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Antioxidant Activity and Phenolic Contents of Ajwain, Mustard, Fenugreek and Poppy Seed

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Abstract: This study was conducted to identify and compare natural antioxidant based plant sources. Polyphenols are the main constituents of the natural antioxidants obtained from fruits, leaves, and seeds etc. In this work, four types of seeds from *Trachyspermum ammi* (Ajwain), *Brassica alba* (Mustard), *Trigonella foenum graecum* (Fenugreek) and *Papaver somniferum* (Poppy) were selected and their extracts were tested for antioxidant activity (AOA) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and chelating methods. Total phenolic contents were found in the range of 1015-6900 mg/100 g ($p<0.5$). Antioxidant activity by DPPH radical scavenging and chelating methods was found to be in the range of 44-95% and 33-77% ($p<0.5$), respectively. This study revealed that *T.ammi* is a potent source of natural antioxidant and can be used to replace synthetic antioxidant. However mustard, fenugreek and poppy have also proved to be the potential sources of phenolics.

Keywords: Antioxidant activity, chelating effect, DPPH, phenolics content, polyphenols, seeds.

1. INTRODUCTION

Oxygen is the third most abundant element that makes up nearly 21% of the earth's atmosphere. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, causing significant damage to cell structure [1]. The increase of ROS in the human body can damage DNA structure and lipid peroxidation causing diseases especially cardiovascular disease, certain forms of cancer, premature aging, mental disorder and Parkinson. Antioxidants play a significant role in the prevention of oxidative damage due to imbalance between the human body and reactive oxygen species (ROS) [2]. Antioxidant also enhances the shelf life of food, maintain important nutritional values of the food and protect the formation of harmful organisms during storage. For this purpose, both synthetic and natural antioxidants are in use since long. However, the use of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are restricted in food items due to carcinogenic effect and other harmful health issues There has been a growing interest over the past few years for food scientists and chemists and now even the researchers are interested in the exploration of more natural antioxidant based plants., Extracts from different types of plant species tested for their antioxidant activities for *in vitro* studies proved that these plant extracts have a better impact on health as compared to BHT [3]. Extracts from plants like green tea, soybeans, peanuts, cotton seeds, canola etc. have been extensively studied by different researchers. High potency of antioxidant activities in ethanolic extract of mustard seeds is also well established [4]. Similarly, researchers also studied some other types of plants

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for their health benefits in the light of Holy Quran. A number of plant species have been cited on the basis of their curative measures, for example ginger for cough, pomegranate for intestinal bleeding, and banana for diarrhea and for other conditions as well [5]. *T. ammi* (Ajwain) is also a very common seed spice, belong to family Apiaceae, mostly cultivated in Egypt; however it is also found in Iraq, Iran, Afghanistan, Pakistan, and India. Traditionally, it has great importance not only in food but also as a medicine for the cure of stomach and gastro intestinal illnesses. The ajwain seeds contain ajwain oil (2-4.4%), thymol is the most important element of this oil [6]. During the extraction of *Trachyspermum ammi* L. (Carom) and *Foeniculum vulgare* Mill. (Fennel) seeds by using water, methanol and acetone as extraction medium, the highest polyphenols were obtained from *T.ammi* as compared to other seeds and methods [7].

Phenolic compounds have been used and studied as part of the natural antioxidants on the basis of structure and functionality. Most of the fruits, vegetables, leaves, cereals etc. contain phenolic contents with antioxidant activity. The *Brassica* family, vegetables/seeds have great beneficial impacts on health and are partially correlated to their photochemical possessing AOA. *Brassica* family species also have a great economical importance globally. They also played a vital role in routine diet and mostly available in the form of seeds, fresh and processed vegetables, mostly available in Asian countries. All types of Brassica plants contain phythochemicals having antioxidant activity based on phenolic compounds which can be utilized for the protection of food stuff [8]. Similarly, other more efficient sources of extraction of phenolic compounds are leafy vegetables, special cereals, some spices, and edible fruits. Although oilseeds are gaining popularity but still less attention is given as compared to other resources of phenolic compounds. Antioxidants from oilseeds and synthetic were compared and found several advantages and disadvantages related to their application and cost [9].

