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Evaluation of Antioxidant Prospective of *Diospyros malabarica* Methanolic Extract for Improving Oxidative Stability of Mustard Oil

RABIA REHMAN[1]*, ASMA RAZA[1], LIVIU MITU[2]*
[1]Institute of Chemistry, University of the Punjab, Lahore-54590, Pakistan
[2]Department of Nature Sciences, University of Pitesti, 1 Targu din Vale Str., 110040, Romania

Abstract*. Antioxidants are substances which inhibit auto-oxidation of oils and fats by donating an atom of hydrogen to free radicals produced in the initial stages of autooxidation. In the course of the past two decades, a lot of work is done on application of natural plants extracts as preservative in edible oils due to the drift towards reducing the use of synthetic food additives. The aim of this research project was to estimate antioxidant efficacy of pharmaceutical plant species of Pakistan origin famous as "Gaab tree" (Diospyros malabarica), in local language and its use as a stabilizing agent for mustard oil. To carry out this study, its fruit and leaves methanolic extracts were prepared and their antioxidant efficiencies were analyzed by means of various tests like: TPC, TFC, TAC. They were found to be enriched with potential antioxidants. For finding antioxidant potential, DPPH, ABTS, FRAP analysis and β-carotene bleaching tests were also carried out. Results indicated their oxidative potentials were similar to synthetic antioxidants, like: BHA and BHT. So these extracts can be used as natural stabilizers, like synthetic one, for minimizing the use of synthetic chemicals for food storage.*

Keywords*: Diospyros malabarica, Extract, Antioxidants, Oil stabilization, Food storage*

1. Introduction

An antioxidant is a substance that has considerable potential to prevent or suspend the oxidation of an oxidizable substrate when it is added or applied to that substrate even in a minor quantity [1]. The oxidation of fatty acids in fats and oil causes development of free radicals which impart unpleasant tasting and smell which changes the food product as unhealthy and undesirable for costumers. The composition of foods changes during handling and fatty acids become more vulnerable to attack of oxygen. Moreover when fatty foods or oils are processed, sometimes naturally occurring antioxidants in them are also damaged which make them more liable to oxidation [2]. Over the years some synthetic antioxidants, like: BHA, BHT and propylgallate are applied in food processing to preserve its freshness and check its decay by oxidation. They are often used in mueslis, frying oils, preserved foods, and animal fodder [3]. However in recent years a major health concern has arisen because of harmful effects of these synthetic antioxidants as they are reported to be carcinogenic. For instance it has been reported that BHA is capable to boost urothelial cancer at elevated dosage levels (2–3 % in take) and epithelial tumor at minor doses (1–0.75 % in take) at earlier stages in rats [4], while in research on humans, BHA is found to be involved in skin hives, rapid edema and respiratory disorder [5]. Similarly, irrespective of its usefulness BHT employs a negative influence on the lungs, kidneys, cardiac cells, digestion of fats, liver damage and coagulation of blood cell. It is also capable of producing physical or functional defects in the human embryo [6].

Keeping in view all these health concerns, customers mostly recognize natural antioxidants as better preservatives than synthetic ones. Phenols represent most impactful class of natural antioxidants. They are extracted from material of plant origin mostly. They are found to shield easily oxidizable components of food from oxidation. Most important are herbs and spices [7]. Some medicinal plants of Algerian origin, Chinese origin and Iranian origin are also found to possess wonderful antioxidant activities [8-10].

**email: grinorganic@yahoo.com, ktm7ro@yahoo.com;*

Diospyros malabarica is a medicinal plant which belongs to family Ebenaceae of plants. It is found in tropical regions of Pakistan, India, Thailand, Japan, Nigeria, South Africa and Philippines. It grows slowly; the tree easily spreads up to 32-36 m in height while it has a black shaft of 70 cm thickness. It has green elongated leaves. Its fruits are covered with a brownish dust which falls off at maturity (Kinho). It is used to cure ailments like cancer, rheumatism, liver sickness and atherosclerosis. It also controls free radicals and slows down ageing [11], it is enriched with phenols and ascorbic acid [12].

The purpose of this work was to scrutinize antioxidant potential of fruits and leaves of *D. malabarica* tree and their application as a preservative in raw mustard oil to maintain its oxidative stability.

2. Materials and methods

Raw, decolorized and fragrance-free mustard oil was bought from a local oil refinery of Jhang, Punjab Pakistan. Fresh fruits and leaves of *D. malabarica* plant were gathered from botanical garden of Punjab university, Lahore, Pakistan.

2.1 Chemicals

BHA and BHT were used as synthetic antioxidants. They were purchased from Fluka chemicals. Other chemicals used to evaluate antioxidant activity of *D. malabarica* samples were TPTZ, DPPH, ABTS and Folin–Ciocalteu. All these reagents were of analytical grade, purchased from Merck and used as such without additional refining.

