Antioxidant Potential and Stabilization Studies of Sunflower Oil Using *Sorbaria tomentosa* Extract and its Cu(II)/Zn(II) Chelates

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Sorbaria tomentosa,, commonly known as "Berre" is native to Himalaya and Hindukush range in Pakistan. Qualitative phytochemical screening as well as quantitative antioxidant potential of its ethanolic extract was evaluated. Antioxidant potential of the extract was determined using standard methods like DPPH, FRAP, total phenolics, total flavonoids, ABTS radical cation scavenging assay and β-carotene linoleic acid emulsion system. Cu(II) and Zn(II) chelates of ,,Sorbaria tomentosa,, were also prepared and their antioxidant potential was compared with the extract as well as with synthetic antioxidants (BHT and BHA). It was observed that ,,Sorbaria tomentosa,, is a good source of natural antioxidant that worked efficiently compared to respective chelates. Keeping in view less efficiency of chelated extracts, stabilization studies of sunflower oil were conducted with ethanolic extract (250, 500, 1000 ppm) of ,,Sorbaria tomentosa,,. Various parameters like PV, FFA and IV were estimated to evaluate stabilization of oil. ,,Sorbaria tomentosa,, extract (1000 ppm) showed almost same role during stabilization like BHA at ambient condition during a storage period of 45 days.

Keywords: Sorbaria tomentosa, phytochemicals, antioxidant potential, chelation, stabilization

The quality and marketing of any food product depends upon customer choice, flavor, appearance aspect and fragrance. A large number of edible food contains unsaturated fatty acids that lead to the deterioration of food. To overcome such deterioration, several antioxidants have been utilized in past [1]. These antioxidants can be present naturally in food products or can be added during food processing. It's worth mentioning that large number of endogenous antioxidants may be lost during processing of food, and hence the addition of exogenous antioxidants is required [2]. Natural antioxidants are oftenly available from plants, tissues of animals, microorganisms and can be extracted as pure or raw substances [3]. These are also used as scavengers of reactive oxygen species (ROS) and may regulate the body antioxidant defense system [4]. Edible oils contain largeamount of unsaturated fatty acids which are easily oxidized causing oxidative deterioration and hence decrease in the nutritional contents of oils [5]. To cope up this problem of oil, several synthetic antioxidants such as BHT, BHA and TBHQ are added to oils. However, several reports revealed that these substances cause health problems [6]. Scientists are trying to replace these synthetic antioxidants with natural antioxidants (essential oils and plant extracts), as they play an important role in the prevention of oxidization of fatty acids [7]. *Sorbaria tomentosa* (S.T.) is native to Pakistan (Himalaya range, Kagan, Hindukush range of Swat), Tajikistan, Nepal and Afghanistan and is normally used for ornamental purpose. Ethnomedical studies reveal that its flowers paste with milk is used to cure burns, wounds, for asthma and rashes, etc [8, 9]. The accessible approach through literature reveals that the antioxidant potential of other species of *Sorbaria* named *Sorbaria sorbifolia* has been reported [10]. Similarly, anti-tumor potato disc assay, cytotoxicity and radish seed phytoxicity activity of methanolic extract of *Sorbaria tomentosa* has also been reported [8]. However, it is surprisingly noted that no work has been reported about the antioxidant potential of *Sorbaria tomentosa* and its efficacy to stabilize edible oils. Therefore, the present work is mainly aimed to report the antioxidant potential of *Sorbaria tomentosa* and its Cu(II) and Zn(II) chelates. Stabilization of sunflower oil using its extract is also part of this manuscript.

Experimental part

Collection and drying of plant material

Sorbaria tomentosa was collected from Kalam, Khaber Pakhtunkhwa-Pakistan and was dried at ambient conditions in shade for the removal of moisture contents and stored in polythene bags.

Extraction of plant material

The powdered material of whole plant (2.0 Kg) was weighed and subsequently soaked in ethanol (95 %) for a week. After one week, solvent was evaporated using rotary evaporator at 50°C under reduced pressure. Dark brown colored extract (198 g) was recovered and stored in black glass vials for further experimental purpose.