In Pakistan, India, Middle East and some other Eastern countries Fenugreek (*Trigonella foenum graecum*) leaves and seed (herbaceous plant) are abundantly cultivated. Due to its aroma and good flavor it is greatly consumed as a spice in several food preparations. It also has a great history of medicinal use. Scientists are still working on different types of solvents and methods for the maximum yield of the polyphenols. It has also been reported that extraction from Fenugreek seed by methanol, ethanol, dichloromethane, hexane, acetone and ethyl acetate produced only methanolic (25.89 %) and ethanolic (25.32%) extracts in excellent yield. This may be due to the polarity and composition (chemical) of the phenolic content [10].

Poppy Seeds (*Papaver somniferum*) with good taste, are nutritious oilseeds used in Indian cuisine in the form of Khus Khus (white small size granules) and historically, it has been utilized for the treatment of several diseases like asthma, stomach disorder and eyesight improvement. The antioxidant potentials from poppy seeds extract were less studied and only few scientists have reported that among 15 aqueous spice extracts, the highest TPC was found in the extracts of clove buds and cinnamon bark, however, the lowest TPC was found in the poppy seed extracts [11]. This indicates that this type of solvent also plays a vital role in the q extraction quantity and quality of Polyphenols.

Pakistan is purely an agriculture based country and natural antioxidants are the global need for many food, pharmaceutical and packaging industries, only few studies have been conducted in Pakistan on quantification of polyphenolic contents from such types of seeds. The main objective of this study is to determine and compare the antioxidant activity (AOA) and total phenolic content (TPC) from extracts of *P.somniferom, T.Ammi, Brasica Alba* and *T.foenumgraccum* extracted from the seed source of plant.

2. MATERIALS AND METHODS

Seeds of Ajwain, Mustard, Fenugreek and Poppy were purchased in the month of June from local market of Karachi, Pakistan. These were properly washed with water to remove dust particles, dried and stored in plastic bags at room temperature. DPPH radical, choloroform, methanol, sodium sulphate, reagents and chemicals (analytical grades) were purchased from BDH/Merck from their local suppliers.

2.1. Extraction and Estimation of Total Phenol from Seeds

One kilogram of each type of seed under study was ground to coarse size in a blender. To obtain

rapid result and maximize the efficiency of the process, the extraction of oil was done by chloroform-methanol ($CHCl_3$:MeOH) mixture using Bligh and Dyer method [12].

The crushed sample of about 500gm was mixed in ratios of 1:2 (v/v) $CHCl_3$:MeOH.MeOH and homogenized skillfully. After this, the whole solution was again re-homogenized with $CHCl_3$ (1-litre) and double distilled water (1L). The mixture was centrifuged at 1000 rpm for 5 minutes at room temperature to separate the solid from liquid (with 95% separatory funnel). Rest of the 10% solution was collected by adding 100-200 ml of $CHCl_3$, by vigorously shaking and collecting at the bottom of the separating funnel and filtered by standard filter paper. Oil fraction was recovered by Rotavapor **(**Buchi R-200**)** at 40^{o}C, whereas, for removal of alcohol polyphenol and methanol, mixture was concentrated and re solubilized in the distilled water. Oil from seed was collected with some moisture which was removed by adding anhydrous sodium sulphate (Na_2SO_4) to retain the quality and stability of the extracted material. After removing the moisture, clean, dry oil was collected by decanting and stored at 4^{o}C till further study.

2.2. Estimation of Total Phenol from Seeds

The quantitative analysis of total phenol from extracted crude/oil was performed by spectrophotometeric method with slight modification, previously used by scientist [13]. The reaction mixture based on crude extract/oil mixed with (80:20, v/v) MeOH: water (1:5 w/v) with 10% Folin-ciocalteu reagent was dissolved in water. Then the whole mixture was vortexed for 2-3- minutes, incubated at 30 ^{o}C for 10 minutes. Then the whole sample was analyzed by UV type spectrophotometer at 765nm. The total phenols were measured in mg/100g. The extracts were stored in desiccators after noting the yield. However, other activities were measured by utilizing different methods within a week.

