

Antioxidant Properties of Agro-Industrial Waste and Their use as Natural Preservative for Sunflower Oil

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ABSTRACT

The everyday waste like the peels of *Aloe vera*, cucumber (*Cucumis sativus*), grape fruit (*Citrus paradisi*), sour orange (*Citrus aurantium*), pomegranate (*Punica granatum*) and leaves of olive tree (*Olive europaea*) were evaluated for antioxidant activities and preservation of crude sunflower oil at a temperature of 80 °C for 7 days. Highest IC₅₀ were recorded for the methanolic extract of pomegranate peel (0.109±0.01 mg/mL) and olive leaves (0.122±0.01mg/mL). After 7 days of incubation in oven the sunflower oil contained butylated hydroxytoluene (BHT) in showed free fatty acid value (FFA) contents 3.20±0.06% and peroxide value (POV) 35.5±0.05 meq/kg. The extract of Olive leaves and pomegranate peel showed lower FFA content and POVs as compare to BHT. *A.vera*, grape fruit and sour orange peel extracts showed intermediary efficacy in the oil substrate and cucumber peel extract resulted satisfactorily. However, the inhibition of oil oxidation was totally dependent on the concentration therefore all the extracts were effective at 800 µg/mL except BHT. These findings revealed that the natural antioxidants can replace synthetic antioxidants for the preservation of foods and edible oil.

Highlights:

- Plants leaves and fruits peels contains phytochemicals.
- They possess free radical scavenging activity.
- They can be used as natural preservatives in food products.

KEY WORDS: Antioxidant activity, Oven test, peels and leaves extract, sunflower oil

INTRODUCTION

Lipid peroxidation leads to the formation of off-flavor low molecular weight compounds, thus, oxidative stability is an important factor to determine the shelf life and quality of oil [1]. Reactive Oxygen Species (ROS) are produced in food because of food's biological nature [2]. ROS react with food's carbohydrates, vitamins, proteins and fatty acids adversely effecting important amino acids, fatty acids and vitamins which produce carcinogenic compounds. Oxidation of food by ROS produce oxidized dimers and trimmers changing the function of sugars, vitamins and proteins, thus, making it unacceptable for consumers [3]. The shelf life of products especially of oils and lipid containing foods is enhanced by the addition of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylated hydroxyquinone (TBHQ) however, the synthetic antioxidants have used against cancer [4-5]. Due to restrictions on synthetic antioxidants, the use of plant derived antioxidants as natural preservatives for enhancing the shelf life and oxidative stability of edible oils has attracted a great scientific focus [6]. It is reported that most of the identified that the by-products of food industry are rich in bioactive compounds including phytochemicals [7]. Some of the plants phytochemicals serves as antioxidants and their use can prevent many chronic diseases to occur [8]. The phytochemicals are divided into different groups: [9] in which flavonoids very important and can be used against various diseases [10]. Thus, the research is still active to discover new antibiotics, antioxidants and other chemotherapeutic agent from plants, an alternative source of the available medicines [11]. Various phenolic compounds with potential antioxidant activities have been reported from the extract of many agricultural wastes like almond hulls, rice hulls and buckwheat hulls [12]. Food industry

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produces large amount of by-products which are discarded as wastes. The objective of this research is to investigate agro-industrial wastes for their antioxidant potential and to check the efficacy of these by-products as natural preservative in sunflower oil model in comparison with synthetic antioxidant BHT.

2. MATERIAL AND METHODS

2.1 Collection of samples

The samples pomegranate (*Punica granatum*) peels, grape fruits (*Citrus paradisi*) peels, sour oranges (*Citrus aurantium*) peels, cucumber (*Cucumis sativus*) peels, aloe vera (*Aloe vera*) leaf peels and olive leaves (*Olive europaea*) were collected from the local market and preserved in polyethylene bags till further use [13].