2.2 Preparation of crude extract

Cleaning of the fruits and leaves of *D.malabarica* was done cautiously with distilled water. The fruits were unpeeled and sliced into tiny pieces and seeds were removed. The fruits pulp and leaves were left to dehydrate at ambient temperature for 15-20 days. These completely dried out fruits pulps and leaves were now pulverized into fine powder. 5 g of powdered fruits pulp and leaves were soaked in 150 mL methanol separately and agitated mechanically on an orbital shaker at ambient temperature. Methanol was used on hit and trial basis and stood out as one of the finest solvent to extract antioxidants [13]. Once shaking was done after 46-48 h, the sample solutions were filtered and filtrate was left to evaporate methanol at normal temperature. When methanol got vaporized totally, it left behind a solid extract as fine powder [14]. The yields of the extracts were recorded and extract was put in storage in for additional valuation.

2.3 Evaluation of antioxidant potential of D. malabarica plant

Antioxidant potential of *D. malabarica* fruits (DFE) and leaves extracts (DLE) was evaluated by using following assays;

(i) Total Phenolic Contents (TPC) estimation

A previously reported method was used to assess TPC values in which Folin–Ciocalteu (FC) reagent was used. 0.2 mL dilute extract of *D. malabarica* fruits (DFE) and leaves (DLE) were taken separately and respective standards of Gallic acid were taken and 0.2 mL of fresh FC reagent (10 % v/v) was added to each. Then 2 mL of 7.5 % Na_2CO_3 was added to each sample and 7 mL of dd H_2O was added later. The samples were later placed in the darkness for finishing of reaction. The value absorbance of every sample was detected using Vis-Spectrophotometer 712. Gallic acid standards were used to draw calibration curve and to determine total phenolic content of DLE and DFE as mg/100 g of dried weight [14].

(ii) Total flavonoids content (TFC)estimation

Kim's method was used for measuring total flavonoids.1 mL of DFE, DLE and 1 mL of different standards of catechin were taken in 10 mL flasks and diluted with 4 mL of distilled H_2O. Straightaway, 0.3 mL of 5% $NaNO_2$ was poured to each sample. 0.3 mL of 10% $AlCl_3$ was added to them after 5 min.

After 1 min, 2 mL of 1 M NaOH was added in each sample. Promptly, 2.4 mL of distilled water was added to these samples. Then samples were stirred and values of absorbance were noted at 510 nm. Standardization curve was drawn with catechin. The results were stated as catechin equivalents (CE) as mg/100 g of dried wt. [15].

(iii) Total anthocyanin contents (TAC) estimation

De Silva's method [16] was used for concluding TAC of fruit and leaves extracts; 95 mL methanol was added to 5 g of extract samples. The blends were subjected to orbital shaking for 1/2 h. After filtration, the filtrate was transferred into a 100 mL flask. It was diluted up to mark with methanol; 0.1 % v/v HCl sol. (in methanol) was used to make 50 times dilution of given samples. Values of absorbance was taken at 528 nm; 0.1 % v/v HCl was counted as blank. Quantification of anthocyanin content (QAC) was described in terms of cyanidine-3-glucoside chloride (abs. at 718 nm). Final calculations were made using following equation-(1):

$$\boldsymbol{QAC} = \frac{\boldsymbol{A(sample)\times 50{,}000}}{\boldsymbol{718 \times dwt}} \quad (1)$$

dwt = weight of the dry leaves and fruits

(iv) Ferric reducing antioxidant power (FRAP) assay

Benzie and Strain's method was followed for measuring reducing power of antioxidants extracted from *Diospyros malabarica* fruit and leaves samples. For this, 0.01 mL dilute extract samples were mixed with 0.3 mL of recently prepared FRAP mixture. Samples were further diluted with 0.03 mL demineralized H_2O and values of absorbance were taken at 593 nm. Standards (0.2-1 mmol/L) of $FeSO_4.7H_2O$ were also tested and their absorbance was noted. Antioxidant potential was calculated by using this standard curve. Final result was stated as conc. of *D. malabarica* extracts possessing reducing power (equivalent to mmol/L of $FeSO_4.7H_2O$) [17].

(v) 2,2-diphenyl-1-picryl-hydrazyl (DPPH) protocol

Brand-William's method was used to carry out this protocol [18]. (Brand-Williams, Cuvelier, & Berset, 1995)60 μmol/L standard solution of DPPH was set up; 0.1 mL of different concentration of fruit and leaves extract (DFE and DLE) along with Butylated hydroxy anisole (BHA) and Butylated hydroxytoluene (BHT) were prepared in separate test tubes; 3.9 mL of DPPH was poured into these samples. All samples were covered and left for 1/2 h. After wards values of absorbance were recorded at 517 nm. Absorbance of control (simply DPPH) was measured too. Lastly observations were specified by % inhibition by eq.-(2):

$$\%\boldsymbol{Inh} = \frac{\boldsymbol{Ao-A1}}{\boldsymbol{Ao}} \times \mathbf{100} \quad (2)$$

where A_o is the absorbance of the control & A_1 is the absorbance of test samples.

