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Analysis of antioxidant activity in extracts of *Calotropis procera* (Ait.) R.Br.

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ABSTRACT

Objectives: To analyze total phenols, flavonoids and antioxidant potential of the root and leaf extracts and latex of field grown as well as tissue cultured *Calotropis procera* plants.

Methodology and results: Six types of plant materials comprising of leaves, latex and roots from field grown as well as *in vitro* raised plants were tested to determine their antioxidant activity. Total phenols and flavonoids were determined by Follin Ciocalteu reagent and aluminum chloride method, respectively. Free radical scavenging activity was determined by DPPH. Total phenols and flavonoids content were high in latex of field-grown plants and lowest in the extracts of *in vitro* roots and *in vivo* leaves. The highest antioxidant capacity was exhibited by extracts of lyophilized latex (IC_{50} = 0.060 mg.ml^{-1}) and the lowest (IC_{50} = 0.27mg.ml^{-1}) was in root extracts of field grown plants.

Conclusion and application of findings: Occurrence of more total phenols in the naturally growing *Calotropis procera* plants as compared to the *in vitro* raised plants suggests that the plants synthesize phenolic compounds under stress conditions in their natural habitat for defense purposes. The observations reported in this paper could be of applied value in utilization of latex, which showed strong antioxidant potential as this plant is growing wildly in the Indian desert. Many studies indicate that total phenols and flavonoids contribute significantly to the total antioxidant potential of many fruits and vegetables. Our findings add clarity to the available knowledge in this area of work.

Key words: *Calotropis procera,* antioxidant, DPPH

INTRODUCTION

Scientific evidence suggests that under oxidative stress conditions, oxygen radicals such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (·OH) and peroxyl radicals (H_2O_2) are produced in biological systems. These oxygen radicals are called Reactive Oxygen Species (ROS) and they can lead to oxidative damage to cellular components such as proteins, lipids and DNA. These oxygen radicals play important roles in degenerative processes such as ageing (Ames *et al*., 1993), cardiovascular diseases, cancer, Alzheimer's disease and other neurodegenerative diseases, (Ames, 1983; Gey, 1990; Smith *et al*., 1996).

A number of clinical studies suggest that the antioxidants in fruits and vegetable are key factors in reducing the incidence of chronic diseases including heart disease and some cancers (Salah *et al*., 1995; Gerber *et al*., 2002;

Kris-Etherton *et al*., 2002; Serafini *et al*., 2002). Antioxidants are reported to boost the function of immune cells against homeostatic disturbance and their free radical scavenging activity has been substantially investigated (De la Fuente & Victor, 2000).

Calotropis procera (Ait.) R.Br. (Family: Asclepiadaceae) locally known as "*aak*" in India is being used as herbal medicine by people living the desert areas. A comprehensive review on traditional uses and phytochemistry of *C. procera* has been published (Mueen *et al*., 2005). The present investigation aims to quantitatively estimate the total phenols and flavonoids and antioxidant potential in the extracts of roots, leaves and latex of field grown as well as tissue cultured plants of *Calotropis procera.* These compounds are important due to their medicinal values.

MATERIALS AND METHODS

Extract preparation: Extracts prepared from fresh materials were used for analyzing total phenols, flavonoids and antioxidant activity. Six types of plant materials were tested. These included: (1) leaf (VVL), (2) lyophilized latex (VVLt), (3) root (VVR) from matured plants growing in the field, (4) leaf (VtL), (5) dry latex (VtLt) and (6) root (VtR) from four months old *in vitro* raised plants. The latex was lyophilized in a lyophilizer (Alpha 1-2 LD, Vaccubrand GMBH). All the extracts were prepared in the ratio of 1:10 in 80% methanol.

All solvents used were of analytical grade; 1,1-diphenyl -2- picryl hydrazyle (DPPH) and quercitine were procured from Sigma Chemical Co. (St. Louis, US); Gallic acid and Ascorbic acid were procured from Merck Co. (Germany), Follin Ciocalteu, Aluminum chloride, Methanol, Sodium carbonate and Potassium acetate were purchased from Qualigens Fine Chemical Co. (India).

Total phenols: Total phenols were determined by Follin Ciocalteu reagent method (McDonald, 2001). An aliquot of each plant extract (0.5ml of 1:10 mg.l^{-1}) or gallic acid (standard phenolic compound) was added to Follin ciocalteu reagent (5ml 1:10 diluted with distilled water) and 4ml of 1M solution of Na_2CO_3. The mixture was allowed to stand for 30 minutes at room temperature (37˚C) and absorbance was measured at 710 nm. Total phenolic contents of extracts were expressed as mg Gallic acid equivalent (GAE)/gm dry weight. All samples were analyzed in triplicates.

Total flavonoids: The total flavonoids content was analyzed by aluminum chloride method (Chang *et al.,* 2002). Each plant extract (0.5 ml of 1:10 gm.l^{-1}) was mixed with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand for 30 minutes at room temperature (37˚C) and absorbance was measured at 415 nm. Total flavonoids contents were expressed as mg Quercetin equivalents (QE) g $^{-1}$ dry mass. Samples were analyzed in triplicates.