Chemicals

All the chemicals and solvents were of analytical grade and purchased from Merck Germany. BHT and 2, 2,-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

2.3 Processing of samples

The samples were washed to remove dust residue and dried in vacuum oven at 40 °C to lower the moisture content. The samples were grinded and 50 gm of each sample was extracted with 200 mL methanol, overnight at room temperature using an orbital shaker. The sample mixture was then filtered through Whatman No. 1 filter paper to remove the extract residues. The residues were re-extracted twice with the same solvent and extract was collected. The extract was then concentrated to dryness at 42 °C in a rotary evaporator. The dried extracts were stored in a refrigerator until further analysis [13].

2.3 Determination of antioxidant activity

The antioxidant activity of the samples was monitored through DPPH assay. DPPH absorbs at 517 nm but it decreases in the presence of a scavenger i.e.; antioxidant. This assay is based on the principle that DPPH is reduced to DPPH₂ when it gains a hydrogen atom from the antioxidant molecule, resulting in a color change from purple to yellow. Briefly, 0.004% (w/v) of DPPH was prepared in 95% methanol. Different concentrations of extracts and standard ascorbic acid (50 to 2000 µL equivalent to 50 and 2000 ppm) were added to 3 mL of DPPH. The control was prepared similarly as above containing methanol instead of extract. Methanol was used for baseline correction of spectrophotometer. After 30 minutes absorbance was measured at 517 nm. The decrease in the absorbance of reaction mixture indicates higher scavenging activity of the sample. The DPPH scavenging potential of the sample peels was calculated by the following formula [14].

$$\% \text{ DPPH Scavenging effect} = \left[\frac{(A \text{ controle} - A \text{ sample})}{A \text{ comtrole}} \right] \times 100$$

2.4 Fortification of sunflower oil with antioxidant concentrates

The crude concentrated MeOH plant extracts were added to 50 gm of sunflower oil in concentration of 400 ppm, 600 ppm and 800 ppm. A reference sample with synthetic antioxidant BHT was prepared according to its legal limit of 200 ppm. Fortified oil samples with extracts and the reference sample with BHT were stirred at 45 °C for 15 minutes to allow uniform dispersion of the samples. The control sample of oil was prepared without any extract. The sunflower oil samples were placed in an electric oven for a week at 80 °C. All the oil samples were prepared in triplicates. The oxidative stability of oil was assessed after every 24 hours by measuring its peroxide value (POV) and free fatty acid value (FFA) by standard method of AOAC, 1990.

2.2 Statistical analysis

Data given are mean of triplicate readings with standard deviation performed with SPSS version 20.0. Differences of $p < 0.05$ were considered significant.

3 RESULTS AND DISCUSSION

3.1 Free radical scavenging activity

Antioxidants present in foods may be soluble in different solvents like water, fats or they may be insoluble in either, sometimes they are not available freely as they are bound to cell walls to react with DPPH, their kinetics may be different due to different reaction rates and hence, the reaction will not often completed in a specific time. Antioxidants are isolated frequently with different solvents and the antioxidant activity and extract yield of the sample is greatly dependent on the polarity of antioxidant compounds and nature of the solvent. Hence, the

absorbance of DPPH solution when reduced to 50% of the initial absorbance by the antioxidant sample is considered as the end of the reaction and this value is called the half maximal inhibitory concentration, also known as IC₅₀ value [15]. A lower IC₅₀ value depicts strong antioxidant activity of a compound. Anti-radical power (ARP) is actually a free radical scavenging potential of an antioxidant. It is the inverse of IC₅₀.

$$\text{ARP} = 1/\text{IC}_{50}$$

All the extracts utilized in this assay had shown DPPH radical scavenging activity as shown in Table 1. Ascorbic acid was used as a reference antioxidant and had IC₅₀ value at 0.04±0.03 mg/mL. Among the extracts pomegranate peel extract exhibited highest DPPH radical scavenging activity which showed IC₅₀ value of 0.109±0.01 mg/mL. The lowest IC₅₀ was shown by cucumber peel (1.63±0.01 mg/mL). Significant difference has been observed among the IC₅₀ value of different extracts. The mean IC₅₀ values of olive leaves, grape fruit peel, sour orange-peel and *A. vera* skin were 0.122±0.01, 0.64±0.12, 0.73±0.04 and 1.2±0.05 mg/mL, respectively.