2.3. Radical Scavenging Activity by DPPH Essay

The DPPH scavenging activity was performed according to the method of Negi *et al*. with slight changes [14]. 0.1 ml of the crude extract was mixed in a concentration range of 50-250 µg/100 µL in the MeOH was then treated with methanolic solution of DPPH (1.4 mL; 0.2 mM) then mixed and homogenized with 1.5 ml of distilled water (D.W), and then it was kept in a dark place for half an hour. The absorbance was measured at 516 nm against the blank in a spectrometer (UV VIS Shimadzu). The DPPH activity of the crude extracts was estimated by decrease of DPPH values in toluene. Similarly, all extracted oil in concentration range of 100-250 µg/100 µL (in toluene) was then treated with toluene solution of DPPH (1.5 mL; 0.2 mM) and the decrement in absorbance was noted by the same procedure. The procedure used by Le *et al.* has been utilized to calculate the percentage of DPPH [15]. Low absorbance gave high radical scavenging activity after accepting an electron to become more stable. The violet color of the methanolic DPPH solution decreased with the absorbance of solution at 516 nm and recorded.

2.4. Chelating Effect

The antioxidant activity was also measured by chelating method for the methanolic extract of seeds by slight modification in the method used by Re *et al.* [16]. This method has reduction ability for all types of seed extracts from Ferrous to Ferric ions. The reaction mixture contain 0.25 mL of Ferrous sulfate ($FeSO_4$ solution, 1 mM), 0.25 mL of extract (50-250 µg/mL in methanol), 1 mL of Tris-HCl buffer (pH 7.4), 1-mL of 2,2′- bipyridyl solution (0.1% in 0.2M HCl) and 2.5 mL of methanol. To measure the absorbance of the whole solution spectrophotometer (522 nm) was used with Na_2EDTA (disodium ethylenediaminetetraacetate) used as standard for the evaluation of Fe2+ chelating effect.

2.5. Statistical Analysis

Statistical Analysis was used for determining correlation between total phenolics and antioxidant activity. All results were based on triplicate presented as mean±SD. Anova and Tukey's test were applied to take significant differences among the mean values ($p<0.05$).

3. RESULTS AND DISCUSSION

In Pakistan, the data based on quantitative and qualitative analysis of total phenolic compounds and antioxidant activities from different plant sources are very less, although benefits are known not only for food but also for Pharmaceutical and packaging industries. The total phenolic contents and antioxidant activities (50-250 µg/mL) of

Table 1. Total Phenols and antioxidant activity of the crude polyphenolic extracts derived from oilseeds. ($p<0.05$). Results are expressed as std deviation (n=3).

Samples µg/100µL	Total Phenols mg / 100g	Antioxidant Activity	
		% DPPH Scavenging	% Chelating Effect
P. somniferum			
50		44.0±0.98	33.0±1.89
100		53.0±1.45	39.0±1.90
150	1015	59.0±2.30	41.0±1.92
200		63.0±1.78	49.6±1.89
250		66.5±0.56	56.4±1.67
T. ammi			
50		66±1.45	49.3±2.40
100		74±0.98	55.0±3.00
150	6900	83±2.34	60.2±3.09
200		90±2.78	66.7±3.12
250		95±3.00	77.7±1.67
Brassica alba			
50		57.0±1.53	35.9±2.08
100		59.2±0.85	40.6±2.99
150	2950	66.5±2.51	46.5±2.76
200		73.5±2.08	53.5±1.67
250		79.4±1.52	65.4±2.10
T. foenumgraecum			
50		50.4±0.45	39.0±2.10
100		55.3±1.56	44.0±2.08
150	1900	62.0±2.08	49.5±1.50
200		67.0±1.89	53.5±1.79
250		73.0±1.78	61.0±1.54

