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Phytochemical study of Sesame (*Sesamum indium)* seed oil and defatted seed cake

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Abstract
In this study, the oil and defatted seed cake of two varieties of Sesame (HT-1 and HT-2) were used to evaluate phytochemical characteristics like fatty acids, mineral content, total phenol, flavonoids, total tocopherols, caretonoids, antioxidant activity etc. Oil, Free fatty acid, Peroxide value, Iodine value, Saponification value, and Unsaponifiable matter, were determine according to AOAC (Association of Official Agricultural Chemists) method. Free fatty acids were found to be higher inHT-1 (1.7±0.3) (as % oleic acid). Peroxide value was found to be less in HT-2 (2.5±0.2meq/kg) as compared to HT-1(2.6±0.1meq/kg). Iodine value was higher in HT-2 (117±0.5g/100g) compared to HT-1(111±0.7g/100g). In mineral composition, iron was found higher in defatted seed of HT-1(5.7±0.2 mg/100g). Saponification value was higher in HT-1 (196±0.1 mg/g KOH) as compared to HT-2. In different fatty acids, highest composition found as oleic acid in HT-2 (41.8±0.4%). From the results, it was inferred that total phenolics, flavonoid content and total tochopherols in phenolic extract of seed oil of Sesame was higher in HT-2 (24.3±0.6 mg GAE/100g, 6.7±1.2 mgCAE/100g, 46.4 ±3.5 mg/100g respectively) while in methanolic extract of defatted seed cake were corresponding 24.5±3.7 mg GAE/g, 7.0±0.6 mg CAE/g, 6.7±0.7 mg/100g. Antioxidant activity in phenolic extract of seed oil was higher in HT-2 (96 %). The result showed that HT-2 variety of Sesame is a good source of antioxidants that can be used for more frequent as antioxidant in food, feed as well as in functional foods.

Keywords: Sesame, mineral, phenols, antioxidant activity

Introduction
Oils and fats are indispensable part of the human diet and more than 90% of global production is used as food or as ingredients in food products. Antoniassi *et al.,* (1998) [1] evaluated both quality and identity of oils. Amongst the identified traditional oil seeds, Sesame assumes particular significance due to its high quality polyunsaturated fatty acids in seeds oil content and rich in phenolic contents are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Sesame oil, with almost 85% unsaturated fatty acids, is the most oxidatively stable dietary oils. Lipid oxidation is a free radical process responsible for foodstuffs deterioration as well as in numerous human ailments like atherosclerosis, arthritis, cancer, and AIDS (Pourmorad *et al*., 2006) [15]. As per Velioglu *et al*., 1998 [19], antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Today's synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones (TBHQ) and propylgalate (PG) are suspected to cause or prompt carcinogensis (Ito *et al*., 1986) [8]. Therefore, demand arises for replacing these synthetic antioxidants with natural alternatives. The chemical composition of sesame shows that the seed is an important source of oil (44–58%), protein (18–25%), carbohydrate (13.5%) and ash (5%) (Kahyaoglu and Kaya, 2006 [9]; Kamel-Eldin and Appelpvist, 1994 [10]; Mohamed and Awatif, 1998 [12]; Shyu and Hwang, 2002 [17]; Yoshida, 1994 [21]. Tocopherols are particularly important functional component in foods.

Material and methods
The commercially available chemicals from Qualigens, Merk and Ranbaxy, of highest purity, were used for various experimental procedures. The seed of varieties HT-1 and HT-2 of Sesame were procured from oil seed section, Department of Genetics and Plant Breeding, CCS, Haryana Agricultural University, Hisar.

After cleaning, the seeds were ground into fine powdered form. Oil, Free fatty acid, Peroxide value, Iodine value, Saponification value, and Unsaponifiable matter, were determine according to AOAC (1990) [3] method.

Oil

Dried and ground samples (100g) each of seed kernel were weighed in a thimble and placed in soxhlet apparatus. Dry pre-weighed solvent flask containing petroleum ether and condenser were attached for each sample in three replicates. The heating rate was adjusted to give a condensation rate of 2-3 drops/sec. and extracted for 16 hrs. Removed the thimble and retained petroleum ether. The excess of petroleum ether was evaporated from the solvent flask on a hot water bath and dried the flasks in a desiccators and weighed.