ARP mean value of different extracts *P. granatum*, *O. europaea* leaves, *C. paradisi* peel, *C. aurantium* peel, *A. vera* leaf skin and *C. sativus* peel were 9.17±0.21, 8.19±0.07, 1.56±0.09, 1.4±0.03, 0.83±0.11 and 0.61±0.05 mg/mL⁻¹ respectively.

Moure et al. (2001) [16] stated that the antioxidant activity is dependent on the phenolic content of plants. However, it has been stated that not the amount but the kind of phenolic compounds determine the antioxidant property of plants [17]. Among the extracts pomegranate possesses high ARP i.e., 9.17 mg/mL⁻¹ as compare to other extracts. This may be attributed to higher total polyphenols and flavonoids content in peel rather than pomegranate juice [18]. More than one hydroxyl groups on flavonoid structure depicts strong antioxidant activity. Cucumber peel exhibited least ARP i.e., 0.61mg/mL⁻¹, this may be explained by the fact that not all the phenolic compounds can scavenge DPPH radical [19].

3.2 Oxidative stability of sunflower oil with extracts during storage

Oxidation in sunflower oil was measured by two rancidity parameters POVs and FFA values. POVs determine the primary oxidation of oil while FFA value determines the secondary oxidation. Primary oxidation results in the formation of peroxides and hydroperoxides due to the reaction between unsaturated fatty acids and oxygen in presence of heat and light. This initiation of oxidation can be terminated by free radicals quenchers such as synthetic antioxidants or natural antioxidants. The quenching ability is due to the presence of phenolic moiety in these compounds which act as potent chain terminating antioxidants [20]. If this termination doesnot occur it will lead to the formation of secondary oxidation products such as aldehydes, ketones etc. and result in the formation free fatty acids.

In this study the oil samples were stored for a period of one week at a temperature of 80 °C and assessed for POVs and FFA values after every 24 hours. After one week analysis it is evident from the data (Table 3 & 4) that the plant extracts had significantly retarded the POVs and FFA value. During storage the POVs and FFA values were reduced by BHT from 58.5±0.13 meqkg⁻¹ (control) to 35.3±0.05 meqkg⁻¹ and 9.5±0.04% (control) to 3.20±0.06%, respectively. These results similar to results reported in literature [21] that showed the decrease in the rancidity of fried potato chips by the addition of BHA and BHT to cooking oil during storage. The plant extracts showed significant changes in the POVs and FFA values. Pomegranate peel and olive leaves were reported to be strong antioxidants as compare to BHT as pomegranate peel extract at concentration of 400 µg/mL, 600 µg/mL and 800 µg/mL showed POVs 34.1±0.12, 30.0±0.09 and 22.1±0.04 and FFA values of 2.70±0.11%, 2.20±0.02% and 1.60±0.15%, respectively.

Olive leaves (*O. europaea*) extract at same concentration showed POVs 35.1±0.05 meqkg⁻¹, 32.5±0.09 meqkg⁻¹ and 25.7±0.15 meqkg⁻¹ while the FFA values were 3.34±0.09%, 2.78±0.07% and 1.98±0.08%, respectively. Thus, pomegranate peel and olive leaves reduced the POVs from 58.5±0.13 meqkg⁻¹ (control) to 22.1±0.04 meqkg⁻¹ and 25.7±0.15 meqkg⁻¹, respectively. The reduction in FFA values were from 9.50±0.04% (control) to 1.60±0.15% and 1.98±0.08% of pomegranate peel and olive leaves, respectively. Our findings are similar to the findings of Babovic et al (2010) as they had detected that the rosemary extract possessed strong antioxidant activity than BHA when both are added at a concentration of 200 mg/kg to sunflower oil at elevated temperature of 98 °C [22].

