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Antioxidant Activity of a Solution of Thymol in Ethanol

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Abstract

***Background*:** Antioxidants are combinations that protect the body against cell membranes injury or cell genetic material damage from free radical activity. Free radicals are the source of many diseases such as cancer and skin aging.
***Materials and Methods*:** In this study, thymol antioxidant activity has been compared and evaluated using three stable radical scavenging methods: 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), measurement of phenolic compounds and hydrogen peroxide sweeper.
***Results*:** The study results showed that the amount of IC_{50} for ethanol thymol in radical scavenging method DPPH is equivalent to 0.538±0.02 µg/ml, in the phenol method, the total phenol amount is 0.36±0.06 mg and in the sweeper hydrogenated water method, it is 0.39±0.09 µg/ml.
***Conclusion*:** The value of IC_{50} in the three methods indicates that component factors, radical production source or scavenging reaction is performing almost with the regular grade which is dependent on Thymol concentration at a given time. The tested material has good antioxidant properties and it can be used as a natural antioxidant and in some materials as additives.

Introduction

Today, so many studies have been conducted to replace chemicals with natural materials in order to remove or reduce chemical and synthetic compounds in food stuff. Great efforts have been put in this regard to find natural antioxidants from plant sources. Oxidation reactions caused by free radical activity have a pathological role in many diseases including heart disease, kidney failure, diabetes, cancer and premature aging prevention [1-4]. It seems that antioxidant compounds protect cell membranes against damage from free radicals [3-5]. Free radicals are very strong reactors with a tendency to get electrons and pair their own electrons. Therefore, they cause the other molecules to get damage or lose their function. The damages induced by oxidative reactions in genes of proteins and other molecules intensify cardiovascular diseases, cancer, aging, cataract, and liver hemorrhage.

The important point is that there should be a balance between oxidants and antioxidants to maintain the body's optimal physiological conditions. The excessive production of oxidants (particularly in chronic bacterial, viral and parasitic infections) causes imbalances or so-called oxidative stresses. Nowadays, synthetic antioxidants such as BHT-BHA-THPQ are used in the industry to delay fat oxidation, but due to poor nutrition and carcinogenic effects of these compounds, the use of natural antioxidants has attracted the attention of researchers. There are a lot of radical inhibitors in the living bodies most of which such as vegetables, fruits and natural drinks are in the food basket of each family. Consumption of natural antioxidants or supplements containing antioxidants avoids aging skin. Meanwhile, antioxidants avoid the destruction of the hereditary material and thus prevent cancer.

Besides, accelerating the excretion of toxins and cellular wastes, they prevent from lots of cell destruction induced diseases, including diseases such as dementia, Alzheimer's, advanced liver, heart and kidney problems [6-10]. One of the other radical inhibitors is *Zataria multiflora* whose name is driven from a plant of the mint family and it is publicly propagated in Iran, Afghanistan and Pakistan. The highest percentage of the essence composition of this plant is thymol (Fig. 1).

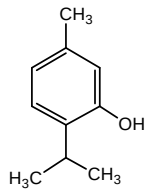

Figure 1. Chemical structure of thymol

The purpose of this study is to examine antioxidant properties of thymol. Therefore, the antioxidant effect of thymol on Qtabgrdan oil has been investigated and has been proven to be very effective [23]. Also, Japanese

researchers have also conducted some studies on the microbial effects of thymol [10].

Materials and Methods

Pure thymol, which is a granule and white solid substance, along with all chemicals and solvents required for research with the highest purity percentage and reagent 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), was purchased from Floka Company. The antioxidant activity level will be measured through stable radical scavenging method of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), in DPPH method according to the activity of hydrogen atoms and electrons of thymol and some pure compounds through discoloration of amethystine ethanolic thymol DPPH [10]. The scavenging ability of free radical DPPH was measured using Okada method. 30 µl of ethanol solution DPPH (1mM) and then the value of 2.7 ml of 96% ethanol were added to 20 µl of thymol solution with different concentrations (0.05-0.60 µg/ml), and the mixture has been severely shaken. Then, the mixture was put in the laboratory environment for 30 minutes and then its absorption was measured using a spectrophotometer at 517 nm. Inhibition percentage was measured through comparing with control solution, which had only reagent DPPH with no Thymol in it, and the curve of inhibition percent changes was calculated against the concentration logarithm and IC_{50}. This was done three times and the mean of results was calculated and reported.

