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COMPARISON OF ANTIOXIDANT ACTIVITY OF SOME MEDICINALLY IMPORTANT PLANTS FROM PAKISTAN

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ABSTRACT

Background. Chemical composition and medicinal potential of *Centella asiatica* (*C. asiatica*), *Cedrus deodara* (*C. deodara*) and *Artemisia persica* (*A. persica*) prompts the need to investigate antioxidant attributes of these species as alternative source of natural antioxidants. The main aim of the present investigation was to evaluate the antioxidant potential of methanolic extracts of these plants towards stabilization of sunflower oil as oxidation substrate.
Material and methods. Total phenolic and total flavonoid contents, DPPH radical scavenging activity, peroxide value, iodine value, conjugated dienes, trienes and *P*-anisidine value were determined by recommended methods of AOCS.
Results. It was observed that all the plant extracts possessed antioxidant activity, but generalized statement was in favour of *Centella asiatica*. DPPH scavenging activity of *Centella asiatica* extract was just like BHT emphasising the antioxidant potential of *Centella asiatica* in fats and oil containing foods.
Conclusion. *Centella asiatica* extracts can be explored as a source for antioxidant components for food preservation. However, further studies may be carried out to isolate and identify more specific natural and safe antioxidants by combining various analytical techniques.

Key words: antioxidant activity, medicinal plants, Pakistan

INTRODUCTION

The edible oils and fats are generally oxidised in the presence of oxygen and light resulting in formation of reactive oxygen species which have been associated with cancer, cardiovascular diseases, inflation and aging [Siddhuraju and Beeker 2003]. Oxidation phenomenon in fats and fats containing food is the major factor in deterioration and rancidity of food stuff especially during storage [Pezzuto and Park 2002]. Consumption of oxidised lipids is associated with oxidation of biological membranes, genotoxicity and tocopherol inhibition [Sikwese and Duodu 2007]. Such detrimental effects of lipid oxidation are compensated by adding chemicals known as antioxidants. Antioxidants encounter the reactive oxygen species and reduce the risk associated with lipid oxidation. Synthetic antioxidants like butylated hydroxyanisole (BHA) and

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butylated hydroxytoluene (BHT) are frequently used but toxicity of BHA and BHT is a question upon their safety [Ku and Mun 2007]. Tertiary butylhydroquinone (TBHQ) and BHA have been banned and removed from generally recognised as safe (GRAS) list [Farag et al. 1998]. Therefore, an increasing interest is being observed in the search of safe antioxidants [Amakura et al. 2002, Orhan et al. 2003].

Many plants are traditionally used as folk medicines and to increase shelf life of food in many countries [Hulin et al. 1998, Raza et al. 2009, Wójciak et al. 2011, Anwar and Przybylski 2012]. Plants are a good source of natural antioxidants and most of such properties of plant extracts are due to phenolics, flavonoids and essential oils. Previous works of many scientists indicate that it is a necessary step to explore the hidden potential of plants regarding antioxidants [Raza et al. 2009, Rashid et al. 2010, Kapusta-Duch et al. 2012]. Determination of total phenolics (TP) and total flavonoids is a matter of interest to explore the antioxidant activity of plants as TP and TF are widely associated with physiological, antioxidant and pharmaceutical roles respectively [Sultana et al. 2008, Othman et al. 2007]. Phytochemicals present in *Centella asiatica* (*Apiaceae*), *Cedrus deodara* (*Pinaceae*) and *Artemisia persica* (*Asteraceae*) encourage exploring antioxidant potential of these plants [Schaneberg et al. 2003, Oyedeji and Adayan 2005]. Current comparative experimental design evaluated the total flavonoids, phenolics and lipid oxidation inhibition of plant extracts of *Centella asiatica*, *Cedrus deodara* and *Artemisia persica* because of their frequent use in folk medicines in Pakistan.

MATERIAL AND METHODS

Chemicals and reagents

1,1-Diphenyl-2-picryl hydrazyl (DPPH) from Walko chemicals, Japan, BHT from Sigma-Aldrich USA, $NaNO_2$, $AlCl_3$, NaOH and Folin-Ciocalteu reagent. All the chemicals were of analytical grade.

Collection of plant material

Plant materials were collected from northern areas of Pakistan in February 2011 except *Centella asiatica* which was collected from botanical garden of Government College University (GCU), Lahore in March 2011. All the plants were identified from the botanical museum of GCU.

