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Phytochemicals screening and free radical scavenging potential of leaf and flower extract of *Calotropis procera*

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Abstract
The aim of the present study was to evaluate the phytochemical constituents and to assess *in vitro* lipid peroxidation inhibitions and antioxidant activities of different fractions of dried leaves and flowers of *Calotropis procera*. Qualitative analysis of phytochemicals constituent like steroids, saponin, flavonoid, tannin, coumarin, terpenoids and cardiac glycosides was performed by the well-known tests protocol available in the literature. Antioxidative efficacy was studied through DPPH, reducing power assay and lipid peroxidation inhibition assay in liver homogenate. The phytochemicals screening revealed the extract richness in Tannins, Flavonoid, steroids coumarin, and terpenoids. Strong antioxidant scavenging activities were observed in different fractions of leaf and flower extract. Leaf and flower extract of *Calotropis procera* showed lipid peroxidation inhibition against Thiobarbituric acid. From the results of the present investigation, it could be concluded that *Calotropis procera* extracts can be explored as a potential source for isolation of natural antioxidant.

Keywords: Phytochemicals screening, DPPH, Lipid peroxidation inhibition, antioxidant, *Calotropis procera.*

Introduction
For thousands of years ago nature has been the best source of medicinal and therapeutic agents and a vast variety of modern drugs have been isolated from natural source. Medicinal plants contain phytochemicals that have great therapeutic value. These plants have been used for the centuries as potential medication for different human and animal's diseases.

Production of free radical are considered as a part of normal metabolic pathways but continuous and uncontrolled production of the free radicals may results in tissues damage which may lead to several diseases of heart by impairment of lipid metabolism, cancer by damaging DNA and other age related diseases by proteins deterioration[1]. Natural antioxidants derived from the plant such as tannins, phenolic acids, flavonoids has been previously reported to protect living organism from oxidative damage and concomitant lipid peroxidation, and other DNA and protein damages because of their free radicals scavenging properties[2]. Antioxidant potential of any substance may be determined by various methods of assessment like metal ions chelating ability, free radical scavenging activity, reducing ability and lipid peroxidation inhibition.

Calotropis procera is a medicinal plant, commonly known as Aak and used in many Ayurvedic formulations. The use of plants, their extract and pure isolated compounds provide the base for modern pharmaceutical compounds [3]. Plants produce wide range of bioactive molecules and most of these bioactive molecules provide defense against various diseases and infections [4].

It has been previously determined the antioxidative potential of *C. procera* by using its root extract [5]. Similarly antioxidant activity of leaf extract of *C. procera* to chelate metal ions has been proved [6]. While, antioxidant potential of *C. procera* fruit extract through DPPH was also evaluated in different studies [7]. Patel *et al*. (2014) [8] studied the comparative antioxidant activity by DPPH (1,1-Diphenyl-2-picryl hydrazyl) of *C. procera* and *C. gigentia* by using their methanol extract and it was reported that *C. procera* possess high antioxidant properties due to more phenols and flavonoids as compared to *C. gigantea*. It has been proved that methanol extract of *C. procera* latex exhibited positive activity to scavenge free radicals [9]. Presently, water, hexane, ethanol and ethyl acetate fractions of *C. procera* leaf and flower extracts are investigated to determine the phytochemical constituent and antioxidant properties of the plant through techniques like DPPH, Lipid peroxidation and reducing power ability.

Materials and Methods

Plant Material

Fresh leaves and flowers of *Calotropis procera* were collected and authenticated by Karachi University Herbarium. Leaves and flowers were washed under tap water and dried under shade. Dried leaves and flowers were ground into fine powder and stored in polythene bags at room temperature.

Preparation of Extract

To obtain a crude extract, flower and leaf materials were soaked in 80% ethanol for about 10 days then filtered through filter paper. The extracts were concentrated by using rotary evaporator. Hexane, ethyl acetate, water and ethanol fractions of leaf and flower samples of *C. procera* were separated by using separating funnel technique and concentrated on rotary evaporator.

Phytochemicals screening

Phytochemicals studies of different fractions of leaf and flower extract was carried using standard methods to detect the presence of cardiac glycosides, saponins, flavonoids, tannins, terpenoids, coumarin, steroid etc [10- 12].

