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December 30, 2012

Archives • 2012 • vol.3 • 167 - 173

Evaluation of antioxidant activity of *Cleome brachycarpa* Vahl ex DC, an under-exploited desert plant of United Arab Emirates

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Abstract

The native plant flora of UAE is almost under-explored for their potential benefits. The *cleome brachycarpa* plant were grown on a nursery bed with normal watering and growth conditions for 90 days and were then harvested as a whole plant. This study was carried to evaluate the total phenolic content and *in-vitro* antioxidative activities of the ethanolic (80%) extract of the plant. When ethanol (80%) was used as an extraction solvent, a yield of 4% was achieved. Results showed that total phenolic content of ethanolic extract obtained from the stem (1031 mg/kg) and root (1032 mg/kg) of the plant was higher than that of leaves (1020 mg/kg) ($p < 0.05$). *In-vitro* antioxidative activity of different plant showed different activities. Leaf extract showed higher 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activities and ferric reducing antioxidant power (FRAP) compared to the extract obtained from stem and shoot. However, there was no difference in the DPPH radical scavenging activities and FRAP among the extract stem and root. The higher antioxidative activities with concomitant higher total phenolic was found in all three parts of the plant. Thus, the extract obtained from un-exploited plant of UAE was found to possess good antioxidative activity extract and could be a promising source of natural antioxidant to be applied in animal feed and as a natural food additive.

Keywords: *Cleome brachycarpa*, antioxidative activity, medicinal plants, native plants.

Introduction

Arabian Peninsula is the birth place of herbal drugs, and the use of folk medicine has existed there since time immemorial (Al-Yahya, 1984). Emirates have a range of flora, consisting of a large number of medicinal herbs, shrubs and trees. But apart from its wide flora, even though the country has wide range of deserts without any plants, the usage of herbal medicine is still striving for existence. The use of medicinal plants for an extended period without any precautions is also very common in this area even for the toxic ones. World Health Organization (WHO) reported that about 4000 million people in developing countries believe in the efficiency and regular use of herbal remedies for almost all kinds of diseases (Ghazanfar, 1994). Although modern medicine may be available in these countries herbal medicines have often maintained popularity because of historical and cultural reasons. With the desert and its wildlife, the bedu of the UAE were familiar with medicinal properties of many plants. Even today local people make good use of medicinal herbs. Even though the medicinal and aromatic treatment system is prevailing in parts of Arabian Peninsula, it is not common way of treatment in the UAE. The main reason is that there is a lack of knowledge about plants and plant products which have lots of medicinal properties. The native plant flora of UAE is almost unexplored for the potential benefits which might occur in these native plants. Therefore, it is necessary to evaluate the native medicinal herbs for the presence of phenolic compounds and other bioactive compounds which may possess antioxidant and antimicrobial properties.

Around 678 terrestrial plant species are currently known from the United Arab Emirates, many of which show adaptations to survive in extreme desert environments. In a recent study by Sabitha et al. (2012), a total of 132 plants species were found to possess medicinal properties. It belongs to 114 genera and 49 families. Among the many medicinal plants of United Arab Emirates, the herbs belong to the family Cleomaceae are the categorized as under exploited ones. Even though the Cleome plant is common to the Emirates; it is reported to be threatened for overgrazing and overexploitation, but not medicinal purpose. Although traditional medicine has been practiced in the UAE for many years; literature on most of the plants used for the treatment of diseases is very little (Sabitha et al., 2012). There is no complete list of medicinal plants of the region. The recent work published by Zayed Complex for Herbal Research and Traditional Medicine listed 29 species of plants, all of which are not indigenous (ZCHRTM, 2005). Authors have studied the antioxidant metabolism underlying the defense mechanism in medicinal plants including horticulturally important plants (Jaleel et al., 2006, 2007, 2008, 2009), but little attention is gained in terms of native plants and antioxidant status.

Cleome brachycarpa is a perennial (or annual) herbs often perennating with a woody base, viscid-pubescent, sub-erect or erect, up to 50 cm tall and well branched mostly from below portions. *Cleome brachycarpa* plant is used against abdominal discomfort (Atta-ur-Rahman et al. http://www.iccs.edu/). The plant is listed in arid zone medicinal species in UNESCO arid zone research (UNESCO, 1960). Even though many medicinal values are elucidated since many years, the actual phytochemical content in terms of active principles components is not yet revealed in native plants. So, it is needed to evaluate the plant for its phenolic contents, the antioxidative activity and other phytochemical contents. Because of the accelerated local, national and international interest in recent years, the demand for medicinal and aromatic plants has increased manifolds and pharmaceutical industry views plant wealth as a source of income. Due to easy availability, no side-effects and sometimes only source of health care, the demand for medicinal plants is increasing in both developing and developed countries (Sabitha et al., 2012).