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Preliminary Phytochemical Screening of Plant Extract

Phytochemical screening of plant extract was performed by different qualitative tests such as alkaloids determination by Hager's test [11]. cardiac glycosides by keller killiani, salkowiski, and baljet test [12], steroids by sulphuric acid test [13], flavonoids by alkaline reagent and lead acetate test [12], terpenoids by salkowiski and sulphuric acid test [13, 12], proteins by ninhydrin, biuret and xanthoproteic test [14, 13] carbohydrates by Fehling solution and Molisch test [13, 12] saponins by foam and froth test [15], phenols by ferric chloride test [13] and tannins by lead acetate and ferric chloride test [13, 16].

Antioxidant Potential of Pure Extract of „Sorbaria Tomentosa" and its Chelates

Evaluation of Total Phenolic Contents (TPC)

TPC of *Sorbaria tomentosa* extract was determined by the reported method of [17]. Absorbance of three sample solutions [extract, Cu(II) chelate, Zn(II) chelate] was measured on Spectrophotometer at 765 nm by using gallic acid solution as standard. The results were expressed as gallic acid equivalents (GAE) (g/100 mL) of *Sorbaria tomentosa* extract. All samples analyses were done in three replications.

Measurement of Total Flavonoid Contents

Total flavonoids in plant extract were determined by colorimetric method according to protocol reported by [18]. Absorbance of three sample solutions [extract, Cu(II) chelate, Zn(II) chelate] were determined at 510 nm. Results were expressed as total flavonoids of *Sorbaria tomentosa* extract in milligram of catechin/100 g of dry content weight. All the samples were analyzed in three replications.

DPPH Assay

The DPPH radical scavenging effect of *Sorbaria tomentosa* extract was determined by a reported assay [19]. Measurement of absorbance was carried out at 515 nm against ethanol (blank sample). Results were expressed by following formula:

$$(\%) \text{ of Radical inhibiting effect} = [A_{\{control\}} - A_{\{sample\}}]/A_{\{control\}} \times 100$$

$A_{\{control\}}$ = Control solution absorbance comprising of DPPH solution and ethanol

$A_{\{sample\}}$ = Sample solution absorbance

β-Carotene-Linoleic Acid Emulsion System

The antioxidant effect of *Sorbaria tomentosa* aliquot was determined in an aqueous emulsion of *β*-carotene and linoleic acid by method reported by [20]. Absorbance was measured for each sample instantly at 470 nm and then after 15, 30, 45 and 60 minutes at 50°C. For each extract duplicate analysis was done.

Ferric Reducing Antioxidant Power Assay

FRAP method was performed according to the described assay of [21]. The reaction mixture contained 10 µL of *Sorbaria tomentosa* extract/chelates, distilled H_2O (30 µL) and freshly prepared FRAP reagent (300 µL). Measurement of absorbance was done against each solution at about 593 nm. Results were expressed in terms of concentration of $FeSO_4.7H_2O$.

ABTS Radical Cation Inhibition Activity

This assay was performed by mixing 0.1 mL of plant extract/metal chelate/BHA/BHT and 0.9 mL of ABTS radical cation solution. The resulting mixture was kept for few seconds and then placed in dark for five minutes. Absorbance was determined at 734 nm against

water : ethanol (50:50) as blank [22]. Results were calculated by the formula given below:

$$(\%) \text{ of ABTS radical cation inhibition effect} = \{A_{(control)} - A_{(sample)}\}/A_{(control)} \times 100$$

$A_{(control)}$ = ABTS radical cation solution and water (control solution) absorbance

$A_{(sample)}$ = ABTS radical cation solution and test extract solution absorbance

Preparation of Metal Chelates of Plant Extract

For metal chelation of extract, the extract solution was prepared in ethanol (20 mL). Metal salt ($ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$) solution were prepared in water (20 mL). Then both the solutions were mixed in round bottom flask and stirred for about 3 to 6 hours at room temperature. Precipitates were formed, which were allowed to settle down over night and subsequently separated by centrifugation. Finally, the resulting precipitates were stored in dried vials for determination of their antioxidant potential [23]. FT-IR studies was conducted to check the formation of metal chelates.

Stabilization of Sunflower Oil

Samples Preparation

Refined bleached deodorized (RBD) sunflower oil (SFO) was obtained from a local refinery in Lahore. Ratio of sample to oil was kept as 1:1. SFO was taken and 250, 500 and 1000 ppm of *Sorbaria tomentosa* ethanolic extract was added in it. Similarly, BHA and BHT (200 ppm) were also added separately in SFO for comparison. Control was prepared by adding only SFO without extract [24]. During the storage period of 45 days all samples were analyzed and should be kept air tight.

