

Full Length Research Paper

Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of akk [*Calotropis procera* (Ait.) Ait. F.]

Naveed Ahmad^{1,2}, Farooq Anwar^{1*}, Sohail Hameed² and Mary C. Boyce³

¹Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan.

²National Institute for Biotechnology and Genetic Engineering, (NIBGE), P. O. Box 577 Jhang Road Faisalabad, Pakistan.

³School of Natural Sciences, Edith Cowan University, Perth, Western Australia.

Accepted 6 July, 2011

In the present study antioxidant and antimicrobial activities of extracts from leaves and flowers of local Akk (*Calotropis procera*) were examined. The antioxidant components were extracted using 80% aqueous methanol, 80% aqueous ethanol and 80% aqueous acetone solvents. The antioxidant yield from leaves and flowers of *C. procera* ranged from 8.48 to 14.07 g/100 g dry weight. The total phenolic and flavanoid content were considerable with total phenolic yields (expressed as gallic acid equivalents) reported in the range of 0.11 to 0.32 g/100 g dry weight, and total flavonoid content (expressed as catechin equivalents) reported in the range 0.01 to 0.10 g/100 g dry weight. *C. procera* extracts exhibited a reasonable DPPH radical scavenging activity (IC₅₀ 8.81 to 37.30 mg/ml) and inhibition of linoleic acid peroxidation (13.63 to 41.53%). Antimicrobial activity of the extracts was evaluated against selected strains of bacteria and pathogenic fungi. The present results revealed that among the samples analyzed, maximum antioxidant and antimicrobial activities were exhibited by 80% ethanol extract of leaves whereas lowest by 80% acetone extract of flowers. From the results of the present investigation, it could be concluded that *C. procera* extracts can be explored as a potential source for isolation of antioxidant and antimicrobial agents. However, an appropriate solvent extraction system should be used to recover potent antioxidant components from *C. procera*.

Key words: Akk extracts, solvent extraction, phenolic components, antioxidant assays, antimicrobial potential, microorganisms.

INTRODUCTION

Reactive oxygen species and free radicals formed during oxidation have been reported to contribute to diseases such as cancer, diabetes, cardiovascular diseases and ageing (Halliwell and Gutteridge, 1999). Antioxidants have the ability to protect the body from oxidative damage (Ozsoy et al., 2008) by scavenging the free radicals and inhibiting peroxidation and other radical-mediated processes. Although a number of synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) are commercially available,

due to safety concerns, their use is often restricted in the food industry (Liu and Yao, 2007). In the recent years, significant attention has been directed towards exploring plant-based natural antioxidants, especially the phenolics and tocopherols (Chaovanalikit, 2004; Katalinic, 2006; Aneta, 2007). Such natural antioxidants are not only reported to have anti-carcinogenic potential and protect the foods from oxidative deterioration, nevertheless these are also associated with other health beneficial effects, such as lowering the incidence of aging, inflammation, cardiovascular diseases and certain cancers (Frankel, 1995; Katalinic, 2006; Iqbal et al., 2007; Liu and Yao, 2007).

On the other hand, human health has serious threats being faced due to intake of food contaminated with food-borne microorganisms. The presence of these

*Corresponding author. E-mail: fqanwar@yahoo.com. Tel: 92-41-9200161-67. Ext. 3309. Fax: 92-41-9200764.

microorganisms can mess up the food stuff and reduce the quality of processed foods (Soliman and Badaea, 2002). To develop the microbial resistance, it is necessary to search new potential effective substances against these pathogenic microorganisms, which steers to antibiotic formation.

It is widely accepted that some biologically or chemically active compounds isolated from plant materials have ability to inhibit the growth of pathogenic organism (Farnsworth, 1966; Levens et al., 1979; Kuhnt et al., 1994; Meyer and Afolayan, 1995; Hussain et al., 2008, 2011). *Calotropis procera*, a member of the family Asclepiadaceae, locally known as "akk", is an erect, much branched perennial shrub with milky latex throughout the parts (Khanzada et al., 2008; Meena et al., 2010). The plant, commonly known as giant milkweed, is also known by several other names such as sodom apple, calotrope, French cotton and small crown flower (English) (Howard, 1989; Liogier, 1995; Parrotta, 2001). The species, a native of West Africa is now widely distributed and found in parts of the American, Asian and Australian continents (Rahman and Wilcock, 1991).

A comprehensive review on traditional and medicinal uses and the phyto-chemistry of *C. procera* is published by Ahmed et al. (2005). As such the plant has been extensively employed to cure several diseases like leprosy, ulcers, bronchial asthma and skin infections, piles and disorders of the spleen, liver and abdomen in the native medicine systems of Sudan, Unan, Arab and India (Watt and Breyer-Brandwisk, 1962; Kartikar and Basu, 1994).

Latex, derived from this plant, has been reported to be used as an abortifacient (The Wealth of India, 1950). Similarly, a multitude of biological properties, for example, spasmogenic, antidyseric, antisyphilitic, anti-rheumatic, antifungal, mullusccide, diaphoretic have been ascribed to different parts of this useful plant (Sharma, 1934; Watt and Breyer-Brandwisk, 1962; El-Badwi, 1997). However, there has been no detailed examination of its antioxidant and antimicrobial activity. The purpose of the present investigation was to evaluate the antioxidant and antimicrobial activities of the extracts from selected parts of *C. procera* grown in the central Punjab region of Pakistan using different extraction solvents.

