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COMPARATIVE ANTIOXIDANT POTENTIAL AND TOTAL POLYPHENOLIC CONTENTS OF DIFFERENT PARTS OF *Datura stramonium*

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This study was carried out to evaluate the total polyphenolic contents and antioxidant potential of different parts of *Datura stramonium*. Extracts of different parts of *Datura stramonium* were prepared by reflux and mercerization methods with methanol and water. Total polyphenolic contents were evaluated by Folin-Ciocalteu method. Antioxidant potential was determined by using DPPH, super oxide and Nitric oxide radical scavenging tests. Reductive power and antioxidant potential in linoleic acid system were also evaluated in all parts of plant. The seeds, leaves, stem and flower extracts of *D. stramonium* exhibited a wide range of TPC (total polyphenolic contents) and antioxidant potential. All parts of plant under study showed appreciable amount of total polyphenols. Amongst different parts, flower extracts prepared by reflux using methanol as solvent, exhibited the highest polyphenolic contents (880 mg GAE/g of extract) and showed maximum antioxidant potential. Methanolic extracts of flowers at 100µg/mL concentration inhibited the maximum DPPH radical (99%), Super oxide radical (96%), Nitric oxide radical (86%) and Linoleic acid peroxidation (56.45%). Methanolic extract of flowers also showed maximum reducing potential. These results indicate that flowers of *D. stramonium* are promising source of natural antioxidants.

Keywords: *Datura stramonium*, polyphenols, antioxidant activity, reflux, mercerization methanol

INTRODUCTION

Polyphenolic compounds have attracted the attention of scientist as natural antioxidants. They have shown immense prospective as potential protecting factors against many dreadful diseases including cancer, heart diseases, infectious diseases etc. (Aslam *et al*., 2012; Baskar *et al*., 2012). The antiradical potential of remedial plants is mostly associated with their phenolic acids, flavonoids, tannins, and vitamins C and E contents. Plant polyphenols especially flavonoids have ability to act as excellent free radical scavengers (Otang *et al*., 2012; Ayesha, 2013). Flavonoids stop lipid peroxidation. It is also suggested that flavonoids have anti-inflammatory, anti-allergic, anti-viral and anti-cancer properties (Souza *et al*., 2008; Koffi *et al*., 2010; Blazekovic *et al*., 2010; Krishnaiah *et al*., 2011).

Physicians and food experts recommend the consumers to increase the intake of food rich in antioxidant compounds like polyphenols due to their beneficial effect on human health. There is inverse relationship between the intake of polyphenols and occurrence of heart diseases (Rakic *et al*., 2006). Strong evidences about the benefits of polyphenols has urged the search for natural antioxidants leading to the identification of natural resources and extraction, isolation of antioxidant molecules (Katalinic *et al*., 2006: Jahan *et al*., 2011).

D. stramonium, is an uninhabited flowering plant belonging to the family Solanaceae, having potential as an anticancer (Koffi *et al*., 2010), antiradical (Kumar *et al*., 2008), antilipidemic, anti-inciting, anti-rheumatoid and hypoglycemic (Christhudas *et al*., 2013) agent. Antioxidant properties of *D. stramonium* leaf extracts have been reported in some of the basic studies. However, no detailed studies on its antioxidant potential in various parts, through different tests (based on diverse principles) have been carried out. Therefore, this study was planned to compare antioxidant potential and polyphenolic content from different parts of *D. stramonium* in order to find the new sources of natural antioxidants. These findings will demonstrate a significant role in recovery of antioxidant polyphenols, which are reported to possess a great potential against many diseases linked with oxidative stress, which may ultimately lead to development of innovative nutraceutical products.

MATERIALS AND METHODS

***Collection of plant material*:** Fresh plant material of *Datura stramonium* was collected from Botanical Garden of University of Agriculture, Faisalabad. The plant parts were dried under ambient condition. The dried parts (seeds, leaves, stem and flowers) were ground to a fine powdered form by using a commercial blender.

***Preparation of plant extracts*:** The extracts of seeds, leaves, stem and flowers of *D. stramonium* were prepared with water and methanol by mercerization and reflux methods.

***Mercerization*:** The dry powdered plant materials (25g) were macerated with water (150mL) and methanol (150mL) at ambient temperature for 48 hours. After completion of

extraction time the extracts were filtered and solvent was evaporated under reduced pressure.