Phenolic compounds were measured through the Folin-Ciocalteu colorimetric method to determine flavonoids [11]. Phenolic compounds were measured according to the methods in which Folin-Ciocalteu is used as the reagent and Gallic acid as the standard. We mixed 0.1ml of thymol solution with different concentrations (0.10-0.60 mg/ml) and 50 ml of distilled water and one ml Folin-Ciocalteu reagent and have completely shaken. After 5 minutes, 3ml of 2% sodium carbonate solution were added and the mixture was continuously shaken for 2 hours. Then, the sample absorbance was measured at a wavelength of 760nm. The same procedure we used again for 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml of all standard solutions of Gallic acid to prepare the standard curve.

In this research, the capability of samples was determined in the Hydrogen Peroxide sweeper, through the Ruch method [1]. Hydrogen Peroxide Solution (40mM) was prepared in phosphate buffer (pH=7.2), then, the solution of thymol with different concentrations was prepared in distilled water (0.10-0.60 mg/ml). 4ml of thymol solution with different concentrations in distilled water was added to 1ml of solution Hydrogen Peroxide (40 mM). Hydrogen Peroxide absorbance was determined at 230 nm after 15 minutes comparing with the control solution containing phosphate buffer, but without Hydrogen Peroxide [12-14]. Hydrogen peroxide sweeper percentage was calculated in extracts and standard materials as follows:

$$[H_2O_2] \text{ Sweeper Percentage} = [(A_0-A_1)/A_0] \times 100$$

Where absorbance A_0 is in control and absorbance A_1 is in the presence of thymol and standard sample. Test results were expressed as mean. All the measurements have been repeated three times. Data analysis has been separated through Duncan's multiple range tests. Values of IC_{50} were calculated based on linear regression analysis between percentage of inhibition and the respective concentrations.

Results

In this test, radicals of other radical species which are hydrogen donors react with antioxidants or DPPH and will become reduced and their color will turn from dark purple into bright yellow and their absorption will decrease by 515 Nm. At a wavelength of 517, free radicals produced from antioxidant, DPPH reduction determines the overall reaction of stoichiometry (the number of colorless molecules by a reducing agent molecule) of antioxidants or other radical species. DPPH remaining is measured after 60 minutes at the highest absorption. The much greater this value is; the much less the antioxidant activity will be in free radicals removal. DPPH stable radical trapping model is widely used in evaluating the free Trapping ability of various samples [15]. Radical trapping activity of all samples will enhance through the increase of concentration. IC_{50} of DPPH radical trapping activity was 0.538±0.02 µg/ml (Fig. 2).

We did this three times through this method and calculated and reported the mean of results. In addition, figure 3 shows the ratio of thymol response to the thymol absorption in the presence of DPPH. This suggests that thymol solution with different doses of its response is the lowest in the concentration of 0.538 µg/ml. The total amount of phenol was determined through Folin-Ciocalteu and has been expressed as milligrams equivalent to Gallic acid per gram thymol by referring to the standard curve. The total amount of phenol in thymol is 0.36±0.06 mg/g (Fig. 4).

Phenols and polyphenols are widely available in food products from plant sources and it has been revealed that these materials have distinct antioxidant activity [16]. The thymol response test based on absorption to concentration ratio is as follows. Total amount of phenol present in thymol represents that thymol solution with different doses of its response has the concentration 0/36 µg/ml (Fig. 5).