In Vitro Antioxidant Study

Lipid peroxidation inhibition assay

Preparation of tissue homogenate

Tissue homogenate was prepared by taken fresh tissue of a normal albino rat; the tissue was minced with ice chilled phosphate buffer saline pH 7.4 and homogenized. The homogenate was centrifuged at 3000 rpm for 15 minutes; clear supernatant was collected for lipid peroxidation inhibition assay.

Effect of *C. procera* on inhibition of lipid peroxidation activity was studied *in vitro* according to the guidelines of Halliwell and Gutteridge (1999) [13].

DPPH free radical scavenging activity

1, 1-diphenyl -2-picrylhydrazyl (DPPH) radical scavenging assay performed as described by Kumar *et al*. (2013) [5].

Reducing power assay

The reducing power capacity of *C. procera* flowers and leaves extracts were determined by following the methods of Oyaizu (1986) [15] and Mishra *et al*. (2013) [14].

Results

Phytochemical Screening

Table 1: Leaves Extract of Calotropis Procera

Phytochemical	Water	Hexane	Ethanol	Ethyl acetate
Terpenes	+	-	-	+
Glycosides	-	+	-	+
Saponins	-	+	-	-
Flavonoids	+	-	+	-
Tannins	+	+	+	+
Terpenoids	+	+	+	-
Coumarin	+	-	+	-
Steroid	-	+	+	+

Table 2: Flowers Extract of Calotropis Procera

Phytochemical	Water	Hexane	Ethanol	Ethyl acetate
Terpene	+	-	-	-
Glycosides	-	-	+	+
Saponins	+	+	-	-
Flavonoids	+	-	+	+
Tannins	-	-	-	-
Terpenoids	-	-	-	-
Coumarin	+	+	+	+
Steroid	-	+	-	-

Lipid peroxidation inhibition assay

Leaves and flowers extract of *C. procera* showed concentration dependent Lipid peroxidation inhibition activity in vitro and exhibited capability of protecting tissue from peroxidative damage. In liver homogenate both leaves and flower extract exhibited significant dose dependent protective efficacy against lipid peroxidation activity.

Highest LPOI activity of Flowers extract was found in water extract is (89.58%), ethyl acetate (65.23%) and hexane (54.99%) respectively (Figure 1). However highest LPOI activity of leaves extract in water (75.11% with 10 gm/ ml) and ethyl acetate (75.11% with 8 mg/ ml) as compared to ethanol (56.94% with 6 mg/ ml) and hexane (54.98% with 10 mg/ ml) (Figure 2), while BHA exhibited 85% activity as compared to ascorbic acid showing 75% lipid peroxidation inhibition activity.

DPPH free radical scavenging activity

Free radical scavenging potentials of C. procera of leaves and flowers extract at different concentrations (1–10 mg/mL) were measured by the DPPH radical scavenging assay, and the results are shown in Figure 3 and Figure 4. The degree of discoloration indicates the scavenging potentials of the extracts.

Flower extract exhibited highest DPPH scavenging activity as compared to leaf. Highest activity of flower extract is found in ethanol extract (88.19%) followed by water extract (83.05%), ethyl acetate (77.2%) and hexane (76.38) respectively. However, leaves extract with various solvents showed different values of scavenging activity irrespective of their concentration. Amongst all of the solvents water extract exhibited highest activity (75.97%) following ethyl acetate (75.11), ethanol (56.94%) activity and hexane extract showed (54.98%). While BHA and ascorbic acid were used as standard showing 85% and 75.56% activity as compared to test extracts.

Reducing power assay

Measurement of reducing potential can also be used to express antioxidant activity of the plant extracts. Flower showed highest reducing power as compared to leaf. Amongst all of the test extracts, flower extract showed different absorbance pattern of reducing power irrespective of their concentrations. While, flower water extract was found to have concentration dependent pattern of reducing power. Highest reducing power was observed in flower water extract with 1.827 absorbance value followed by hexane with 1.469 absorbance, ethanol with 0.572 absorbance and ethyl acetate showed lowest absorbance respectively. However, ascorbic acid showed less reducing power as compared to water, hexane and ethanol extract (Fig. 5).