DPPH- free radical scavenging activity: The stable 1, 1 diphenyl-2-picryl hydrazyl (DPPH) was used for *in vitro* determination of free radical scavenging activity of the extracts (Koleva *et al*., 2002). Different concentrations of each extract were mixed with methanolic solution of DPPH (0.004%). The mixture was allowed to stand for 15 minutes. The scavenging of free radicals by extract was evaluated spectrophotometrically at 517 nm against the absorbance of DPPH radicals. The percentage discoloration was calculated as follows:

DPPH radical scavenging activity (%) = [$AC_{517} – AE_{517}$ / AC_{517}] x 100

where;

AC_{517} is absorbance of a DPPH solution without extract;

AE_{517} is the absorbance of the tested plant extract with DPPH.

The degree of discoloration indicates the free radical scavenging efficiency of the substances. Ascorbic acid was used as the free radical scavenger reference compound. The absorbance measurements were recorded on Spectroscan-50, UV-VIS spectrophotometer (Biotech. Engineering Management Co. UK.).

RESULTS

The results showed remarkably high total phenols and flavonoids content in the latex of field grown plants (VVLt), at 9.4 and 3.72 mg.g^{-1} dry weight, respectively (Fig. 1). The lowest phenol content was in the extract of roots (VtR) of *in vitro* raised plants with 3.1 mg.g^{-1} dry weight while the lowest flavonoid content was in leaves of field grown plants (VVL) at 1.24 mg.g^{-1} dry weight.

The antioxidant capacity of different extracts of *Calotropis procera* was measured as ascorbic acid equivalent gm^{-1} dry wt. (AAE) using DPPH free radicals.