Hydrogen Peroxide Sweeper by thymol can be attributed to its Phenolic substances which give electrons to H_2O_2 and thereby neutralize it and turn to water [17, 18]. Thymol's ability in the effective sweeping of hydrogen peroxide, which was obtained according Roth approach, was compared with the ability of Ascorbic Acid as standard. Sweeper Hydrogen peroxide is somehow concentration-dependent. Sweeper activity of thymol was fairly well. Its IC_{50} is 0.39±0.09 µg/ml.

Although hydrogen peroxide is not considered highly reactive, it will sometimes cause cell toxicity due to the hydroxyl radicals' production in cell. Thus, it is very important to remove H_2O from all food systems. The role of free radicals in many diseases has been well proven. Reactive oxygen species will be produced in several biochemical reactions in our bodies. If cellular

components do not effectively sweeper such reactive oxygen species, the disease state will be created [19]. Thymol response test based on the absorption to concentration ratio is as follows. The total amount of phenol using hydrogen peroxide sweeper by thymol indicates that thymol solution with various doses of its response in the concentration 0.39 µg/ml has the least effect.

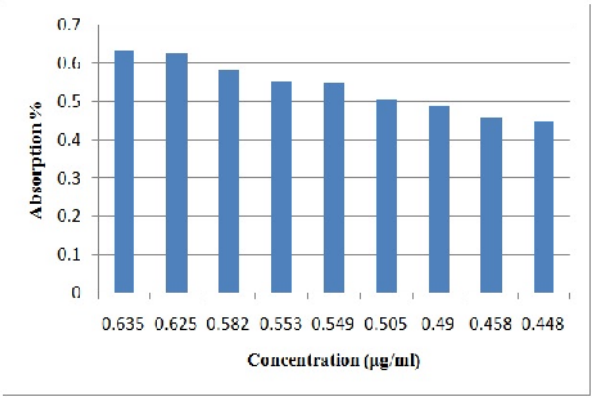

Figure 2. the ratio of concentration to thymol absorption in the presence of DPPH

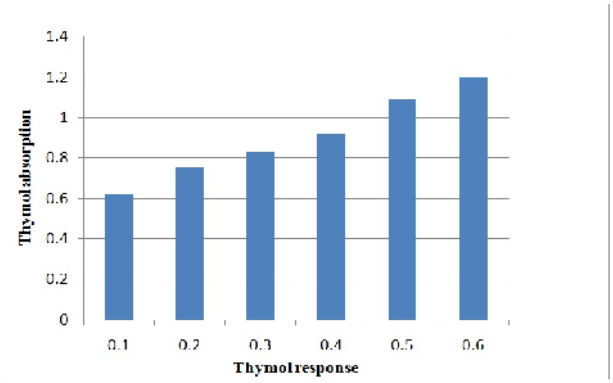

Figure 3. The ratio of thymol response to thymol absorption in the presence of DPPH

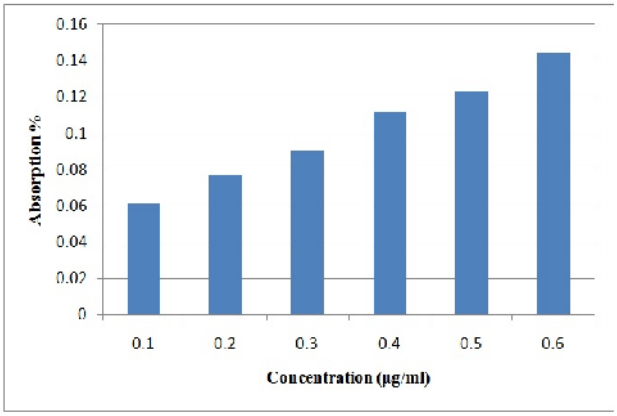

Figure 4. The ratio of thymol concentration to thymol absorption to determine the total phenol through Folin-Ciocalteu method