A concentration dependent absorbance of reducing power was found in leaf extract with water. The highest value of reducing power was observed in water extract (1.093). While, other

three extracts showed improper values irrespective of their concentrations. The absorbance value of ascorbic acid was found at lower side as compared to test extracts (Fig. 6).

Figure 1
Effect of different flower extracts of C. *procera* on Lipid peroxidation inhibition (%).

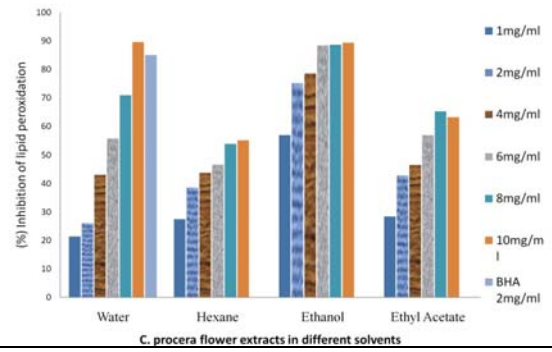


Figure 2
Effect of different Leaves extracts of C. *procera* on Lipid peroxidation inhibition (%).

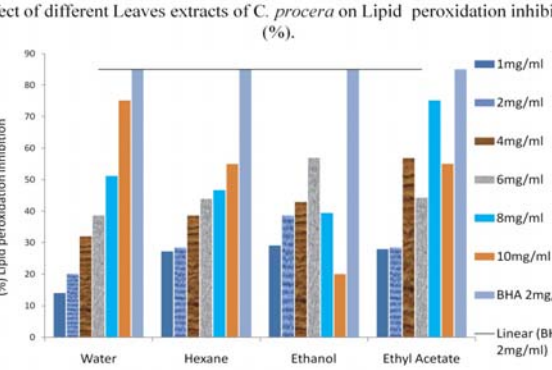


Figure 3
DPPH free radical scavenging activity of flower extracts of C. procera

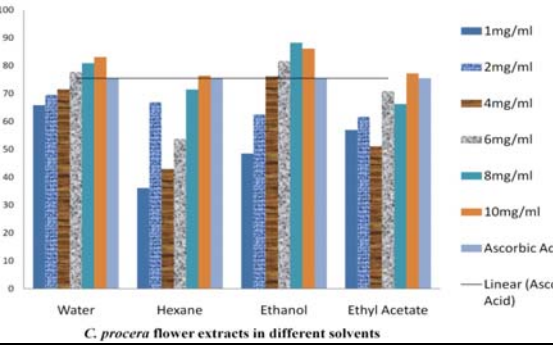


Figure 4
DPPH free radical scavenging activity of leaves extracts of C. procera

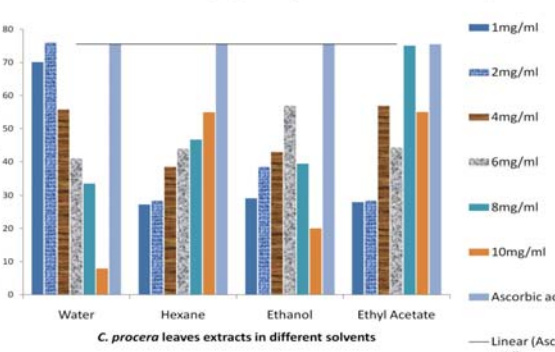


Figure 5
Reducing power of flower extracts of C. procera

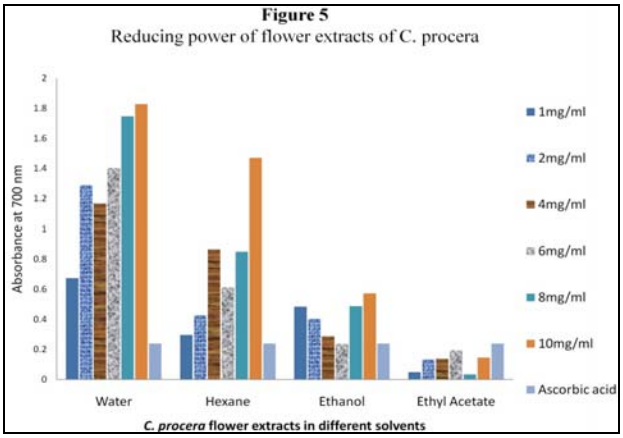
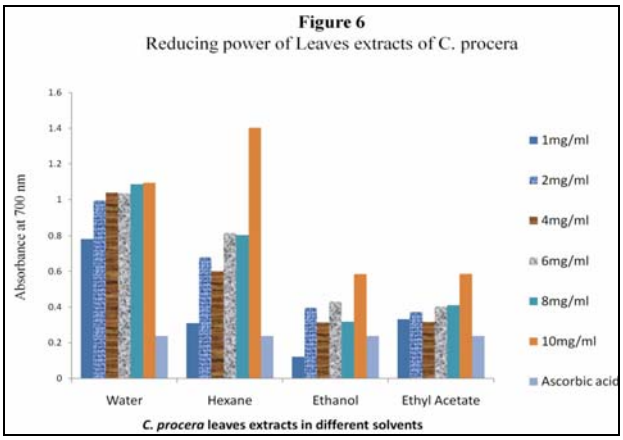


Figure 6
Reducing power of Leaves extracts of C. procera



Discussion

Plants are the natural antioxidants source against free radicals and their reactive derivatives (ROS), which are responsible for various diseases of heart by damaging lipids, cancer by damaged DNA and ageing by the damage of protein [1]. In the present study to counter act this action *C. procera* leaf and flower extracts were utilized *in vitro* as an antioxidant agent by analyzing DPPH scavenging activity, reducing power and lipid peroxidation inhibition activities. While, *C. procera* was found to be effective to inhibit or reduce the lipid peroxidation chain reaction [5], free radical and reducing power [16].

Flower and leaf extracts of *C. procera* with various solvents exhibit effective lipid peroxidation inhibition (LPOI) activity. Flower extract exhibited highest lipid peroxidation inhibition as compared to leaf extract. Flower extract showed concentration dependent increased in lipid peroxidation inhibition in water, hexane and ethanol extract except ethyl acetate extract (Figure 1).

There is a concentration dependent LPOI activity in leaf water and hexane extracts (Figure 2).

Similarly, leaf extracts of *C. procera* with various solvents showed effective DPPH scavenging activity in all concentrations.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging is a very sophisticated method for determination of in vitro antioxidant activity. DPPH is a nitrogen containing free radical which is largely being used to determine antioxidant property of any substance. It is also known that DPPH reacts quickly with substances which have phenol group in their structure. It is a dark purple colored solution which contains an unpaired electron, responsible for transfer of electron from antioxidant to DPPH radical, Purple color of DPPH solution is