Extract preparation

Both stems and leaves of each plant were air dried at ambient temperature. The dried plant materials were completely ground to powder form and screened using 40 mesh size. Forty grams of ground plant materials were extracted with methanol using Soxhlet apparatus separately for each plant. Extracts were filtered and concentrated to dryness under reduced pressure at 40°C using rotary evaporator. Each extract was resuspended in methanol to make 2 mg/ml stock solutions [Sanchez-Moreno 2002].

Determination of Total Flavonoid Contents (TF) and Total Polyphenol Contents (TP)

The TF contents of all plant extracts were determined by following the method of Zhishen et al. [1999] with little modification. Equal quantities of each sample (0.5 ml) were mixed with 2 ml of deionized water followed by addition of 0.15 ml of 15% $NaNO_2$ solution and stayed for five minutes. Then 0.15 ml of 10% $AlCl_3$ solution was added to each extract and again allowed to stand for 5 minutes followed by 2ml of 4% NaOH solution. Final volume to 5 ml was made by adding double deionized water and allowed to stand for 15 minutes. Absorbance of test mixtures was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at 510 nm against water as blank. Results expression was µg lutin trihydrate/g dried extract. Folin-Ciocalteu method [Slinkard and Singleton 1977] was utilized to determine TP contents of all extracts with Gallic acid as an internal standard. Dilutions of extracts of every plant were made by adding 45 ml of distilled water to 0.5 ml of extracts followed by addition of 1 ml of Folin-Ciocalteu reagent. After three minutes 3 ml of 2% Na_2CO_3 was added and resulting mixture was allowed to stand for 120 minutes with discontinuous shaking. After shaking absorbance was taken at 760 nm and concentration of TP compounds in all extracts was expressed as µg of Gallic acid equivalent per gram of dry matter on UV-1700, Shimadzu, Japan spectrophotometer.

Initial screening of plant extracts for antioxidant activity

DPPH assay on TLC was performed to get the rough idea of antioxidant activity for further experimental proceeding was adopted. According to Bektas et al. [2005], a 1:10 dilution of each methanolic plant extract was made in methanol. Five µl of each dilution was applied to TLC plates and developed with a mixture of methanol and ethyl acetate (1:1). After development of TLC plates, each plate was sprayed with 0.2% of DPPH reagent in methanol and allowed to stand for 30 minutes. Bleaching of purple colour of DPPH reagent by yellowish spots was the indication of positive antioxidant activity.

DPPH free radical scavenging activity

Free radical scavenging activity of plant extracts was determined using method of Miliaukas [Miliauskas et al. 2004]. Dilutions of extracts were made by adding 10 mg of each extract in 10 ml of methanol followed by the addition three ml of 6×10^{-5} M freshly prepared DPPH in methanol. 77 µl of mixture was incubated for 15 minutes at room temperature in dark and absorbance was taken at 515 nm. BHT was used as control under same protocol. % age radical scavenging was calculated using following formula:

$$\text{DPPH radical scavenging (\%)} = [(A_B - A_E)/A_B] \times 100$$

where:

A_B – absorbance of blank sample at t = 0,

A_E – absorbance of tested extracts after 15 minutes incubation.

Antioxidant activity in sunflower oil as oxidation substrate

Refined, bleached and deodorised (RBD) sunflower oil (SFO) were collected and 100 mg of crude plant extracts were added to 120 ml of RBD sunflower oil and stirred for 20 mints at room temperature to homogenise. Samples were stored for 90 days at ambient conditions. A controlled sample without plant extracts was also stored under same conditions. SFO was selected due to its high degree of unsaturation and wide use in cooking. Peroxide value (PV), Iodine value (IV), conjugated dienes (CD), conjugated trienes (CT) and *para*-anisidine value were monitored after every 15 days in triplicate. PV and IV were determined following the recommended methods of AOCS [1989]. For CD and CT IUPAC [IUPAC 1987] methods were followed. Samples were diluted with iso-octane and absorbance was measured at 232 nm and 268 nm respectively for CD and CT using spectrophotometer (Hitachi, U-2001, model 7400, Tokyo, Japan). For *P-anisidine*, samples were treated with *P-anisidine* reagent to generate coloured compound and absorbance was measured at 350 nm (Hitachi, U-2001, model 7400, Tokyo, Japan) by standard method of IUPAC [1987]. All the measurements were carried out in triplicate and statistically analysed by analysis of variance (ANOVA) at 95.0% confidence level using Minitab 16.0.

