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Distribution of Antinutrients and Antioxidant Properties in the plant of Thornapple (*Datura stramonium L*) *Solanaceae*

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The distribution of antinutrient and antioxidant properties were studied on sun dried thorn apple plant that was separated into the leaf, seed, whole fruit and stem. The antinutrient contents of phytate was highest in the whole fruit (35.39 mg/g) and least in the leaf (9.59 mg/g); oxalate and saponin were observed to be highest in the seed (3.06 and 23.56) mg/g respectively as the leaf contained the highest amount of tannin and cyanide (0.071 and 4.22) mg/g respectively. The results obtained for antioxidant phytoconstituents and activities of various parts showed that the leaf contained a higher value of total phenol content (0.397 mg/g) than other parts with the seed (0.277 mg/g); the stem (0.144 mg/g) and the whole fruit having the least (0.100 mg/g) amount. The ferric reducing antioxidant property (FRAP) was also observed to be highest in the stem (96.69%); the leaf (68.90%); whole fruit (62.70%) with the least in seed (25.60%) for 0.25mg/mL sample solution. The free radical scavenging ability of the extracts against (1,1-diphenyl–2 picrylhydrazyl) DPPH was observed highest in the seed (83.40%); leaf (78.90%); whole fruit (50.40%) while the stem was the least with 11.50%. The results however showed that both the leaf and the whole fruit are potential poisons for animals and humans because of high level of cyanide contents and every part of the plants was significantly higher in one phytoconstituent or the other.

Keywords: Total phenol, reducing power, scavenging ability, mineral chelation, *Datura*

Introduction

There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury (Pourmorad et al*.,* 2006). Besides, well known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices, some natural antioxidants (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements (Schuler 1990), the search is a continuous one even into the less edible plants. *Datura stramonium,* more commonly known as jimson weed or thorn apple, is a wild-growing flowering plant belonging to the family Solanaceae and is a medicinal plant with antinociceptive (Abdollahi et al., 2003) antioxidant (Couladis et al., 2003), hypolipidemic (Rasekh et al., 2001), anti-inflammatory, anti-rheumatoid (Tariq et al., 1989), and hypoglycemic (Gharaibeh et al., 1988) properties. Due to the potent combination of anticholinergic substances it contains, *Datura* intoxication typically produces effects similar to that of an anticholinergic delirium contrasted to hallucination): a complete inability to differentiate reality from fantasy; hyperthermia; tachycardia; bizarre, and possibly violent behavior; and severe mydriasis with resultant painful photophobia that can last several days. Pronounced amnesia is another commonly reported effect (Freye, 2009). Dietary antioxidants are gotten or sourced from diet. They are considered beneficial because of their protective role against oxidative stress, which is involved in the pathogenesis of multiple diseases such as cancer, (Alia et al., 2003; Gülçin, 2010; Gülçin et al., 2010)

Antinutrients are natural or synthetic compounds which form insoluble complexes with calcium, zinc, iron and copper, for example phytic acid, they can also bind to essential vitamins and minerals that may adversely affect nutrients by interfering with digestion or absorption and also key to bad health (Ben Balzer's Paleolithic, 2008).

Proteins can also be anti nutrients, such as trypsin inhibitors and lectins found in legumes. Another form of anti nutrients is the group of polyphenolic compounds that include tannins. These compounds, chellate metals such as iron, zinc reduce the absorption of nutrients, but they also inhibit digestive enzymes and may also precipitate proteins (Ben Balzer's Paleolithic, 2008).

Nevertheless, the large fraction of modern diets that come from a few crops, particularly cereals has raised concerns about the effects of anti nutrients in these crops in human health. The possibility now exists to eliminate anti nutrients entirely using genetic engineering and traditional methods of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant foods through reducing certain anti nutrients such as phytic acid and poly phenols and oxalic acid.

The aim of this study is however to quantify these studied antinutrients and antioxidants in the various parts of the plant as indication for utilization and further investigation of the parts with the highest or the least amounts of these phytoconstituents.

