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Comparison of total phenols and antiradical activity of flower, leaf, fruit and latex extracts of milkweed (*Calotropis procera*) from Jiroft and Bam cities

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Abstract

Plants are rich sources of phenolic compounds (flavonoids, tannins and anthocyanins) and dietary antioxidants. This study was conducted in Jiroft and Bam regions of Kerman, Iran, to compare the phenolic compounds and antiradical activity of flowers, leaves, fruits and latex extracts of Milkweed (*Calotropis procera*) in these cities. The phenolic content was measured by the Folin–Ciocalteau method and the antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl). Total phenols content were high in leaves extracts (9.72 and 9.02 mg Gallic acid/g dry weight in Bam and Jiroft plants, respectively) and lowest in latex of plants (3.1 and 3.59 mg Gallic acid/g dry weight in Bam and Jiroft plants, respectively). The highest antioxidant capacity was exhibited by extracts of leaves (DPPH= 74.31%, IC_{50} = 0.18 $mg.ml^{-1}$) and the lowest DPPH=26.23%, IC_{50} = 0.42$mg.ml^{-1}$) was in dried latex extracts of plants. The phenols and scavenging potential of leaf and fruit extracts of plants growing in Bam city were higher compared to Jiroft plants. In contrast, phenolic compounds and scavenging activity of flower and latex extracts of plants growing in Jiroft city were higher than Bam plants. The obtained results showed that flower, leaf, fruit and latex extracts of plant of *Calotropis procera* possess antiradical activity and could be used as natural antioxidant ingredients in food and drug industries.

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Introduction

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 and Victor, 2000).

Calotropis procera is a traditional medicinal plant growing wild from West Africa to South East Asia. The plant is erect, tall, large, much branched and perennial with milky latex throughout. The milky juice of the plant is used in India as a purgative, and the flowers are used as a digestive, a stomachic and a tonic and have an anti-asthmatic effect. The root bark is useful in treating skin diseases, enlargement of the abdominal viscera, intestinal worms and ascites (Khan and Malik, 1989). Further, the root of *C. procera* is used as a carminative in the treatment of dyspepsia (Kumar and Arya, 2006). The aqueous extract of the latex inhibits cellular infiltration and protects against the development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma (Choedon *et al.*, 2006). The chloroform extract of the root exhibits protective activity against carbon tetrachloride induced liver damage (Basu *et al.*, 1992). This plant is also known for its free radical scavenging and antioxidant property that is comparable to standard antioxidant, Vitamin C (Mueen Ahmed *et al.*, 2004). Plant derived natural products such as phenolic compounds (flavonoids), terpenoids, steroids, glycosides, saponins, volatile oils etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant activity (Ahmed, 2007; Amarowicz, 2007; Halliwell, 2005; Mueen, 2003). Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases.

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). The present investigation presents the first report on comparative analysis of the in-vitro total phenols and antioxidant potential in the extracts of flowers, leaves, fruits and latex of *Calotropis procera* plants from Jiroft and Bam cities in Kerman province (Iran). This could help in finding novel antioxidant compound(s).

Materials and methods

Plant materials

Extracts prepared from materials were used for analyzing total phenols and antioxidant activity. Eight types of plant materials were tested. These included: (1) leaf, (2) flower, (3) fruit (4) latex from plants growing in the Jiroft city (5) leaf (6) flower, (7) fruit (8) latex from Bam city. Latex was collected from the aerial parts of plant *Calotropis procera* and dried under shade. The plants flower, leaf and fruits of *Calotropis procera* were collected from Jiroft and Bam local farms in spring and summer.

Chemicals

All solvents used were of analytical grade; 1, 1-diphenyl -2- picryl hydrazyle (DPPH) was procured from Sigma Chemical Co.; Gallic acid, Follin Ciocalteu, Methanol, Sodium carbonate were purchased from Merck Co. (Germany).

Extract preparation

Dried plant materials were ground. The dried powder of *Calotropis procera* (1g) was soaked in 10 ml methanol–water (80:20 v/v). Extraction carried out at ambient temperature (20 °C) for 24 h using a laboratory shaker. The ratio of methanol and water which lead to the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by and biglari *et al.* (2008). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the sample constituents stored at 4 °C until use (Arabshahi-Delouee and Urooj 2007).

Estimation of total phenolic compounds

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard and

Singleton 1977). Briefly, 20 µl of extract solution were mixed with 300 µl of Na2CO3 solution (20%), then 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40¬°C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as Gallic acid equivalents by using the following linear equation were obtained from calibration curve:

A= 0.98 C + 9.321 × 0.001 (1) R2= 0.9965

Where A is the absorbance and C is concentration as Gallic acid equivalents (µg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Bios (1958) method. Briefly, 1 ml of a 1 mM methanol solution of DPPH was mixed with 3 ml of extract solutions in methanol (containing 50–400 µg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The phenolic compounds and antioxidant activity of flowers, leaves, fruits and latex extracts of *Calotropis procera* from Jiroft and Bam cities were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at P<0.05 significant level using the SAS software (2001) program.

Results and discussion

Total phenolic compounds

The results showed remarkably high total phenols content in the leaves of Bam and Jiroft grown plants at 9.72 and 9.02 mg.g^{-1} dry weight, respectively (Fig. 1). The lowest phenol content was in the extract of latex with 3.1 mg.g^{-1} dry weight in Bam plants.