converted into yellow as the radical is scavenged by the antioxidant; this property of DPPH is used for spectrophotometric analysis [17].

Flower extract exhibited highest DPPH scavenging activity as compared to leaf. The antioxidant activity of flower extract usually increases with the increase of concentration (Fig. 3).

Leaf extract with various solvents showed different values of scavenging activity irrespective of their concentration (Figure 4).

In vitro antioxidant activity was also determined by the method of reducing power capacity. The main principle of reducing power assay is that compounds which have reduction potential may react with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}) which then react with ferric chloride to form ferrous complex. The colour intensity of the complex may be determined in terms of absorbance at 700 nm. Higher absorbance indicates higher reduction potential. The reducing ability (absorbance) of a given sample determines its antioxidant potential with the ability to donate hydrogen atom to free radical chain [5, 7].

Different solvent extracts of *C. procera* flower and leaf were also analyzed to determine the reducing power. Flower showed highest reducing power as compared to leaf. Amongst all of the test extracts, flower extract showed different absorbance pattern of reducing power irrespective of their concentrations. While, flower water extract was found to have concentration dependent pattern of reducing power (Figure 5).

A concentration dependent absorbance of reducing power was found in leaf extract with water (Figure 6).

Conclusion

It is concluded from the above study that water, hexane, ethanol and ethyl acetate fractions of *C. procera* flowers and leaf extracts in different solvents have a significant potential to inhibit free radical activity. Results divulge that the flower extract is more potent than leaf extract as antioxidant. Therefore, *C. Procera* could be proposed as a potential source of segregation of natural antioxidant and could be efficient for various treatment strategies.

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Conflict of interest: Authors have no conflict of interest.

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