***Reflux*:** The dry powdered plant materials (25g) were refluxed for 1 hour with water and methanol (150mL). The extracts obtained were concentrated under reduced pressure. The crude extracts were stored in refrigerator for further analysis.

***Determination of total polyphenolic contents*:** Total polyphenolic compounds in seeds, leaves, stem and flower extracts of *D. stramonium* were determined by Folin-Ciocalteu method (Pourmorad *et al*., 2006). The extracts (0.001g/ml) were mixed with Folin-Ciocalteu reagent (10 times diluted, 5ml) and sodium carbonate (20%, 4ml), after one hour absorbance was noted at 765nm. The standard curve was prepared with gallic acid (0.01-0.1mg/ml) and results expressed as mg of gallic acid equivalent (mgGAE/g of plant extract).

***DPPH scavenging assay*:** The DPPH free radical scavenging assay was carried out by following the procedure of Yen and Chen (1995). Stock solution of DPPH radical (0.1mM, 1mL) was added to 3mL of *D. stramonium* extracts at various concentrations (20-100 µg/mL) and this mixture was incubated for half an hour in dark. The absorbance was noted at 517 nm. A decrease in absorbance of reaction mixture showed a good free radical inhibition potential of extracts. BHT and Vitamin C were used as standards. The solution without plant extract was used as control.

***Superoxide radical scavenging assay*:** Super oxide radical scavenging activity in different extracts of *D. stramonium* was evaluated according to the method described by Vaidya *et al*. (2008). Five concentrations (20-100 µg/ml) of standards (BHT and ascorbic acid) and *D. stramonium* extracts were prepared. Plant extract (1ml) was mixed with 1mL of sodium carbonate (5%), 0.4 ml of NBT (150µm) and 0.3 ml of EDTA (0.5%) and first absorbance was observed at 560 nm. The reaction was started by addition of hydroxyl-amine hydrochloride (0.4, 1%) to the above solution. The mixture was kept at 25°C for 5 minutes. Reduction of NBT was measured at 560 nm. BHT and ascorbic acid were used as standards. A parallel control was also treated in the similar manner.

***Nitric oxide scavenging assay*:** Nitric oxide scavenging activity of *D. stramonium* extracts was evaluated according to the method described by Jahan *et al*. (2011). Sodium Nitro-prusside solution (1 mL, 5mM) prepared in buffer (0.2 M, pH 7) was mixed with various concentrations (20-100 µg/ml) of plant extracts and kept at ambient temperature for 30 minutes. After completion of time, 1.5 ml of incubated solution was separated and 1.5 ml of Griess reagent was added (1% Sulphonamide, 2% Phosphoric Acid, 0.1% coupling reagent). The absorbance was noted at 500 nm. BHT and ascorbic acid were analyzed as standards. Inhibition percentage of Radical assays was calculated with the following formula.

$$\% \text{ Inhibition of Radical} = [A_0 - A_1/A_0] \times 100$$

A_0 = Absorbance of control

A_1= Absorbance of sample

***Measurement of reducing power*:** Five concentrations (20-100 µg/ml) of *D. stramonium* extracts were prepared. The reaction mixture contained plant extract, phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The control contained all the reaction reagents except plant extract. The mixture was kept at 50°C for twenty minutes. Then TCA (1% w/v, 2.5 mL) was added to stop the reaction, followed by centrifugation at 3000 rpm for 30 minutes. Supernatant layer (2.5 mL) was removed and blended with 25 mL of water and $FeCl_3$ (0.5 m L, 0.1% w/v) and absorbance was noted at 700 nm (Jahan *et al*., 2011).

***Antioxidant activity in linoleic acid system*:** It was evaluated by the method of Anwar *et al*. (2013). The reaction mixture contained extracts (500µg), emulsion of linoleic acid (2.5 mL) and buffer (2 ml, 0.04 M, pH 7). It was kept at 37°C for 72 hours for fast oxidation process. After 24 hours, 2 mL of the incubated sample was removed and 0.5 mL of $FeCI_2$ (0.02 M) and 0.5 mL of 30% (w/v) ammonium thiocyanate was added. Amount of peroxide was evaluated by measuring the absorbance at 500 nm. Samples were analyzed at every 24 hours. BHT and vit. C were used as standards. All the tests were performed in triplicate.

$$\% \text{ Inhibition of Lipid Peroxidation} = 100\text{-}[A_1/ A_0 \times 100]$$

***Statistical analysis*:** The results were expressed in terms of Mean ± S.D. of means.

RESULTS AND DISCUSSION

***Total polyphenolic contents*:** Phenolic compounds are normally present in many plants and exhibit multiple therapeutical potentials (Kahkonen *et al*., 1999). Many medicinal plants are great sources of phytochemicals such as phenolic and polyphenolic compounds, many of which have strong antioxidant actions which are often used in food products and in numerous curative treatments (Wong *et al*., 2006; Liu *et al*., 2008).