crude extracts and oils from *Trachyspermum ammi (ajwain)*, *Brassica alba (mustard)*, *Trigonella foenum graecum* (Fenugreek) and *Papaver somniferum* (opium poppy) seeds were presented in Tables **1** and **2**. The highest total phenols were found in T.Ammi (6900 mg/100g), *Brassica alba* (1015mg/100g), *T. soenumgraecum* (1900 mg/100g) and the least in *Papaver somniferum* (1015 mg/100g). However, in Table **2**, oilseed extract shows less amount of total polyphenols in order *T.ammi* > *Brassica alba* > *Trigonella foenum graecum* > *Papaver somniferum* seeds. Percentage DPPH radical is used to scavenge the free hydrogen from phenolic as DPPH-H, after that the color was changed from purple to yellow at 517nm from 9660 to 1640 due to addition of electron [17]. However our experimental data, (Figs. **1** to **4**) show that AOA of T.Ammi, *Brassica alba, soenumgraecum, Papaver somniferum* were concentration dependant. Hence a strong correlation has been observed between AOA and concentration. Since at 250 µg/mL T.ammi showed the highest AOA (95%±3.00) for T.ammi, whereas the lowest (66%±1.45) at 50 µg/mL (Table **1**) was found for poppy seed. However, the antioxidant activities extracts of *T.ammi*, *Brassica alba*, *Trigonella foenum graecum* and *Papaver somniferum* seeds were obtained in the range of (50.45±3.00- 95±3.00) for crude polyphenolics as per Table1 extract whereas for oily extracts the range lies between (37.42±2.09 – 88.8.0±2.09) by DPPH and 16.00±2.09 – 16.00±2.09) by chelating method as mentioned in Table **2**. The higher DPPH values of crude *T.ammi* show greater potential as compared to oil extract, this may be due to the solubility and purity of the compound. Therefore, with respect to synthetic antioxidant e.g. BHA (78%–97%), our values are significantly estimated on higher side so they can be replaced with the synthetic ones [18]. There is no linear

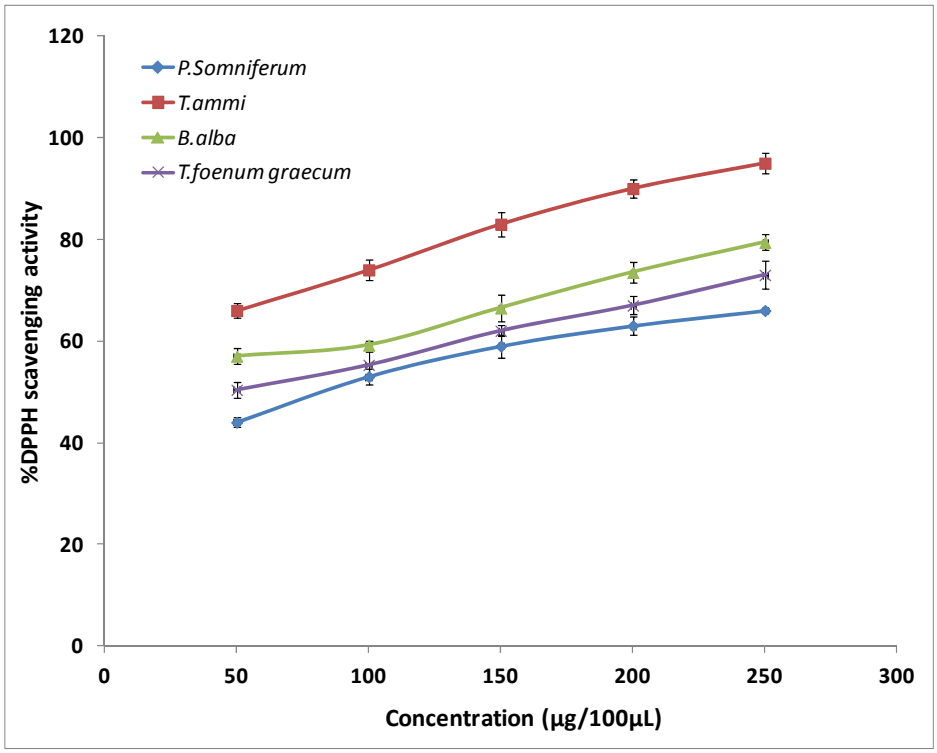

Fig. (1). % DPPH scavenging activity of the crude polyphenolic extracts derived from *T. ammi, B. alba, T. foenum-graecum* and *P. somniferum* seeds ($p < 0.05$).