Calculation

Oil content in sample (% dry wt. basis)= $\frac{(b-a)\times 100}{Wt.of sample\ (g)}$

Iodine value

Two grams of oil sample was taken in a 500 ml glass stoppered conical flask containing 10 ml of $CHCl_3$. The flask was swirled until the sample entirely dissolved. Iodine monochloride (25 ml) was added to it and again swirled the mixture. The flask was allowed to stand in dark place for one hour. After that, 15 ml of KI solution and 100 ml of water were added. Flask was shaken vigorously and titrated with standard sodium thiosulphate solution. Two blanks were made in the same manner by omitting the sample. Blanks were also allowed to stand with test mixture for the same length of time.

Calculation

$$\text{Iodine number } \left(\frac{g}{100}\right) = \frac{(B-S)(N)(12.7)}{W}$$

B = Volume of standard sodium thiosulphate solution (ml) used for blank.
S = Volume of standard sodium thiosulphate solution (ml) used for sample.
N = Normality
W = Weight of sample (g)

Saponification value

The oil (2 g) was taken into flask and added 25 ml of 0.5 N alcoholic KOH solution. Reflux condenser was attached and heated on boiling water for 60 min. Flask was swirled frequently during heating. After that a drop of 1 % phenolphthalein was added and titrated with 0.5 N HCl. Operations were performed with blank as well.

Calculation

Saponification value = $\frac{(B-A)\times 28.05}{Wt.of\ oil(g)}$

A = Volume of 0.5 N HCI used for sample
B = Volume of 0.5 N HCI used for blank

Saponification and removal of unsaponifiable material

Seed oil (5 g each) was refluxed separately with potassium hydroxide in 95% ethanol (2 M, 50 ml) for one hour. The mixture was allowed to cool, diluted with water (50 ml) and extracted with diethyl ether (3 x 50 ml). Sufficient quantities of water and ether were necessary for a good phase separation. The combined ether extracts were washed with distilled water (3 x 100 ml) and allowed to stand overnight. The ether was removed under vaccume at 40ºC and the residue dried by evaporation with acetone to obtain the unsaponifiable matter.

Peroxide value

The oil (5 g) was taken into 500 ml conical flask. Acetic acid - $CHCl_3$ mixture (30 ml) was added to the flask. Saturated KI solution (0.5 ml) was added to it and allowed to stand for 1 min. About 450 ml of water was added to the flask and then titrated against standard 0.01 N sodium thiosulphate by using starch indicator to liberate all iodine free $CHCl_3$ layer until the blue colour just disappeared. Blank was titrated similarly in the absence of oil.

Calculation

Peroxide value = $\frac{A\times N\times 1000}{Wt.of\ oil(g)}$ meq / kg oil

A = ml $Na_2S_2O_3$ (Test - blank); N =Normality of $Na_2S_2O_3$ solution

Fatty acid spectrum

Methyl esters were prepared by the method of Luddy *et al*. (1968) [11]. Methyl esters of fatty acids were separated using Chemito 8610 HT Gas chromatograph equipped with FID and a BPX70, 0.25ml fused silica column (SGE Pvt. Ltd., Ringwood, Victoria, Australia) was used. The carrier gas was hydrogen and injection was operated in the split mode, the split ratio being approximately 50: 1. Injector and detector temperature were 270ºC and 280ºC respectively. The oven temperature was held at 70ºC for 1 min. and then programmed at 30ºC/min. to 170ºC followed by further programming at 30ºC/ min. to 200ºC and held at this temperature for 6 min. Data was captured and analysed with, Chemito 5000 integrator (Tashniwal Instruments, India Ltd.)

Free fatty acids

Free fatty acids were determined as per the method of (Rao *et al.*, 1972) [16]. Fifty ml of denatured alcohol was added to one g of oil sample in a 250 ml conical flask. Few drops of phenolphthalein were added, titrated against 0.1 N NaOH till a permanent light pink colour appeared which persisted for at least 1 min. The percentage of free fatty acids was calculated by using the following formula:

Free fatty acids (in terms of oleic acid) = $\frac{100\times 282\times V}{Wt.of\ oil\times 10\times 1000}$

V = Volume of 0.1N sodium hydroxide used.

