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Variation in Antioxidant Activity and Phenolic and Flavonoid Contents in the Flowers and Leaves of Ghaneri (*Lantana camara* L.) as Affected by Different Extraction Solvents

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Abstract: The current study reports variation in antioxidant activity and phenolic and flavonoid contents in the flowers and leaves of a medicinal herb Ghaneri (*Lantana camara* L.) as affected by different extraction solvents (80% methanol, 80% ethanol, absolute methanol and absolute ethanol). The antioxidant activity was assessed by measuring the reducing power, inhibition in linoleic acid peroxidation and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging activity. Of the extracts tested, flower extract produced by 80% methanol possessed maximum amount of total phenolics (21.4 g GAE/100 g DW), total flavonoids (13.8 g CE/100 g DW), reducing power (0.095 for extract concentration of 1.0 mg mL⁻¹), inhibition of linoleic acid peroxidation (83.3%) and DPPH radical scavenging activity (72.3%). A significant difference was observed for phenolic and flavonoid contents and antioxidant activity in relation to different extraction solvents and plant parts analyzed (p<0.05) thus prompting the need for utilization of appropriate solvent media and material for isolation of maximum amount of antioxidants. The results of this study explore the potential of *L. camara* as a rich source of natural antioxidants for development of functional foods and nutraceutical applications.

Key words: *Lantana camara*, solvent extracts, phenolics compounds, antioxidant activities, radical scavengers, reducing power

INTRODUCTION

It is widely perceived that free radicals such as superoxide anion (O⁻²), hydroxyl radical (•OH) and peroxy radical (ROO•), generated as result of oxidation, are linked with several chronic health diseases such as, cancer, inflammation, aging and atherosclerosis (Conforti *et al.*, 2008; Moulehi *et al.*, 2012). In order to suppress the process of lipid oxidation, antioxidants have gained much recognition (Silva *et al.*, 2007). Growing evidence has shown an inverse correlation between the intake of dietary antioxidants and reduced risk of chronic diseases and aging (Saeed *et al.*, 2005; Amensour *et al.*, 2010). However, the applications of most widely used synthetic antioxidants in the food industry, for example butylated

hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been restricted recently because of their safety and carcinogenic potential (Ksouri *et al.*, 2009; Borneo *et al.*, 2009).

Traditionally, plants have been used as a source of food and phytomedicine to treat various diseases (Gilani and Atta-ur-Rahman, 2005; Khan *et al.*, 2012). In fact, many medicinal plants are known to be an excellent source of bioactives with multiple biological and antioxidant properties (Borneo *et al.*, 2009; Alam *et al.*, 2012). The health promoting effects of plant-based antioxidants are attributable to their protective effects by counteracting reactive oxygen species (Muanda *et al.*, 2011; Yusufoglu and Alqasoumi, 2011). A variety of antioxidants such as, tocopherols, phenolic acids and

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flavonoids have been detected in a variety of agricultural and food products including cereals, fruits, vegetables, herbs and oil seeds (Netzel *et al.*, 2007). Plant phenolics are the most important class of natural compounds which have been extensively studied because of their powerful anti-carcinogenic and antioxidant potential among others (Liu *et al.*, 2009; Amensour *et al.*, 2010).

The phenolic contents and antioxidant activity of medicinal plants and herbal extracts have been widely investigated (Katalinic *et al.*, 2006; Ilahi *et al.*, 2013). According to Djeridane *et al.* (2006) ethanol extractable components from *Artemisia campestris* L. and *Artemisia arvensis* L. contain appreciable amount of phenolics and free radical scavengers. According to Silva *et al.* (2007) methanol and ethanol extracts from leaf, bark, fruits and seeds of the species namely *Byrsonima crassifolia*, *Bauhinia macrostahya*, *Cecropia palmata*, *C. odorata*, *C. exaltata*, *D. kunthii*, *Davill arugosa*, *D. subcymosa*, *Inga edulis* and *Stryphnodedron barbadetimam* are potential sources of antioxidants. Amensour *et al.* (2010) reported that methanol and ethanol extracts from the leaves of Rockrose (*Cistus ladaniferus*) is a potential source of free radical scavengers and antioxidants.

Several of the phytochemical related studies reveal that the contents and distribution of phenolic compounds in plant extracts vary depending upon the nature of extraction media and plant material/parts (leaves, flowers, bark, seeds and fruits) used. Methanol and ethanol, separately or as aqueous mixture, are mostly used to extract phenolic compounds from plant materials (Zielinski and Kozłowska, 2000). Sajid *et al.* (2012) investigated that antioxidant activity and phenolic contents of *Pongamia pinnata* (L.) Pierre varied notably in relation to different parts (bark, leaves and seeds) and extraction solvents employed. Of the solvents used, aqueous methanol was established to be the most effective solvent to recover higher amount of phenolics from the bark of *P. pinnata*. Similarly, Shabir *et al.* (2011) reported that 80% methanol extracts of leaves had higher antioxidant activity and phenolics compared to the extracts from flowers and bark of Gulmohar (*Delonix regia*) (Bojer ex Hook.). Ahmad *et al.* (2011) also revealed considerable variation in total phenolic and total flavonoid contents among different solvent extracts of *Calotropis procera* (Ait.) Ait. F. Meanwhile, Manzoor *et al.* (2012) appraised significant variation in phenolic contents and antioxidant activity between peel and pulp of peach (*Prunus persica* L.) fruit using aqueous methanol (80% methanol).