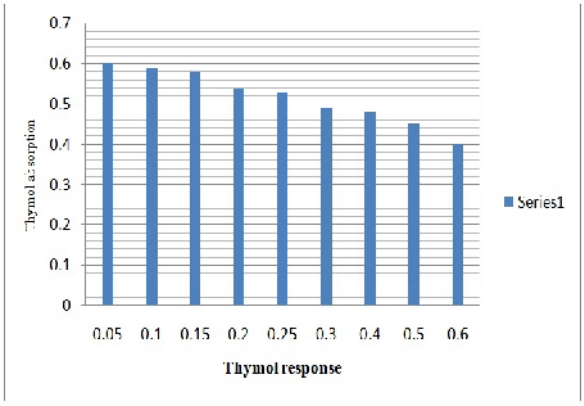

Figure 5. The ratio of thymol response to thymol absorption to determine the total phenol through Folin-Ciocalteu method

Discussion

In this study, the antioxidant activity of thymol has been examined, using various methods, including antioxidant activity of thymol using stable radical scavenging 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), phenolic compounds measurement, hydrogen peroxide sweeper measurement, measurement of flavonoids, ferric reducing, trapping nitric acid, chelation of metal and activity through FTC method. The value of IC_{50} in this test was equal to 36.1±0.18 which showed good effects. The results of recent studies on free radicals confirms the fact that antioxidant-rich foods play a vital role in prevention from cardiovascular disease, cancer [20] and neurodegeneration diseases, including Parkinson's and Alzheimer's disease [21] as well as prevention from inflammation and problems caused by cellular and skin aging [22]. Antimicrobial and antioxidant activity of plant *Salvia glutinosa* has been previously reported. In the plant *Salvia glutinosa* is the main substance of Sesquiterpene (D germakern) [12]. Our study results show the antioxidant effect of thymol extracted from sunflower [23]. In previous studies, the desirable antimicrobial and antioxidant activity of plant *Tanacetum pinnatum* whose main composition is comfar was confirmed. In this study, the antioxidant activity of thymol has been investigated using three methods including thymol antioxidant activity investigation using stable radical scavenging 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Phenolic compounds measurement and measurement of beta-carotene linoleic acid.

Value of IC_{50} in this test was equal to 1205.0±722.8 µg/ml. The comparison with previous studies shows that the aromatic compounds and monoterpenes with –OH functional groups have a good antioxidant effect [24].

Considering that thymol effects, including its antibacterial effects and different function, have not been studied, it has become necessary to review them as a new antioxidant compound. Antioxidant effect has been studied in this research. Today, so many studies have been conducted to replace chemicals by natural substances in order to eliminate or reduce chemical and

synthetic compounds in food stuff and great efforts have been put in this regard to separate natural antioxidants from plant sources. While maintaining and processing food, lipid oxidation will lose the nutritional and digestive quality of food, and create oxidized products such as free radicals. Free radicals produced in food systems lead to the spontaneous oxidation and production of undesirable chemical compounds and thus the pungency and unsavory of food. Moreover, free radicals in biological systems will cause so many diseases, particularly cancer [25].

Antioxidants are compounds, which effectively prevent from fat oxidation [26]. In 2005, Goli et al investigated the antioxidant effect of the extract extracted from pistachio green skin in soybean oil. The results of BHA and BHT of synthetic antioxidants of 600 ppm from the extract showed that 200 ppm concentration of synthetic antioxidants has the highest antioxidant effect.

Therefore, pistachio green skin was introduced and reported as a source with antioxidant effect which is due to the phenolic compounds in it [27].

Anyway, we mentioned some cases and the study results shows that radical trapping activity in all thymol samples will enhance through the increase of concentrations. IC_{50} in radical trapping activity of DPPH is 0.538±0.02 µg/ml. Total amount of phenol has been determined through Folin-Ciocalteu method and is expressed as mg equivalent to Gallic acid per gram thymol with reference to the standard curve. The total amount of phenol in thymol is 0.36±0.06 µg/ml thymol. Hydrogen peroxide sweeper is somehow concentration-dependent.

Sweeper activity of thymol is relatively favorable and IC_{50} is 0.39±0.09 µg/ml. The studied antioxidant activity of thymol is caused by its containing phenolic compounds. In this study, using three different methods revealed that thymol has antioxidant properties. This research can be considered a starting point for application of thymol in oil and other non-food substances.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

No conflict.

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