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Comparison of antioxidant activity of essential oil of *Centella asiatica* and Butylated hydroxyanisole in sunflower oil at ambient conditions

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Abstract. Present analytical investigation was designed to compare the antioxidant activity of essential of *Centella asiatica* and butylated hydroxyanisole (BHA). Essential oil of *Centella asiatica* was extracted through steam distillation exhibited positive antioxidant activity during DPPH on TLC indicating the antioxidant potential of essential oil. Free fatty acid values (FFA), Peroxide values (PV), Iodine values, Conjugated dienes (Cd) and Conjugated trienes (Ct) were determined to monitor the antioxidant activity of essential oil and BHA in sunflower oil. Refined, bleached and deodorized (RBD) sunflower oil (SFO) was utilized for this purpose. Essential oil delivered a strong antioxidant activity by prohibiting the increase in above-mentioned oxidative parameters. Controlled samples and BHA containing samples were also investigated for comparison.

Key words: Antioxidant activity, *Centella sciatica*, Essential oil, Sunflower oil

Introduction

Lipid peroxidation is considered a principal mean of deterioration in the quality of food stuffs. It not only imparts rancid and undesireable flavours to fat products, but also generates reactive oxygen species which are linked to carcinogenesis, inflammation, aging and cardiovascular disorders (Pezzuto & Park 2002). Lipids containing polyunsaturated fatty acids are readily oxidized by molecular oxygen, and such oxidation proceeds by a free radical chain mechanism (Aruoma 1998). It also decreases organoleptic value of foods and imparts rancid and unpleasant flavors to the raw and end-use oil and fat products, thus making them unacceptable to consumers (Min & Lee 1998). The oil industry has to pay special attention in this context, as oils,fats and fatty foods suffer from stability problems (Wu & Nawar 1986). Traditionally, chemically synthesized compounds, such as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT), are used as antioxidants in oil products. However, some of these compounds have been questioned for their safety (Bran 1975, Whysner et al. 1994). The use of BHA and BTH is proved to be carcinogeneic. Therefore, there is an increasing interest in the antioxidative activity of natural compounds (Amakura et al. 2002, Orhan et al. 2003). Ter-butyl hydroquinone (TBHQ) is not allowed in Japan, Canada and Europe. Similarly, BHA has also been removed from the generally recognized as safe (GRAS) list of compounds. (Frag et al. 1998). Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods (Hulin et al. 2002). Most of their properties are due to essential oils produced by their secondary metabolism (Adam et al. 1998). Plant essential oils as antioxidants were researched in detail with the view to investigating their protective role for highly unsaturated lipids (Deans et al. 1993).

Centella asiatica (L) Urb (*Apiaceae*), commonly called pennywort or gotu kola, is a perennial creeping weeds commonly found in moist places (Fosberg et al. 1979, Whistler 1988, Van Wky et al. 1997). The composition of essential oil of *Centella asiatica* and other phytochemicals reported by various scientists can lead us to an idea to exploit the essential oil of *Centella sciatica* as an antioxidant in case of lipid oxidation. (Schaneberg et al. 2003, Oyedeji et al. 2005)

In Pakistan *Centella sciatica* is used for wound treatment and to alleviate skin diseases. No earlier reports on antioxidant activity of *Centella asiatica* in vegetable oils are available in context of Pakistan.

Present research work is designed to investigate the antioxidant activity of essential oil of *Cantella asiatica* or brahmi booti. It is very essential to develop natural antioxidants to meet up the challenges and demands especially in case of lipid oxidation.

Material and methods

Refined, bleached and deodorized (RBD) sunflower oil samples were used to investigate the antioxidant activity of essential oil

of *Centella asiatica*. Sunflower oil was selected due to its high use in food as it is a rich source of linoleic acid and it easily can undergo rancidity due to high degree of unsaturation. (Shahidi et al. 1992)

Collection of plant material

The plant material of *Centella asiatica* was collected from Botanical garden of GC University, Lahore, Pakistan in February 2007.

Extraction

Extraction of essential oil was carried out by using steam distillation.500 gm of whole plant material was used for 3 hours to obtain essential oil. Ten Kg of plant was used to collect oil. The oil was filtered through 1mm sieve and stored at 4 c^{o} till use. Percentage yield was 0.18%.

Initial Screening of Antioxidant Potential Essential Oil

DPPH assay on TLC

DPPH assay with TLC was used to measure the antioxidant activity of essential oil. Method of Bektas was followed (Bektas et al. 2005); 1:10 dilution of essential oil was made in methanol. Five microlitres of this dilution was applied on the TLC plate. Plate was developed by methanol and ethyl acetate in ratio of 1:1. Then the plate was sprayed with 0.2% of DPPH reagent in methanol and stayed for 30 min at room temperature. Purple color of DPPH reagent bleaching by yellow spots is the indication of positive antioxidant activity.