(vi) ABTS protocol

Shalaby's method was used for performing this assay [19]; 0.007 M ABTS and 0.245 M $K_2S_2O_8$ solutions were made; 0.02 L from each sample was taken into a 0.1 L flask (in equal ration) and placed into dark for 18 h. Both reagents reacted to form $ABTS^+$ ions. At the end of incubation of 18 h the flask was taken out and soln. was made up to 0.1 L with methanol; 100 μL of concentrations (0.2, 0.4, 0.6 mg/mL) of DFE & DLE along with BHA and BHT were taken and 900 μL of $ABTS^{\cdot+}$ was mixed into each. Lastly values of absorbance were recorded at 734 nm. It was also recorded for control (simply $ABTS^{\cdot+}$). Final results were made by eq. (3):

$$\boldsymbol{Antioxidantactivity}\ (\%) = \frac{\boldsymbol{Ablank-Asample}}{\boldsymbol{Ablank}} \times \mathbf{100} \quad (3)$$

where Ablank is the absorbance of the ABTS.

(vii) β–carotene bleaching assay

This test was performed by following method in literature [20]. 2 mg β–carotene was dissolved into 10 mL of $CHCl_3$. After mixing, 2 mL of the soln. was mixed into 0.04 mL of linoleic acid and 0.4 mL of Tween 20; $CHCl_3$ was evaporated into a rotatory evaporator and 100 mL of dd H_2O was poured to above sample. This emulsion was mixed vigorously by an orbital shaker. Then 5 mL of this mixture was added to 0.2 mL of 1mL/L soln. of BHA, BHT, DFE and DLE. The absorbance of all solutions was noted at 470 nm after regular intervals of 20 min.

Stabilization studies

(i) Sample preparation

Methanolic extracts of *Diospyros malabarica* fruit and leaves samples (0.25, 0.5 and 1mL/L conc.) were mixed into crude mustard oil. Similar oil samples were prepared with artificial antioxidants, BHT and BHA (0.2 mL/L) for relative analysis [21]. An equivalent control sample of RBD mustard oil (sans any antioxidant) was also set up. All the samples were stored at room temperature for 39 days. Stabilization of all oil samples (with or without antioxidants) was checked after regular intervals of every 12 days till period of 39 days was complete.

(ii) Measurement of free fatty acid value (FFA), peroxide value (PV) and iodine value (IV)

Antioxidant power of *Diospyros malabarica* fruit and leaves samples for stabilizing of RBD mustard oil was assessed by testing free fatty acid, peroxide and iodine values. Every oil sample was analyzed for antioxidant action at constant intervals of 13 days up to 39 days by using the AOAC certified procedures [22-24]. All determinations were performed in triplicate sets and results were stated as mean ± standard deviation. Significant differences ($P < 0.05$) were verified using one way ANOVA.

3.Results and discussions

Measurement of antioxidant activity

Total phenolic and total flavonoid contents of methanolic extract of *Diospyros malabarica* fruit sample were 43.4 ± 0.01 and 64.7 ± 0.01 mg equivalent of gallic acid / 100g dry wright *Diospyros malabarica*, correspondingly. Similarly TPC and TFC values for DLE were 37.1 ± 0.01 and 77.6 ± 0.01 mg equivalent of gallic acid / 100g dry wright *Diospyros malabarica*, respectively. The existence of these phenolic and flavonoids in DFE and DLE may impart antioxidant power to them. It is stated that antioxidant potential of phenolic compounds is primarily owing to reducing power, H-donation ability, quenching singlet oxygen and metallic chelation [25, 26]. The amount of total anthocyanin contents (TAC) in DFE and DLE was 2.089 ± 0.01 μg/mL and 0.6329 ± 0.01 μg/mL, respectively. The studies have shown that the anthocyanidins own antioxidant activities as high as flavones [27]. In *FRAP assay*, the reducing power of methanolic extract of DFE and DLE was 0.447 ± 0.01 mmole /L and of DLE is 0.436 ± 0.01 mmole /L of $FeSO_4$, respectively which specifies the existence of antioxidant power in these extracts [28].

