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Antioxidant Activity of the Leaves and Stems in Different Growth Stage of Methanol Extract From *Datura stramonium* Plant from Sistan and Baluchestan.

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ABSTRACT

Stramonium is a species of Datura genus and were grown in the botanic garden of the University of Sistan and Baluchestan and were studied the collected samples in flowering period. The methanol extracts of the samples were extracted by Soxhlet apparatus and were concentrated in a rotary. Antioxidant activity of the samples was measured by using DPPH and FRAPS methods. Methanol extracts as compared with synthetic antioxidant BHT is better than other extracts showed good effect.DPPH method showed highest antioxidant activity for flowers and roots in after flowering period and for leaves in during flowering period (IC50= 48.95 and 45.84 and 35.78 respectively) compared with the amount for leaves and stems in after flowering period and for stems in before flowering period (IC50= 31.50 and 6.59 and 11.38, respectively).FRAP assay showed highest antioxidant activity for flowers and roots in after flowering period and for leaves in during flowering period (mMFe+2/sample=60.75 and 40.78 and 56.92 respectively) compared with the amount for leaves and stems in after flowering period and for stems in before flowering period (mMFe+2/sample=30.54and 19.61 and 30.50, respectively.

Keywords: Datura plant, Methanol extract, Antioxidant, DPPH method

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INTRODUCTION

Daturastramonium, more commonly known as wild-growing flowering plant belong to the family Solanaceae and is a medicinal plant with antinociceptive [1] antioxidant [2]. Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. These free radicals which are produced by environmental pollutants, radiation, chemicals, toxins and deep fried and spicy foods as well as physical stress, can cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins [3,4].

Increased free radicals formation may produce a continuous level of oxidative damage [5,6] which leads to many diseases such as atherosclerosis, cancer, stroke, asthma, arthritis and other age related diseases[5,7]. Fortunately, the generated free radicals are removed from the body through the antioxidant defense mechanisms. Antioxidants are considered as possible protection agents reducing oxidative damage of human body from reactive oxygen species (ROS) and retard the progress of many chronic diseases as well as lipid per oxidation [8].Therefore, there is a lot of ongoing research on such substances for their potential usefulness as dietary supplements and as adjuvants for use in therapeutic management of free radicals related disorders. Synthetic antioxidants like butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT), tertiary butyrate hydroquinone and gallic acid esters, have been suspected for liver damage and carcinogenesis [9].Hence, strong restrictions have been placed on their application and there is an attitude to substitute them with naturally occurring antioxidants ([10,11].Several methods have been developed to determine the antioxidant potential of food products. The trolox equivalent antioxidant capacity (TEAC) using ABTS (2,2-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid) as an oxidant, the ferric reducing antioxidant power (FRAP), the DPPH (2,2-diphenyl- 1-picrylhydrazyl) free radical scavenging potential, the oxygen radical absorption capacity (ORAC), the total radical absorption potentials (TRAP), and the photochemiluminescence (PCL) assays are some of the most commonly used [12,13].

Antioxidants can reduce radicalsprimarly by two mechanisms: single electron transfer and hydrogen atom transfer.ABTS FRAP, and DPPH are methods that measure the former, and ORAC and TRAP represent the latter. The mechanism of PLC is still unclear [13].In this study the antioxidant activities of the methanol extracts of some parts of *Daturastramonium* plant were investigated in three different periods including before flowering, during flowering and after flowering periods. For determination of the antioxidant activities in this plant two methods were used, namely, DPPH and FRAP.

MATERIALS AND METHODS

Plant material

Plant samples (*Daturastramonium.L*) were cultivated in the botanic garden of the University of Sistan and Baluchestan. These samples were collected in three separate periods; before flowering, during flowering and after flowering, after which they were dried at room temperature in darkness and powdered using a grinder. Finally, they were kept in a refrigerator at 4° C in complete darkness.

Preparation of extract

In order to extract the natural products for investigating the antioxidant activity of the plant, 150 mL of methanol was added to 15 grams of the powdered sample in a flask. Then, soxhlet method (Extraction) was performed for 12 hours and the extract was concentrated using the rotary method. For drying and removing the microbes in the extracts, they were placed inside the incubator.