Extraction technique has a significant effect on extraction efficiency. The extracts of different parts of *D. stramonium* were prepared with water and methanol by mercerization and reflux methods. Present study showed the comparison between two extraction techniques; mercerization and reflux. These two methods are commonly used for extraction of polyphenols. Methanol extracts showed the presence of more TPC than aqueous extracts. It may be due to its high polarity and organic nature. Many researchers used this solvent for the extraction of polyphenols from medicinal plants (Choi *et al*., 2002; Kaur *et al*., 2006; Akter *et al*., 2008; Khalaf *et al*., 2008; Manian *et al*., 2008; Ouzounidou *et al*. 2012; Vekiari *et al*., 2012).

The results of total polyphenolic contents (TPC) in mg GAE/g of extract of *D. stramonium* are presented in Fig.1.

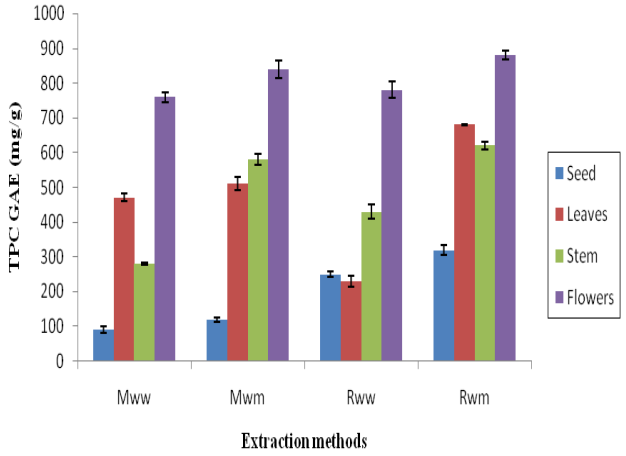

Figure 1. Total polyphenolic contents of different parts of *Datura stramonium*.

Mww: Mercerization with water, Mw ≤ m: Mercerization with methanol, Rww Reflux with water: Rwm; Reflux with methanol.

TPC in seeds, leaves, stem and flower extracts of *D. stramonium* prepared with water and reflux method were 250, 230, 430 and 780 mg GAE/g of extract, respectively. While TPC in seeds, leaves, stem and flower extracts refluxed with methanol was 320, 680, 620 and 880 mg GAE/g of extract respectively. Similarly TPC in seeds, leaves, stem and flowers mercerized with water 90, 470, 280 and 760 mg GAE/g and with methanol was 120, 510, 580 and 840 mg GAE/g, respectively.

Significant difference ($p<0.05$) was observed in the amounts of total polyphenols extracted from different parts of plant. All parts offered considerable amount of total polyphenols. Among different parts, flower extracts of *D. stramonium* exhibited the highest amount of total polyphenolic contents followed by leaves, stem and seed extract.

***DPPH, superoxide and nitric oxide radical scavenging activity*:** Antioxidant capacities, principally radical scavenging activities, are very essential due to the injurious role of free radicals in foods and in biological systems. DPPH radical scavenging assay is important assay for assessment of free radical quenching potential of medicinal plants due to its ease and simplicity (Khalaf *et al*., 2008; Ranilla *et al*., 2010). The DPPH scavenging activity of ascorbic acid, BHT and different extracts of *D. stramonium* is shown in Fig. 2. The DPPH scavenging activity of seeds, leaves, stem and flower extracts of *D. stramonium* at 100µg/ml was 72%, 96%, 95% and 99%, respectively. While the DPPH scavenging activity of ascorbic acid and BHT were 88% and 76%, respectively. The results revealed that *D. stramonium* flowers extract possess the strongest antioxidant potential than both natural and synthetic antioxidants used in this experiment.