DPPH assay is centered on the principle that when DPPH accepts a hydrogen (H) atom from antioxidant, it is reduced to $DPPH_2$ [29]. As a result the purple colour of solution is changed to yellow with simultaneous decrease in absorbance at 517 nm and resultant increase in % inhibition. This change measured by noting absorbance gives the antioxidant potency of sample under analysis [30]. Result of our study revealed that radical scavenging action of *Diospyros malabarica* fruit samples extract (DFE) increased progressively and exceeded that of BHT. At 0.2 mg/mL, % inhibition value of DFE was 37.54 ± 0.72 %, which is lower than both BHA and BHT. However, at 0.4 mg/mL it raised above value of

BHT, i.e 56.22 ± 0.59 % and 1 mg/mL of BHA and DFE were almost equally strong (81.14 ± 0.88 % and 80.81 ± 0.63 %, respectively) to scavenge free radical. However, % inhibition values of DLE were lower than BHA, BHT and DFE. Still at higher concentration (1mL/L), DLE show considerable inhibition and was almost comparable to BHT (Table 1, Figure 1).

General results proposed subsequent trend of radical scavenging power of given samples;

BHA > DFE > BHT > DLE.

Table 1. DPPH assay results as % inhibition for DFE, DLE, BHA and BHT

Conc. mg/mL	BHA	BHT	DFE	DLE
0.2	59.18 ± 0.66	52.62 ± 0.99	37.54 ± 0.72	23.27 ± 0.59
0.4	61.31 ± 0.84	55.90 ± 0.72	56.22 ± 0.59	33.60 ± 0.62
0.6	72.29 ± 0.85	58.36 ± 0.76	68.52 ± 0.86	48.03 ± 0.99
0.8	79.34 ± 0.83	67.54 ± 0.74	75.90 ± 0.78	55.40 ± 0.72
1	81.14 ± 0.88	72.78 ± 1.09	80.81 ± 0.63	60.32 ± 0.84

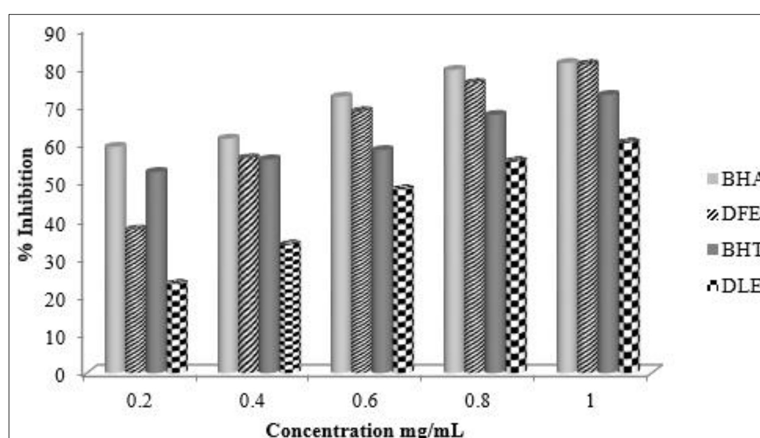

Figure 1. Comparison of % inhibition of extract samples with standards BHA and BHT

ABTS assay demonstrates free radical scavenging action of DFE & DLE through fading of its indigo shade. Literature discloses, phenols react quickly with ABTS radical ions therefore greater concentrations of phenolic compounds in extract would enhance radical scavenging action of plant [31]. Antioxidant action of DFE & DLE was tested by this procedure and matched with artificial antioxidants (BHA and BHT) at varying conc. such as 0.2 mg/mL, 0.4 mg/mL and 0.6 mg/mL (Figure 2). It is clear from data in Table 2 that at 0.2 mg/mL antioxidant activity of DFE (i.e. 13.3mg/mL ± 0.420) was lower than BHT (20 mg/mL± 0.55) but equal to BHA (i.e. 13.3 ± 0.20). Increasing concentration of DFE (0.6 mg/mL) increased its radical scavenging action (23.3 ± 0.65) which is comparable to BHT (47 ± 0.5), though it is lower than BHA (60.7 ± 0.40). In case of DLE, values (10 ± 0.20) were lower than BHA, BHT and DFE. Only at 0.6 mg/mL values of DFE and DLE became equal.

Overall trend was: **BHA > BHT > DFE > DLE**.

Table 2. ABTS assay results for DFE, DLE, BHA and BHT

Conc. mg/mL	ABTS activity (%)			
	BHA	BHT	DFE	DLE
0.2	13.3 ± 0.20	20 ± 0.55	13.3 ± 0.42	10 ± 0.20
0.4	26.6 ± 0.87	29 ± 0.47	20 ± 0.50	16.6 ± 0.42
0.6	60.7 ± 0.40	47 ± 0.5	23.3 ± 0.65	23.3 ± 0.5