Determination of Antioxidant activity:

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay:

The DPPH radical scavenging activity of the extracts from *Daturastramonium* in three separate periods including before flowering, during flowering and after flowering was measured according to the procedure described by Brand-Williams [14].In summary, 1 mL of samples of several concentrations of the extracts in methanol were individually added to a 1 mL solution of DPPH radical in methanol (last

concentration of DPPH was 0.1 mM).The mixture was shaken strongly, stirred and allowed to stand in the dark place at room temperature for 30 min. Then, the absorbance of the resulting solution was measured at 517 nm using a UV-Vis Spectrophotometer (Specords 100 spectrophotometer).

The percentage of DPPH radical scavenging by the extract was calculated by the following formula.

$$\text{Scavenging free radicals DPPH} = \left(\frac{A_C - A_s}{A_C}\right) * 100$$

In this regard, A_C and A_s are the absorbance of control and absorption [15].

FRAP – ferric reducing antioxidant power assay:

The total antioxidant capacity of the *Datura stramonium* in three separate periods including before flowering, during flowering and after flowering was determined using a modification of the FRAP (ferric-ion reducing antioxidant power) assay [16]. FRAP reagent was provided using 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM of 2,4,6-tri-2-pyridyl-s-triazine(TPTZ) constituted in 40 mM of HCl. All three solutions were mixed together in the ratio 10:1:1 severally. The FRAP assay was performed by incubating the sample and reagent at 37°C for 10 min. The absorbance of the reaction mixture was recorded at 595 nm by UV-Vis. The antioxidant activity of the samples was determined against a standard of known FRAP value; ferrous sulfate.

Determination of total phenol contents in the extracts

The concentration of phenol in plant extracts was determined using spectroscopy method [17].Methanol solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was provided by combining 0.5 ml of methanol solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2 ml 5% NaHCO3.Blank was concurrently provided**,** containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2ml of 5% of NaHCO3. The samples were then incubated in an incubator at 45 ^{o}C for 30 min. The absorbance was decided using spectrophotometer at λmax equal to 765 nm. The samples were provided in triplicate for each analysis and the mean value of absorbance was obtained. The same process was repeated for the standard solution of Gallic acid and the calibration line was interpreted. Based on the measured absorbance, the concentration of phenol was read (mg/ml) from the calibration line; then the content of phenol in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

RESULTS AND DISCUSSION

The amount of total phenol compound and antioxidant activity in both method (DPPH and FRAP) showed significant difference between some parts of the plant in different periods.

Table 1: The results of phenol assay in different samples

Samples	mg GA/gr sample
Leaf after flowering	31.50
Stems before flowering	11.38
Root after flowering	35.78
Leaf during flowering	45.84
Stems after flowering	6.59
Flower	48.95

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Table 2: The results of DPPH assay in different samples

Samples	IC50
Leaf(after flowering)	135.44
Stems(before flowering)	230
Roots(after flowering)	81.35
Leaf(flowering)	52.86
Stems(after flowering)	310
Flower	16.74

Table 3: The results of FRAP assay in different samples

Samples	(mMFe^{+2}/sample)
Leaf(after flowering)	30.54
Stem(before flowering)	30.50
Root(after flowering)	40.78
Leaf(flowering)	56.92
Stem(after flowering)	18.61
Flower	60.75