MATERIALS AND METHODS

Collection and pretreatment of plant material

Samples of leaves and flowers were assayed from the fully mature plants of *C. procera* grown in the vicinity of National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Pakistan.

The specimens were identified and authenticated by a taxonomist at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The specimens were cut into small pieces and then ambient air-dried and stored in polythene bags at -4°C.

Chemicals and reagents

Linoleic acid, 2,2-diphenyl-1-picrylhydrazyl, gallic acid, Folin-Ciocalteu reagent, ascorbic acid, trichloro-acetic acid, sodium nitrite, aluminium chloride, ammonium thiocyanate, ferrous chloride, ferric chloride, potassium ferricyanate and butylated hydroxytoluene (99.0%), were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals (analytical grade), that is, anhydrous sodium carbonate, ferrous chloride, ammonium thiocyanate, chloroform and methanol, used in this study were procured from Merck (Darmstadt, Germany). All culture media and standard antibiotic discs were procured from Oxoid Ltd. (Hampshire, UK).

Extraction from plant materials

The dried leaves and flowers material were ground into a powder (80 mesh) in a grinding mill (Tector-Cemotec 1090 sample mill, Hognas, Sweden). The ground material (20 g) were separately extracted with 200 ml of 80% methanol (80:20, methanol: water), 80% ethanol (80:20, ethanol: water) and 80% acetone (80:20, acetone: water) by using an orbital shaker (Gallenkamp, UK) for 8 h at room temperature. The extracts were filtered through Whatman No.1 filter paper to isolate the insoluble residues. The residues were re-extracted twice with fresh aliquots of the same solvent and three extracts for each solvent combined. The solvent was removed under vacuum at 45°C, using a rotary evaporator (N-N series, Rikakikai Co. Ltd, Tokyo, Japan) and the resulting residue stored at -4°C until used for further analyses.

Antioxidant activity of plant extracts

Determination of total phenolic contents (TPC)

The amount of TP was assessed using Folin-Ciocalteu reagent procedure with a slight modification as described by Chaovanalikit and Wrolstad (2004). The dried residue was reconstituted in water (1 mg/ml) and 1.0 ml mixed with 0.5 ml of Folin-Ciocalteu reagent and 7.5 ml deionized water. After 10 min at room temperature 1.5 ml of 20% (w/v) sodium carbonate was added. The mixture was heated in a water bath at 40°C for 20 min and then cooled in an ice bath. The absorbance was recorded at 755 nm using a spectrophotometer (Hitachi U-2001, model 121-0032). Gallic acid standards (5 to 100 ppm) were treated in a similar manner. TPC was calculated relative to gallic acid and therefore expressed as gallic acid equivalents (GAE) per gram of dry residue. All samples were analyzed in triplicate.

Determination of total flavonoid contents (TFC)

TF contents were determined following the procedure by Dewanto et al. (2002). The dried residue was reconstituted in water (100 mg/ml) and 1.0 ml was placed in a 10 ml volumetric flask. Distilled water (5.0 ml) was added followed by 0.3 ml of 5% NaNO₂. After 5 min, 0.6 ml of 10% AlCl₃ was added and 5 min later, 2 ml of 1 M NaOH was added and volume made up with distilled water. The solution was thoroughly mixed and the absorbance was read at 510 nm. A similar approach was adopted for the catechin standards (5 to 100 ppm). The amount of TF was calculated using a calibration curve for catechin (5 to 100 ppm) and expressed as catechin equivalents (CE) per dry matter. All samples were analyzed in triplicate and results averaged.

Antioxidant activity determination in linoleic acid system

The antioxidant activity of extracts was determined by measuring

the % inhibition of peroxidation in a linoleic acid system as described by Osawa and Namiki (1981) with a little modification. The dried extracts (5 mg) for each treatment was added to a solution containing linoleic acid (0.13 ml), 99.8% ethanol (10 ml) and 0.2 M sodium phosphate buffer, pH 7, (10.0 ml). The resulting mixture was then diluted to 25.0 ml with distilled water. The solution was incubated at 40°C and the degree of oxidation was measured following thiocyanate method (Yen et al., 2000). Briefly, 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml of sample solution and 0.2 ml of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. After stirring (3 min), the absorption was measured at 500 nm. A control was performed with linoleic acid but without extracts. Synthetic antioxidants; butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) were used as a positive control. The maximum peroxidation level observed as 432 h (18 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation was calculated to express antioxidant activity. Percent inhibition of linoleic acid peroxidation:

$$= 100 - [(Abs. \text{ increase of sample at 360 h} / Abs. \text{ increase of control at 432 h}) - 100]$$

Determination of reducing power

The reducing power of the extracts was determined according to the procedure described by Yen et al. (2000). Equivalent volume of plant extract containing 12.5 to 50.0 mg of dry matter 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 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 diluted with 5.0 ml of distilled water and ferric chloride (1.0 ml, 0.1%), and absorbance was read at 700 nm (Hitachi U-2001). The measurements were run in triplicate and the results were averaged.

DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically as described by Tepe et al. (2005). Aliquots (50 µl) of various concentrations (0.10 to 5.0 mg/ml) of the extracts were added to 5 ml of 0.004% methanol solution of DPPH. After incubating the samples for 30 min at room temperature, the absorbance was read against a blank at 517 nm:

$$I(\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}})$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the tested compound) and A_{sample} is the absorbance of the tested compound. Extract concentration providing 50% inhibition (IC_{50}) was determined from a graph plotting percentage inhibition against extract concentration.

Antimicrobial activity

Microorganisms tested

C. procera leaves and flowers extracts were individually tested against a set of microorganisms, including Gram positive bacteria *Bacillus pumilus* JF313263, Gram negative bacteria *Escherichia coli* DH5 α , pathogenic fungal strain *Aspergillus niger* and *Fusarium oxysporum*. The pure bacterial and fungal strains were obtained

from NIBGE, Faisalabad, Pakistan. The purity and identity of the strains were further verified by the Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan. Bacterial strains were grown overnight at 37°C in nutrient agar (NA, Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 30°C using potato dextrose agar (PDA, Oxoid).

Disc diffusion method

The antimicrobial activity of *C. procera* leaves and flower extracts was assessed by agar disc diffusion method (National Committee for Clinical Laboratory Standards, 1997). The discs (6 mm in diameter) were saturated with 15 µl extracts of 100 mg/ml concentration placed on the inoculated agar. Amoxycillin (25 to 100 mg/ml/disc) (Oxoid) and Flumequine (25 to 100 mg/ml/disc) (Oxoid) were used as positive control for bacteria and fungi, respectively. Disc without samples was used as a negative control. Antimicrobial activity was calculated by measuring the inhibition zone.

Micro-dilution broth method

Determination of minimum inhibitory concentration (MIC), which represents the concentration of extracts that completely inhibits the growth of microorganisms, was done following micro dilution broth susceptibility assay, as reported by NCCLS (1999). A series of dilutions were prepared in the range 5 to 100 mg/ml of extract in a 96-well microtitre plate, including one growth control (NB/SDB + Tween 80) and one sterility control (NB/SDB + Tween 80 + test oil). 160 µl NB and SDB for bacteria and fungi, respectively, were added onto the micro plates with 20 µl of the tested solution. A 20 µl of the suspension of tested microorganisms containing 5×10^5 colony-forming units CFU/ml (confirmed by viable count) of standard microorganism was inoculated onto the micro-plates. The plates were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi. Amoxycillin and Flumequine were used as positive control for bacteria and fungi, respectively. The growth was indicated by the presence of a white 'pellet' on the well bottom. The MIC was calculated as the highest dilution showing complete inhibition of the tested strains.

Statistical analysis

Three different samples of flowers and leaves *C. procera* were assayed. All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. Statistically significance difference was denoted by probability value of $p \leq 0.05$. All data are presented as mean values \pm standard deviation (SD) for triplicated determinations.

RESULTS AND DISCUSSION

Antioxidant yield

The antioxidant yield (g/100 g dry weight) from the leaves and flowers of *C. procera* extracted with aqueous methanol (80%), aqueous ethanol (80%) and aqueous acetone (80%) are shown in Table 1. The antioxidant yield from leaf material varied significantly ($p < 0.05$) with the extracting solvent used. For example, aqueous

Table 1. Extract yields (g/100 g of dry weight) from *C. procera* leaves and flowers.

Extracts	Leaves	Flowers
80% Methanol	10.87 ± 0.32 ^b _b	13.10 ^{ab} ± 0.39 ^b _a
80% Ethanol	12.06 ± 0.24 ^a _b	14.07 ± 0.28 ^a _a
80% Acetone	8.73 ± 0.17 ^c _a	8.48 ± 0.25 ^c _a

Values are mean ± SD for three different samples of each part analyzed individually in triplicate. Different superscript letters within the same column indicate significant ($p < 0.05$) differences of means among the extracting solvents; Subscript letters within the same row indicate significant ($p < 0.05$) differences of means between the plant parts.

Table 2. Total phenolic content (GAE g/100 g of dry matter) and total flavonoid contents (CE g/100 g of dry matter) of *C. procera* leaves and flowers extracts.

Extracts	Total phenolics		Total flavonoids	
	Leaves	Flowers	Leaves	Flowers
80% Methanol	0.15 ± 0.01 ^{ab} _b	0.21 ± 0.01 ^b _a	0.05 ± 0.02 ^{ab} _a	0.07 ± 0.01 ^{ab} _a
80% Ethanol	0.20 ± 0.02 ^a _b	0.32 ± 0.01 ^a _a	0.08 ± 0.01 ^a _a	0.10 ± 0.01 ^a _a
80% Acetone	0.11 ± 0.02 ^b _a	0.15 ± 0.02 ^c _a	0.01 ± 0.01 ^b _a	0.03 ± 0.02 ^b _a

Values are mean ± SD for three different samples of each part analyzed individually in triplicate. Different superscript letters within the same column indicate significant ($p < 0.05$) differences of means among the extracting solvents; Subscript letters within the same row indicate significant ($p < 0.05$) differences of means between the plant parts.

ethanol gave the maximum yield at 12.06 g/100 g dry weight while aqueous acetone gave the poorest yield at 8.73 g/100 g dry weight. For the flower extractions with the same solvents, while aqueous ethanol extracted the most antioxidants it was not significantly greater than that extracted by aqueous methanol. The higher yields from leaves and flowers with 80% ethanol revealed greater efficacy of this solvent towards recovering antioxidant components from these materials. The present results also depicted a significant ($p < 0.05$) difference of the extract yields between the two plant parts tested, especially, when aqueous methanol and ethanol were used, which might be linked to the availability of different extractable antioxidant components into the solvents.