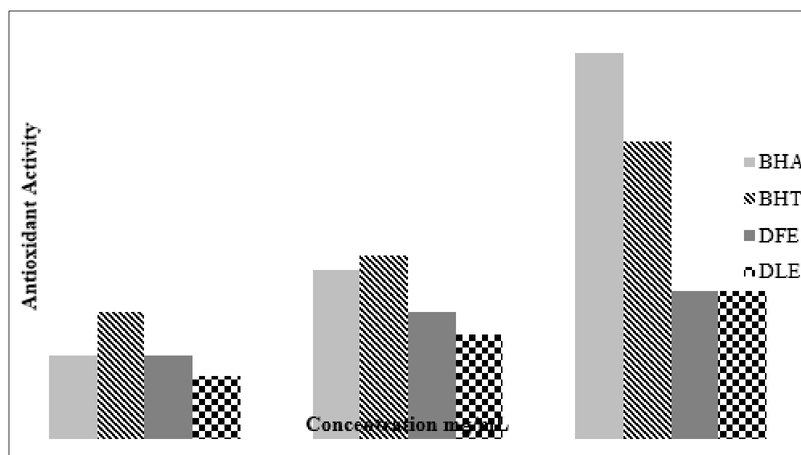

Figure 2. Comparison of antioxidant activity of extracts with standards

β-Carotene bleaching assay is another valuable mode for assessing the antioxidant efficacy [32]. The reduction in rate of decoloration of β-carotene indicates increasing antioxidant activity. Figure 3 illustrates the graph plotted between absorbance of DFE & DLE at 470 nm and time taken via each one to bleach out β-carotene. The data in Table 3 shows that the control sample had highest value of absorbance from 0 to 60 min (i.e. 0.097 ± 0.001- 0.093 ± 0.001). Values of BHA and DFE were close to each other from start (i.e. 0.050 ± 0.001 and 0.048 ± 0.001, respectively) to end of reaction (i.e. 0.025 ± 0.001 and 0.031 ± 0.001, respectively). Best absorbance values were given by BHT from start to end (i.e. 0.024 ± 0.001 - 0.018 ± 0.001). The results suggested that as time increases, concentration of antioxidants in DFE is increased which is clear from drop in rate of fading in color of β-carotene. Eventually absorbance values fall from 0.048 ± 0.001 to 0.031 ± 0.001. Likewise a gradual fall was observed in values of synthetic antioxidants BHA (0.050 ± 0.001 to 0.025 ± 0.001) and BHT (0.024 ± 0.001 to 0.018 ± 0.001). The observed fall in values of DLE was quite high (0.068 ± 0.001 to 0.051 ± 0.001) as compared to BHA, BHT and DFE still these samples were way stable as compared to control. Control sample (i.e. simply linoleic acid) had a slight decrease in values from 0.097 ± 0.001 to 0.093 ± 0.001. Consequently, β-carotene assay verified that the DFE & DLE have sufficient competency to be used as natural antioxidant.

Table 3. *β*-carotene bleaching test results

Tester	**Period (min)**			
	0	**20**	**40**	**60**
Control	0.097 ± 0.001	0.096 ± 0.001	0.094 ± 0.001	0.093 ± 0.001
BHA	0.050 ± 0.001	0.038 ± 0.001	0.027 ± 0.001	0.025 ± 0.001
BHT	0.024 ± 0.001	0.022 ± 0.001	0.019 ± 0.001	0.018 ± 0.001
DFE	0.048 ± 0.001	0.045 ± 0.001	0.039 ± 0.001	0.031 ± 0.001
DLE	0.068 ± 0.001	0.066 ± 0.001	0.056 ± 0.001	0.051 ± 0.001

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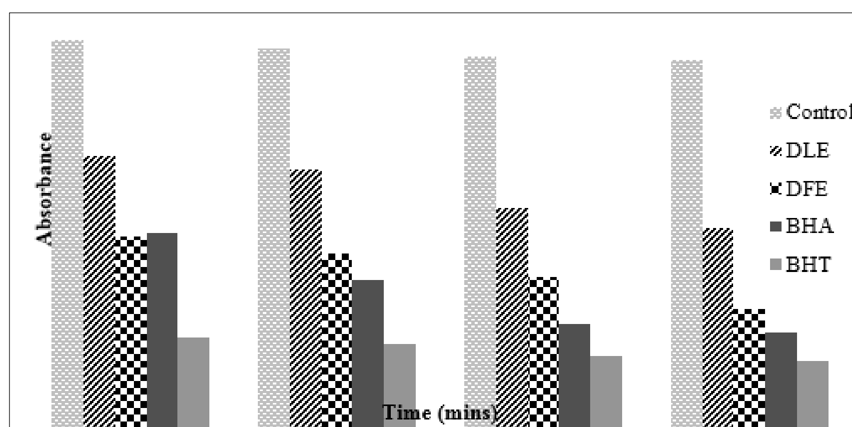

Figure 3. Comparison of β-carotene test of extracts

Stabilization of mustard oil using DFE and DLE

RBD mustard oil was used as oxidative substrates. Mustard oil was stabilized by antioxidants extracted from leaves and fruit of *D. malabarica*. A contrast study was carried out with synthetic antioxidants (BHA and BHT).