Percentage inhibition associated with PV was calculated by simple mathematical relationship using the following formula:

$$\text{\% inhibition} = 100 \times (FVc - FVs)/FVc$$

where:

FVc – final value of parameter for control,

FVs – final value of parameter for sample.

RESULTS AND DISCUSSION

Table. Percentage extraction yield

Name of plant	Extract yield, %
Centella asiatica	11
Artemisia persica	09
Cedrus deodara	07

Maximum extract percentage yield was observed in *Centaella asiatica* followed by *Artemisia persica* and *Cedrus deodara*. Extract yield of plants depends upon the availability of various extractable components due to chemical composition and polarity of molecules [Hsu et al. 2006]. Methanol was used as solvent because of its potential to extract maximum amount of extractable material from plants. Polarity of methanol may be the reason regarding extraction efficiency. Moreover, the capability of a solvent to dissolve maximum number of compounds and metabolites present in plants is considered as a key factor in extraction. Some previous reports suggested the use of methanol as solvent for extraction of plant components [Manzoor et al. 2012, Shabir et al. 2011, Sultana et al. 2009].

Total Flavonoid Contents (TF) and Total Polyphenol Contents (TP)

The TP and TF contents are represented in Table 1.

Table 1. Total phenolics (TP) and total flavonoids (TF) in mg/g dried plant extracts

Name of plant	Total phenolics (TP) mg gallic acid/g dried extract	Total flavonoids (TF) mg rutin trihydrate/g dried extract
Centella asiatica	257.3A ±2.00	165.3A ±1.00
Artemisia persica	155.0B ±3.00	109.0B ±1.00
Cedrus deodara	121.7C ±1.00	41.5C ±2.00

Centella asiatica was found as a rich source of biologically active compounds having maximum values for TP and TF contents among all three plants. Although these values may differ for same plants depending upon various soil and environmental factors. *Centella asiatica* was found to consist of high value for both TP and TF among all three plants. Antioxidant activities are highly dependent upon the structure of phenolic and flavanoid compounds. The TP and TF of all plants were significantly varied.

DPPH assay on TLC

Initial screening by DPPH assay on TLC showed that all the plants possessed antioxidant potential. Bleaching of purple colour by yellow colour was observed with all extracts of plants indicated positive antioxidant activity.

DPPH radical scavenging activity

The DPPH assay results as percentage inhibition of lipid oxidation are shown in Table 2. The DPPH free radical scavenging activity exhibited close resemblance with standard BHT.

However, the plant extract of *Centella asiatica* was highly ranked among all plant extracts regarding % inhibition. Statistically talking, there was no significant difference in the activity of BHT and *centella asiatica*.

Table 2. DPPH percentage inhibition of plant extracts in comparison with BHT as standard

Test sample	Percentage inhibition
BHT (Standard)	89.80A
Centella asiatica	88.00A
Artemisia percica	79.70B
Cedrus deodara	66.70C

Effect of antioxidants on various oxidative parameters of SFO

Increase in PV is a good index of lipid oxidation and represents the formation of hydro peroxides during oxidation. PV is widely monitored by scientists working on antioxidants to assess the extent of oxidation [McGinely 1991, Gulcan and Bedia 2007]. Relative increase in PV was represented in Table 3.

Many scientific reports were available on PV measurement of vegetable oils without and with plant extracts to investigate the oxidative deterioration of lipids and antioxidant potential of plants vegetable oils [Chatha et al. 2011, Raza et al. 2009, Anwar et al. 2006]. Increase in PV of control SFO sample was very high compared to all the plant extracts showing that all plants have antioxidant potential. But the comparative studies of PV of SFO samples having plant extracts showed that the extract of *Centella asiatica* was most potent antioxidant followed by *Artemisia percica* and *Cedrus deodara*. The final values at the end of 90 days storage protocol awarded a logical reason to the strongest antioxidant potential of *Centella asiatica* as SFO sample having *Centella asiatica* extract exhibited least increase in PV except BHT but also close resemblance.

Percentage age inhibition of PV augmentation for BHT containing SFO sample was 94.01%, for *Centella asiatica* was 93.66%, for *Cedrus deodara* it was 93.56% and 93.61% for *Artemisia percica* respectively. Relative decrease in IV was shown in Table 4.