Estimation of Peroxide Value (PV), Free Fatty Acid Value (FFA) and Iodine Value (IV)

Peroxide value, free fatty acid value and iodine value of sunflower oil was investigated by using *Sorbaria tomentosa* ethanolic extract, synthetic antioxidants (BHA and BHT) at regular intervals of 15 days over storage period of 45 days by following the reported AOAC method [25]. Meanwhile, all parameters were also measured for control sample.

Statistical Analysis

All analysis were carried out in three replicates and results were expessed as mean ± standard deviations. Significant difference among all readings ($p < 0.05$) was calculated by applying one way analysis of variance (ANOVA) on statistical package for social sciences (SPSS).

Results and discussion

Percentage yield of the plant extraction in ethanol was determined and it was 9.9 ± 0.01 %. Phytochemical screening of *Sorbaria tomentosa* extract showed the presence of alkaloids, tannins, flavonoids, carbohydrates, saponins and phenols as major phytochemicals while cardiac glycosides and proteins were present in small amount (table 1). All these phytochemicals are bioactive compounds which are responsible for antioxidant activity, antimicrobial and anti-inflammatory activities. Antioxidant potential is attributed to phenols and flavonoids [26].

Table 1
QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *Sorbaria tomentosa* EXTRACT

Phytochemicals	Test	Methanolic extract
Alkaloids	Hager's test	++
Tannins	Ferric chloride test	++
	Lead acetate test	++
Cardiac glycosides	Keller Killiani test	+
	Salkowiski test	+
	Baljet test	-
Steroids	Sulphuric acid test	+
Flavonoids	Alkaline reagent test	+++
	Lead acetate test	+++
Terpenoids	Salkowiski test	+
	Sulphuric acid test	+
Proteins	Ninhydrin test	-
	Biuret test	-
	Xanthproteic test	+
Carbohydrates	Fehling solution test	++
	Molisch test	++
Saponins	Foam test	+++
	Froth test	++
Phenols	Ferric chloride test	+++

Significant amount (+++); moderate amount (++); small amount (+);
absent completely (-);

FT-IR spectra of Sorbaria tomentosa and its Chelates

FT-IR studies showed different stretching frequencies in pure extract and its chelates as shown in (Fig. 1). In *Sorbaria tomentosa* extract, different peaks were observed at 3350 cm^{-1}, 2928 cm^{-1}, 1618 cm^{-1}, 1401 cm^{-1}, 1041 cm^{-1} which showed the presence of ν –OH, ν -CH,

ν -NH_2, ν -CH_2 and ν –C-O (ester) respectively. The various changes/shifts of stretching frequencies in Cu(II)/Zn(II) chelates (Fig. 1) than those observed in pure extract of *Sorbaria tomentosa* showed the involvement of various functional groups of extract in chelation with metal ions [27].

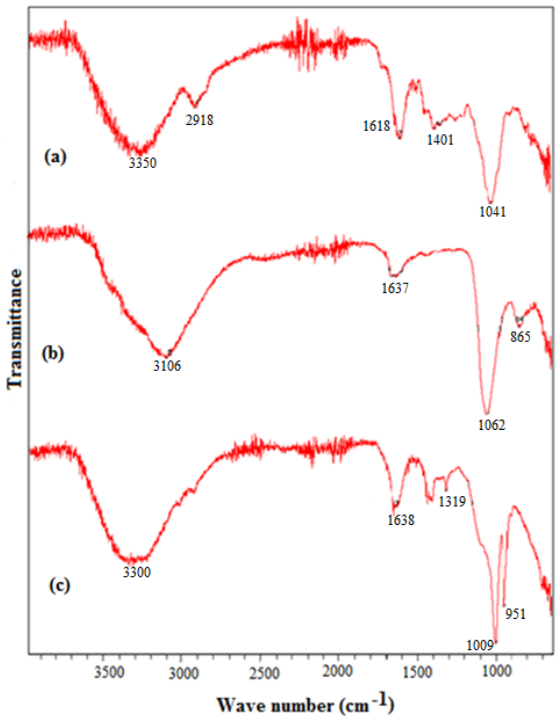

Fig. 1. FT-IR spectrum of (a) *Sorbaria tomentosa* pure extract
(b) Cu(II) chelated *Sorbaria tomentosa* (c) Zn(II) chelated *Sorbaria tomentosa*