Materials and Methods

Sample collection and preparation:

Fresh samples of the plant *Datura strammium* (thorn apple) was collected from Oshukoti area in Akure, Ondo State and the identification and authentication was carried out at the Department of Crop, Soil, and Pest Management (CSP), Federal University of Technology, Akure,Ondo State, Nigeria. All the chemicals used were of analytical grade, while the water was glass distilled. The samples collected were separated into leaf, seed, whole fruit, stem which were sun dried, crushed or pounded to reduce the size for blending and subsequently blended to powder separately with Marlex Excella laboratory blender.

Aqueous extract preparation for antioxidant properties

The aqueous extract of the various parts were prepared by homogenizing them in distilled water (1:20 w/v); the homogenates were then centrifuged at 3,000 rpm for 10 minutes and the respective supernatants were used for the assays on:

Determination of total phenol content

The total phenol content was determined on the extracts using the method reported by Singleton et al. (1999). Appropriate dilutions of the extracts were oxidized with 2.5mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The total phenol content was subsequently calculated using Tannic acid as standard.

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to
reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5mL aliquot was mixed with 2.5mL of
200 mmol l^{-1} sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5mL of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5mL of the supernatant was mixed with an equal volume of water and 1mL of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Lin, et al., (2010). 1.0mL of the extract was mixed with 1mL of 0.4 mmol l^{-1} methanolic solution containing DPPH radicals. The mixture was left in the dark for 30mins and the absorbance was measured at 516nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Determination of phytate

Phytate was determining according to the method of Wheeler and Ferrel (1971) where 4g of sample was soaked in 100mL of 2% HCl for 3hrs and filtered through No 1 Whatman filter paper. 50mL of the filtrate was placed inside conical flask and 10mL of 0.3% of ammonium thiocyanate solution was added as indicator after which 107mL of distilled water was added to give it the proper acidity, this was be titrated against 0.0056g per milliliter of standard Iron (III) Chloride solution that contained 0.00195g of iron per milliliter until a brownish yellow colouration persist for 5minutes.

Determination of oxalate

Oxalate was determine by soaking 1g of the sample in 75mL of 1.5N H_2SO_4 for 1hr and then filtered through No 1 Whatman filter paper. 25mL of the filtrate was taken into conical flask and this was titrated hot between (80-90^{oc}) against 0.1m $KMnO_4$ until a pink colour that persisted for 15 seconds was obtained. (AOAC, 1990)

Determination of saponin

The spectrophotometric method of Brunner (1984) was used for saponin determination whereby 2g of finely grinded sample was weighed into a 250mL beaker and 100mL of Isobutyl alcohol was added. The mixture was shaking with shaker for 5hours to ensure uniform mixing. The mixture was then filtered with No 1 Whatman filter paper into 100mL beaker containing 20mL of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was again filtered though No 1 Whatman filter paper to obtain a clear colourless solution. 1ml of the colourless solution was taken into 50ml volumetric flask using pipette, 2mL of 5% Iron (III) Chloride solution was added and made up to the mark with distilled water. This was allowed to stand for 30min for the colour to develop after which the absorbance was read against the blank at 380nm. The concentration of saponin in mg/g was expressed from the absorbance of the sample and standard saponin

Determination of tannin

The spectrophotometric determination of tannin was done according to the method of Makkar *et al*., 1993) where 0.2g of finely ground sample was weighed into a 250mL sample bottle. 10ml of 70% aqueous acetone was added and shaken for 10mins in ice water bath. The solution was then centrifuged to obtain the supernatant. 0.5mL of the solution was pipetted into test tube and 0.5mL of distilled water was added. Thereafter 0.5ml of folin reagent (diluted 1:1) was added and 2.5mL of 20% Sodium carbonate will be

added. The tubes were shaking and incubate at room temperature for 40min and the absorbance was read against the blank at 700nm.

The concentration of tannic acid in mgTA/g was obtained from the standard tannic acid calibration curve.