Lantana camara L. (Ghaneri), belonging to family *Verbenaceae*, is commonly known as wild or red sage. It is an evergreen aromatic shrub grown in tropics,

sub-tropics and warm temperate regions of the world as a popular ornamental hybrid (Ghisalberti, 2000). It grows up to a height of 1-2 m and occurs luxuriously in elevations up to 2000 m (Bhakta and Ganjewala, 2009).

A wide range of medicinal properties of this plant have been reported in the literature which include the treatment of itches, cuts, ulcers, swellings, bilious fever, cataract, eczema and rheumatism (Abdulla *et al.*, 2009). The leaf extracts of *L. camara* var. *aculeata* have been studied for their phytochemical constituents and termiticidal effects against adult termite workers (Verma and Verma, 2006). Lancamarone, a steroid from the leaves possesses cardiogenic activity while lantamine, an alkaloid derived from the bark and roots has been shown to possess strong antipyretic and antispasmodic properties comparable with those of quinine (Sharma *et al.*, 2000; Bhakta and Ganjewala, 2009).

Even though some reports are available in the literature on the phytochemical investigations and biological activities of *L. camara*, however, detailed studies on the antioxidant compounds and antioxidant activity of different parts of this species in relation to extraction media are missing. Thus, the present study was conducted to appraise variation in phenolics and antioxidant properties of flowers and leaves of locally grown *L. camara* as affected by different extraction solvents.

MATERIALS AND METHODS

Sample collection: Samples of flowers and leaves of a red flowering variety of *L. camara* were collected from the vicinity of the University of Agriculture, Faisalabad, Pakistan. The specimens were further identified and authenticated by Dr. Mansoor Hameed (Taxonomist, Department of Botany, University of Agriculture, Faisalabad). Hot air-dried samples were processed for further analyses.

Reagents and standards: Linoleic acid (99.0%), BHT (99.0%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), catechin (98.0%), gallic acid (98.0%) and Folin-Ciocalteu reagent were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and purchased from Merck (Darmstadt, Germany).

Preparation of extracts: The dried samples of flowers and leaves were ground using a commercial blender (TSK-949, Westpoint, France) and were passed through an 80 mesh sieve. The ground material (20 g each) was extracted with 200 mL each of the solvents; absolute methanol (99.9% purity), absolute ethanol (99.8% purity), 80% methanol (absolute methanol: water 80:20 v/v) and

80% ethanol (absolute ethanol: water 80:20 v/v) for 8 h at room temperature in an orbital shaker (Gallenkamp, UK). The extract was separated from the residue by filtering through Whatman No. 1 filter paper. The residue was extracted twice with the same fresh solvent and then three extractions pooled. The excess solvent was removed under vacuum (45°C) using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd., Tokyo, Japan). The crude extract was weighed to determine the percent yield and then stored at 4°C, until used for analyses (Sultana *et al.*, 2007).

Determination of total phenolics content (TPC):

Amount of total phenolics was assessed using Folin-Ciocalteu reagent as described previously (Sultana *et al.*, 2007). Briefly, 50 mg of each of the extract were mixed with half mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was placed at room temperature for 10 min; 1.5 mL of 20% sodium carbonate (w/v) was added followed by heating the mixture in a water bath at 40°C for 20 min and then cooling it in an ice bath. Subsequently the absorbance was recorded at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amount of total phenolics was calculated using gallic acid calibration curve ($R^2 = 0.9980$) constructed by analyzing a range (10-100 ppm) of standard solutions. The data (results) were expressed as Gallic Acid Equivalents (GAE) g/100 g of dry weight (DW).

Determination of total flavonoid contents (TFC):

TFC were measured following a previously reported method (Dewanto *et al.*, 2002). Briefly, 1 mL plant extract solution (containing 0.1 mg extract material mL⁻¹) was mixed with 4 mL water in a 10 mL volumetric flask. Initially, 0.3 mL of 5% NaNO₂ was added to the volumetric flask; at 5 min, 0.3 mL of 10% AlCl₃ was added; at 6 min, 2 mL of 1.0 M NaOH were added. The extract solution was further diluted with water (2.4 mL). Absorbance of the final mixture was measured at 510 nm. TFC were determined as Catechin Equivalents (CE) g/100 g of DW).

DPPH scavenging assay: The antioxidant activity of the extracts was assessed by measuring their ability to scavenge DPPH free radicals according to a method as described by Bozin *et al.* (2006). The extract samples (with concentration varying between 0.5 and 100 µg mL⁻¹) were mixed with 1 mL of 90 µM DPPH solution and made up with 95% methanol, to a final volume of 4 mL. The synthetic antioxidant, BHT, was used as positive control. After 1 h incubation period at room temperature, the absorbance was recorded at 515 nm. DPPH scavenging capacity (%) was calculated using the equation as follows:

$$\text{DPPH scavenging (\%)} = \frac{A_b - A_s}{A_b} \times 100$$

where, A_b is the absorbance of the control reaction (containing all reagents except the test compounds) and A_s is the absorbance of the test compounds.