Antioxidant Potential of Pure Extract of „Sorbaria tomentosa" and its Chelates

Total Phenolic and Total Flavonoids Contents

Phenols are main ingredients which imparts antioxidant potential to plants. Flavonoid is an important phytochemical found in plants which possess anti-allergic, anti-fungal and anti-inflammatory activities [28]. (Table 2) showed that the extract has greater amount of phenolic and flavonoids contents as compared to its chelated extract. Therefore, it is concluded that *Sorbaria tomentosa* extract contained maximum antioxidants compared to chelated extracts (S.T. > Zn-S.T. > Cu-S.T.) and can be utilized as a source of natural antioxidant easily.

FRAP Assay

This assay involves reduction of Fe^{+3} to Fe^{+2} and finally light blue colored solution is formed upon the addition of antioxidant containing substance [29]. (Table 2) showed that the trend of antioxidant potential was in the order of (S.T. > Cu-S.T. > Zn-S.T.). It reveals that S.T. possesses good antioxidant potential as compared to its chelated extracts (Cu-S.T., Zn-S.T.).

Table 2
TOTAL PHENOLIC, TOTAL FLAVONOID CONTENTS AND FERRIC REDUCING ANTIOXIDANT POWER ASSAY VALUE OF EXTRACT AND CHELATED PRODUCT OF EXTRACT

Samples	TPC (mg of GAE/100 g dry weight)	TFC (Catechin equivalents mg/100 g)	Concentration (mM/L of $FeSO_4$)
Sorbaria tomentosa extract	2.91 ± 0.01	2.201 ± 0.00	0.741 ± 0.00
Cu(II)-chelated *Sorbaria tomentosa* extract	0.56 ± 0.00	1.30 ± 0.00	0.370 ± 0.00
Zn(II)-chelated *Sorbaria tomentosa* extract	1.43 ± 0.01	1.49 ± 0.01	0.527 ± 0.01

DPPH Assay

DPPH is a stable radical and is widely used for the determination of antioxidant potential of extracts [30]. The values of % inhibition were in the range BHT (65.70 − 74.84), BHA (65.67 − 72.49), S.T. (19.40 − 63.11), Cu-S.T. (14.28 − 44.35) and Zn-S.T. (12.58 − 40.29) and are graphically represented in (Fig. 2). This figure shows that % inhibition was increased by increasing the concentration (0.2-1 mg/L) of *Sorbaria tomentosa* extract and respective chelates. The observed order of % inhibition was as follow: BHT > BHA > S.T. > Zn-S.T. > Cu-S.T.;

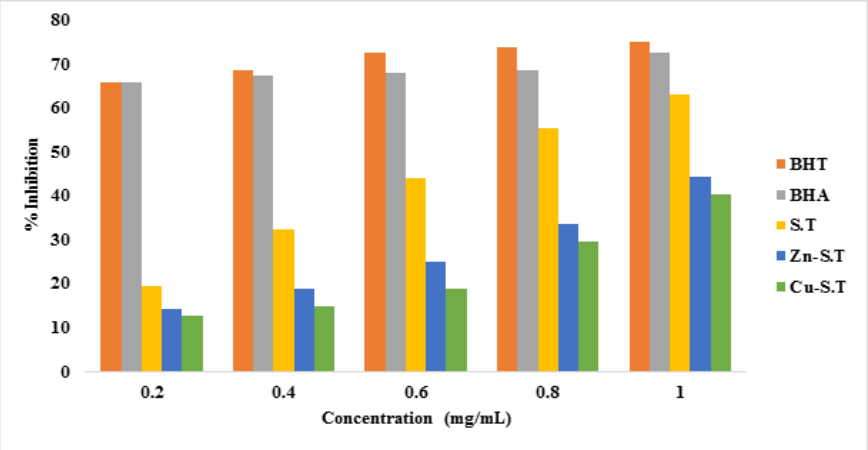

Fig. 2. Radical inhibiting activity of standard antioxidant (BHA and BHT), extract and chelated extract products