Determination of total cyanide

The AOAC (1990) method was used to determine the total cyanide. 4g of the sample was soaked in a mixture containing 40ml of distill water and 2ml of orthophosphoric acid. This was mixed and stoperred and left overnight at room temperature to set free the bounded hydrocyanic acid. The resulting solution was transferred into distillation flask, drop of paraffin was added as antifoaming agent together wit broken chips as antibumps. The flask was fitted to distillation apparatus and 45mL of the distillate was collected in the receiving flask that contained 40ml of distill water containing 0.1g of NaOH pellet. The distillate was then transferred into 50ml volumetric flask and made up to the mark with distill water. 20ml of distillate was collected into conical flask and 1.6mL of 5% potassium iodide solution was added and titrated against 0.01M of Silver nitrate solution until fainted turbidity that persist was obtained. Total cyanide in mg/g was calculated from the titre value obtained.

Results

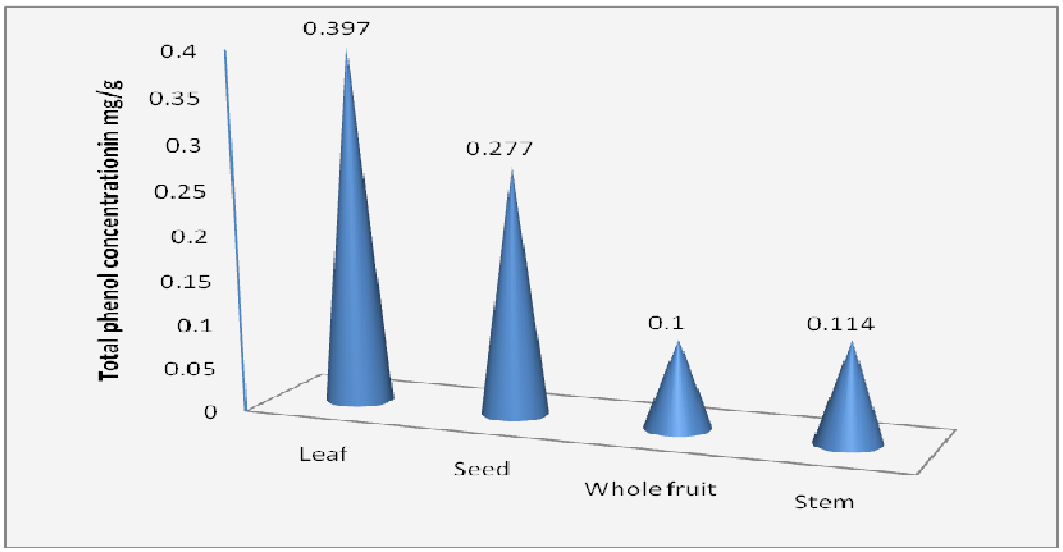

Figure 1: Total phenol contents of the various parts of Datura plant

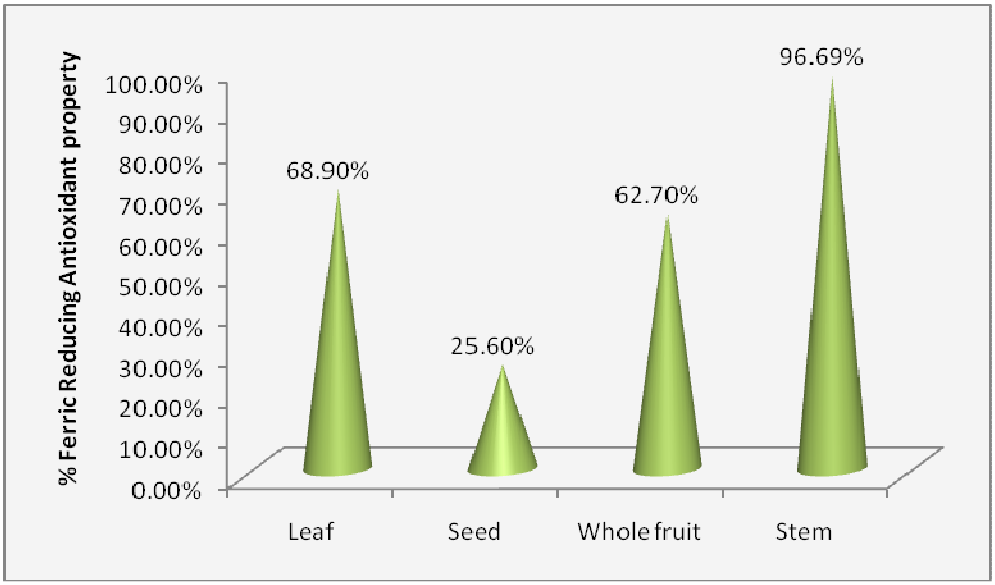

Figure 2: Ferric reducing antioxidant property of the various parts of *Datura* plant

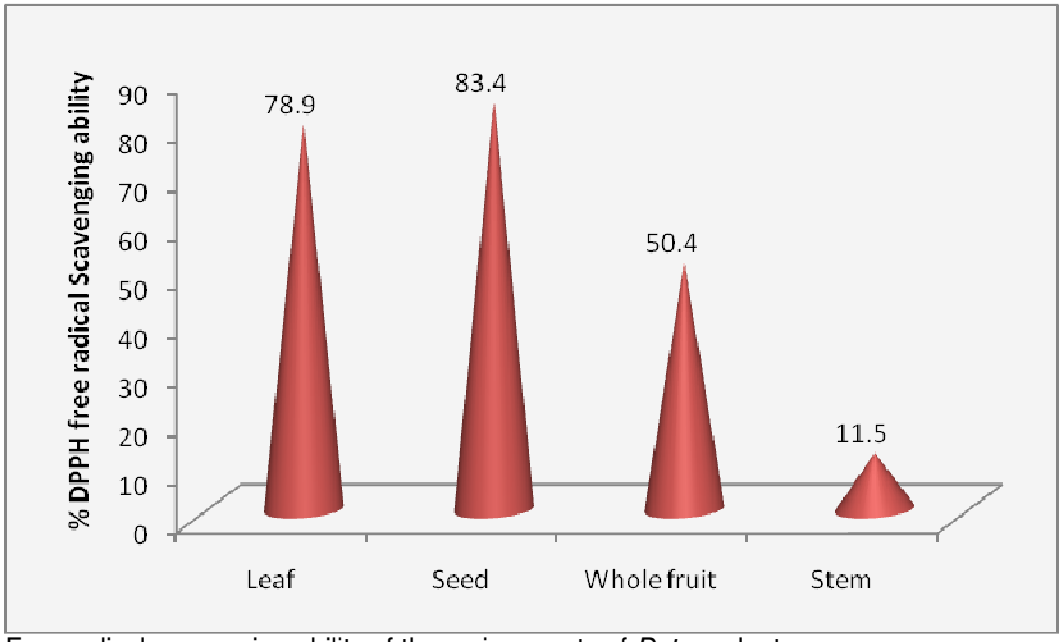

Figure 3: DPPH Free radical scavenging ability of the various parts of *Datura* plant