Ahmad et al. (2009) reported the extraction yield with 90% ethanol from the leaves of *C. procera*, to be 14.17 g/100 g, higher than that determined in the present study with 80% ethanol. Such differences in the extracts yield might be attributed to the availability of different extractable components as well as to the nature of soil and agro-climatic conditions of the regions from where the plant tissues harvested (Hsu et al., 2006).

Our results regarding higher antioxidant extract yield with ethanol are in agreement with the findings of Oktay et al. (2003) who examined higher extract yields from fennel (*Foeniculum vulgare*) seeds with ethanol. Amount of antioxidant components that can be extracted from a plant material depends on different factors such as chemistry and availability of extractable plant components and nature of extracting solvent, as well as extraction techniques employed (Hsu et al., 2006).

Total phenolic (TP) and flavonoid (TF) contents

Plant phenolics are gaining continuing interest as ingredients for functional foods and nutraceutical applications due to their potential health benefits (Aneta et al., 2007). Phenolic compounds from plants are known to act as very good natural antioxidants (Awika et al., 2003). Many studies reported that total phenols and flavonoids contribute significantly to the antioxidant activities of fruits and vegetable (Katalinic et al., 2006). The amounts of total phenolics (TP) and total flavonoid (TF) in the leaves and flowers extracts of *C. procera*, varying from 0.11 to 0.32 GAE (g/100 g) and 0.01 to 0.10 CE (g/100 g) respectively, are given in Table 2. The amount of phenolic components extracted from leaves and flowers using different solvents varied significantly ($p < 0.05$) with regard to the extraction solvent and material used. Ethanol extract (80%) of flowers showed the maximum contents of TP and TF 0.32 and 0.10 g/100 g, respectively. Ethanol is usually preferred for the extraction of antioxidant compounds from plant matrices mainly due to its lower toxicity and good extraction efficacy (Karadeniz et al., 2005; Tung et al., 2007).

This difference in the amount of TP and TF may be due to varied efficiency of the extracting solvents to dissolve endogenous antioxidant compounds of the tissues analyzed. The ability of different solvents to extract TP and TF followed the order: 80% ethanol > 80% methanol > 80% acetone. Of the two parts of the tested plant, flowers were found to contain relatively higher amount of phenolics ($p < 0.05$) and flavonoids ($p > 0.05$). Differences

Table 3. DPPH radical scavenging activity (IC₅₀ value) and inhibition of linoleic acid peroxidation (%) of *C. procera* leaves and flowers extracts.

Extracts	IC ₅₀ Value (mg/ml)		% inhibition of linoleic acid oxidation	
	Leaves	Flowers	Leaves	Flowers
80% Methanol	14.36 ± 0.28 ^b _a	11.19 ± 0.23 ^b _b	26.13 ± 0.52 ^b _b	32.44 ± 0.64 ^b _a
80% Ethanol	11.86 ± 0.35 ^c _a	8.81 ± 0.17 ^c _b	30.84 ± 0.61 ^a _b	41.53 ± 0.83 ^a _a
80% Acetone	37.30 ± 0.75 ^a _a	29.68 ± 0.59 ^a _b	13.63 ± 0.41 ^c _b	17.7 ± 0.53 ^c _a

Values are mean ± SD for three different samples of each part analyzed individually in triplicate. Different superscript letters within the same column indicate significant ($p < 0.05$) differences of means among the extracting solvents; Subscript letters within the same row indicate significant ($p < 0.05$) differences of means between the plant parts.

in the amounts of TP and TF in the two parts tested could be explained by the fact that presence of phenolics is affected by plant tissues, maturity at harvest, growing conditions, soil conditions and post-harvest treatment (Jaffery et al., 2003).

No earlier reports are available on the total phenolics and flavonoids contents of *C. procera* flowers extract with which to compare the results of our present analysis. However, according to Ramesh et al. (2009), total phenolics and flavonoids in methanol (80%) extract of leaves of *C. procera* from India were determined to be 3.8 and 2.1 mg/g, respectively. These amounts are quite low compared to our present data. In another study by Patel et al. (2010), the amount of TP 14.94 mg/g determined in the leaves of *C. procera* were found to be comparable to our result of 80% methanol extract while total flavonoids (28.05 mg/g) were found to be higher than our present data. Such variations of TP and TF among different studies might be ascribed to the use of different extraction solvent and as well as to the varied agro-climatic factors of the regions from where the plant materials were harvested.

DPPH radical scavenging assay

DPPH is a very stable organic free radical with deep violet color which gives absorption maxima within the 515 to 528 nm. Upon receiving proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and became yellow. It is widely accepted that as the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, DPPH radical scavenging activity and hence antioxidant activity of a plant extract or a related compound also increases (Sanchez-Moreno et al., 1999).