Grape fruit peel and sour orange peel also inhibited oxidation efficiently as compare to BHT. Grape fruit peel and sour orange peel showed intermediate antioxidant activity given in Table 3 & 4. After 7 day storage grape fruit peel extract at concentration of 600 µg/mL and 800 µg/mL showed POVs 33.3±0.01 meqkg⁻¹ and 38.9±0.15 meqkg⁻¹ and FFA values 3.14±0.12% and 2.68±0.06%. The efficient concentration of sour orange peel (*C. aurantium*) as compare to BHT was observed to be 800 µg/mL at which POV was 30.1 meqkg⁻¹ and FFA was 2.98%, after 7 days testing at 80 °C. El-Bagoury et al. (2004) [23] reported that POV and FFA of sour orange peel at 600 µg/mL extract was observed to be 33.1± 0.09 meqkg⁻¹ and FFA was 2.98±0.09%, respectively as compare to

control with POV 81.2 meqkg^{-1} and 4.32% respectively, after 3 days at $100 \text{ }^{\circ}\text{C}$ in cotton seed oil. Our research suggested that the inhibitory effect of extract is concentration dependent as increase in concentration considerably reduced sunflower oil oxidation. Nagwa, Amal, Marvet & Marwa (2012) [24] have also been analyzed that the antioxidant activity from natural source sage (*Salvia officinalis* L.) in mayonnaise at ambient temperature over a period of four months. The ethanolic extract of sage showed to retard the peroxide value of mayonnaise but the decrease in POVs was concentration dependent.

Similarly, *A. vera* leaf skin and cucumber peel also reported that the increase in concentration of extracts is responsible for decrease in oxidation of oil as compare to control after every 24 hours analysis. After 7 days at elevated temperature *A. vera* leaf skin $400 \text{ } \mu\text{g/mL}$, $600 \text{ } \mu\text{g/mL}$ and $800 \text{ } \mu\text{g/mL}$ had mean POVs $41.5 \pm 0.05 \text{ meqkg}^{-1}$, $35.5 \pm 0.03 \text{ meqkg}^{-1}$ and $31.6 \pm 0.09 \text{ meqkg}^{-1}$ and FFA $3.78 \pm 0.14\%$, $3.37 \pm 0.03\%$ and $3 \pm 0.04\%$, respectively.

Cucumber peel (*C. sativus*) extract at $800 \text{ } \mu\text{g/mL}$ had comparable results with standard BHT. At 7th day mean POVs and FFA value for $800 \text{ } \mu\text{g/mL}$ were $34.5 \pm 0.03 \text{ meqkg}^{-1}$ and $3.10 \pm 0.07\%$, respectively. The antioxidant capacity of extracts as natural preservatives in sunflower oil in inhibiting peroxidation was in correspondence with the free radical scavenging potential in which pomegranate peel showed strong antiradical potential while cucumber peel reported weak antiradical potential. Moreover, phytochemicals derived from plants have been tested as natural preservatives in corn, olive, rapeseed, cottonseed, fish, sunflower and peanut oils [25]. Pistachio hulls extracts have shown to inhibit soybean oil oxidation at $60 \text{ }^{\circ}\text{C}$ at a concentration of 0.06% (w/w) as inhibited by 0.02% BHA and BHT. Therefore, 600 ppm hydromethanolic extracts of *moringa oleifera* leaves was potentially good in delaying the oxidation in sunflower oil stored at room temperature for a duration of 60 days [26]. Extracts of Tergo variety of wheat has revealed to inhibit fish oil oxidation as inhibited by tocopherol [27]. This antioxidant activity of plant extracts is attributed to different phenolic compounds present in them. The research suggested the presence of hydroxycinnamates, flavanones, flavones glycosides, poly-methoxylated flavones and many other glycosides and amines in orange peel ultra-filtered molasses [28]. These compounds contributed to the antioxidant activity of orange peel.

Table: 1 DPPH scavenging activity of plant extracts

Values are expressed as \pm S.D of triplicate data.