Though medicinal plants in UAE constituted only about 18% of the total plant species, it is highly probable that the medicinal properties of the remaining 82% have not been yet discovered or documented. Therefore the present investigation

was undertaken to evaluate total phenolic contents and *in vitro* antioxidative activity of one of the under exploited desert plant of the UAE, *Cleome brachycarpa*.

Materials and Methods

Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, 2-diphenyl-1-picryl hydrazyl (DPPH) Sigma Chemical Co. (St. Louis, MO, USA). Ethanol and acetone were obtained from Merck (Damstadt, Germany).

Plant materials

The seeds of *Cleome brachycarpa* Vahl ex DC. were sown on nursery beds, and transplanted after one month to growing pots. The plants were given normal watering and growth conditions. As these plants are adapted to growing in natural and wild habitats, no further fertilizer was used. The plants were harvested in whole after 90 days of growth. The plants were dried at 60°C in hot air oven for 12 hours and powdered by using blender and powdered samples were stored in sealable plastic bags until analysed.

Extraction of crude phenolics from the dried plant leaves

Dry plant powder was subjected to extraction of phenolic compounds according to the method of Santoso et al. (2004) with slight modifications. The powder (10 g) was mixed with 150 mL of absolute ethanol. The mixture was stirred at room temperature (28–30°C) using a magnetic stirrer (IKA-Werke, Staufen, Germany) for 6 h. The mixture was then centrifuged at 5000 x g for 10 min at room temperature using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA). The supernatant was filtered using Whatman filter paper No. 1 (Whatman International, Ltd, Maidstone, England). The filtrate was then evaporated at 40°C using an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd, Tokyo, Japan). To remove the residual ethanol, the extract was purged with nitrogen gas. The extract was then dried using a freeze dryer to obtain the dry extract. Dried extract was powdered using a mortar and pestle and kept in an amber bottle and stored in a dessicator until use.

Determination of total phenolic content

Quantification of total phenolic content in ethanolic plant extract was carried out according to the method of Slinkard & Singleton (1977). The ethanolic plant extract powder will be dissolved in 25% ethanol (v/v) to obtain concentration of 0.5% (w/v). The solution (0.5 mL) was added to 100 μL of Folin–Ciocalteu reagent (two-fold diluted with deionized water) and mixed thoroughly. After 3 min, 1.5 mL of 2% sodium carbonate solution was added. The reaction mixture was mixed thoroughly and placed in the dark for 40 min and the absorbance was read at 760 nm. The total phenolic content was calculated from the standard curve of tannic acid (0–0.1 mg/mL) and expressed as mg tannic acid per gram of dry ethanolic plant extract after blank substraction. Blank was prepared in the same manner, except that distilled water was used instead of Folin–Ciocalteu reagent.

Study on in-vitro antioxidative activity of plant extracts

Ethanolic plant extracts in the powdered form was comparatively determined for their antioxidative activities by different *in vitro* assays. Prior to assay, dry plant extracts were dissolved in 25% ethanol (v/v) to obtain concentration of 0.5% (w/v). Thereafter, the obtained solution was subjected to the assays for antioxidative activities.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Wu et al., (2003) with a slight modification. Sample (1.5 ml) was added with 1.5 ml

of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Sample blank was prepared in the same manner except that ethanol was used instead of DPPH solution. A standard curve was prepared using ascorbic acid in the range of 0-100 ppm. The activity was calculated after the sample blank substraction and expressed as mg ascorbic acid equivalents (AAE)/ml of phenolic solution.

Ferric reducing antioxidant power (FRAP)

FRAP will be assayed according to Benzie and Strain (1996). Stock solutions include 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$. $6H_2O$ solution. A working solution was prepared freshly by mixing 25 ml of acetate buffer (300 mM, pH 3.6), 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3$. $6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min in a water bath (Memmert, D-91126, Germany) and was referred to as FRAP solution. A sample (150 µl) was mixed with 2850 µl of FRAP solution and kept for 30 min in dark at room temperature. The ferrous tripyridyltriazine complex (coloured product) was measured by reading the absorbance at 593 nm. Sample blank was prepared by omitting $FeCl_3$ from FRAP solution and distilled water was used instead. A standard curve was prepared using ascorbic acid in the range of 0-100 ppm. The activity was calculated after the sample blank subtraction and expressed as mg ascorbic acid equivalents (AAE)/ml of phenolic solution.