Determination of mineral contents

The sample was digested by wet oxidation. 0.2 gm of each extract was transferred to a conical flask. 1mL of perchloric acid and 5 mL nitric acid was added, mixed well and kept overnight at room temperature followed by digestion on low temperature at 70-80ºC and then at higher temperature until the volume of the solution reduced to about 1mL. After the digestion of all, the mixture volume was made to 10 mL with distilled water and analyzed by using atomic absorption spectrometer.

Determination of total phenolics content

The total phenolics were determined by the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) [18] method using gallic acid as standard for which a calibration curve was obtained with solutions of 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/ml of gallic acid. A 1.0 ml of diluted extract (all fraction were diluted with methanol to adjust the absorbance within the calibration limits), 1.0 ml of 1mol/L Folin–ciocalteau reagent (diluted to 1:2 ratio) and 2.0 ml of Na_2CO_3 (20% w/v) were

mixed and the volume was made to 50 ml. After 8 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes. Then the absorbance of supernatant solution was measured at 730 nm using Spectronic 20 spectrophotometer against a blank prepared similarly with the same solvent but omitting the extract. The concentration of phenolics thus obtained was multiplied by the dilution factor and the results were expressed as the equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

Determination of flavonoid content

The aluminium chloride colorimetric assay, as described by Zhishen *et al.,* (1999) [22] was used. Briefly, 1ml of extracts or obtained solution of catechin (0.02, 0.04, 0.06, 0.08 and 0.01 mg/ml) was added to test tubes containing 4ml of double distilled water. To the mixture was added 0.3ml 5% $NaNO_2$. After 5 minute, 0.3ml 10% $AlCl_3$ was added. Immediately, 2ml 1M NaOH was added and the total volume was made upto 10ml with double distilled water. The solution was mixed thoroughly and the absorbance of both the samples, blank and standard was read at 510 nm using UV visible spectrophotometer Spectronic 20. Total flavonoid content was expressed as mg catechin equivalents per gram of the extract (mg CAE/g).

Total tocopherol

Aliquots (10, 15, 20 and 25 mg/ml) of a solution of alpha tocopherol in the ethanol were transferred to a volumetric flask and the volume was adjusted to 8ml with ethanol. Poured 1ml of 2, 2' -dipyridyl reagent and 1ml ferric chloride to above prepared diluted solutions and the mixture shaken for 10 seconds. The absorbance of the mixture was read at 520 nm against ethanol as a blank (Philip *et al;* 1954) [14]. Then the standards curve was drawn. The above described procedure was followed by using 10, 20, 30, 40 mg sample solutions. The content of alpha tocopherol in the extract was calculated from regression equation of the standard curve 200± 10 mg of the oil sample were weighed accurately into a 10 ml volumetric flask. 5 ml of toluene were added by pipette and the oil taken into solution. 3.5 ml of 2,2′bipyridine (0.07 % w/v in 95% aqueous ethanol) and 0.5 ml of $FeCl_3 6H_2O$ (0.2% w/v in 95% aqueous ethanol) were added in that order. The solutions were made up to 10 ml with 95% aqueous ethanol. After standing for one min. the absorption at 520 nm was determined using as a reference a blank solution, prepared as above but omitting the oil. Solutions were protected from strong light during colour develop.

Total carotenoids

Total carotenoids were found by the method of Vasconcellous *et al*. (1980) [20]. Oil (0.5 g) was taken in 100 ml conical flask. The oil sample was dissolved in cyclohexane (2.5% w/v) and the absorbance was read at 417 nm and following equation was used to estimate total carotenoids.

$$\text{mg carotene/kg oil} = \frac{(\text{absorbance at 417 nm}) \times \text{sample in volume in ml}}{0.204 \times (\text{sample weight in g})}$$

2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method. The effect of extracts on DPPH radical was estimated according to the method of Hatano *et al*., (1988) [7]. 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that shows a maximum absorption at 517 nm in methanol. When DPPH encounters proton donating substances such as an antioxidant and a radical species, the absorbance at 517 nm disappears because the DPPH radical is scavenged. On the basis of this principle, the radical scavenging effect of each fraction was measured. Briefly 0.3, 0.6, 0.9, 1.2, 1.5 mg of methanol extract of varieties HT-1, HT-2 of Sesame were added to 1ml of 2,2'-diphenyl-2-picrylhydrazyl radical (DPPH: $0.025gL^{-1}$ in methanol) final volume was made to 10 ml with methanol and mixed by vortex for 5 minute. The absorbance of the sample was measured at 517 nm in every 10 minutes till a steady state is reached (40 min) using the spectrophotometer Spectronic 20. Similarly, a control sample was also prepared. For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the percentage of decline of the absorbance after 2 hrs relative to the control, corresponding to the percentage of DPPH that was scavenged.