(i) Peroxide value (PV)

PV is estimation of preliminary oxidation of lipids and oils that arises owing to the production of peroxides [33]. Figure 4 displays the continuous rise in PV throughout storing period (at ambient temp.) of mustard oil samples under study. On 39th day, peroxide value of all samples reached to extreme. Originally, PV of control mustard oil sample was 1.2 ± 0.14 meqO_2/kg which reached to 11.5 ± 0.15 meqO_2/kg on 39th day. Control sample presented highest rate of oil deterioration within storage period because it was deprived of antioxidants. DFE (1mL/L) had minimum PV value in its mustard oil sample. Originally, PV of this sample was 1.2 ± 0.14 meqO_2/kg which elevated up to 7.4 ± 0.12 meqO_2/kg on 39th day. This is a proof of antioxidant occurrence in DFE which inhibit oil from deterioration. PV values of DLE were 2nd highest after control, the rise was from 1.2 ± 0.14 meqO_2/kg to 11 ± 0.12 meqO_2/kg for DLE (1mL/L), however it was comparable to that of BHA. Results of this study are constant with the verdicts of other workers who testified that lipid peroxides can be considerably avoided by the adding natural antioxidants as preservatives in raw oils [34, 35]. PV for other samples of DFE & DLE (0.25 mL/L and 0.5 mL/L) and BHA, BHT (0.2 mL/L) were observed which are denoted in graph in (Table 4). This data refers to following trend for mustard oil sample:

BHT (0.2 mL/L) > DFE (1 mL/L) > DFE (0.5 mL/L) > BHA (0.2 mL/L) ≈ DLE (1 mL/L) >
DFE (0.25 mL/L) > DLE (0.5 mL/L) > DLE (0.25 mL/L) > Control oil.

Analogous studies of DFE at 1 mL/L with artificial antioxidants BHA and BHT were conducted in mustard oil.

Table 4. PV's of DFE & DLE VS BHA, BHT & control oil

Days	Control oil	BHA (0.2mL/L)	BHT (0.2mL/L)	DFE (0.25mL/L)	DFE (0.5mL/L)	DFE (1mL/L)	DLE (0.25mL/L)	DLE (0.5mL/L)	DLE (1mL/L)
0	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14	1.2±0.14
13	4.2±0.1	2.6±0.12	2.4±0.13	2.8±0.1	2.4±0.12	2.2±0.1	2.4±0.12	2.3±0.11	2.2±0.14
26	7.1±0.12	5.7±0.1	5.5±0.14	5.6±0.12	4.8±0.1	3.6±0.12	6.8±0.14	6.4±0.14	4.8±0.1
39	14.3±0.14	11±0.12	6.9±0.15	11.5±0.15	9.0±0.1	7.4±0.12	12.4±0.16	11.6±0.15	11±0.12

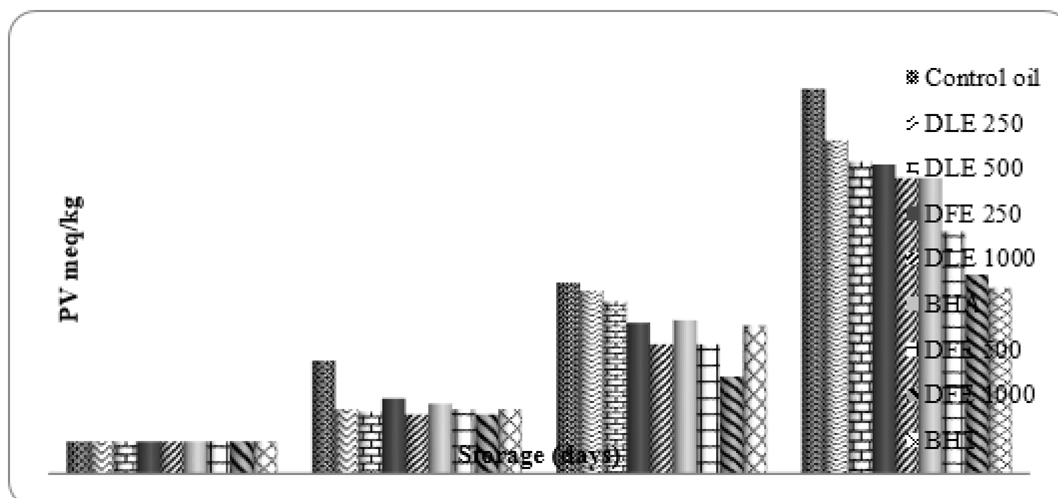

Figure 4. Comparison of peroxide values of natural (DFE and DLE) and synthetic stabilizers (BHA and BHT)

(ii) Free fatty acid value (FFA)

Interaction of fats and oils with moisture leads to hydrolysis of triglycerides and release of free fatty acids [36]. Figure 5 demonstrates that FFA value of mustard oil observes a steady increase on storage at room temperature. Control undergoes maximum oxidation and it has greatest FFA value. FFA of control mustard oil sample from zero to 39th day was 0.124 ± 0.001 to 1.41 ± 0.001 %. DFE (1 mL/L) in mustard oil presented FFA value from 0.124 ± 0.001 to 0.705 ± 0.001 %. DLE (1 mL/L) in mustard oil presented FFA value from 0.124 ± 0.001 to 0.787 ± 0.001 %. A related pattern of increase in FFA value of oil over storage by decay products of hydroperoxides was reported by Miyashita [37].