Results and discussion

Though medicinal plants in UAE constituted only about 18% of the total plant species, it is highly probable that the medicinal properties of the remaining 82% have not been yet discovered or documented. The work on the extraction and quantification of phenolic compounds and antioxidative activity from the UAE medicinal plants are scanty. Therefore the present investigation was undertaken to evaluate the total phenolic content and the invitro antioxidative capacity of one of the under exploited desert plant of the UAE, *Cleome brachycarpa*.

Total phenolic content of the ethanolic plant extract from leaf, stem and root is shown in Fig. 1. The results showed that the total phenolic content in the leaf was higher than that found in stem and root part of the plant extract. Total phenolic content leaf, stem and root was found to be 1398, 1087 and 1032 mg/100g of dry extract, respectively. Therefore, there was a variation in the total phenolic contents between the different part of the plant. Phenolic compounds are phytochemicals abundant in plants and have been effectively used as an alternative additive in different food systems as an antioxidants (Maqsood et al., 2010a, 2010b, 2011a, 2011b). Plant extracts rich in polyphenols are important candidates, as they are easily obtained from natural sources (Maqsood et al., 2012, 2013). In general, phenolic compounds play a role as antioxidants through different mechanisms of action, such as scavenging of free radicals, quenching of reactive oxygen species, inhibition of oxidative enzymes or through interaction with biomembranes (Maqsood et al., 2013). Type and amount of phenolic compounds varied with plant, maturation, season, etc (Sultana et al. 2007).

There seem to be good coorelation between total phenol content and antioxidant capacity (DPPH radical scavenging activity and FRAP). Similar linear correlation between these two variables have been obtained in many studies (Katalinic et al., 2006). Others (Ou et al., 2003) have not found a linear correlation between total phenols and antioxidant power. Differences in the response and the degree of the linearity (that is in the "r" coefficient) seem to be related to the fact that different studies use different methods for the determination of antioxidant capacity. These methods differ in terms of their assay experimental conditions and principles.

DPPH radical scavenging activity of different part of *Cleome brachycarpa* extract is shown in Figure 2. The decrease in absorbance of the DPPH radical caused by antioxidant is due to the scavenging of the radial by hydrogen donation. It is visually noticeable as a colour change from purple to yellow. A lower value of EC50 indicates a higher antioxidant activity. From the results of our study, it was noticed the ethanolic plant extract from the leaf part displayed higher DPPH radical scavenging activity than those displayed by the stem and root part of the plant ($p<0.05$) (Fig. 2). When compared between stem and root, it was found that the stem extract showed higher DPPH radical scavenging activity than root extract of the plant. Similarly the FRAP assay showed a notable different activity in different parts of *Cleome brachycarpa* plant (Fig. 3). There was no difference in the FRAP activity of the leaf and stem part of the plant ($p>0.05$). however their activity was higher than that showed by root extract ($p<0.05$).

The antioxidant potential in both roots and leaves of the different investigated species is significant depending on the species, root or leaf extract revealed stronger activity in scavenging free radicals and reducing transition metal ions (Matkowski et al., 2008). The discrepancies between the results of different antioxidant assays, frequently observed in other studies (Zhao et al., 2007) were not pronounced in our study. In another antioxidant study of *Salvia miltiorrhiza* (Zhao et al., 2007) the high ability to both scavenge free radicals and to reduce ferric ions is reported. In S. verticillata, even the subspecific taxonomic diversity affects the antioxidant potential (Tepe et al., 2007). To date, there have been no thorough data on antioxidant properties of *Cleome brachycarpa*. Thus, the highest activity of *Cleome brachycarpa* leaf extract is even more noteworthy. In contrast, the root extract of *Cleome brachycarpa* was the weakest antioxidant. Matkowski et al., (2008) also reported that the root extracts of *Salvia miltiorrhiza* displayed stronger activity than leaf extracts in DPPH and P–Mo assays, whereas in trolox equivalent antioxidant assay (TEAC) assay, the leaf extract of S. verticillata was stronger. The TEAC seems to depend more on the flavonoids in leaf extracts, but on phenolic acids in the roots (Matkowski et al., 2008). In the present study, total phenolic content correlate to the antioxidant activity measured by DPPH and FRAP. The DPPH assay, carried out in an organic solvent is able to test both hydrophilic and lipophilic free radical scavengers. Yet, the leaves of *Cleome brachycarpa* species have been less studied with respect to both chemical composition and biological activities. The variation in the sum of phenolic conpounds was significant between roots, stem and leaves. The flavones can also play an important role in leaf extracts, since the leaves are generally rich in them (Matkowski et al., 2008).

