی فریک؛ DPPH، ۲،۲ دی فنیل-۱- پیکریل هیدرازیل

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