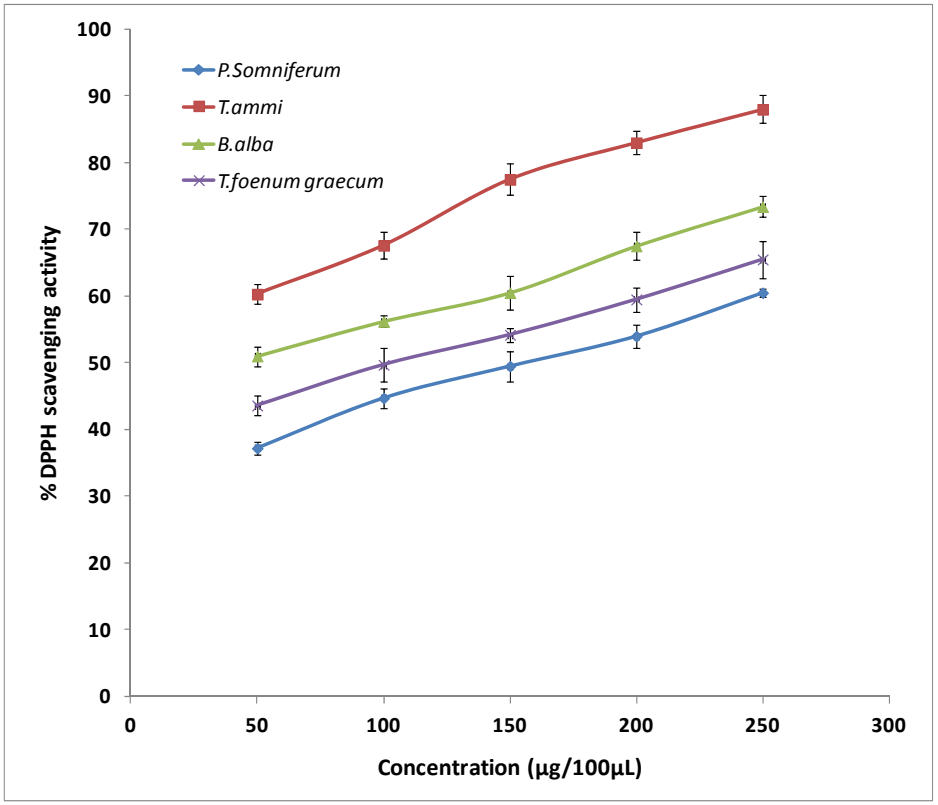

Fig. (2). % DPPH scavenging activity of the oils extracted from *T. ammi, B. alba, T. foenumgraecum* and *P. somniferum* seeds ($p < 0.05$).

Table 2. Total Phenols and antioxidant activity of the oils extracted from oilseeds. (p< 0.05),-not found, No. of samples analyzed n=3.

Samples µg/100µL	Total Phenols mg/100g	Antioxidant Activity	
		% DPPH Scavenging	% Chelating Effect
P. somniferum			
50		37.2±0.98	-
100		44.7±1.45	-
150		49.5±2.30	16±2.09
200	78	54.0±1.78	20±2.88
250		60.5±0.56	25±3.09
T. ammi			
50		60.3±1.45	19.0±2.70
100		67.6±1.98	23.5±1.23
150		77.5±2.34	30.5±1.67
200	1990	83.0±1.78	36.5±1.89
250		88.0±2.09	41.0±1.12
Brassica alba			
50		51.0±1.50	12.5±1.20
100		56.2±0.87	18.8±2.78
150		60.5±2.55	22.5±2.00
200	550	67.5±2.09	29.5±1.05
250		73.4±1.55	35.5±1.34
T. foenumgraecum			
50		43.6±1.45	-
100		49.7±2.56	-
150		54.2±1.08	17.6±2.08
200	220	59.5±1.80	23.3±1.29
250		65.5±2.78	30.0±3.00

correlation between AOA and phenolic compound as indicated by DPPH method since values of R^2 lies between 0.52-0.65.