Plants	Part used	IC ₅₀ (mg/mL)	ARP(1/ IC ₅₀)
<i>Punica granatum</i>	Fruit peel	0.109 ± 0.01	9.17 ± 0.21
<i>Olive europaeae</i>	Leaves	0.122 ± 0.01	8.19 ± 0.07
<i>Citrus paradisi</i>	Fruit peel	0.64 ± 0.12	1.56 ± 0.09
<i>Citrus aurantium</i>	Fruit peel	0.73 ± 0.04	1.4 ± 0.03
<i>Aloe vera</i>	Leaf skin	1.2 ± 0.05	0.83 ± 0.11
<i>Cucumis sativus</i>	Fruit peel	1.63 ± 0.01	0.61 ± 0.05
Standard			
Ascorbic acid		0.04 ± 0.03	25 ± 0.03

Values within a column are significantly different at $p < 0.05$.

Table: 2 Effect of storage conditions on POVs and FFA of control and BHT added oil at $80 \text{ }^{\circ}\text{C}$

Storage time Days	Control		BHT(200 $\mu\text{g/mL}$)	
	FFA(%) oleic acid	POV(meq/kg)	FFA(%) oleic acid	POV(meq/kg)
0	0.26 ± 0.06	2.1 ± 0.07	0.26 ± 0.01	2.1 ± 0.09
1	0.95 ± 0.02	5.1 ± 0.03	0.31 ± 0.04	2.7 ± 0.04
2	1.89 ± 0.07	9.6 ± 0.04	0.63 ± 0.13	3.2 ± 0.01
3	2.80 ± 0.23	16.8 ± 0.03	0.98 ± 0.08	7.8 ± 0.01
4	4.20 ± 0.01	22.3 ± 0.07	1.40 ± 0.04	11.7 ± 0.04
5	5.90 ± 0.12	30.5 ± 0.05	1.93 ± 0.03	18.9 ± 0.08
6	7.40 ± 0.05	39.8 ± 0.16	2.67 ± 0.02	27.1 ± 0.03
7	9.50 ± 0.04	58.5 ± 0.13	3.20 ± 0.06	35.5 ± 0.05

Values are expressed as \pm S.D of triplicate data.

Values within a column are significantly different at $p < 0.05$.

Table: 3 Effect of storage conditions on POVs and FFA on plants extract added oil at 80 °C

Storage time days	<i>Olive europaea</i> leaves		<i>Punica garanatum</i> peel		<i>Citrus aurantium</i> peel	
400 µg/mL	FFA (%)	POV (meq/kg)	FFA (%)	POV (meq/kg)	FFA (%)	POV (meq/kg)
0	0.26±0.06	2.1±0.05	0.26±0.09	2.1±0.03	0.26±0.07	2.1±0.04
1	0.56±0.03	2.9±0.07	0.39±0.15	2.7±0.06	0.57±0.05	3.60±0.09
2	0.98±0.13	3.7±0.04	0.57±0.07	4.1±0.04	0.89±0.08	5.80±0.07
3	1.45±0.04	4.50±0.14	0.89±0.05	7.6±0.09	1.47±0.03	10.1±0.11
4	1.76±0.11	9.4±0.05	1.21±0.09	12.7±0.06	1.96±0.01	16.2±0.02
5	2.1±0.08	14.9±0.09	1.63±0.12	19.5±0.08	2.65±0.12	23.9±0.14
6	2.65±0.04	23.1±0.12	2.10±0.08	26.4±0.17	3.23±0.09	32.5±0.06
7	3.34±0.09	30.5±0.05	2.70±0.11	35.1±0.12	3.67±0.06	40.1±0.08
600 µg/mL						
1	0.58±0.12	2.7±0.06	0.39±0.09	2.5±0.05	0.62±0.08	3.31±0.12
2	0.81±0.15	3.6±0.04	0.55±0.07	3.9±0.07	0.89±0.06	5.21±0.09
3	1.24±0.09	6.3±0.06	0.82±0.03	6.7±0.05	1.32±0.09	8.60±0.04
4	1.61±0.05	11.3±0.03	1.01±0.06	11.1±0.03	1.76±0.11	13.0±0.08
5	1.89±0.08	17.8±0.05	1.24±0.11	16.8±0.04	2.3±0.15	19.4±0.06
6	2.29±0.04	24.6±0.02	1.65±0.09	2.9±0.08	2.75±0.08	28.2±0.09
7	2.78±0.07	32.5±0.09	2.20±0.02	30±0.09	3.56±0.09	36.3±0.11
800 µg/mL						
1	0.49±0.01	2.5±0.02	0.34±0.09	2.2±0.13	0.56±0.09	2.90±0.05
2	0.68±0.09	3.1±0.04	0.45±0.02	2.6±0.09	0.82±0.04	3.40±0.03
3	0.87±0.06	4.9±0.08	0.63±0.06	4.8±0.07	1.05±0.02	5.8±0.05
4	1.07±0.04	8.5±0.01	0.87±0.08	8.9±0.14	1.50±0.09	10.6±0.02
5	1.29±0.07	13.4±0.11	1.10±0.09	12.3±0.05	1.99±0.11	16.7±0.11
6	1.56±0.09	18.9±0.09	1.29±0.17	16.7±0.09	2.50±0.12	25.4±0.13
7	1.98±0.08	25.7±0.15	1.60±0.15	22.1±0.04	2.98±0.09	33.1±0.09