Determination of antioxidant activity in sunflower oils

Storage of samples

Seven refined, bleached and deodorized sunflower oil samples (SFO) were stored in triplicate in transparent polyethylene bottles of 250 ml capacity each. Out of total twenty one bottles, seven bottles contained 120 ml blank deodorized, refined and bleached SFO (blank). Other seven bottles contained 200 ppm of BHA per 120 ml deodorized, refined and bleached sunflower oil samples (SFO). Remaining seven bottles contained 200 ppm of essential oil per 120 ml of RBD sunflower oil. (Duh and Yen, 1997)

All these samples were stored at ambient conditions. All the investigations in triplicate were made on weekly basis. Data was analyed by GraphPad Prism 3.0 (Graph Pad Software, Inc.San Diego, USA).

Analysis of rancidity parameters in Sunflower oil

Free fatty acid (FFA) values and Peroxide values (PV) and Iodine values (IV) were determined by following the recommended methods of AOCS (AOCS, 1989). Conjugated dienes (CD) and Conjugated trienes (CT) were determined according to recommended methods of IUPAC (IUPAC 1987). For the determination of Conjugated dienes (CD) and and Conjugated trienes (CT) sunflower oil samples were diluted with iso-octane to bring the absorbance with in the limits. The absorbance was measured at wavelength 232 nm and 268 nm for conjugated diene and triene values respectively (Hitachi, U-2001, Model 7400 spectrometer, Tokyo, Japan IUPAC, 1987). All these parameters were good indicators of lipid oxidation. Many scientists monitored the phenomenon of lipid oxidation to judge the extent of oxidation and antioxidant potential of plant extracts (Anwar et al. 2006, Anwar et al. 2006, Freja et al. 1999, Gulcan et al. 2007).

Results and Discussions

Formation of free fatty acids might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture (Freja et al. 1999). Table 1 shows the changes in Free Fatty Acid values. Free Fatty Acid (FFA) value of blank RBD sunflower oil was found to be 0.055 $\pm$ 0.01. After one week the FFA values were promoted to 0.076$\pm$ 0.001. While the FFA value for the BHA containing sample after one week of storage protocol was found to be 0.058 $\pm$ 0.001. This value was same as essential oil containing sample. After the completion of seven week storage protocol FFA value for blank solution was increased to 0.298 $\pm$ 0.002. This change in FFA contents was significant according to statistical analysis. FFA value for BHA containing

Table 1. Free Fatty Acid Values in Terms of % ages of various RBD sunflower oil samples.

No of weeks	Blank sample	BHA sample	Essential oil samples
1st	0.076 $\pm$ 0.001	0.058± 0.001	0.058± 0.001
2nd	0.095 $\pm$ 0.001	0.061± 0.001	0.062± 0.002
3rd	0.142 $\pm$ 0.002	0.069± 0.003	0.070± 0.001
4th	0.196 $\pm$ 0.002	0.077± 0.001	0.077± 0.001
5th	0.235 $\pm$ 0.003	0.086± 0.001	0.085± 0.002
6th	0.284 $\pm$ 0.001	0.095± 0.002	0.094± 0.003
7th	0.298 $\pm$ 0.002	0.101± 0.002	0.102± 0.002

Original value of blank= 0.058$\pm$ 0.001
t-test significant in case of blank
t-test non-significant in case of BHA and essential oil containing SFO samples.

sample was increased from 0.058 ± 0.001 (1st week value) to 0.101± 0.002 at the end of seven week experimental protocol. Changes in BHA containing RBD sunflower oil samples showed the significant blockage of oxidation phenomenon as compared to blank RBD sunflower oil sample. Similar findings were observed in case of essential oil containing RBD sunflower oil sample. In this case FFA value was jumped from 0.058± 0.001 (1st week value) to 0.102± 0.001(7th week value). These findings explored the strong antioxidant ability of essential oil of *C. asiatica*.

Peroxide value is a widely used measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation. (Gulcan et al. 2007). Changes in Peroxide values are showed in Table 2. Peroxide value of blank RBD sunflower oil sample was 0.85± 0.01. It was increased to 7.01± 0.01 at the end of seven week trial. These changes were significant indicating the noticeable phenomenon of lipid oxidation. Peroxide value of BHA containing RBD sunflower oil was found to be 0.87± 0.01 after one week. It was subjected to 0.97± 0.01 at the completion of seven week analysis. Investigations in case of essential oil containing RBD sunflower oil samples expressed the peroxide value increase from 0.88±0.02 (1st week value) to 0.99±0.01.

Changes in case of BHA and essential oil containing RBD sunflower oil samples were very minor indicating the strong antioxidant activity of BHA and essential oil of *C asiatica*. Decrease in iodine value (IV) is an authentic tool to monitor lipid oxidation (Naz et al. 2004). Magnitude of variation in iodine values was measured according to recommended methods of AOCS (AOCS, 1989). Changes in Iodine values were presented in Table 3.