β-Carotene Linoleic Acid Emulsion System

β-Carotene linoleic acid is a useful method for the evaluation of antioxidant activity. It has been reported that the rate of β-carotene decolorization can be reduced in the presence of antioxidants [31]. (Fig. 3) shows the graph between absorbance versus time at 470 nm. It can be concluded from this graph that with the passage of time (0-60 min), *Sorbaria tomentosa* pure extract showed absorbance values (0.085-0.050), Cu(II) chelate (0.090-0.067) and Zn(II) chelate (0.088-0.058). This reveals that in case of *Sorbaria tomentosa* absorbance values decreased indicating reduction in β-carotene decolorization hence proving it better antioxidant than its chelated forms. However, it has lesser potential when compared with synthetic BHT and BHA having absorbance values in the range (0.090 - 0.067) and (0.088 - 0.058) respectively.

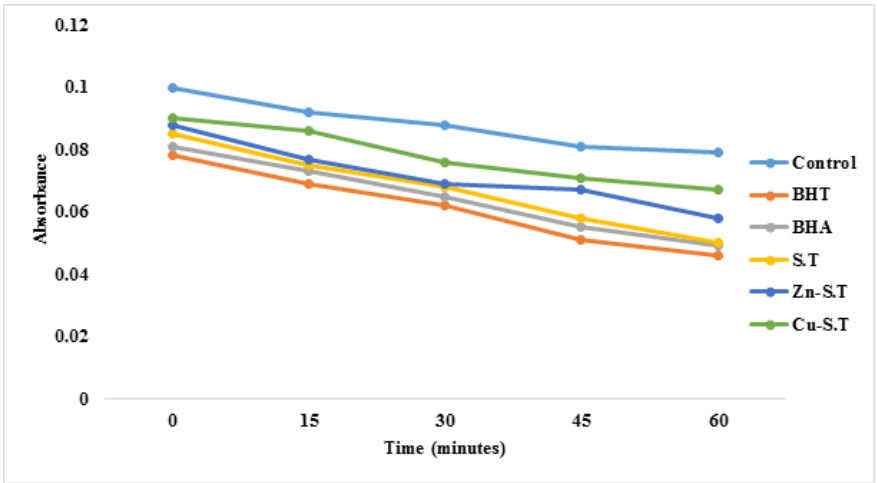

Fig. 3. A graph between absorbance of extract, chelated products and standard antioxidants with the passage of time (0-60 min)

ABTS method

ABTS radical cation scavenging activity was also determined for the assessing the antioxidant potential S.T. extract and its respective chelates. The dark bluish green color of ABTS radical was decreased on addition of the extract and respective chelates. (Fig. 4) represents a graph between concentration and % inhibition (antioxidant activity). This figure depicts similar trend as outlined in above methods thus revealing following order:

BHT > BHA > S.T. > Zn-S.T. > Cu-S.T.;

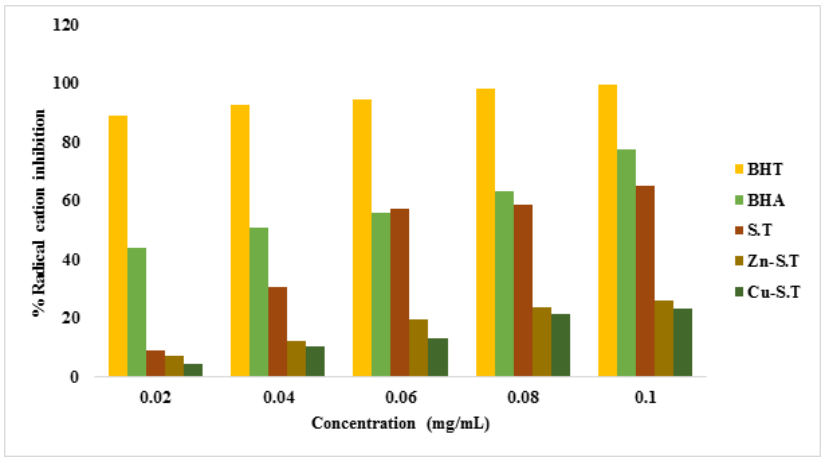

Fig. 4. Radical cation scavenging activity of standards (BHA and BHT), extract and chelated products of extract

Generally, it can be concluded from all the above methods that the order of antioxidant potential of investigated substances was as follow: S.T. > Zn(II)-S.T. > Cu(II)-S.T.; This may be justified that as the ethanolic extract of S.T. contains lot of phenols and flavonoids

(table 2), hence they can donate H^+ ion easily thus exhibiting more antioxidant potential. Cu(II) and Zn(II) forms stable chelates with plant extract thus scavenging free flavonoids and polyphenols which are source of better antioxidant potential in uncomplexed extract. Therefore, after keeping in view decreased antioxidant potential of chelated extracts only uncomplexed extract of S.T. was used for further stabilization studies.