Values are expressed as ± S.D of triplicate data
 Values within a column are significantly different at p < 0.05.

Table: 3.8 Effect of storage conditions on POVs and FFA on plants extract added oil at 80 °C

Values are expressed as ± S.D of triplicate data.

Storage time days	<i>Aloe verapeel</i>		<i>Cucumis sativus</i> peel		<i>Citrus paradisi</i> peel	
400 µg/mL	FFA (%)	POV (meq/kg)	FFA (%)	POV (meq/kg)	FFA (%)	POV (meq/kg)
0	0.26±0.07	2.1±0.09	0.26±0.03	2.1±0.11	0.26±0.08	2.1±0.04
1	0.61±0.09	3.2±0.06	0.66±0.08	3.9±0.09	0.52±0.07	3.4±0.02
2	0.90±0.04	5.1±0.06	0.99±0.12	5.9±0.05	0.96±0.04	5.3±0.15
3	1.53±0.13	11.1±0.09	1.68±0.18	11.6±0.05	1.59±0.03	9.9±0.07
4	2.0±0.07	18.9±0.12	2.10±0.09	19.2±0.03	1.98±0.16	15.8±0.14
5	2.58±0.12	26.4±0.17	2.63±0.15	28.3±0.07	2.68±0.09	23.1±0.05
6	3.24±0.16	33.1±0.08	3.18±0.03	37.5±0.04	3.1±0.13	31.2±0.06
7	3.78±0.14	41.5±0.05	3.89±0.07	45.3±0.08	3.56±0.07	38.5±0.09
600 µg/mL						
1	0.50±0.03	2.9±0.08	0.58±0.04	3.2±0.11	0.42±0.04	2.8±0.06
2	0.87±0.01	3.4±0.05	0.81±0.06	6.5±0.04	0.71±0.06	5.2±0.04
3	1.36±0.05	8.5±0.09	1.57±0.08	10.2±0.07	1.26±0.03	9.0±0.02
4	1.81±0.12	12.5±0.13	1.89±0.09	16.4±0.09	1.74±0.11	13.8±0.03
5	2.44±0.06	19.2±0.04	2.32±0.11	26.9±0.03	2.15±0.04	19.1±0.05
6	2.81±0.04	27.5±0.07	2.78±0.17	34.6±0.05	2.58±0.15	26.5±0.01
7	3.37±0.03	35.5±0.03	3.58±0.05	40.7±0.15	3.14±0.12	33.3±0.01
800 µg/mL						
1	0.43±0.14	2.7±0.05	0.48±0.06	2.8±0.12	0.39±0.03	2.6±0.09
2	0.70±0.04	3.1±0.15	0.77±0.07	4.1±0.05	0.71±0.14	3.8±0.06
3	1.13±0.08	7.2±0.09	1.39±0.08	9.8±0.07	1.28±0.16	6.5±0.07
4	1.65±0.05	11.4±0.07	1.79±0.03	14.1±0.03	1.53±0.07	11.1±0.02
5	2.11±0.12	17.2±0.05	2.21±0.15	20.6±0.09	1.86±0.08	17.6±0.16
6	2.57±0.09	25.8±0.08	2.59±0.18	27.8±0.02	2.30±0.09	23.3±0.09
7	3.00±0.04	31.6±0.09	3.10±0.03	34.5±0.07	2.68±0.06	28.9±0.15

Values within a column are significantly different at p < 0.05.