Calculation

The percentage of DPPH, which was scavenged (%$DPPH_{sc}$) was calculated using: % $DPPH_{sc}$= $\{(A_{cont}-A_{samp})/A_{cont}\}$ x 100
Where $A_{cont}$is the absorbance of control and $A_{samp}$is the absorbance of sample.

Measurable Analysis

The trial estimations were completed in triplicate and results were displayed as mean of three recreates ± standard deviation.

Result and discussion

Soxhlet extraction of the seed oil with petroleum ether (60-80ºC) gave straw colour with 46.5±0.3% and 48.3±0.1% in varieties HT-1 and HT-2 of Sesame. The yield of methanol extract in whole seed were 5.8±0.2%, 4.8±0.2% and the yield of methanol extract in defatted seed were 10.2±0.1%, 10.3±0.3% in both varieties.

Chemical properties

The chemical properties of oil are amongst the most important properties that determines the present status of the oil. Free fatty acid and peroxide values are considered as valuable measures of oil quality. The mean peroxide values were 2.6±0.1 meq/kg and 2.5±0.2 meq/kg in varieties HT-1 and HT-2 of Sesame. The peroxide value was low in HT-2 variety which is a good index for the stability of the oil and its susceptibility to rancidity during storage. Nzikou *et al* (2009) [13] reported the lower peroxide value of 0.06±0.7 meq/kg in Sesame. The mean iodine value 111±0.7g /100g and 117±0.5g /100g in varieties HT-1 and HT-2 of Sesame indicating a high degree of unsaturation when compared to most plant oils. In codex standard for named vegetable oils the reported iodine value was 104-120 g/100g in Sesame. The saponification values were 196±0.1 mg/g KOH, 173±1.0 mg/g KOH for varieties HT-1 and HT-2 of Sesame. There values are in agreement with the value reported in codex standard for named vegetable oils which is correspondingly186-199 mg/g KOH of oil in Sesame. The unsaponifiable matter was 1.2±0.0%, 1.3±1.5% in HT-1and HT-2 respectively. Nzikou *et al* (2009) [13] reported that the unsaponifiable matter was 1.8±0.2% in Sesame oil. The concentration of free fatty acid was found as 1.7±0.2%, 1.2±0.3% (as% oleic acid) in varieties HT-1 and HT-2 of Sesame.

Minerals

The Sesame seeds contained significant amount of important minerals. The Iron concentration was (3.6±0.3 mg/100g) in

variety HT-1 and (2.8±0.1 mg/100g) in variety HT-2. But the corresponding value in defatted seed for Iron were (5.7±0.2 mg/100g) in variety HT-1 and (3.4±0.3 mg/100g) in variety HT-2. Thus, in defatted seed the Iron concentration was higher than that of the whole seed.

Fatty acid composition

The major saturated fatty acids in seed oil were palmitic acid (10.6%, 9.7%), stearic acid (5.7%, 5.4%) with respect to HT-1 and HT-2. The main unsaturated fatty acids were oleic acid (41.2%, 41.8%) and linoleic acid (40.8%, 41.6%) acids with respect to HT-1 and HT-2 (Table 1). The results obtained are in agreement with those of the literature of Carvalho (2012) [5].

Table 1: Relative composition (%) of fatty acids in seed oil of varieties HT-1 and HT-2

Fatty acid	HT-1	HT-2
Palmitic ($C_{16:0}$)	10.6± 0.1	9.7±0.1
Stearic acid ($C_{18:0}$)	5.7± 0.2	5.4±0.1
Oleic acid ($C_{18:1}$)	41.2± 0.2	41.8±0.2
Linoleic acid ($C_{18:2}$)	40.8 ±0.1	41.6±0.4
Total saturated fatty acids (%)	16.3	15.1
Total Unsaturated fatty acids (%)	82.0	83.4

Values are mean of three replicates ± standard error

Table 2: Antioxidant activity (%) of crude seed oil extract of the varieties HT-1 and HT-2 of Sesame (BHA & BHT were taken as standard).