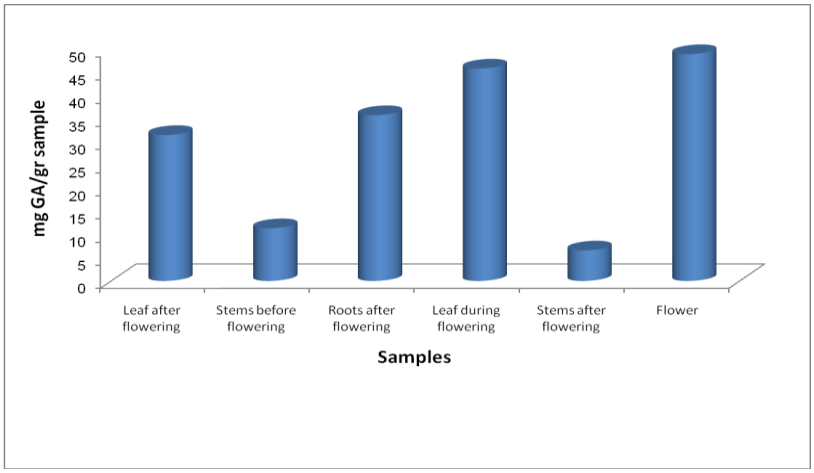

Figure 1: The measurement of phenol in methanol extract from different samples

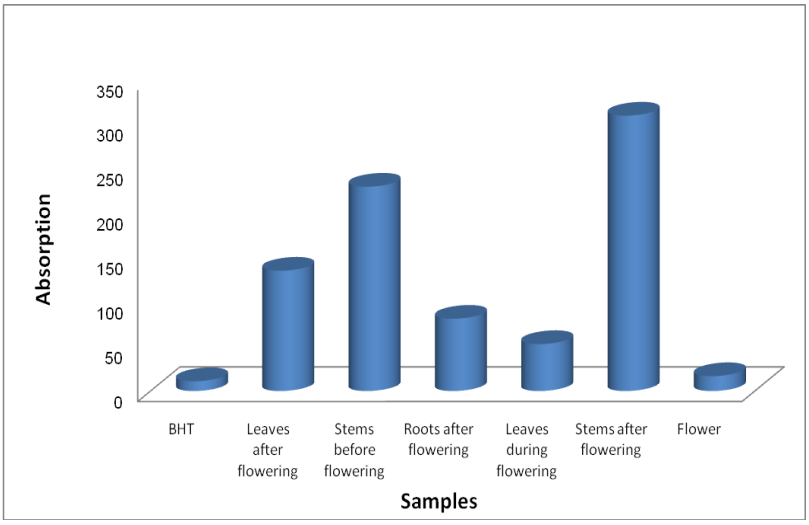

Figure 2: Absorption comparison of BHT in different samples

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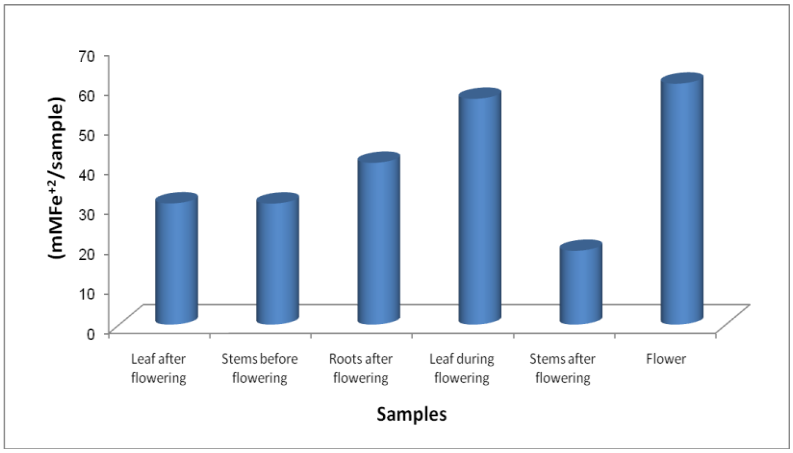

Figure 3: The measurement of FRAP assay in different samples

The amount of total phenol for flower and roots in after flowering period and for leaves in during flowering period was the highest (48.95 and 45.84 and 35.78 respectively) compared with the amount for leaves and stems in after flowering period and for stems in before flowering period (31.50 and 6.59 and 11.38, respectively) according to table 1.

Also, DPPH method and FRAP assay showed significantly different antioxidant activity for different parts of plant in different periods. DPPH method showed highest antioxidant activity for flower and roots in after flowering period and for leaves in during flowering period (IC50= 48.95 and 45.84 and 35.78 respectively) compared with the amount for leaves and stems in after flowering period and for stems in before flowering period (IC50= 31.50 and 6.59 and 11.38, respectively) according to table 2.

FRAP assay showed highest antioxidant activity for flowers and roots in after flowering period and for leaves in during flowering period ($mMFe^{+2}$/sample=60.75 and 40.78 and 56.92 respectively) compared with the amount for leaves and stems in after flowering period and for stems in before flowering period ($mMFe^{+2}$/sample=30.54and 19.61 and 30.50, respectively) according to table 3.

CONCLUSION

Production of oxidants in the human body can cause cancer. To eliminate and neutralize the free radical oxidant, special antioxidant compounds are used. There are many synthetic chemical antioxidant compounds that have high antioxidant activity. But these compounds can cause harmful side effects in the body. Today, researches in the natural antioxidants have been focused on some plants because of their high amount of antioxidant activity. So, we investigated *Daturastramonium* plant in this study. Two methods were used for this purpose and an overview of various antioxidant properties and plant extracts obtained. *Datura* plant extracts were extracted with methanol to compare the antioxidant activity of compounds extracted from different parts of the plant. Results showed that the leaf extract has the most powerful antioxidant.

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