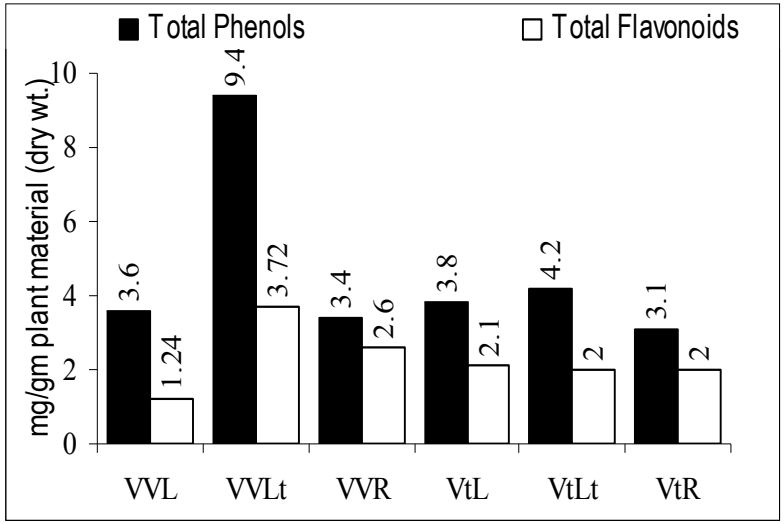

Figure 1: Total Phenol and Flavonoid contents

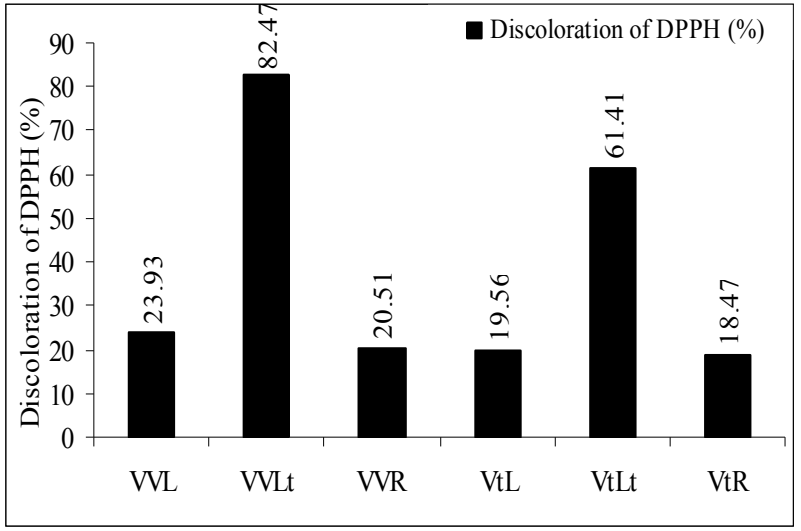

Figure 2: Total Antioxidant capacity

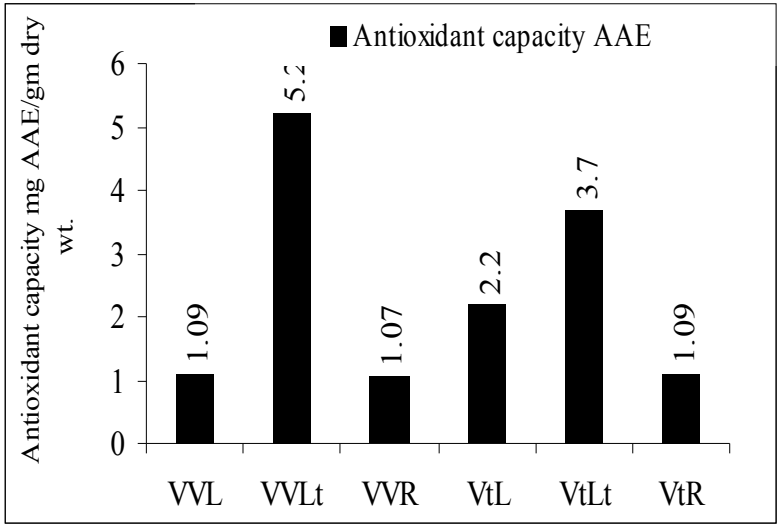

Figure 3: Percentage inhibition of DPPH free radicals

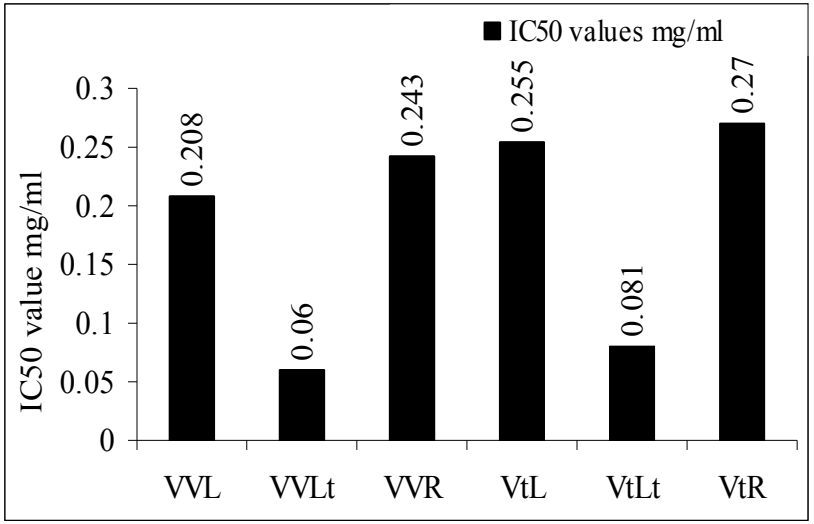

Figure 4: DPPH radical scavenging activity of different extracts of *Calotropis procera*.

The highest antioxidant capacity was exhibited by extracts of VVLt (Fig. 2) at 5.2 mg.g $^{-1}$ dry wt., followed by VtLt, VtL, VVL and VtR showing the same antioxidant capacity. VtLt, VtL, VVL and VtR were not showing similar antioxidant capacity but from VtLt to VtR the antioxidant capacity was in descending order i.e. VtLt >VtL > VVL/VtR (only VVL and VtR were equal). The poorest antioxidant capacity was found in root extracts of field grown plants (VVR) at 1.07 mg.g^{-1} dry wt.

The percentage inhibition/discoloration of free radicals by different extracts was investigated against DPPH. The highest percentage of discoloration (82.47 %) of DPPH was observed in the extract of VVLt and lowest (18.47 %) in VtR (Fig. 3). Regression equations to derive the IC_{50} values (concentration of extracts required to scavenge 50% DPPH free radicals.) showed inverse relationship between IC_{50} value and percentage scavenging potential of a sample. The strongest DPPH radical scavenging activity was exhibited by extracts of VVLt (fig.4) with IC_{50} = 0.060 mg.ml^{-1} while the lowest activity was found in VtR with IC_{50} = 0.27 mg.ml$^{-1.}$

DISCUSSION

The present investigation provides a comprehensive profile of the antioxidant activity of extracts of different plant parts of an important medicinal plant, *C. procera*, with respect to its phenols and flavonoids content. Many reports of natural antioxidants of plant origin have been published and their importance in health, food and preventive medicine has been well documented (Halliwell *et al*., 2005).

Our data shows significant antioxidant potential exists, more importantly in the latex of field grown and tissue-cultured plants of *C. procera*. These observations could be of applied value in utilization of latex, as this plant grows wildly in the Indian desert. The scavenging potential of latex of plants growing in their natural conditions was also higher when compared to *in vitro* raised plants. Although many studies support that total phenols and flavonoids contribute significantly to the total antioxidant potential of many fruits and vegetables (Katalinic *et al*., 2006), our observations that scavenging potential as well as total quantity of phenols and flavonoids are maximum in latex, adds further to the available knowledge in this area of work.

Although reports are available on anticancer, antidiabetic and anti-inflammatory properties of latex of *C. procera* (Roy *et al*., 2005; Alencar *et al*., 2006, Choedon *et al*., 2006), the present investigation presents the first report on comparative analysis of antioxidant potential of extracts from its leaf, root and latex of *in vitro* raised and naturally growing plants. More total phenols found in the naturally growing plants of *C. procera* as compared to *in vitro* raised plants further suggests that the plants synthesize phenolic compounds under stress conditions in their natural habitat for defense purposes.

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