Similarly, in chelating method four types of seeds show higher potential to chelate Ferrous ion (Fe^{+2}) into Ferric (Fe^{+3}). Our result showed that due to impurities in polyphenols no linear correlation was observed between total phenolic and AOA (R^2=0.30 to 0.85) even by chelating method. However, the correlation was observed in extracts of 26 commonly used oilseeds, nuts and milk products were also found in the range of R^2 093-0.99 by group of researchers. The same research group found *areca* nut (10841 gallic acid equivalent mg/100g) with highest antioxidant activity by DPPH (28622 mg/100g trolex Eq) and FRAP (4220341 mg/100g Ferrous sulfate equivalent) methods to be in-line with mustard seed TPC estimated at about 725 mg/100mg. This was in accordance with antioxidant activity by DPPH (1155 mg trolex/100g) and FRAP (27666 mg ferrous sulfate equivalent/100g), mustard seed which also proved to be a potent source of the antioxidant the highlighted sentence needs revision, author assistance required [19]. Our result also provides mustard seed as potent source of antioxidant as per Tables 1 and 2. However, the utilization of natural polyphenols on commercial scale is still not in very much practiced due to low recovery of polyphenols and incurred high cost. Whereas in other research based on 24 medicinal plant extracts without any correlation between TPC and AOA showed significant phenolic contents in *Trigonella foenum-graecum* (194.63mg/100g) and less in *Papaver somniferum* (44.42mg/100g), our result also follows the same pattern (1900-1050 mg/100g) as shown in graphs [20]. It is already explained

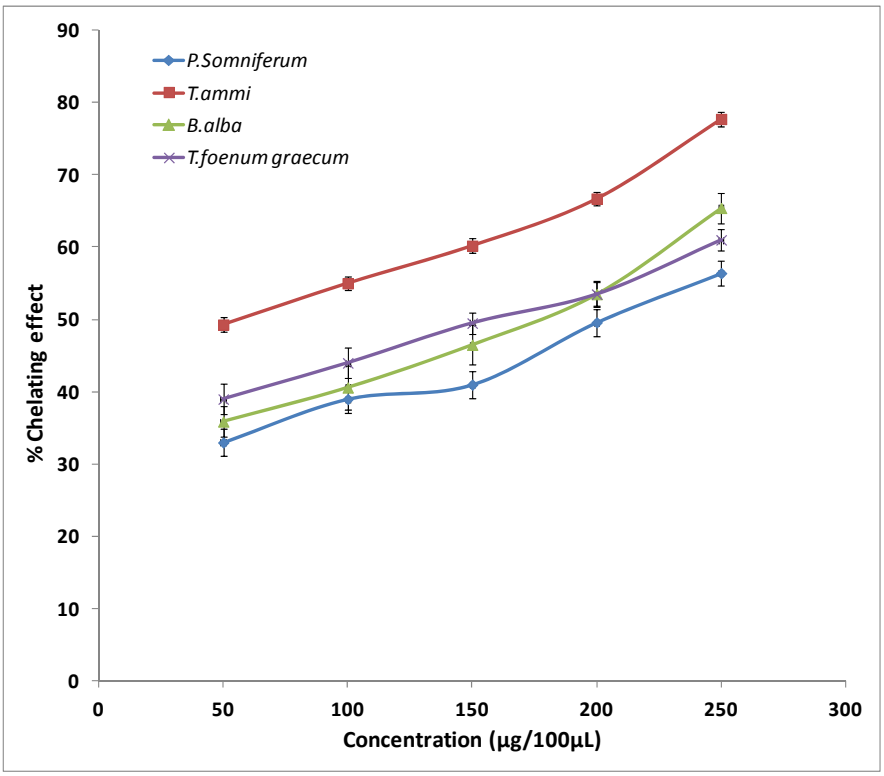

Fig. (3). % Chelating effect of the crude polyphenolic extracts derived from *T. ammi, B. alba, T. foenumgraecum* and *P. somniferum* seeds ($p < 0.05$).