Table: 4 Effect of storage conditions on POVs and FFA on plants extract added oil at 80 °C

Storage time days	<i>Oliveuropaea</i> leaves		<i>Punica garanatum</i> peel		<i>Citrus aurantium</i> peel	
400 µg/mL	FFA (%)	POV(meq/kg)	FFA (%)	POV(meq/kg)	FFA (%)	POV (meq/kg)
0	0.26	2.1	0.26	2.1	0.26	2.1
1	0.56	2.9	0.39	2.7	0.57	3.60
2	0.98	3.7	0.57	4.1	0.89	5.80
3	1.45	4.50	0.89	7.6	1.47	10.1
4	1.76	9.4	1.21	12.7	1.96	16.2
5	2.1	14.9	1.63	19.5	2.65	23.9
6	2.65	23.1	2.10	26.4	3.23	32.5
7	3.34	30.5	2.70	35.1	3.67	40.1
600 µg/mL						
1	0.58	2.7	0.39	2.5	0.62	3.31
2	0.81	3.6	0.55	3.9	0.89	5.21
3	1.24	6.3	0.82	6.7	1.32	8.60
4	1.61	11.3	1.01	11.1	1.76	13.0
5	1.89	17.8	1.24	16.8	2.3	19.4
6	2.29	24.6	1.65	2.9	2.75	28.2
7	2.78	32.1	2.20	30	3.56	36.3
800 µg/mL						
1	0.49	2.5	0.34	2.2	0.56	2.90
2	0.68	3.1	0.45	2.6	0.82	3.40
3	0.87	4.9	0.63	4.8	1.05	5.8
4	1.07	8.5	0.87	8.9	1.50	10.6
5	1.29	13.4	1.10	12.3	1.99	16.7
6	1.56	18.9	1.29	16.7	2.50	25.4
7	1.98	25.7	1.60	22.1	2.98	33.1

3.3 Conclusion

In this study, the peels and leaves which are wastes of food industry are utilized as preservatives in sunflower oil. Each of them possessed different levels of antioxidant activity at different concentrations. Further study should be conducted in these plant extracts to analyze the essential antioxidant compounds and effective concentration responsible for preservation of different food products. In addition, they can be preferred over synthetic antioxidants as they are natural and minimize adverse health effects related with synthetic antioxidant.

REFERENCES

- [1] Nagwa, M. R., Amal, A. H., Mervat, I. F., & Marwa, M. E. M. (2012). Assessment of the antioxidant activity of Sage (*Salvia officinalis* L.) extracts on the shelf life of mayonnaise. *World Journal of Dairy and Food Sciences*, 7, 28–40.
- [2] Wettasinghe, M., & Shahidi, F. (2000). Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chemistry*, 70:17–26.
- [3] Min, D. B., & Choe, E. O. (2002). Effects of singlet oxygen oxidation on the flavor of foods and stability of vitamins. *Food Science and Biotechnology*, 11:582–586.
- [4] Siddhuraju, P., Becker, K. (2007). The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L.) see extracts. *Food Chemistry*, 101, 10–19.
- [5] Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. *Trees. Food Chemistry*, 104, 1106–1114.
- [6] Anwar, F., Sidiqq, A., Iqbal, S., & Asi, M. R. (2007). Stabilization on sunflower oil with *Moringa oleifera* leaves under ambient storage. *Journal of Food Science*, 69, 67–72.
- [7] Schieber, A., Stintzing, F. C., & Carle, R. (2001). By-products of plant food processing as a source of functional compounds – recent developments. *Trends in Food Science and Technology*, 12, 401–413.
- [8] Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of fruits. *Journal of Agricultural and Food Chemistry*, 50, 7449–7454.
- [9] Shan, B., Cai, Y. Z., Sun, M., Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry* 53, 7749–59.