HT-1		HT-2		BHA		BHT	
Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)
0.01	38	0.01	40	0.001	19	0.001	49
0.02	64	0.02	67	0.002	42	0.002	69
0.03	81	0.03	83	0.003	57	0.003	79
0.04	89	0.04	90	0.004	69	0.004	86
0.05	93	0.05	94	0.005	74	0.005	89
0.06	94	0.06	95	0.006	78	0.006	91
0.07	95	0.07	96	0.007	80	0.007	92
0.08	96	0.08	97	0.008	82	0.008	93
0.09	97	0.09	98	0.009	82	0.009	94
0.10	97	0.10	98	0.010	82	0.010	94

Phenolic contents and antioxidant activity

It is well known that phenolic compounds are widely distributed in oils. The results showed that the content of total phenols in methanolic extracts of crude oil were 17.5±2.0mg GAE/100g, 24.3±0.6 mg GAE/100g in varieties HT-1 and HT-2. The content of total flavonoids and total tocopherols was 10±1.8 mg CAE/100g, 6.7±1.2 mg CAE/100g and 34.2±2.5 mg/100g, 46.4±3.5 mg/100g in HT-1 and HT-2 respectively. The antioxidant activity (EC50) exhibited by crude seed oil extracts were 0.015±0.3 mg/ml (HT-1) and 0.014±0.1 mg/ml (HT-2). The corresponding maximum antioxidant activity exhibited 95 % (HT-1) and 96 % (HT-2) at the concentration of0.07 mg/ml of the extract respectively (Table 2)

Table 3: Antioxidant activity (%) of defatted seed cake extract of varieties HT-1, HT-2 of Sesame (BHA & BHT were taken as standard)

HT-1		HT-2		BHA		BHT	
Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)	Conc. in mg/ml	Activity (%)
0.01	42	0.01	45	0.001	19	0.001	47
0.02	67	0.02	54	0.002	42	0.002	69
0.03	83	0.03	63	0.003	57	0.003	79
0.04	89	0.04	70	0.004	69	0.004	86
0.05	91	0.05	76	0.005	74	0.005	89
0.06	92	0.06	82	0.006	78	0.006	91
0.07	93	0.07	88	0.007	80	0.007	92
0.08	93	0.08	92	0.008	82	0.008	93
0.09	93	0.09	92	0.009	82	0.009	94
0.10	93	0.10	92	0.010	82	0.010	94

In methanolic extracts of defatted seed cake, the content of total phenolics was 20.0±2.60 mg GAE/g, 24.5± 3.7 mg GAE/g in varieties HT-1 and HT-2 respectively. The content of total flavonoids and total tochopherols was 8.3±0.6mg CAE/g, 7.0±0.6 mg CAE/g and 7.2±0.7 mg/100g, 6.7±0.7 mg/100g in both varieties respectively. The antioxidant activity (EC_{50}) exhibited by methanol extract of defatted seed cake of varieties HT-1, HT-2 were 0.015±0.1 and 0.014±0.2 mg/ml of the extract respectively. The corresponding maximum antioxidant activity defatted seed cake extract of HT-1 and HT-2 were 93 % and 88 % at the concentration of 0.07 mg/ml respectively (Table 3)

Table 4: Chemical constituents and Antioxidant activity of crude oil extract of varieties HT-1 and HT-2 of Sesame.

Parameters	HT-1	HT-2	BHA(Std)	BHT(Std)
Total phenol(mg GAE/100g)	17.5±2.0	24 3±0.6	_	_
Total flavonoids (mg CAE/100g)	10±1.8	6.7 ±1.2	_	_
Total tocopherol(mg/100g)	34.2±2.5	46.4 ± 3.5	_	_
Carotenoid content(mg/kg)	8.2±1.0	7.6±0.9	-	-
DPPH (EC_{50})(mg/ml)	0.015±0.3	0.014±0.1	0.0026±0.2	0.0011±1.0
DPPH (%)Conc. (mg/ml)	95(0.07)	96(0.07)	82(0.009)	94(0.009)

Values are mean of three replicates ± standard error

Table 5: Chemical constituents and Antioxidant activity of methanol extract of defatted seed cake of variety HT-1 and HT-2 of Sesame.