Decrease in IV was observed in all plant extracts containing SFO samples but much lower than the CTR sample. Periodic decrease in IV of SFO samples was also found minimum in SFO sample with extract of *Centella asiatica* in comparison with BHT containing SFO sample. At the end of storage protocol the IV

Table 3. Relative increase in PV (meq/kg) for SFO samples stabilised with plant extracts

SPD	CTR	BHT	*Centella asiatica*	*Cedrus deodara*	*Artemisia percica*
15	1.45A ±0.02	0.87B ±0.03	0.87B ±0.02	0.88B ±0.02	0.87B ±0.02
30	3.95A ±0.03	0.92B ±0.02	0.93B ±0.03	0.95B ±0.03	0.95B ±0.03
45	7.08A ±0.04	0.98B ±0.02	0.99B ±0.03	1.10B ±0.03	1.09B ±0.03
60	10.06A ±0.03	1.06B ±0.04	1.11B ±0.04	1.13B ±0.04	1.11B ±0.04
75	15.63A ±0.04	1.13B ±0.04	1.18B ±0.03	1.21B ±0.03	1.19B ±0.03
90	19.88A ±0.04	1.19B ±0.04	1.26B ±0.03	1.28B ±0.03	1.27B ±0.03

Initial value of PV SFO (CTR) at zero time = 0.65 ±0.03.
SPD represents storage period in days.
CTR is used for control.

Table 4. Relative decrease in IV (g I_2/100 g oil) for SFO samples stabilised with plant extracts

SPD	CTR	BHT	*Centella asiatica*	*Cedrus deodara*	*Artemisia percica*
15	143A ±1.04	145B ±1.11	145B ±1.13	144B ±1.02	145B ±1.02
30	139A ±1.03	145B ±1.13	144B ±1.22	143B ±1.03	143B ±1.12
45	133A ±0.94	144B ±0.98	142B ±1.04	139B ±1.81	140B ±0.90
60	128A ±2.11	142B ±1.31	140B ±1.20	137B ±2.01	138B ±1.01
75	124A ±1.25	140B ±1.37	137B ±1.05	134B ±1.52	135B ±1.55
90	120A ±1.34	139B ±1.30	136B ±1.14	129B ±1.26	133B ±1.35

Initial value of IV for SFO (CTR) at zero time = 146 ±1.10.
SPD represents storage period in days.
CTR is used for control.

were found to be 120 ±1.34, 139 ±1.30, 136 ±1.14, 129 ±1.26 and 133 ±1.35 for control, BHT, *Centella asiatica*, *Cedrus deodara* and *Artemisia percica* SFO samples respectively. *Centella asiatica* plant extract containing SFO sample exhibited the highest capability to take a rain check on IV in the vicinity of BHT. The CD and CT is also an important oxidation indicator representing the primary oxidation products [Halliwell and Gutteridge 1985]. Relative increase in CD and CT was shown in Tables 5 and 6 respectively.

The CD of controlled SFO sample at the start of storage period was increased from 0.11 ±0.04 to 2.89 ±0.11 at the end of 90 days storage. The CD at the end of storage period for *Centella asiatica* extract containing SFO was found to be 1.28 ±0.21. The increase in CD for *Centella asiatica* extract containing SFO was in close proximity to BHT value, indicating the maximum antioxidant activity among all plant extracts.

Similar trend in CT and *Centella asiatica* extract was observed and once again found to be most effective source of antioxidants. The *P-anisidine* values were shown in Table 7.

P-anisidine reagent form yellowish complex with aldehydes present in oil as a result of oxidation [Shahidi 1997]. Significant increase was observed in case of control SFO sample. The increase in *P-anisidine* in case of SFO samples with plant extracts seems to be non-significant. The least increase in *P-anisidine* value was observed in SFO sample with BHT followed

Table 5. Relative increase in CD [ε_{1cm} ($\lambda_{232\ nm}$)] of SFO stabilised with plant extracts