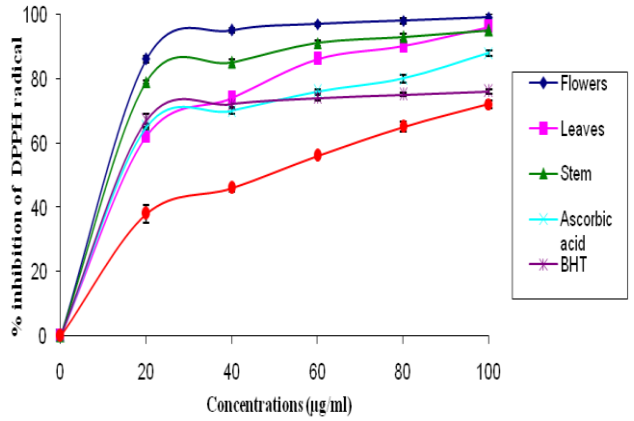

Figure 2. Percentage inhibition of DPPH radical by different parts of *D. stramonium*.

Superoxide radicals are very damaging to various biomolecules in cells and are associated with risk factor of cardiovascular, neurodegenerative, inflammation and other diseases (Shyur *et al*., 2005; Kumar and Sharmilla, 2007). The results of superoxide scavenging activity of *D. stramonium* are presented in Fig.3. Percentage inhibition of superoxide radical by seeds, leaves, stem and flower extracts of *D. stramonium* at 100µg/ml was 73%, 66%, 68% and 74%, respectively. The superoxide radical scavenging activity of flower extract is greater than BHT but lower than ascorbic acid. The results showed that superoxide radical scavenging activity of *D. stramonium* extracts increased by increasing concentration (Sokmen *et al*., 2005; Kumar *et al*., 2008; Sangameswaran *et al*., 2009; Lee *et al*., 2009).

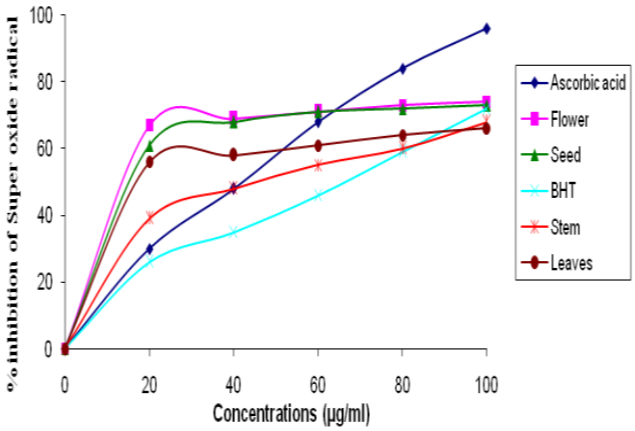

Figure 3. Percentage inhibition of superoxide radical by different parts of *D. stramonium*.

Nitric oxide (NO) is lipophilic and extremely diffusible solute that forms inside the cell. Surplus production of nitric oxide leads to a number of diseases for instance neurodegenerative and muscles diseases. Fig 4 represents

the results of %inhibition of Nitric oxide by seeds, leaves, stem and flower extracts of *D. stramonium*, BHT and Ascorbic acid. Flower extract showed the highest percentage inhibition of nitric oxide radical as 80%, followed by leaf extract 75%, seed extract 74% and lowest was stem extract 73%. Percentage inhibition of nitric oxide by BHT and ascorbic acid was 86% and 82% respectively. Results showed that flower extract of *D. stramonium*has greater potential to inhibit NO radical than all other extracts, and exhibits highest antioxidant potential.

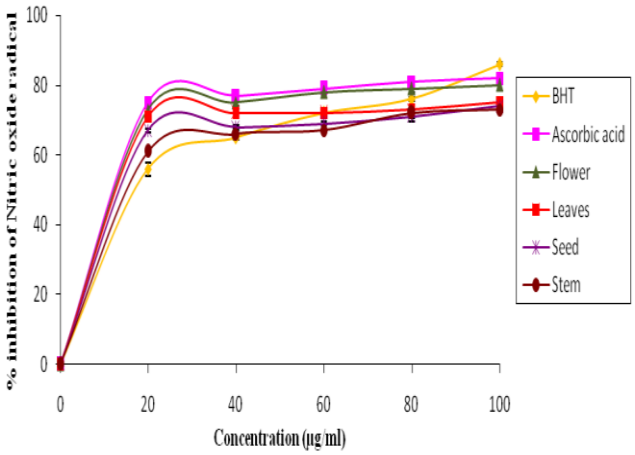

Figure 4. Percentage inhibition of nitric oxide radical by different parts of *D. stramonium*.