Parameters	HT-1	HT-2	BHA (Std)	BHT (Std)
Total phenol(mg GAE/g)	20.0±2.6	24.5±3.7	_	_
Total flavonoids (mg CAE/g)	8.3±0.6	7.0± 0.6	_	_
Total tocopherol (mg/100g) of defatted seed cake)	7.2±0.7	6.7±0.7	_	_
Carotenoids content (mg/kg)	8.2±1.0	7.6±0.9		
DPPH(EC_{50})(mg/ml)	0.015±0.1	0.014±0.2	0.0026±0.2	0.0011±1.0
DPPH (%)Conc. (mg/ml)	93(0.07)	88(0.07)	82(0.01)	94(0.01)

Values are mean of three replicates ± standard error

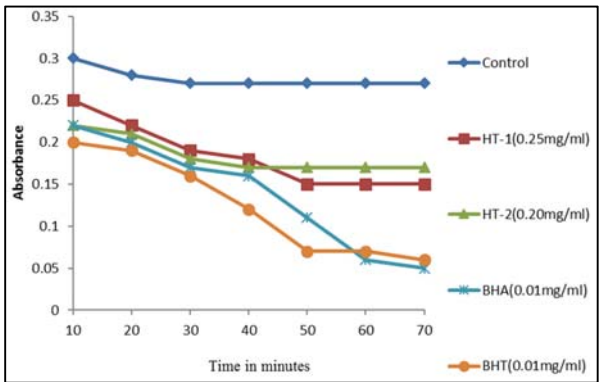

Fig 1: Reaction kinetics between DPPH and oil extract of varieties HT-1 and HT-2 of Sesame (BHA & BHT were taken as standard).

Kinetic study of phenolics

For the reaction kinetics between DPPH and phenolic content of the oil, various absorbance values were observed with time and were noted after every 10 min till a plateau was reached. Further reaction was done after 40 min of incubation. The kinetic curve of oxidation inhibition was constructed to clarify the mechanism of antioxidant action of phenolic compounds present in the oil (Fig.1) and defatted seed cake (Fig.2). For comparison the activity of synthetic antioxidant BHA and BHT were determined. These results may be explained by the studies that show, differently from the synthetic antioxidants BHA and BHT, the phenolic compounds of natural origin may present pro-oxidant activity at low conc. Thus result showed that the antioxidants of the studied methanolic extract of sesame seed oil may be effective in blocking the chain reactions by interaction with the peroxyl radicals.

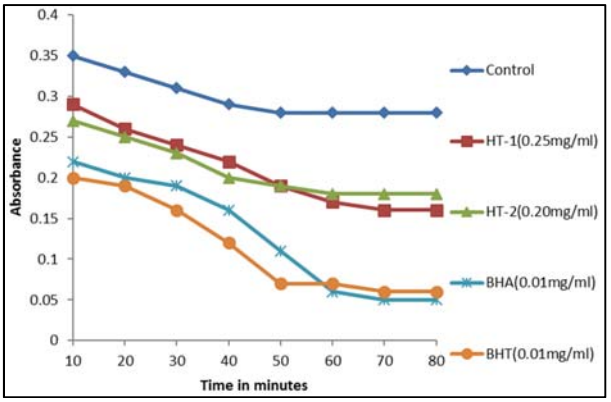

Fig 2: Reaction kinetics between DPPH and defatted seed cake extract of varieties HT-1 and HT-2 of Sesame (BHA & BHT were taken as standard).

Conclusion

This study provide information about nutritional and health impact of sesame oil which serve as nutritional sources of natural antioxidants for health promotion and oxidative stress based disease prevention. The antioxidant activity shown by methanolic extracts of seed oil and defatted seed cake varied with the concentration of phenols, flavonoids and tocopherol contained in it. There is an increase in antioxidant activity of seed oil and defatted seed cake extract with increase in concentration of phenols, flavonoids and tocopherols. Therefore, Sesame oil can be used as a potent antioxidant for netraceutical use.

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