C. procera leaves and flower extracts exhibited good radical scavenging activity, with IC₅₀ (the extract concentration which scavenged 50% of the DPPH free radicals) values ranging from 11.86 to 37.30 mg/ml and 8.81 to 29.68 mg/ml, respectively (Table 3). Ethanol extract of flowers showed the lowest IC₅₀ value of 8.81 mg/ml corresponding to the highest free radical scavenging activity and thus antioxidant activity. The

present results indicated significantly ($p < 0.05$) higher radical scavenging activity of ethanolic flower extract compared with other solvent extracts. Among the extracts tested, acetone extract from leaves showed the highest IC₅₀ value, 37.30 mg/ml, indicating its minimum potential to scavenge free radicals, most probably due to presence of less amount of phenolics compared to other extracts. This is also evident from the results of Table 2 that acetone extracts had low amount of TP. Free radical scavenging activity of different extracts followed the order: 80% ethanol extract > 80% methanol extract > 80% acetone extract. Of the two tissues tested, the flowers extracts relatively exhibited lower IC₅₀ value, showing their better ability to scavenge free radicals than the leaves extract. On the other hand, all the tested extracts offered lower antioxidant activity, when compared with a synthetic antioxidant BHT. The DPPH radical scavenging capacity of the tested extracts might be explained in due part to the presence of phenolic components (Siddhuraju et al., 2002).

No earlier reports are available in the literature regarding the DPPH radical scavenging activity of *C. procera* flower extract with which to compare the results of our present experiment. However, DPPH radical scavenging activity (IC₅₀ value 0.255 mg/ml) of methanol (80%) extract from leaves of *C. procera* from India as investigated by Ramesh et al. (2009) was found to be lower than that of 80% methanol leaf extract in our present analysis.

Antioxidant activity in linoleic acid system

Linoleic acid, a polyunsaturated fatty acid (PUFA) upon oxidation produces peroxides which oxidize Fe²⁺ to Fe³⁺ and the later forms complex with SCN⁻. The intensity of the color of the complex formed corresponding to the concentration is read spectrophotometrically by measuring absorbance at 500 nm. A higher concentration of peroxides formed during reaction offers a higher absorbance value correlating to lower antioxidant activity.

The antioxidant activity of the leaves and flowers extracts of *C. procera* was also assessed by monitoring their ability to inhibit lipid peroxidation (Table 3). The level

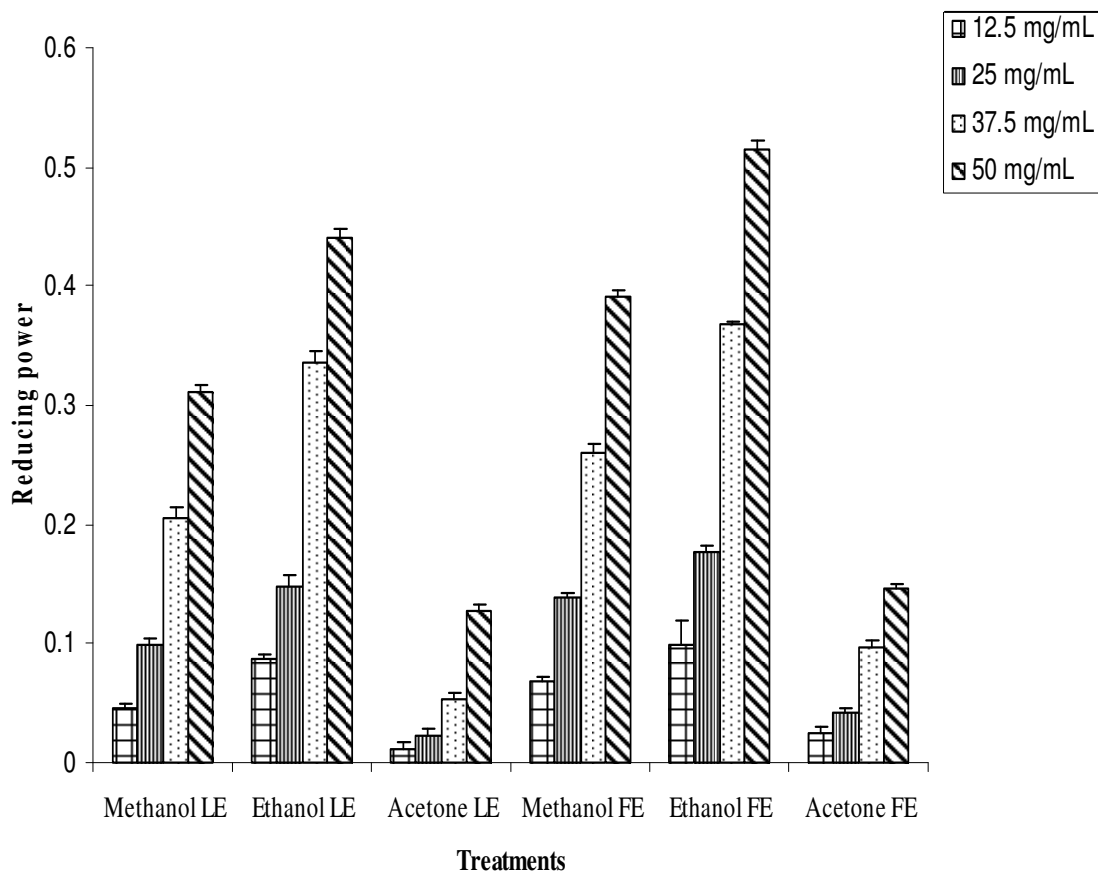


Figure 1. Reducing power (absorbance data at 700 nm) of *C. procera* leaves and flowers extracts. LE: Leaves extract, FE: Flower extract.