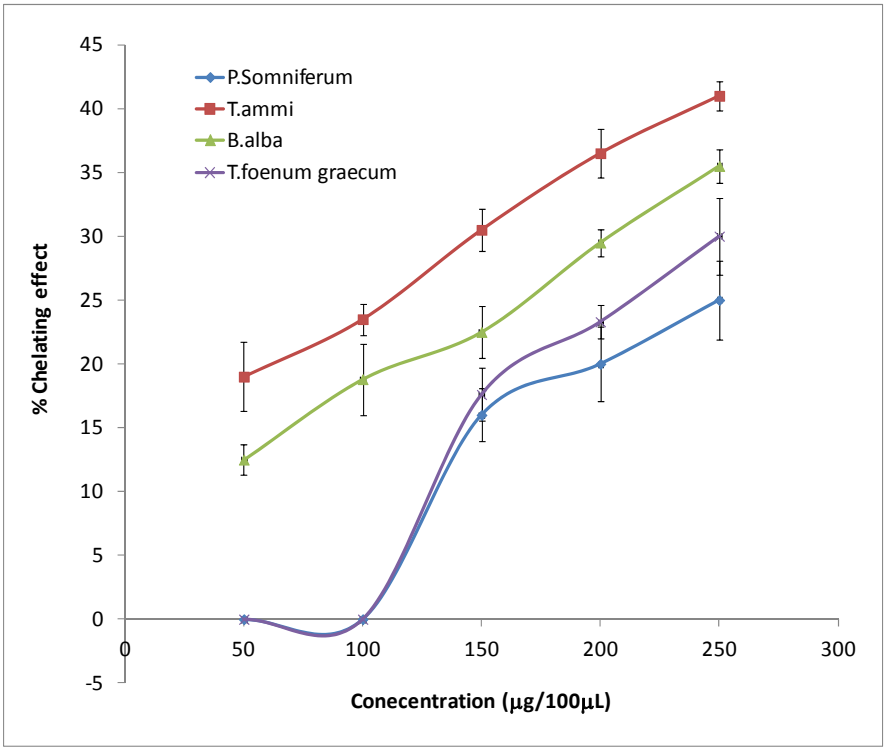

Fig. (4). % Chelating effect of the oils extracted from of *T. ammi, B. alba, T. foenumgraecum* and *P. somniferum* seeds ($p < 0.05$).

that the quantity of phenols depends on the type, climate, cultivation method, type of solvent etc. The IC_{50} values for the extract and oil of *T. ammi*- and *B. alba* were less than 50 µg/100 µL, respectively, while for *T. foenumgraecum* extract and oil were 50 µg/100 µL and 100 µg/100 µL, respectively and for *P. somniferum* were ~100 µg/100 µL and ~150 µg/100 µL respectively. The percentage chelating effect followed the same order as DPPH (Figs. **1-4**).

4. CONCLUSION

On the basis of above experimental data it could be concluded that *Trachyspermum ammi*, *Brassica alba*, *Trigonella foenum graecum* and *Papaver somniferum* seeds can be utilized as great sources of antioxidant based plant species in South Asian region. Overall, the result suggested that the order of phenolic contents and AOA are in ascending order of *T. ammi>B. alba>T. foenumgraecum* and least by *P. somniferum*. The antioxidant activities of crude extracts of seeds were higher as compared to the oil fractions. This study also suggests that natural antioxidant can be utilized for food safety and for increasing the shelf life of the food and securing the health effects. The main challenge faced by the researchers is the isolation, purification, stabilization and incorporation of natural antioxidants in different types of plastic films to increase the shelf life of food.

CONFLICT OF INTEREST

There is no conflict of interest reported by the author(s) regarding this research work.

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