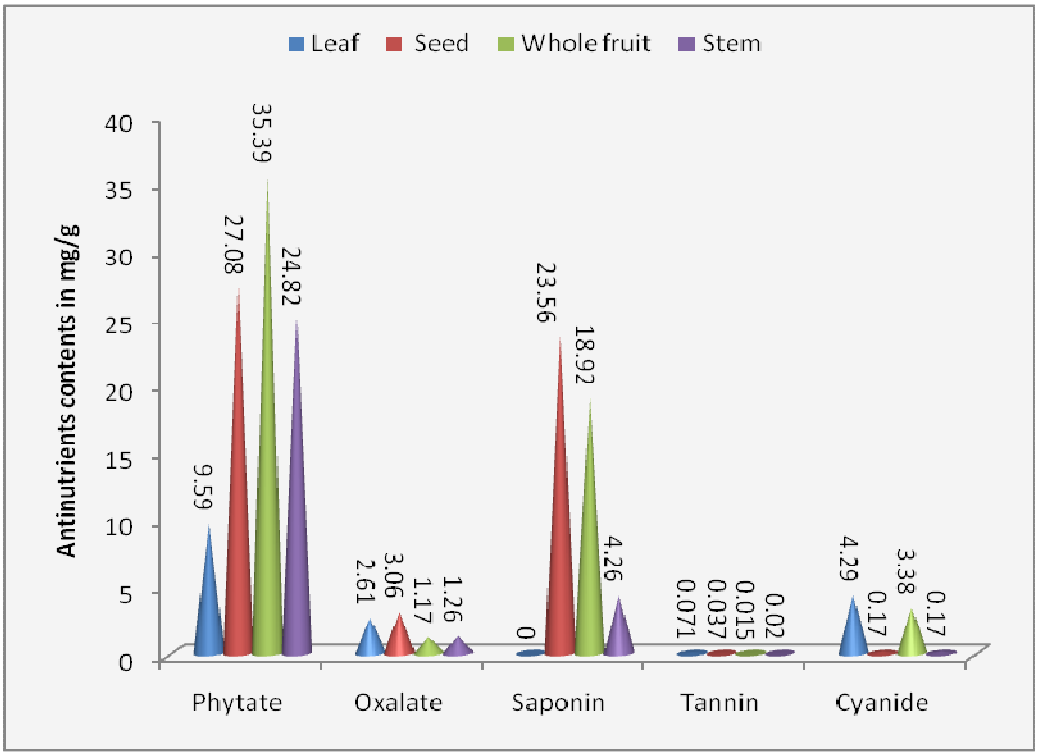

Figure 4: Anti nutrients composition of the various parts of *Datura* plant

Discussion

The results of the distribution of total phenol content in the extracts of the plant showed that all the parts of the plant contained this parameter with the leaf having the highest phenol content than other parts as seen from Figure 1. The least amount of total phenol obtained for the fruit is however three times higher than that obtained for *Jathropha curcas* (Oseni and Akindahunsi, 2010). The ferric reducing antioxidant property was also discovered in all the parts of the plant with the stem

having the highest percentage ferric reducing ability as seen in Figure 2. Similarly from Figure 3 all the parts of the plant showed DPPH free radical scavenging ability with the seed having the highest DPPH scavenging ability, this however is four times higher than that obtained for *Jathropha curcas* (Oseni and Akindahunsi, 2010)

The various parts of the plants also showed high amount of anti-nutrients studied as seen from Figure 4; the fruit contained the highest amount of phytate which has been studied to inhibit the bioavailability or absorption of certain metals in infants and to an extent in adults, the implication of which may result in reduced psychomotor and mental development with long term negative consequences on school performance (Davisson *et al*., 1994). The seed had the highest amount of saponin which is found in many plants (MacDonald *et al.,* 2005) and often referred to as "natural detergents" as it causes red blood cell haemolysis and as such toxic (Mudzwiri, 2007)). Oxalate was also found to be highest in the seed, oxalate is one of the antinutritional factor widely distributed in plant foods (Gupta, *et al*., 2005). The leaf contained the highest amount of tannin which may be responsible for bitterness, pungency, astringent properties, hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The cyanide which was also highest in the leaf is one of the most rapid poisons known with a lethal dose of 0.5-3mg/kg body weight because of its ability of linking with metals (Fe^{2+}, Mn^{2+} and Cu^{2+}) that are functional groups in many enzymes thereby inhibiting vital cellular processes. The presence of high contents of these anti-nutrients might be responsible for the toxic and poisonous natures of this plant that makes the plant to be abhorred by domestic animals where ever it is found and abnormal behaviour of human when it is ingested.

Conclusion

Datura stramonium has proved to be very rich in both antinutrients and antioxidants which can be harnessed for both human and animal uses in the areas of food and pharmaceuticals. The various parts of the plants have been studied to contain varying amounts of these phytochemicals which form the bases for utilizations and further investigations.

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