of inhibition of linoleic acid oxidation by leaves and flowers extracts were found to be moderate, 13.63 to 30.84% and 17.71 to 41.53%, respectively. As expected 80% ethanolic extract of leaves and flowers offered higher inhibition of peroxidation (41.53%) than other extracts which might be attributed to the presence of higher amount of phenolic compounds in these extracts. Flowers extracts generally exhibited superior activity towards inhibition of linoleic acid oxidation than the leaves, revealing their better antioxidant activity. All the tested extracts exhibited much lower inhibition of linoleic acid peroxidation than that given by BHT (92.01%). The order of inhibition of linoleic acid oxidation offered by leaves and flowers extracts of *C. procera* was noted to be as: 80% ethanol > 80% methanol > 80% acetone. Due to lack of data on the percentage inhibition of linoleic acid peroxidation of *C. procera* leaves and flower, we could not compare present results with literature.

Reducing power of extract

Measurement of reducing potential can also be used to

express antioxidant activity of the plant extracts. In this assay ferric ions are reduced to ferrous ions with change in color from yellow to bluish green. The intensity of the color depends on the reducing potential of the antioxidant compounds present in the extract. Greater the intensity of the color, greater will be the absorption; consequently, greater will be the antioxidant activity (Zou et al., 2004).

The reducing power of bioactive compounds is strongly related to the antioxidant activity (Siddhuraju et al., 2002; Yildirim et al., 2001). In the present work, a regular pattern of increase in reducing power as a function of extract concentration was observed for almost all the tested samples (Figure 1). The reducing potential of the leaves and flowers extracts was measured for concentrations ranging between 12.5 to 50 mg/ml, the consequent absorbance values recorded were in the range of 0.01 to 0.52. The maximum absorbance value (0.52) was recorded for 80% ethanol extract of flower, while the minimum (0.01) for 80% acetone extract of leaves. The reducing power of different extracts varied statistically ($p < 0.05$). We did not find any earlier study on the reducing power of *C. procera* leaves and flower extracts with which to compare the data of the present

Table 4. Antimicrobial activity of *C. procera* leaves and flowers extracts.

Parameter	Zone of inhibition (mm) against tested organisms							
	<i>Bacillus pumilis</i>		<i>Escherichia coli</i>		<i>Aspergillus niger</i>		<i>Fusarium oxysporum</i>	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
80% Ethanol extract	19.20 ± 0.25 ^b _a	17.60 ± 0.42 ^b _b	14.90 ± 0.31 ^b _a	12.30 ± 0.27 ^b _b	5.30 ± 0.21 ^b _a	3.90 ± 0.18 ^{bc} _b	7.70 ± 0.26 ^b _a	5.80 ± 0.19 ^{ab} _b
80% Methanol extract	17.40 ± 0.45 ^c _a	14.70 ± 0.36 ^c _b	12.70 ± 0.31 ^c _a	10.90 ± 0.29 ^c _b	4.90 ± 0.18 ^{bc} _a	3.20 ± 0.21 ^c _b	6.40 ± 0.17 ^c _a	6.10 ± 0.23 ^{ab} _a
80% Acetone extract	10.20 ± 0.24 ^d _a	9.80 ± 0.31 ^d _a	9.80 ± 0.27 ^d _a	7.60 ± 0.29 ^d _b	4.20 ± 0.12 ^c _b	5.70 ± 0.21 ^b _a	5.50 ± 0.16 ^d _a	4.30 ± 0.26 ^b _b
Amoxicillin	22.70 ± 0.56 ^a		17.90 ± 0.43 ^a		-		-	
Flumequine	-		-		28.70 ^a ± 0.71		24.20 ^a ± 0.86	

Values are mean ± SD for three different samples of each part analyzed individually in triplicate. Different superscript letters within the same column indicate significant ($p < 0.05$) differences of means among the extracting solvents; Subscript letters within the same row indicate significant ($p < 0.05$) differences of means between the plant parts.

Table 5. Minimum inhibitory concentration (MIC) value of *C. procera* leaves and flowers extracts against tested microorganisms.