Determination of antioxidant activity in linoleic acid system:

The antioxidant activity of the extracts was also determined by measuring the inhibition of linoleic acid peroxidation (Iqbal *et al.*, 2005). Five milligram of extract was added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7). The mixture was diluted up to 25 mL volume by distilled water and incubated at 40°C up to 360 h. Extent of oxidation was measured by peroxide value using the thiocyanate method as described by Yen *et al.* (2000). Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of aqueous solution of ammonium thiocyanate (30% w/v), 0.2 mL of sample solution and 0.2 mL of ferrous chloride (FeCl₂) solution (20 mM in 3.5% HCl; v/v) were added sequentially. After 3 min of stirring, the absorption was measured at 500 nm. A control sample containing all reagents but without the sample extracts was also prepared. The synthetic antioxidant BHT was used as a positive control. Percent inhibition of linoleic acid peroxidation was calculated with the following equation:

$$100 - \frac{\text{Abs. increase of sample at 360 h}}{\text{Abs. increase of control at 360 h}} \times 100$$

Determination of reducing power:

The antioxidant activity of the extracts was also evaluated by measuring their ability to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺). The reducing potential was assessed by monitoring the change in color of the test solution from yellow to bluish green depending upon the concentration of reductant species in the sample extracts. The intensity of bluish green color directly correlated with the reducing potential and thus indicating antioxidant activity of the compounds present in the sample (Yen *et al.*, 2000). Briefly, in this test the extract (2.5-10.0 mg) was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50°C for 20 min. Then 5 mL of 10% trichloroacetic acid was added and the mixture centrifuged at 980 g for 10 min at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 mL) was taken and diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) and the absorbance was noted at 700 nm.

Statistical analysis: Three samples for each of the flowers and leaves of *L. camara* were assayed. Each sample was analyzed individually in triplicate and data reported as mean and SD ($n = 3 \times 3 = 9$). Data were analyzed using two way analysis of variance (ANOVA) using MINITAB 2000 Version 13.2 statistical software (MINITAB Inc. Pennsylvania, U.S.A) at 5% significant level.

RESULTS AND DISCUSSION

Yield of extracts: Data for percent yield of extractable antioxidant components from flowers and leaves of *L. camara* are shown in Table 1. The extract yield from flowers and leaves with different extraction solvents ranged from 36.4-48.9% and 34.7-41.1%, respectively. Among different extraction solvents, the highest yields were achieved from flowers with 80% methanol while the lowest by absolute ethanol. Likewise, percent yield of leaves extract was highest with 80% methanol (43.2%) while lowest (36.4%) for absolute ethanol. Earlier Ali-Emmanuel *et al.* (2003) reported percent yield of *L. camara* alcoholic extract to be 12.7% which is much lower than our result. In the present study, the highest extract yield was obtained in aqueous-methanol, indicating greater efficacy of this solvent system to solubilize and recover greater amount of extractable antioxidant components from the test plant materials.

Total phenolic contents: Total phenolic contents of *L. camara* flowers and leaves extracts are shown in Table 2. The present results depicted somewhat significant ($p < 0.05$) variation of total phenolic contents in relation to two parts and the extraction solvents employed. The extracts from flowers

(12.1-21.4 GAE/100 g DW) have higher TPC compared with leaves (11.7-18.4 GAE/100 g DW) indicating that variations exist for distribution of such antioxidant compounds among different parts of the same plant. Among the different solvents employed, 80% methanol and 80% ethanol were found to be the most while absolute ethanol the least efficient for extraction of TP from leaves (17.9-18.4 g GAE/100 g DW) and flowers (19.5-21.4 g GAE/100 g DW). Methanol (80%) extract of flowers possessed the higher TPC (21.4 g GAE/100 g DW) compared to the leaves extract (18.4 g GAE/100 g DW) which may account for the stronger antioxidant activity of the former compared with the latter. This variation of TP in relation to two parts of the plant tested and the extraction solvents employed might be explained due to the reason that recovery of phenolic compounds is influenced by the type of plant material, the chemical nature of the extractable compounds as well as the effectiveness of extraction solvents to solubilize such compounds (Shabir *et al.*, 2011; Sajid *et al.*, 2012).

It could be understandable from the present data that among the solvent systems used, aqueous mixtures of solvents (80% methanol and 80% ethanol) have shown greater efficacy towards extraction of TP from both the plant parts tested. Meanwhile, methanol (80%) extracts, although not statistically different, have exhibited slightly higher contents of TP compared with aqueous ethanol extracts. Considering this little numerical difference of TP observed between the two aqueous solvent systems, it could be advocated that aqueous mixtures of both the solvents are good to extract phenolic compounds from this species and the little numerical differences observed is practically not significant. Hence, aqueous ethanol can be considered as the solvent of choice for isolation of phenolics from this species since it is less toxic than its counter part.

According to extensive studies, acetone, methanol and ethanol in pure form or mixed with water in different proportions are commonly used to extract bioactive compounds from plant materials, depending on their chemical nature and the intended applications (Dai and Mumper, 2010; Shabir *et al.*, 2011; Sajid *et al.*, 2012). Interestingly, ethanol/water formulations/extracts as ingredients of functional foods or nutraceuticals are safe for human consumption as compared with other organic solvent extracts. In the herbal medicine industry, ethanol extraction is commonly employed to yield crude phytochemicals extracts from plants. Hence, to maximize recovery of plant phenolics for human consumption, establishment of optimal and specific extraction strategies using binary solvent system of ethanol and water would be worthwhile (Wang *et al.*, 2011; Wendakoon *et al.*, 2012).