- [10] Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology*, 47, 85–89.
- [11] Akinpelu, D. A., & Onakoyo, T. M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in Southwestern. *African Journal of Biotechnology*, 5, 1078–108.
- [12] Takeoka, G. R., & Dao, L. T. (2002). Antioxidant constituents of almond [*Prunus dulcis* (Mill.) D.A. Webb] hulls. *Journal of Agricultural and Food Chemistry*, 51, 496–501.
- [13] Ghosia Lutfullah, Hina Tila, Arshad Hussain and Abid Ali Khan (2014). Evaluation of Plants Extracts for Proximate Chemical Composition, Antimicrobial and Antifungal Activities. *American-Eurasian J. Agric. & Environ. Sci.*, 14 (10): 964–970, 2014
- [14] Gülçin, İ., Oktay, M., Kireççi, E., & Küfrevioğlu, Ö. İ. (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chemistry*, 83, 371–382
- [15] Dutra, R., Leite, M., & Brbosa, N. (2008). Quantification of phenolic constituents and antioxidant activity of *Pterodonmarginatus* vogel seeds. *International Journal of Molecular Sciences*, 9, 606–614.
- [16] Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., Nunez, M. J., & Parjo, C. J. (2001). Natural antioxidants from residual sources. *Food Chemistry*, 72, 145–171.
- [17] Rababah, T. M., Hettiarachchy, N. S., & Horax, R. (2004). Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola and ginkgo extracts, vitamin E and tert-butylhydroquinone. *Journal of Agricultural and Food Chemistry*, 52, 5183–6.
- [18] Elfalleh, W., Nasri, N., Thabti, I., M'rabet, A., Yahya, Y., Lachiheb, B., Guasmi, F., & Ferchichi, A. (2009). Physico-chemical properties and DPPH-ABTS scavenging activity of some local pomegranate (*Punica granatum*) ecotypes. *International Journal of Food Sciences and Nutrition*, 60, 197–210.
- [19] Ivanova, D., Gerova, D., Chervenkov, T., & Yankova, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacol*, 96, 145–50.
- [20] Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32, 67–103.
- [21] Rehman, Z. U. (2003). Evaluation of antioxidant activity of methanolic extract from peanut hulls in fried potato chips. *Journal of the Science of Food and Agriculture*, 35, 805–8112.
- [22] Nada, B., Irena, Z., Snezana, S., Jasna, I., Slobodan, P. (2010). Oxidative stabilisation of sunflower oil by antioxidant fractions from selected lamiaceae herbs. *Chemical Industry & Chemical Engineering Quarterly*, 16, 287–293.
- [23] El-Bagoury, A. A. (2004). Utilization of extracts from pomegranate and sour orange wastes as natural antioxidants in retarding cotton seed oil oxidation. *Journal of Agricultural Science*, 29: 1939–1950
- [24] Nagwa, M. R., Amal, A. H., Mervat, I. F., & Marwa, M. E. M. (2012). Assessment of the antioxidant activity of Sage (*Salvia officinalis* L.) extracts on the shelf life of mayonnaise. *World Journal of Dairy and Food Sciences*, 7, 28–40.
- [25] Yanishlieva, N. V., & Marinova, E. M. (2001). Stabilisation of edibleoils with natural antioxidants. *European Journal of Lipid Science and Technology*, 103, 752–767.
- [26] Anwar, F., Sidiqq, A., Iqbal, S., & Asi, M. R. (2007). Stabilization on sunflower oil with *Moringa oleifera* leaves under ambient storage. *Journal of Food Science*, 69, 67-72.
- [27] Yu, L., Haley, S., Perret, J., & Harris, M. (2002). Antioxidant properties of hard winter wheat extracts. *Food Chemistry*, 78, 457–461.
- [28] John, I. M. (2004). Fractionation of orange peel phenols in ultra filtered molasses and mass balance studies of their antioxidant levels. *Journal of Agricultural and Food Chemistry*, 52, 7586-7592.