Parameter	Minimum inhibitory concentration (mg/ml) against tested organisms							
	<i>Bacillus pumilis</i>		<i>Escherichia coli</i>		<i>Aspergillus niger</i>		<i>Fusarium oxysporum</i>	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
80% Ethanol extract	28.00 ± 0.56 ^c _b	31.00 ± 0.82 ^c _a	21.00 ± 0.61 ^c _b	26.00 ± 0.54 ^c _a	35.00 ± 0.68 ^c _b	41.00 ± 0.84 ^c _a	33.00 ± 0.74 ^c _b	38.00 ± 0.73 ^c _a
80% Methanol extract	33.00 ± 0.75 ^b _b	38.00 ± 0.95 ^b _a	25.00 ± 0.69 ^b _b	29.00 ± 0.76 ^b _a	42.00 ± 1.12 ^b _b	49.00 ± 1.10 ^b _a	38.00 ± 0.82 ^b _b	43.00 ± 1.09 ^b _a
80% Acetone extract	39.00 ± 0.87 ^a _b	43.00 ± 0.95 ^a _a	30.00 ± 0.81 ^a _b	38.00 ± 0.74 ^a _a	48.00 ± 1.23 ^a _b	57.00 ± 1.19 ^a _a	51.00 ± 1.23 ^a _b	56.00 ± 1.17 ^a _a
Amoxicillin	18.00 ± 0.54 ^d		16.00 ± 0.49 ^d		-		-	
Flumequine	-		-		21.00 ± 0.58 ^d		25.00 ± 0.69 ^d	

Values are mean ± SD for three different samples of each part analyzed individually in triplicate. Different superscript letters within the same column indicate significant ($p < 0.05$) differences of means among the extracting solvents; Subscript letters within the same row indicate significant ($p < 0.05$) differences of means between the plant parts.

analysis. The reducing power data of different tested extracts followed the similar trends as were observed for linoleic acid inhibition assay.

Antimicrobial activity

The antimicrobial activity of the extracts produced from *C. procera* leaves and flowers against

pathogenic microorganisms are shown in Tables 4 and 5. The results obtained from disc diffusion method, indicated that *B. pumilis* was found to be the most sensitive microorganism showing greater inhibition zones, varying from 10.2 to 19.2 mm and 9.8 to 17.6 mm when exposed to the leaves and flowers extracts, respectively (Table 4). As expected, the leaves and flowers extract against *B. pumilis* showed the lowest MIC values ranging

from 28 to 39 mg/ml and 31 to 43 mg/ml, respectively (Table 5). Relatively smaller inhibition zones 9.8 to 14.9 mm and 7.6 to 12.3 mm and corresponding higher MIC values, 21 to 30 mg/ml and 26 to 38 mg/ml for extracts of leaves and flowers, respectively, indicated their weak antimicrobial activity against *E. coli*. It was also observed that leaves extracts showed greater antimicrobial activity against fungal and bacterial

strains than the flowers extracts (Table 4). Fungal strain *F. oxysporum* was found to be most sensitive to leaves and flower extracts showing inhibition zones in the range of 5.5 to 7.7 mm and 4.3 to 6.1 mm, respectively (Table 4) and exhibited lower MIC values, 33 to 51 mg/ml and 38 to 56 mg/ml, when compared with *A. niger* which showed inhibition zones in the range of 4.2 to 5.3 mm and 3.2 to 5.7 mm and MIC values of 35 to 48 mg/ml and 41 to 57 mg/ml, respectively (Tables 4 and 5). Overall, the extracts from leaves of *C. procera* showed better antifungal activity than the flower extract. The variations in the antimicrobial activity of the investigated plant extracts, with regard to the two parts tested might be attributed to the different chemical composition of the extracts. Some earlier studies showed that the changes in chemical composition of an extracts directly affected their biological activities (Celiktas et al., 2007).

In general, extraction by different solvents of *C. procera* leaves and flowers in the present study yielded the extracts with varying chemical composition, antioxidant and antimicrobial activities. These differences can be ascribed to the availability of different extractable components depending upon the nature of extraction solvents and plant part used. Therefore, we concluded that an appropriate extraction process should be employed to recovering maximum quantity of potent antioxidant components from *C. procera* plant material. A further detailed study on the isolation and structural elucidation, and *in-vivo* biological activities of the functional compounds in different parts of *C. procera* is highly recommended.

ACKNOWLEDGEMENT

The authors would like to acknowledge the support of the members of Technical Services Division (TSD), NIBGE, Faisalabad, Pakistan.