Table 1: Percent yield of different solvent extracts from flowers and leaves of *L. camara*

Solvent extract	Flowers	Leaves
80% methanol	48.97±1.24 ^{Aa}	43.25±1.18 ^{Ab}
80% ethanol	45.73±1.37 ^{Aa}	41.12±1.11 ^{Ab}
Absolute methanol	40.50±1.22 ^{Ba}	39.97±1.46 ^{Bb}
Absolute ethanol	36.45±1.09 ^{Ca}	34.72±1.38 ^{Ca}

Values are Mean±SD of three samples analyzed individually in triplicate at $p < 0.05$. Capital superscript within the same column indicate significant difference ($p < 0.05$) among extracting solvents. Small superscripts within the same row indicate significant difference ($p < 0.05$) between the plant parts

Table 2: Total phenolic contents (g/100 g DW) of extracts from flowers and leaves of *L. camara*

Solvent extracts	Flowers	Leaves
80% methanol	21.45±0.43 ^{Aa}	18.37±0.49 ^{Ab}
80% ethanol	19.54±0.39 ^{Aa}	17.95±0.49 ^{Ba}
Absolute methanol	18.37±0.55 ^{Ba}	12.48±0.49 ^{Bb}
Absolute ethanol	12.12±0.49 ^{Ca}	11.75±0.29 ^{Ba}

Values are Mean±SD of three samples analyzed individually in triplicate at $p < 0.05$. Capital superscripts within the same column indicate significant difference ($p < 0.05$) among extracting solvents. Small superscripts within the same row indicate significant difference ($p < 0.05$) between the plant parts

The extraction of bioactive compounds from plant materials is the first step towards ascertaining the potential utilization of phytochemical extracts in the preparation of functional food, dietary supplements or nutraceuticals, cosmetics and pharmaceutical products. The extraction yield of plant phenolics as well as their antioxidant efficacy not only depend upon plant part/type, genetic make up of species, agroclimatic conditions, harvest time, post-harvest processing, nevertheless it is strongly affected by the chemical nature of extractable compounds as well as the nature of extraction media employed (Dai and Mumper, 2010; Wendakoon *et al.*, 2012). Several literature studies reveal that aqueous organic mixtures of solvents such as 80% methanol and 80% ethanol because of their appropriateness, higher polarity and ability to solubilize wide range of polar bioactives, are more effective, among others, to extract natural antioxidants such as phenolic acids and flavonoids from different plant materials (Sultana *et al.*, 2007; Mohammadi and Atik, 2011; Shabir *et al.*, 2011; Sajid *et al.*, 2012).

Due to the variation in composition of active compounds, different plant materials may require different concentrations of methanol and ethanol to achieve maximum recovery of bioactive components (Mohammadi and Atik, 2011; Shabir *et al.*, 2011; Sajid *et al.*, 2012). In the present experiments, the use of 20% water in alcoholic solvents was due to the reason that phenolic extracts of plants are mixture of different classes of phenols which are selectively soluble in these polar binary solvent mixtures. Inclusion of water as co-solvent can be used to enhance the polarity of liquid phase. The use of mixture containing an alcohol and water present the advantage of modulating the polarity of alcoholic solvents. The solubility of phenolics depends mainly on the hydroxyl groups, the molecular size and the length of hydrocarbon so water alcoholic mixtures become ideal and selective to solubilize and extract greater number of bioactive compounds from plant materials (Mohammadi and Atik, 2011). Besides, the use of water as co-solvent would be valuable when the toxicity and cost aspects of extraction procedure are considered (Wang *et al.*, 2011).

On the other hand, for flowers and leaves, the magnitude of effectiveness of aqueous and absolute solvents used is statistically varied, meaning that water has considerable influence on the values of TPC. Meanwhile, when applied ethanol (80%) and absolute ethanol, there is no statistical significance between the results for leaves. This could be explained by the fact that phenolics yield is dependent upon their chemical nature

as well as the nature/type of plant material. It seems that the nature of the phenolic compounds extracted by methanol and ethanol is different. Though we did not make any detailed analysis about the individual compounds of the different extracts produced from *L. camara*, probably the composition of extracted phenolics in both of the solvents is different.

No previous reports are available in the literature on the total phenolic contents of *L. camara* flowers and leaves to compare with the data of the present study. However, similar investigations on other medicinal herbs/plants reported significant concentration of phenolic compounds in such materials. Morales *et al.* (2008) investigated total phenolic contents to be 72.5 g GAE/100 g in the extracts of *Acantholippia deserticola*; these values are much higher in comparison with those of *L. camara*. Phenolic contents in the present analysis of *L. camara* are comparable to those determined for *Capparis atamisguea* (8.2-10.0 g GAE/100 g) (Borneo *et al.*, 2009) and higher than those in *Vitex doniana* (0.2-7.6 GAE g/100 DW) (Muanda *et al.*, 2011). On the other hand, the amount of total phenolics presently found in *L. camara* was lower than those in methanol and ethanol extracts of leaves of *Capparis ladaniferus* (18.4 g/100 g DW) (Amensour *et al.*, 2010).

Total flavonoid contents: The total flavonoids were determined calorimetrically. Flavonoids are one of the most important polyphenols which are characterized by a common structural feature consisting of two phenolic rings connected via an oxygenated heterocyclic pyran ring. These compounds are widely distributed in different physiological parts of plants, especially in flowers, fruits and leaves and account for almost 60% of the total dietary phenolics (Shahidi and Naczki, 2004). Among different classes of flavonoids, flavonols i.e., quercetin, myricetin and kaempferol are most important due to their physiological functions, antioxidant properties and health benefits (Shahidi and Naczki, 2004). Multiple combinations of different types of groups such as hydroxyl, sugars, oxygen and methyl attached with the basic flavonoids moiety produce various classes of flavonoids such as flavanols, flavanones, flavones and flavonols and anthocyanins and thus prompt the need to extract them from plant materials using preferably polar solvents.