Stabilization of Sunflower Oil

Peroxide Value (PV)

Degree of initial oxidation of any fat or oil is determined in terms of PV [32] from zero to 45th day, PV of sunflower oil (SFO) samples (control, BHT, BHA, S.T.). Percentage of PV (meqO_2/Kg) of SFO with control, BHT, BHA, S.T. (1000 ppm), S.T. (500 ppm), S.T. (250 ppm) was 8.2 ± 0.04, 6.1 ± 0.01, 6.5 ± 0.01, 6.7 ± 0.01, 7.5 ± 0.00, 8.0 ± 0.00 at day zero, and 30.01 ± 0.01, 18.6 ± 0.00, 19.5 ± 0.01, 19.8 ± 0.01, 24 ± 0.00, 28 ± 0.04 on 45th day. The order of increasing PV of SFO with samples was: BHT < BHA = S.T. (1000 ppm) < S.T. (500 ppm) < S.T. (250 ppm) and can be visualized from (Fig. 5). This order revealed that S.T. (1000 ppm) and BHA exhibited similar antioxidant potential. These findings are in agreement with our similar previous studies [23, 33, 34].

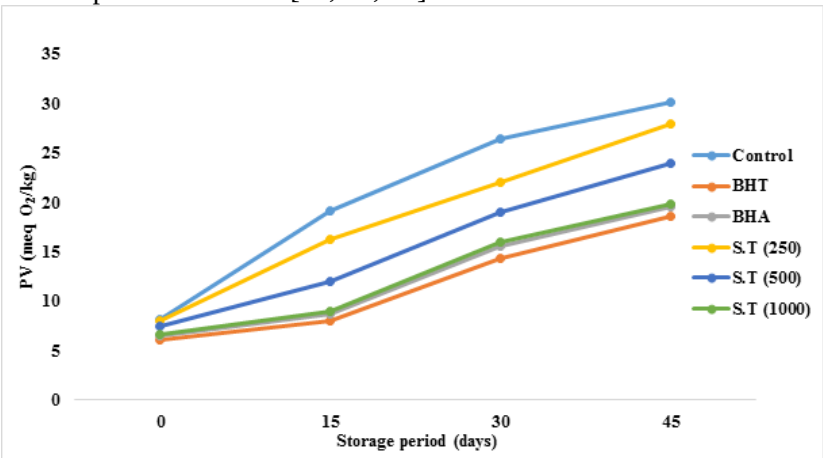

Fig. 5. Effect of *Sorbaria tomentosa* (250, 500 and 1000 ppm) and synthetic antioxidants (BHA and BHT) on peroxide contents (meqO_2/kg) of sunflower oil during storage of 45 days at ambient conditions

Free Fatty Acid Value (FFA)

Triglycerides present in oils get hydrolyzed due to the contact with moisture, resulting in the formation of free fatty acids. More will be the formation of free fatty acids, more will be the rancidity of oil [35]. (Fig. 6) shows the increasing

trend in FFA values for all samples (control, BHT, BHA, S.T.) with passage of time. Maximum increase was observed in SFO with control which was 2.56 ± 0.01 to 4.9 ± 0.00 (%) whereas minimum FFA value was shown by SFO with BHT (2.37 ± 0.01 % to 2.95 ± 0.01 %) from zero to 45th day. SFO with BHA and S.T.(1000 ppm) exhibited FFA comparable values at 45th day which was 3.40 ± 0.00 % and 3.41 ± 0.01 % respectively. The observed trend was: BHT < BHA = S.T. (1000 ppm) < S.T. (500 ppm) < S.T. (250 ppm) < Control, as illustrated in (Fig. 6).

These results are in agreement with our previous findings [23, 33, 34].