The FFA values for conc. of DFE, DLE (0.25 mL/L and 0.5 mL/L) and for BHA, BHT (0.2 mL/L) were tested and presented in graph in Table 5. The results are constant with previous studies which proved decrease in FFA value of oil by adding natural antioxidants [38]. The comparison depicted following trend in mustard oil samples:

BHA (0.2 mL/L) ≈ BHT (0.2 mL/L) ≈ DFE (1 mL/L) > DLE (1 mL/L) > DFE (0.5 mL/L) > DLE (0.5 mL/L) > DFE (0.25 mL/L) > DLE (0.25 mL/L) > Control oil sample.

Table 5. FFA values of DFE, DLE and CONTROL BHA, BHT

Days	Control oil	BHT (0.2mL/L)	BHA (0.2mL/L)	DFE (0.25mL/L)	DFE (0.5mL/L)	DFE (1mL/L)	DLE (0.25mL/L)	DLE (0.5mL/L)	DLE (1mL/L)
0	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001	0.124± 0.001
13	0.864± 0.001	0.427± 0.001	0.425± 0.001	0.455± 0.001	0.423± 0.001	0.412± 0.001	0.705± 0.001	0.423± 0.001	0.422± 0.001
26	1.269± 0.001	0.560± 0.001	0.558± 0.001	0.705± 0.001	0.564± 0.001	0.535± 0.001	0.987± 0.001	0.569± 0.001	0.557± 0.001
39	1.41± 0.001	0.705± 0.001	0.705± 0.001	1.122± 0.001	0.846± 0.001	0.705± 0.001	1.32± 0.001	0.885± 0.001	0.787± 0.001

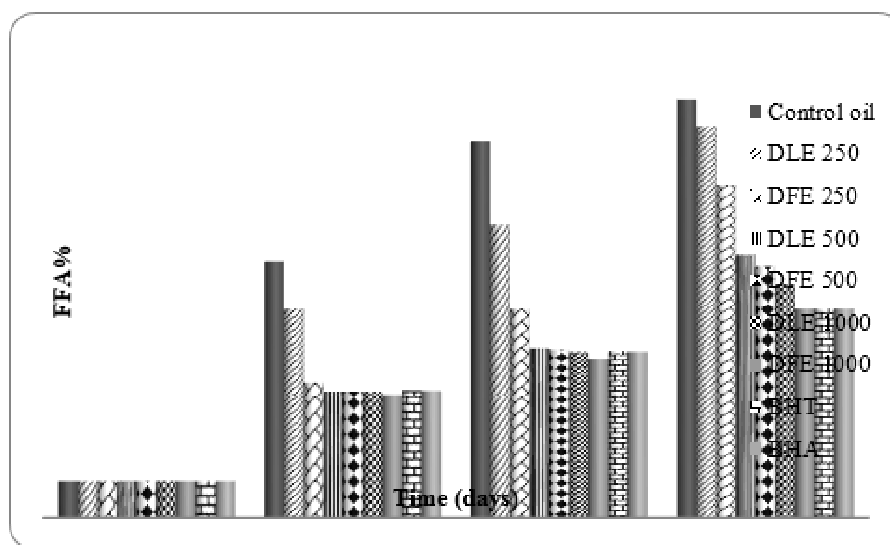

Figure 5. Comparison of FFA values of natural (DFE and DLE) and synthetic stabilizers (BHA and BHT)

(iii) Iodine value (IV)

Iodine value is basically estimation of rate of unsaturation of given oil sample. A larger iodine value marks better quality of oil [39]. Figure 6 shows a gradual fall of iodine value in mustard oil with increasing storage period. Mustard oil sample containing DFE (1mL/L) observed an IV (g I_2/100g of oil) of 158.71 ± 1.05 g I_2/100g to 78.5 ± 1.32 g I_2/100g which was higher than BHA (158.71 ± 1.05 gI_2/100g to 73.43 ±1.20 g I_2/100g) and BHT (158.71 ± 1.05 g I_2/100g to 39.75 ± 1.20 g I_2/100g), while oil samples containing DLE (1ml/L) showed an IV value of 158.71 ± 1.05 g I_2/100g falling to 64.67 ± 1.50 g I_2/100g which was higher than BHA. Expectedly, control presented the lower most value i.e 25.02 ± 1.20 gI_2/100g (Table 6).