The amount of total flavonoids in the flowers and leaves extracts of *L. camara* varied from 8.6-13.8 g CE/100 g DW (Table 3) indicating significant ($p < 0.05$) variation with regard to the extraction solvents

Table 3: Total flavonoid contents (g/100 g DW) of extracts from flowers and leaves of *L. camara*

Solvent extracts	Flowers	Leaves
80% methanol	13.76±0.27 ^{ba}	13.41±0.40 ^{ba}
80% ethanol	10.43±0.21 ^{ba}	10.28±0.36 ^{ba}
Absolute methanol	9.80±0.36 ^{ca}	8.76±0.35 ^{ca}
Absolute ethanol	8.76±0.35 ^{ca}	8.62±0.32 ^{ca}

Values are Mean±SD of three samples analyzed individually in triplicate at p<0.05. Capital superscripts within the same column indicate significant difference (p<0.05) among extracting solvents. Small superscripts within the same row indicate significant difference (p<0.05) between the plant parts

but showed non significant differences between the two parts tested. As such no previous reports on TFC of *L. camara* were found to compare the present data, nevertheless, these levels were comparable with that of another medicinally important species namely *Vitex doniana* (0.7-12.3 g CE/100 g DW) as reported by Muanda *et al.* (2011).

Among the different solvent extracts, 80% methanol extract of flowers showed the highest concentration of TFC (13.8 g CE/100 g DW) while lowest amount (8.6 g CE/100 g DW) of these compounds was observed in absolute ethanol extract of leaves. The overall efficacy order of different extraction solvents used in the present study towards recovery of TFC from *L. camara* flowers and leaves was found to be: 80% methanol > 80% ethanol > absolute methanol > absolute ethanol. Within the solvent systems, aqueous alcoholic solvents (80% methanol and 80% ethanol) were found to be relatively more effective compared with their absolute counterparts. These trends are in agreement with several studies that reveal that binary mixture of absolute methanol and ethanol with water, because of their higher polarity, compatibility and solubilization efficacy can be relatively more effective to extract flavonoids from different plant materials (Sultana *et al.*, 2007; Mohammedi and Atik, 2011; Shabir *et al.*, 2011; Sajid *et al.*, 2012). While looking into the present trends recorded, it seems that the flavonoid compounds present in the leaves and flowers of *L. camara*, due to their specific structural features, are less soluble in absolute alcohols as against their aqueous mixtures resulting in the recovery of significantly higher amounts into the aqueous alcohols. For optimum recovery of plant bioactives, the chemical nature of the compounds to be extracted should be compatible with the nature of extraction solvent (Dai and Mumper, 2010; Wendakoon *et al.*, 2012).

DPPH radical scavenging activity: DPPH, with deep violet color, is a stable organic free radical and gives colorimetric absorption maxima at 515-528 nm. As a part of redox reaction, upon receiving proton from any hydrogen

Table 4: DPPH radical scavenging activity (%) of extracts from flowers and leaves of *L. camara*

Solvent extracts	Flowers	Leaves
80% methanol	72.30±1.44 ^{aa}	64.61±1.93 ^{bb}
80% ethanol	67.07±1.46 ^{ba}	55.38±1.66 ^{cb}
Absolute methanol	70.69±1.55 ^{aa}	61.53±1.53 ^{bb}
Absolute ethanol	58.46±1.75 ^{ca}	49.00±1.40 ^{cb}

Values are Mean±SD of three samples analyzed individually in triplicate at p<0.05. Capital superscripts within the same column indicate significant difference (p<0.05) among extracting solvents. Small superscripts within the same row indicate significant difference (p<0.05) between the plant parts

donor species, such as phenolics, it loses its chromophore resulting into yellow color. Generally, it is assumed that as the concentration of phenolics or the degree of hydroxylation of the phenolic compounds increases DPPH radical scavenging activity increases and with it antioxidant activity (Sanchez-Moreno *et al.*, 1999).

The extracts derived from *L. camara* flowers and leaves exhibited appreciable activity towards scavenging DPPH free radicals with contribution 58.4-72.3 and 49.0-64.6%, respectively (Table 4). The present results indicated significantly (p<0.05) higher DPPH radical scavenging activity of 80% methanol extract of flowers and leaves compared with other solvent extracts while least activity was offered by absolute ethanol extracts of the plant parts tested. A lower DPPH radical scavenging activity exhibited by the absolute ethanol extracts can be linked to lesser amounts of phenolics in these extracts.

Regardless of the choice of solvents, of the two parts tested, the flower extracts exhibited relatively higher DPPH radical scavenging activity depicting their greater ability to scavenge free radicals compared with the leaf extracts. A higher DPPH radical scavenging capacity of the flower extracts can be in due part to the presence of higher amounts of phenolics in this part. It has been reported that plant phenolics mostly act as antioxidants by exercising their hydrogen-donating properties due to their structural features and hydroxyl groups (Siddhuraju *et al.*, 2002; Aberoumand and Deokule, 2008). The presently noted DPPH radical scavenging activity for methanolic extract of *L. camara* leaves (61.5%) is comparable to previously reported values (61.0%) by Bhakta and Ganjewala (2009). Another related study on methanol and ethanol extracts of *C. ladaniferus* leaves showed inhibition of DPPH radicals by 87.7 and 50.1%, respectively (Amensour *et al.*, 2010).