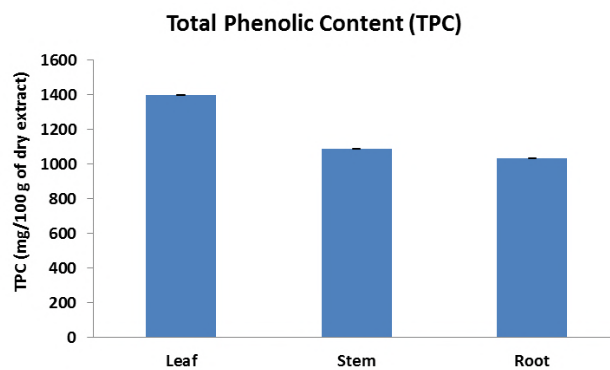

Figure 1. Total Phenol content (TPC) of different parts of ethanolic extract of *Cleome brachycarpa*.

Since testing the plant extract by one antioxidant assay is not enough to come to the real conclusion that the plant extract does possess antioxidative activity, therefore, we screened the antioxidant ability of plant extracts by a second method. Antioxidant ability is a concept that may imply different mechanisms. In this study, we used the FRAP and DPPH radical scavenging assay because of their simplicity and reproducibility. The FRAP assay, which is, according to Benzie et al. (1999) a rapid, reproducible, and easy to perform assay. In this method, the antioxidant activity is determined based on the ability to reduce ferric(III) iron to ferrous (II) iron (Benzie & Strain, 1996). Although this assay was originally developed to measure plasma antioxidant capacity, it can be used to quantify the antioxidant capacity from a wide

variety of biological samples from pure compound to fruits, wines, and animal tissues (Katalinic et al., 2004). As antioxidants are explained to be as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability.

To better understand the distribution of phenolic compounds in different physical parts of *Cleome brachycarpa* and their contribution to the overall antioxidant, further research to evalueate individual phenolic compounds in different separated parts of the plant is needed. Although there are plants with good antioxidant abilities, further concentration and/or purification can help to achieve better antioxidant capacities.

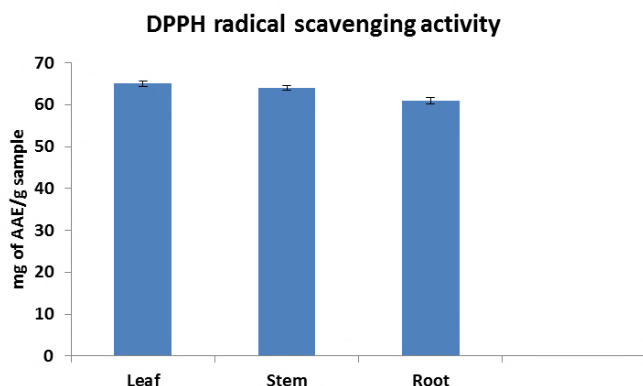

Figure 2. DPPH Radical Scavenging Activity of different parts of *Cleome brachycarpa* The activity was expressed in mg of Ascorbic acid equivalent/g of sample.

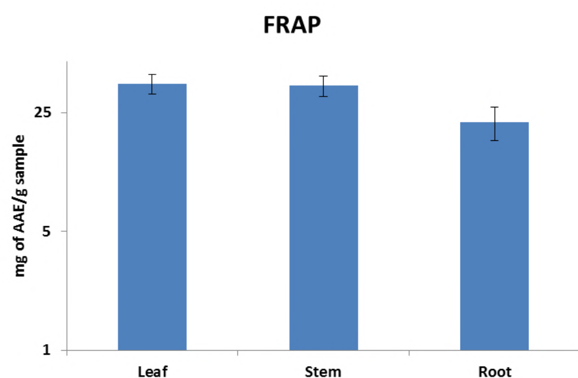

Figure 3. Ferric Reducing Antioxidant Power (FRAP) of different parts of *Cleome brachycarpa*. The activity was expressed in mg of Ascorbic acid equivalent/g of sample.

Conclusion

The results of this study showed that as a whole this plant *Cleome brachycarpa* can be a potential source of natural antioxidants. However, different parts of the plant showed different activities towards different assays. There can be a significance of *Cleome brachycarpa* as an important medicinal plant, which have good antioxidant potentials throughout its plant parts. Further research is needed (specially on those plants found with very high FRAP and DPPH values) to investigate what chemical compounds are present in the plant.

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ISSN: 1827-8620

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ISSN: 1827-8620