SPD	CTR	BHT	*Centella asiatica*	*Cedrus deodara*	*Artemisia percica*
15	0.41A ±0.10	0.31B ±0.10	0.32B ±0.05	0.39B ±0.11	0.25B ±0.03
30	0.76A ±0.12	0.56B ±0.02	0.59B ±0.10	0.63B ±0.04	0.52B ±0.04
45	1.19A ±0.15	0.79B ±0.11	0.83B ±0.16	0.92B ±0.11	0.81B ±0.10
60	1.82A ±0.12	0.92B ±0.10	0.97B ±0.05	1.07B ±0.02	1.02B ±0.11
75	2.42A ±0.21	1.02B ±0.09	1.09B ±0.11	1.25B ±0.03	1.20B ±0.13
90	2.89A ±0.11	1.09B ±0.11	1.18B ±0.21	1.36B ±0.12	1.33B ±0.14

Initial value of CD for SFO (CTR) at zero time = 0.11 ±0.04.
SPD represents storage period in days. CTR is used for control and ±sign indicates standard deviation in triplicate analysis.

Table 6. Relative increase in CT [ε_{1cm} ($\lambda_{268\ nm}$)] of SFO stabilised with plant extracts

SPD	CTR	BHT	*Centella asiatica*	*Cedrus deodara*	*Artemisia percica*
15	0.21A ±0.10	0.11B ±0.10	0.18B ±0.05	0.19B ±0.11	0.19B ±0.03
30	0.47A ±0.12	0.17B ±0.12	0.29B ±0.10	0.32B ±0.04	0.30B ±0.04
45	0.81A ±0.15	0.21B ±0.15	0.38B ±0.16	0.39B ±0.11	0.38B ±0.10
60	1.10A ±0.12	0.26B ±0.12	0.45B ±0.05	0.53B ±0.02	0.51B ±0.11
75	1.54A ±0.21	0.29B ±0.21	0.52B ±0.11	0.69B ±0.03	0.63B ±0.13
90	1.94A ±0.11	0.34B ±0.11	0.62B ±0.21	0.86B ±0.12	0.82B ±0.14

Initial value of CT for SFO (CTR) at zero time = 0.09 ±0.04.
SPD represents storage period in days. CTR is used for control and ±sign indicates standard deviation in triplicate analysis.

Table 7. Relative increase in *P-anisidine* value of SFO samples stabilised with plant extracts

SPD	CTR	BHT	*Centella asiatica*	*Cedrus deodara*	*Artemisia percica*
15	4.56A ±0.02	1.86B ±0.03	2.05C ±0.04	2.54C ±0.44	2.33C ±0.02
30	7.87A ±0.03	2.07B ±0.04	3.44C ±0.03	4.85C ±1.04	3.77C ±0.03
45	11.76A ±0.11	2.26B ±0.10	4.41C ±0.02	5.98C ±0.11	5.33C ±1.03
60	18.45A ±0.05	2.45B ±0.08	6.35C ±0.03	7.03C ±0.13	6..85C ±0.05
75	26.93A ±0.04	2.59B ±0.05	8.88C ±0.03	9.92C ±0.12	9.01C ±1.40
90	38.40A ±0.03	2.70B ±0.12	11.33C ±0.03	12.20C ±0.06	11.80C ±1.50

Initial value of CT for SFO (CTR) at zero time = 1.51 ±0.02.
SPD represents storage period in days. CTR is used for control and ±sign indicates standard deviation in triplicate analysis.

by SFO sample having extract of *Centella asiatica*. The antioxidant potential of plant extracts might be due to the presence of flavonoids and phenolics present in extracts. Table 1 showed high TF and TP contents of plant extracts. The TF and TP contents of *Centella aistica* were maximum [Pittela et al. 2009] hence showed maximum antioxidant activity with potential to fight against the primary and secondary antioxidant products in vegetable oils. Statistical analysis showed that there was significant difference between the control SFO and all other plant extract containing SFO samples including BHT sample. A relationship can be established between antioxidant potential of plants and bioactive compounds present in plants. Apparently, there is no significant difference in antioxidant activities of all three plant extracts, but a generalised trend is established in the favour of *Centella asiatica* regarding antioxidant activity.

CONCLUSION

The above mentioned results and discussion highlighted the hidden potential of antioxidant activity of plants. All the plants were found to be a good source of natural antioxidants however, an aerial comparison of results emphasised that *Centella asiatica* being the richest source of antioxidants. The study can be extended regarding isolation, purification and identification of bioactive compounds. It should be further analysed to identify the bioactive compounds through fractionation combined with modern analytical techniques. *Centella asiatica* as indigenous plant to Pakistan should be used both as salad and/or in the form of its extract during cooking.

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