The overall efficacy order of different extraction solvents used in the present study towards DPPH radical scavenging capacity of *L. camara* flowers and leaves extracts was found to be: 80% methanol > absolute methanol > 80% ethanol > absolute ethanol, indicating notable variations in relation to extraction media.

Interestingly, no significant variations in DPPH radical scavenging activity was observed between 80% methanol and absolute methanol flowers extracts which means that in this typical case, a water content of 20% doesn't matter, being different for ethanol. This can be explained by the fact that not only phenolics possibly some other compounds may also have acted as DPPH free radical scavengers (Sultana *et al.*, 2007). This also suggests that water activity in a binary solvent mixture towards phenolics extraction is also alcohol dependent but still the DPPH scavenging capacity of methanolic extracts of the tested plant parts is superior to the ethanolic extracts.

Reducing power: Measurement of reducing potential can also be used to explain antioxidant activity of the plant extracts. This chemical assay, involves the reduction of ferric ions into ferrous ions resulting change in color from yellow to bluish green. The color intensity, of the complex formed, depends on the redox potential of the antioxidant compounds present in the reaction media. A greater color intensity and thus higher absorption is related to greater antioxidant activity of the extracts (Zou *et al.*, 2004). Table 5 shows reducing potential of different solvent extracts (concentration range of 0.2-1.0 mg mL⁻¹) of *L. camara* flowers and leaves. In the current study, a regular pattern of increasing trend in reducing power with increase in extracts concentration was observed. The reducing potential of different solvent extracts at 1.0 mg mL⁻¹, in terms of absorbance data, ranged from 0.045-0.095 for flowers and 0.038-0.075 for leaves indicating notable (p<0.05) variations among the

Table 5: Reducing powers (expressed as absorbance values at 700 nm) of extracts from flowers and leaves of *L. camara*

Solvent extracts	Concentration (mg mL ⁻¹)		
		Flowers	Leaves
80% methanol	0.2	0.044±0.002 ^{CDa}	0.035±0.003 ^{Cb}
	0.6	0.062±0.001 ^{BCa}	0.051±0.002 ^{Bb}
	0.8	0.078±0.003 ^{ABa}	0.060±0.002 ^{ABb}
	1.0	0.095±0.004 ^{Aa}	0.075±0.003 ^{Ab}
80% ethanol	0.2	0.037±0.002 ^{CDa}	0.027±0.002 ^{Cb}
	0.6	0.055±0.003 ^{Ca}	0.041±0.002 ^{Bcb}
	0.8	0.069±0.002 ^{Ba}	0.055±0.004 ^{Bb}
	1.0	0.085±0.001 ^{ABa}	0.060±0.003 ^{ABb}
Absolute methanol	0.2	0.025±0.002 ^{Da}	0.014±0.002 ^{Db}
	0.6	0.036±0.004 ^{Da}	0.025±0.003 ^{Cb}
	0.8	0.045±0.003 ^{CDa}	0.035±0.002 ^{Cb}
	1.0	0.050±0.002 ^{Ca}	0.045±0.002 ^{Bca}
Absolute ethanol	0.2	0.018±0.002 ^{Ea}	0.012±0.002 ^{Da}
	0.6	0.025±0.001 ^{DEa}	0.020±0.002 ^{CDa}
	0.8	0.035±0.002 ^{Da}	0.031±0.004 ^{Ca}
	1.0	0.045±0.003 ^{CDa}	0.038±0.002 ^{Bb}

Values are Mean±SD of three samples analyzed individually in triplicate at p<0.05. Capital superscripts within the same column indicate significant difference (p<0.05) among extracting solvents. Small superscripts within the same row indicate significant difference (p<0.05) between the plant parts

extraction solvents employed. Of the extracts tested, maximum absorbance value (0.095) was recorded for 80% methanol extracts of flowers while, minimum (0.038) for absolute ethanol extract of leaves. The efficacy order of different extraction solvents used in the present study towards reducing potential of *L. camara* flowers and leaves extracts was found to be: 80% methanol>80% ethanol>absolute methanol>absolute ethanol, indicating notable variations in relation to extraction solvents. Overall, these trends of reducing potential are in line to the results of other antioxidant assays and amounts of phenolics and flavonoids determined. In a previous study conducted by Bhakta and Ganjewala (2009), the reducing power of methanol extracts of *L. camara* was found to be lower than that observed in the present study.

Antioxidant activity in linoleic acid system: The antioxidant principles of plant extracts can also be explained as their ability to inhibit lipid peroxidation. Therefore, the antioxidant activity of the flowers and leaves extracts of *L. camara* was evaluated by monitoring their potential towards inhibition of peroxidation of linoleic acid (Yen *et al.*, 2000). In this test, linoleic acid, upon oxidation produces peroxides which oxidize Fe²⁺ to Fe³⁺. The ferric ion generated thus reacts with SCN⁻ to forming a complex, the concentration of which is determined colorimetrically by recording the absorbance at 500 nm. A higher absorbance is linked with higher concentration of peroxides formed during the reaction, consequently it relates to lower antioxidant activity.

The data related to peroxidation inhibition potential of flower and leave extracts of *L. camara* after incubation period of 360 h is given in Table 6. BHT was used as a positive control to compare the results of this assay. The level of inhibition of linoleic acid peroxidation of the extracts from flowers and leaves was found to be 69.5-83.3 and 67.7-80.5%, respectively indicating significant differences (p<0.05) in relation to plant parts. As expected, in line with the results of other assays, 80% methanol flowers and leaves extracts were found to be most effective to inhibit peroxidation (80.5-83.3%) among others that can be ascribed to the presence of higher concentration of phenolics in these extracts.