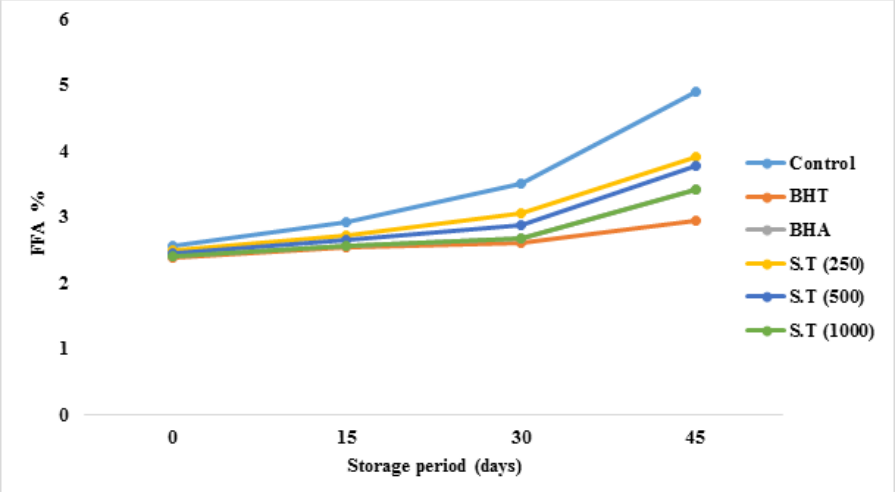

Fig. 6. Effect of *Sorbaria tomentosa (*250, 500 and 1000 ppm) and synthetic antioxidants (BHA and BHT) on free fatty acid contents (%) of sunflower oil during storage of 45 days at ambient conditions

Iodine Value

Iodine value denotes the degree of unsaturation of fatty acids (fats, oils or wax) and expresses the amount of iodine absorbed by fats or oils. More decrease in IV, more will be the rancidity of oil [36]. Iodine values of (control, BHT, BHA, S.T.) were measured and are graphically represented in (Fig. 7). Generally, it was observed that iodine value decreased with the passage of time in all samples. Least decrease in iodine value (gI_2/100g) was observed in SFO-BHT while maximum decrease in IV was observed in control sample during the period of oil storage. From (Fig. 7), the decrease trend of IV can be adduced as follow: BHT < BHA = S.T. (1000 ppm) < S.T. (500 ppm) < S.T. (250 ppm) < Control.

These results are in agreement with our previous findings [23, 33, 34].

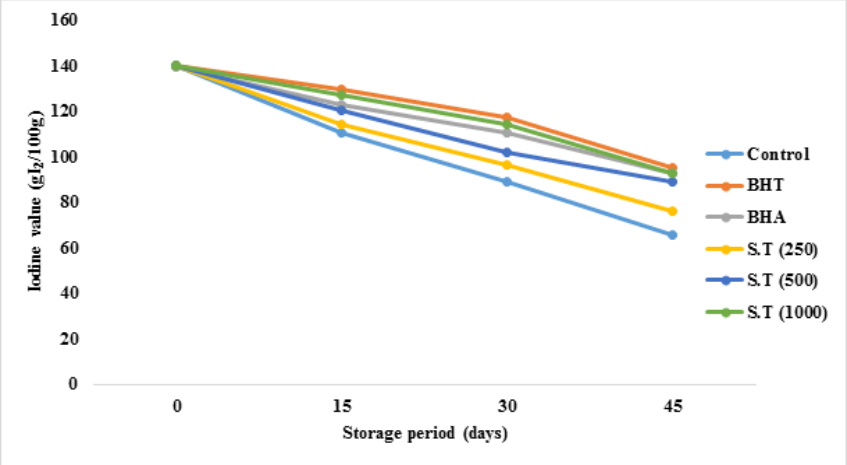

Fig. 7. Effect of *Sorbaria tomentosa* (250, 500 and 1000 ppm) and synthetic antioxidants (BHA and BHT) on iodine value (gI_2/100g) of sunflower oil during storage of 45 days at ambient conditions

Conclusions

Phytochemical studies of *Sorbaria tomentosa* extract showed that it possesses phenols, flavonoids, alkaloids and many other phytochemicals which imparts antioxidant properties to it. Among *Sorbaria tomentosa* and its chelates, the pure extract of *Sorbaria tomentosa* exhibited more antioxidant potential. (S.T.) (1000 ppm) ethanolic extract which was used further for the stabilization of sunflower oil, proved its oil stabilization efficacy comparable with standard synthetic antioxidant like BHA.

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