REFERENCES

- Ahmad N, Rehman ZU, Bukhari MS, Rehman NU, Akhtar N (2009). Extraction efficiency and estrogen or alike activity of ethanolic and aqueous extracts of different parts of *Calotropis procera*. Int. J. Agri. Biol., 11: 621-625.
- Ahmed MKK, Rana AC, Dixit VK (2005). *Calotropis species* (Asclepiadaceae) a comprehensive review. Pharmacog. Mag., 1 (2): 48-52.
- Aneta W, Jan O, Renata C (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940-949.
- Awika JM, Rooney LW, Wu X, Prior RL, Zevallos IC (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J. Agri. Food Chem., 51: 6657-6662.
- Celiktas OY, Nartop P, Gurel A, Bedir E, Sukan FV (2007). Determination of phenolic content and antioxidant activity of extracts obtained from *Rosmarinus officinalis* calli. J. Plant Physiol., 164: 536-1542.
- Chaovanalikit A, Wrolstad RE (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. J. Food Sci., 69: 67-72.
- Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agri. Food Chem., 50: 3010-3014.
- Farnsworth NR (1966). Biological and phytochemical activity of plants. J. Pharm. Sci., 55: 225-276.
- Frankel E (1995). Nutritional benefits of flavonoids: International conference on food factors: Chemistry and Cancer Prevention, Hamamatsu, Japan. Abstract: C6-2.
- Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine. Oxford University Press, Oxford.
- Howard RA (1989). Flora of the Lesser Antilles, Leeward and Windward Islands. Dicotyledoneae. Part 3. Vol 6. Arnold Arboretum, Harvard University, Jamaica Plain, MA. p. 658.
- Hsu B, Coupur IM, Ng K (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chem., 98: 317-328.
- Hussain AI, Anwar F, Poonam SN, Satyajit DS, John EM, Juluri RR, Anisha M (2011). Antibacterial activity of some *Lamiaceae* essential oils using resazurin as an indicator of cell growth. Food Sci. Tech. 44 (4): 1199-1206.
- Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108: 986-995.
- Iqbal S, Bhanger MI, Anwar F (2007). Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. Food Sci. Tech., 40: 361-367.
- Jaffery EH, Brown AF, Kurilich AC, Keek AS, Matusheski N, Klein BP (2003). Variation in content of bioactive components in broccoli. J. Food Comp. Anal., 16: 323-330.
- Karadeniz F, Burdurlu HS, Koca N, Soyer Y (2005). Antioxidant activity of selected fruits and vegetables grown in Turkey. J. Agri. Food Chem., 29: 297-303.
- Kartikar KR, Basu BD (1994). Indian Medicinal Plants, Allahabad, India, 3(2): 1606-1609.
- Katalinic V, Milo M, Kulisi T, Juki M (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem., 94: 550-557.
- Khanzada SK, Shaikh W, Kazi TG, Sofia S, Kabir A, Usmanhane K, Kandhro AA (2008). Analysis of fatty acid, elemental and total protein of *Calotropis procera* medicinal plant from Sindh, Pakistan. Pak. J. Bot., 40(5): 1913-1921.
- Kuhnt M, Probstle A, Rimpler H, Bauer R, Heinrich M (1994). Biological and pharmacological activities and further constituents of *Hyptis verticillata*. Planta Medica., 61: 227-232.
- Leven M, Vanden-berghe DA, Mertens F, Vlietinck A, Lammers E (1979). Antimicrobial activity. Planta Medica., 36: 311-321.
- Lioigier HA (1995). Descriptive flora of Puerto Rico and adjacent islands. Vol. 4 editorial de la Universidad de Puerto Rico, San Juan, PR, p. 617.
- Liu Q, Yao H (2007). Antioxidant activities of barley seeds extracts. Food Chem., 102: 732-737.
- Meena AK, Yadav AK, Niranjan US, Singh B, Nagariya AK, Sharma K, Gaurav A, Sharma S, Rao MM (2010). A review on *Calotropis procera* Linn and its Ethnobotany, Phytochemical, Pharmacological profile. Drug Invention Today, 2(2): 185-190.
- Meyer JJM, Afolayan AJ (1995). Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). J. Ethnopharmacol., 47: 109-111.
- National Committee for Clinical Laboratory Standards (NCCLS) (1997). Approved Standard M2-A6, 5th edn. NCCLS: Wayne, PA.
- National Committee for Clinical Laboratory Standards (NCCLS) (1999). M100-S9. NCCLS: Wayne, PA.
- Oktay M, Gulcin I, Kufrevioglu I (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm-Wiss. U-Technol., 36: 263-271.
- Osawa T, Namiki M (1981). A novel type of antioxidant isolated from leaf wax of *eucalyptus* leaves. Agric. Biol. Chem., 45: 735-739.
- Ozsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of *Smilax excelsa* leaf extracts. Food Chem., 110: 571-583.
- Parrotta JA (2001). Healing plants of Peninsular India. CAB International, Wallingford, UK and New York. p. 944.
- Patel VR, Patel PR, Kajal SS (2010). Antioxidant activity of some selected medicinal plants in western region of India. Adv. Boil. Res.,

- 4(1): 23-26.
- Rahman MA, Wilcock CC (1991). A taxonomic revision of *Calotropis* (Asclepiadaceae). *Nordic. J. Bot.*, 11(3): 301-308.
- Sanchez-Moreno C, Larrauri JA, Saura-Calixto F (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res. Int.*, 32: 407-412.
- Sharma GK (1934). *Calotropis procera* and *Calotropis gigantia*. *Indian J. Vet. Sci.*, 4: 63-74.
- Siddhuraju P, Mohan PS, Becker K (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flower and fruit pulp. *Food Chem.*, 79: 61-67.
- Soliman KM, Badeaa RI (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem. Toxicol.*, 40: 1669-1675.
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem.*, 90: 333.
- The Wealth of India (1950). Council of Scientific and Industrial Research, New Delhi, pp. 20-23.
- Tung YT, Wu JH, Kuo YH, Chang ST (2007). Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. *Bioresource Technol.*, 98(5): 1120-1123.
- Watt JM, Breyer-Brandwisk NG (1962). Medicinal and poisonous plants of southern and eastern Africa, 2nd Edition Livingstone, Edinburgh.
- Yen GC, Duh PD, Chuang DY (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chem.*, 70: 437-441.
- Yildirim A, Oktay M, Bilaloglu V (2001). The antioxidant activity of the leaves of *Cydonia vulgaris*. *Turk. J. Med. Sci.*, 31: 23-27.
- Zou Y, Lu Y, Wei D (2004). Antioxidant activity of a flavonoid rich extract of *Hypericum perforatum* L. *in vitro*. *J. Agric. Food Chem.*, 52: 5032-5039.