Table 6: Percent inhibition of linoleic acid peroxidation of extracts from flowers and leaves of *L. camara*

Solvent extracts	Flowers	Leaves	BHT
80% methanol	83.3±2.12 ^{ab}	80.50±2.01 ^{Ab}	-
80% ethanol	74.09±2.12 ^{Ba}	68.52±1.88 ^{Bb}	90.82±2.14
Absolute methanol	71.30±1.43 ^{Ba}	68.38±1.71 ^{Bb}	-
Absolute ethanol	69.49±1.89 ^{Ca}	67.68±1.35 ^{Bb}	-

Values are Mean±SD of three samples analyzed individually in triplicate at p<0.05. Capital superscripts within the same column indicate significant difference (p<0.05) among extracting solvents. Small superscripts within the same row indicate significant difference (p<0.05) between the plant parts

The variations of peroxidation inhibition data in relation to extraction solvents were more pronounced in case of flower extracts while the leave extracts produced by 80% ethanol, absolute methanol and absolute ethanol revealed insignificant differences. As is depicted, for linoleic acid peroxidation, the result's tendency is somewhat different from the DPPH and reducing power assay. This random antioxidant tendency could be explained in due part to the reason that the antioxidant mechanism in this assays is different from those of other assays employed for antioxidant activity assessment. A weak correlation of percent inhibition with phenolics as well as other assays can also be explicated based on the fact that in addition to phenolics and radical scavengers, certain supplementary compounds, for example, carotenoids and tocopherols etc., also contributed towards percent inhibition of linoleic acid peroxidation (Karadeniz *et al.*, 2005).

All the extracts tested exhibited lower inhibition of peroxidation than that exhibited by synthetic antioxidant BHT (90.8%). In the present analysis, 80% methanol soluble fractions of *L. camara*, although exhibited about 11% less inhibition of linoleic acid peroxidation compared with that by BHT, it reflected a very strong antioxidant potential of this species. In view of this information, it is worthwhile to isolate and purify active compounds from this species to further explore their potential uses as ingredients for development of some functional foods, nutraceuticals and pharmaceuticals.

Correlation of different antioxidant assays with total phenolic and flavonoid contents: It is widely accepted that antioxidant activity of plant materials is strongly linked to the concentration of total phenolics and flavonoids. With this in mind, the results of different antioxidant assays such as, DPPH radical scavenging capacity, measurement of reducing power and inhibition of linoleic acid peroxidation, were correlated with the total phenolic and total flavonoid contents (Fig. 1a-f). A good correlation ($R^2 = 0.810$) between total phenolics and DPPH radical scavenging activity was observed (Fig. 1a) which indicates that phenolics mainly act as free radical scavengers. Plant phenolics may function as chain breakers, free radical scavengers and electron donors. In support to our findings, Aydemir and Becerik (2011) reported a positive correlation between DPPH free radical scavenging activity and total phenolic compounds. Similarly, Wijngaard *et al.* (2009) also recorded a fair correlation ($r = 0.72$) between total phenolics and DPPH radical scavenging capacity of different vegetable and fruits byproducts. On the other hand, a weak correlation ($R^2 = 0.451$) was observed between total flavonoid

contents and DPPH scavenging capacity (Fig. 1d). This may be linked to the assumption that most of the flavonoids in *L. camara* flowers and leaves were in their glycoside form and thus were less effective as compared to their respective aglycone forms (Shahidi and Naczki, 2004). This weak correlation between total flavonoid and DPPH radical capacity is in agreement with the earlier findings (Nickavar *et al.*, 2007).

A very strong and significant linear correlation ($R^2 = 0.935$) was observed between total phenolic contents and reducing power (Fig. 1b) indicating that phenolics mainly contributed to the redox potential of *L. camara* through their electron donor properties. In agreement to our present findings, Li *et al.* (2009) also reported a strong linear correlation between reducing power and total phenolic contents. As expected, a good correlation ($R^2 = 0.847$) was also observed between total flavonoids and reducing power (Fig. 1e). The good correlation between TFC and reducing power can be explained by the reason that compounds like quercetin glycosides, anthocyanins and flavonols might have exhibited higher redox potential.

A weak correlation ($R^2 = 0.582$) was observed between percent inhibition and total phenolics (Fig. 1c) which could be explained on the basis that not only the phenolics but some other compounds such as, tocopherols and carotenoids, also impart antioxidant activity in terms of measurement of percent inhibition of linoleic acid peroxidation (Karadeniz *et al.*, 2005). Similarly, a weak correlation was recorded by Sultana *et al.* (2007) during the investigation of corn cob extracts. In contrast, a strong positive correlation ($R^2 = 0.980$) between total flavonoids and percent inhibition of linoleic acid peroxidation was observed (Fig. 1f) which might have been due to the reason that flavonoid compounds showed oxygen scavenging as well as chelating activity, contributing towards higher correlation as compared to phenolics. These results are in line with the findings of Shabir *et al.* (2011), who also reported a strong correlation between total flavonoids and percent inhibition.