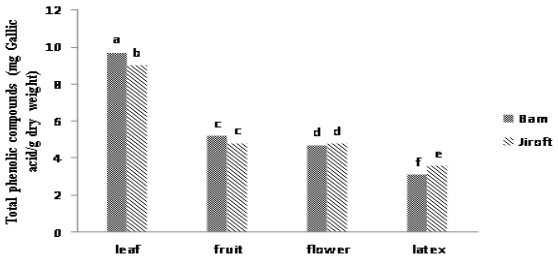

Fig. 1. Total phenolic compound of the extracts of leaves, fruits, flowers and latex of *Calotropis procera* plants from Jiroft and Bam cities (means with same superscripts had no significant difference with each other (P >0.05).

DPPH radical scavenging activity

The percentage inhibition/discoloration of free radicals by different extracts was investigated against DPPH. Figure 2 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the extracts. The highest percentage of discoloration (74.31 %) of DPPH was observed in the extract of leaves and lowest (26.23 %) in latex (Fig. 3). Regression equations to derive the IC50 values (concentration of extracts required to scavenge 50% DPPH free radicals.) showed inverse relationship between IC50 value and percentage scavenging potential of a sample. The strongest DPPH radical scavenging activity was exhibited by extracts of leaves (fig.3) with IC50 = 0.18 mg.ml^{-1} while the lowest activity was found in latex with IC50 = 0.42 mg.ml^{-1}.

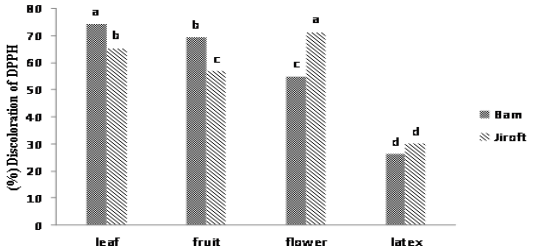

Fig. 2. DPPH radical scavenging activity of leaves, fruits, flowers and latex of *Calotropis procera* extracts from Jiroft and Bam cities (means with same superscripts had no significant difference with each other (P >0.05).

Phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc (Gao *et al.*, 2000). As it can be seen leaf extracts of *Calotropis procera* that contained the highest amount of total phenolic compounds, was found to be the most active radical scavenger followed by fruit, flower and latex extracts. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Arabshahi Delouee and Urooj 2006; Jimenez-Escrig, 2001, Gao *et al.*, 2000; Benzie and Szeto, 1999). So less Antioxidant activity may be due to less phenolic compounds in *Calotropis procera* latex but further work should be done on the isolation and identification of other antioxidant components of leaf, fruit, flower and latex extracts of

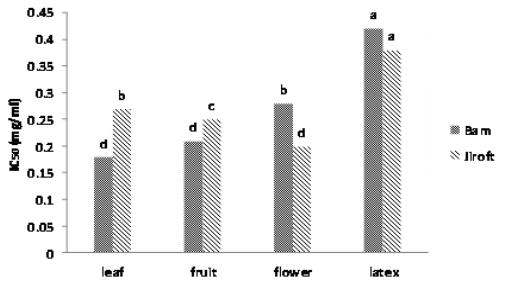

Fig. 3. IC_{50} of leaves, fruits, flowers and latex of *Calotropis procera* extracts from Jiroft and Bam cities (means with same superscripts had no significant difference with each other (P >0.05).

Calotropis procera.

Ramesh *et al.* (2009) analyzed 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 and showed that 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. These results were different from ours. These differences can be due to various factors such as variety, growing condition, maturity, season and geographical origin between the two countries (India and Iran), fertilizers, soil type, amount of sunlight received and experimental conditions (storage, extraction).

The results of Rama Prabha and Vasantha (2011) study showed that the methanolic extract of *C. procera* flowers exhibited the high radical scavenging property and cytotoxic activity. The effectiveness of the flowers might be due to the hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger. A potent scavenger of free radicals may serve as a possible preventive intervention for the diseases. The present study suggests that the flowers of *C. procera* might be a potential source of natural antioxidants.

Yazna Srividya *et al* (2013) illustrated a decrease in the concentration of DPPH radicals due to the scavenging ability of the soluble constituents in the ethanolic fruit extract of *Calotropis procera* and Gallic acid as a reference standard. The IC50 values were found to be 4.10 and 3.3 µg/ml for ethanolic fruit extract of calotropis procera and Gallic acid respectively.

Conclusion

This study 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 content. This plant, a wild growing plant, produces milky white latex that exhibits potent anti-inflammatory and antioxidant properties. Our results shows significant antioxidant potential exists, more importantly in the leaves of *C. procera*. These observations could be of applied value in utilization of extracts, as this plant grows wildly in the Jiroft and Bam deserts. The phenols and scavenging potential of leaf and fruit extracts of plants growing in Bam city were higher compared to Jiroft plants. In contrast, phenolic compounds and scavenging activity of flower and latex extracts of plants growing in Jiroft city were higher than Bam plants. Many studies support that total phenols contribute significantly to the total antioxidant DPPH.

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