The overall trend of IV of different samples of mustard oil and extract was following;

DFE (1mL/L) > BHA (0.2mL/L) > DLE (1mL/L) > DFE (0.5mL/L) > DLE (0.5mL/L) > DFE (0.25mL/L) > DLE (0.25mL/L) > BHT (0.2mL/L) > Control oil.

Thus, Iodine value of all stabilized samples is greater than control oil which signifies aproficient antioxidant potential of DFE under analysis as it has been previously proved that addition of natural antioxidants can maintain iodine values of raw oil [40, 41].

Table 6. IV's OF DFE, DLE, BHA, BHT & control oil sample

Days	Control oil	BHA (0.2mL/L)	BHT (0.2mL/L)	DFE (0.25mL/L)	DFE (0.5mL/L)	DFE (1mL/L)	DLE (0.25mL/L)	DLE (0.5mL/L)	DLE (1mL/L)
0	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05	158.71± 1.05
13	76.45± 0.26	127.55± 1.28	85.01± 0.03	90.25± 0.07	112.54± 0.87	131.96± 1.03	86.98± 0.27	109.98± 0.87	119.96± 1.28
26	53.87± 0.45	95.88± 0.42	64.58± 0.45	70.18± 0.46	80.66± 0.27	100.25± 0.40	66.38± 0.45	74.96± 0.26	86.54± 0.07
39	25.02± 1.53	73.43± 1.20	39.75± 0.20	46.00± 1.21	60.25± 1.40	78.50± 1.32	40± 0.20	59.76± 0.45	64.67± 1.40

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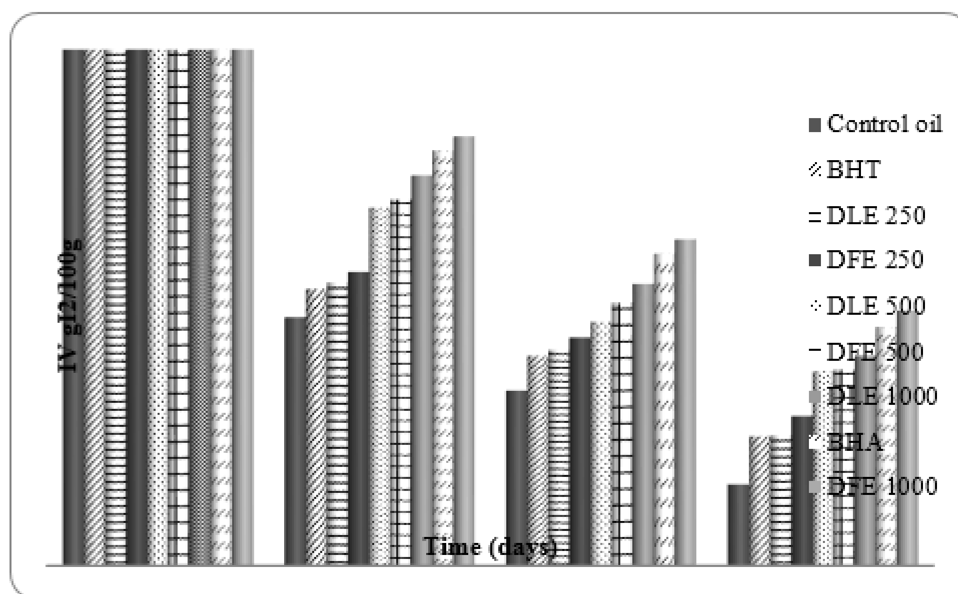

Figure 6. Comparison of IV's of natural (DFE and DLE) and synthetic stabilizers (BHA and BHT)

4.Conclusions

The study proved that stabilization of mustard oil as well as any other oil can be carried out successfully by adding DFE and DLE as antioxidant additives. Results also suggested that at 1 mL/L concentrations DFE had better stabilizing potential than BHA and almost equal to that of BHT and DLE were almost equally strong stabilizers. They effectively constrained oxidative deterioration of mustard oil and hence can be substituted as a natural food additive especially for unsaturated vegetable oils. The phenolic compounds seem to be accountable for the antioxidant potential of DFE and DLE, though further studies are essential to disclose whether they contain other antioxidative constituents. Moreover, in vivo studies and isolation of antioxidant components in DFE and DLE would further merit this study. Its use as a natural food additive would be immensely advantageous as it is cost effective and not hazardous.

List of abbreviations:

Diospyros malabarica fruits pulp extract: DFE
Diospyros malabarica leaves extract: DLE
Total phenolics content: TPC
Total flavonoids content: TFC
Total anthocyanin contents: TAC
2,2-diphenyl-1-picryl-hydrazyl: DPPH
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid: ABTS
Iodine Value: IV
Peroxide value: PV
Free fatty acid value: FFA
Ferric reducing antioxidant power: FRAP
Butylated hydroxyanisole: BHA
Butylated hydroxytoluene: BHT

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