Overall, these correlations confirm that the phenolic compounds are the main constituents contributing to the antioxidant activity of these extracts, though, contribution of some other phytochemicals such as, triterpene, steroids and terpenoids cannot be ruled out. It is also possible that the synergistic effect among the bio-actives lead to increase in overall antioxidant activity of the plant materials. A random correlation of total phenolics and total flavonoids with some assays can be explained on the basis that structure-activity relationship of phenolic and flavonoids is very important and probably, there might have been some phenolics/flavonoids which have

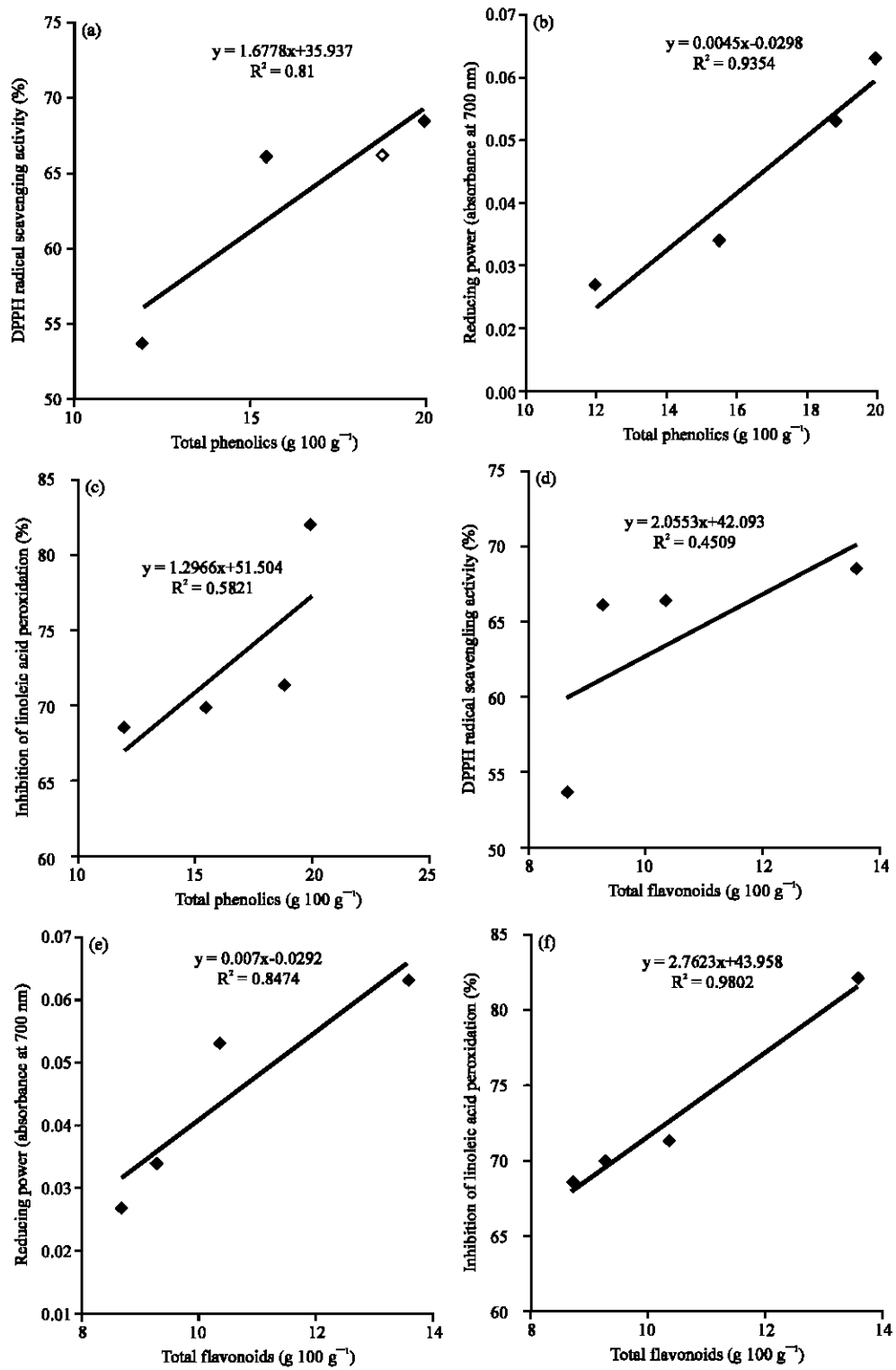


Fig. 1(a-f): Relationship of total phenolics with antioxidant activity, (a) DPPH, (b) Reducing power, (c) Inhibition of linoleic acid peroxidation and total flavonoids with antioxidant activity, (d) DPPH, (e) Reducing power and (f) Inhibition of linoleic peroxidation of extracts from *L. camara*

antioxidant activity and the other do not, or that their actions occur through different mechanisms. Hence, observing considerable variation for correlation among different antioxidant assays, it could be suggested that multiple antioxidant assays should be employed to fully study the antioxidant principles of plant materials.

CONCLUSION

The results of this study have shown significant variation in phenolic compounds and antioxidant activity as a function of extraction solvents and the parts used and this prompted the need to optimize extraction strategies for gaining maximal nutritional benefits of plant extracts. Overall, the extracts derived from flowers and leaves of *L. camara* using 80% methanol possessed higher contents of phenolics and superior antioxidant activity. On the basis of the present investigation, this medicinal herb can be explored as a viable source for the isolation of natural antioxidants and high-valued bioactives which may serve as leads for the isolation of new antioxidants and bioactives for development of functional foods/nutraceutical and pharmaceuticals.

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