***Measurement of reducing power*:** Reducing potential of a compound is said to be the indicator of its antioxidant capacity (Aslam *et al*., 2012). The reducing power of all the extracts tested increased as the concentration increased (Katalinic *et al*., 2006; Liu *et al*., 2008; Krishnaiah *et al*., 2010). The results of reducing power are shown in Fig.5. All the extracts showed an appreciable reducing power. Ascorbic acid exhibits highest reducing power (0.070±0.001) followed by Flower extract (0.067±0.018), seed extract (0.063±0.021), stem extract (0.060±0.018), leaf extract (0.051±0.011) and the lowest reducing power was shown by BHT. The flowers exhibited strong reducing power and the highest TPC which showed the strong correlation between reducing power and TPC. Reducing power of *D. stramonium* is greater than BHT but less than ascorbic acid.

***Antioxidant activity in linoleic acid system*:** Lipid peroxidation is the fundamental step in the onset of many ailments associated to free radicals. It leads to rancidity of food. Iron can hasten lipid peroxidation via Fenton reaction (Aslam *et al*., 2012). Linoleic acidis a polyunsaturated fatty acid. In the absence of any antioxidant it undergoes oxidation and lead to the presence of peroxides. They oxidize Fe^{+2} to Fe^{+3} which can form complex with SCN and can be estimated spectrophotometrically (Jastrzebski *et al*., 2007; Akter *et al*., 2008; Souri *et al*., 2008).

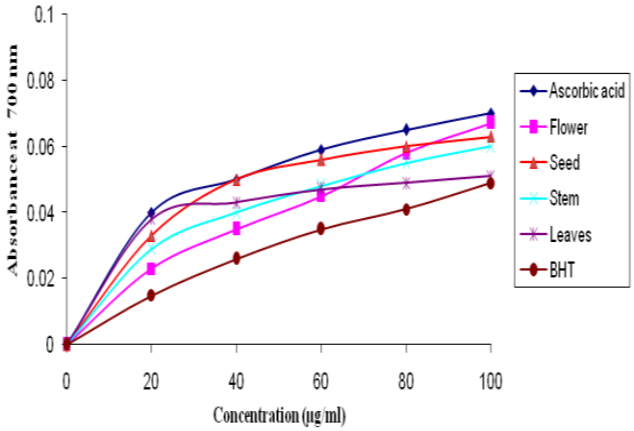

Figure 5. Reducing power of different extracts of *D. stramonium*.

Fig.6 shows the results of percentage inhibition of lipid peroxidation of *D.stramonium*. Percentage inhibition of lipid peroxidation by the extract of flowers of *D. stramonium* at 72 hours was 53.69%, followed by stem extract (50.74%) and seed extract (50.10%) and the lowest inhibition was observed in the leaf extract (42.50%). Percentage inhibition of lipid peroxidation of ascorbic acid and BHT was 43.50% and 56.45%, respectively.

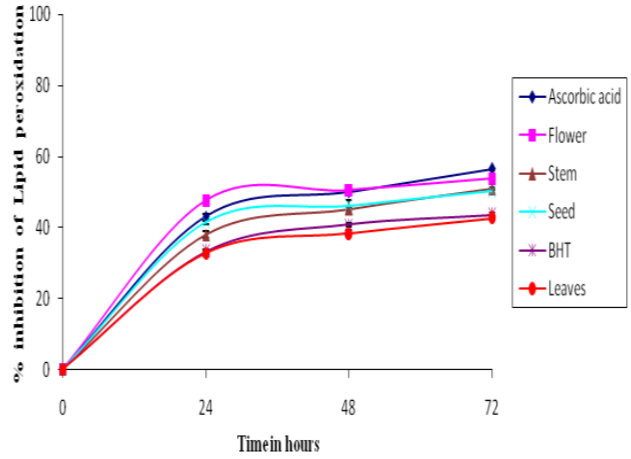

Figure 6. Percentage inhibition of lipid peroxidation by different parts of *D. stramonium*.

In conclusion, the results of this study enlighten the fact that methanolic extracts *of D. stramonium* had strong potential towards all antiradical assays. They had a strong effect on DPPH assay followed by lipid peroxidation inhibition assays and weaker in nitric oxide, super oxide scavenging. Presence of diverse amount of polyphenols in

different extracts may contribute towards their antioxidant capabilities. Hence, extract of flowers could be used in herbal pharmaceuticals as rich source of natural antioxidants.

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