Iodine value for blank RBD sunflower oil sample was calculated as 141± 1.20. It was subjected to 109± 2.50. These variations were statistically significant. While variation in BHA and essential oil containing RBD sunflower oil samples was not huge indicating the presence of antioxidants in the form of BHA and essential oil. RBD sunflower oil samples having 200ppm

Table 2. Peroxide values (meq kg^{-1}) of various RBD sunflower oil samples.

No of weeks	Blank sample	BHA sample	Essential oil sample
1st	1.10± 0.02	0.87± 0.01	0.88± 0.02
2nd	2.48± 0.01	0.89± 0.02	0.90± 0.01
3rd	3.46± 0.01	0.90± 0.01	0.92± 0.01
4th	4.25± 0.03	0.91± 0.01	0.94± 0.01
5th	5.40± 0.01	0.93± 0.02	0.95± 0.01
6th	6.31± 0.02	0.95± 0.01	0.97± 0.03
7th	7.01± 0.01	0.97± 0.01	0.99± 0.01

Original value of blank = 0.85± 0.01
t-test significant in case of blank
t-test non-significant in case of BHA and essential oil containing SFO samples.

Table 3. Iodine vales.

No of weeks	Blank sample	BHA sample	Essential oil sample
1st	139 ±2.50	140±3.25	140±2.50
2nd	135±3.50	140±3.50	139±3.15
3rd	130±2.50	139±2.50	139±2.50
4th	125±1.50	138±3.05	138±2.25
5th	119±3.25	137±2.03	136±208
6th	113±2.55	136±2.05	134±2.05
7th	109±2.50	134±2.50	132±2.50

Original value of blank = 141± 1.20
t-test significant in case of blank
t-test non-significant in case of BHA and essential oil containing SFO samples.

of BHA showed a change from first week value, 140± 3.25 to 134 ± 2.50 at the ending of experiments. The Iodine values of first and last week for essential oil containing RBD sunflower oil samples were 140 ± 2.50 and 132 ± 2.50 respectively. Iodine values for first two weeks in BHA containing RBD sunflower oil samples were the same (Table 3). Conjugated diene (CD) and conjugated triene (CT) is a good measure of oxidative state of oils (McGinely 1991). Conjugated dienes (CD) and conjugated trienes (CT) were determined by measuring the specific extinction co-efficient at 232nm and 268nm respectively. Samples were diluted with iso-octane to bring the absorbance with in the limits (0.2-0.8) and $\lambda_{1cm}^{1\%}$ was determined by recommended methods of IUPAC (100). Variation trends in CD and CT are represented in Table 4 and 5. Significant increase was observed in CD and CT for controlled SFO. *Centella asiatica* sample exhibited prominent antioxidant worth in sunflower oil, which is highly unsaturated vegetable oil (Shahidi et al. 1992).

Conclusion

Above mentioned analytical investigations reveal that essential oil of *Centella asiatica* is an excellent antioxidant for lipid containing foods. Its activity was quite comparable with the synthetic antioxidant BHA. Antioxidant activity of essential oil was probably due to presence of terpenes and phenolics present in essential oil (Oyedeji et al. 2005). After checking the DNA toxicity of essential oil of *Centella asiatica*, it can be cultivated in Pakistan. This can lead to great health and economic benefits. Further studies can lead us to identify the active antioxidant components of essential oil and can be exploited on commercial scale.

Table 4. Conjugated Dienes in terms of molar extinction co-efficient.

No of weeks	Blank sample	BHA sample	Essential oil sample
1st	0.20 ± 0.01	0.18 ± 0.01	0.17± 0.01
2nd	0.35 ± 0.01	0.26 ± 0.01	0.22± 0.01
3rd	0.51 ± 0.02	0.30 ± 0.01	0.28± 0.02
4th	0.63 ± 0.01	0.36 ± 0.02	0.34± 0.01
5th	0.80 ± 0.01	0.41 ± 0.02	0.40± 0.01
6th	0.99 ± 0.02	0.50 ± 0.01	0.47± 0.02
7th	1.13 ± 0.01	0.57 ± 0.01	0.55± 0.02

Original value of blank = 0.15 ± 0.01
t-test significant in case of blank
t-test non-significant in case of BHA and essential oil containing SFO samples.

Table 5. Conjugated trienes in terms of molar extinction co-efficient.

No of weeks	Blank sample	BHA sample	Essential oil sample
1st	0.22 ± 0.01	0.19 ± 0.01	0.19± 0.01
2nd	0.38 ± 0.02	0.28 ± 0.01	0.27± 0.01
3rd	0.51 ± 0.01	0.35 ± 0.01	0.35± 0.02
4th	0.66 ± 0.01	0.43 ± 0.01	0.44± 0.01
5th	0.86 ± 0.02	0.50 ± 0.01	0.50± 0.01
6th	1.05 ± 0.01	0.61 ± 0.02	0.59± 0.02
7th	1.19 ± 0.01	0.71 ± 0.02	0.69± 0.02

Original value of blank = 0.16 ± 0.01
t-test significant in case of blank
t-test non-significant in case of BHA and essential oil containing SFO samples.

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