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In vitro discovery of highly chelatable root extract of thorn apple (*Datura stramonium*)

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This study investigated the *in vitro* chelating ability, antioxidant activity, total phenolic and flavonoid contents of 70% ethanolic extract of thorn apple (*Datura stramonium*). The ethanolic root extract of the plant displayed potent chelating ability (70%) at the lowest concentration (2%) investigated. The chelating ability of the extract showed concentration dependency. There were significant decreases *in vitro* chelating ability of the root extract from 2 to 10% concentrations (P<0.05). The root extract also demonstrated exhibited concentration dependent increases in antioxidant activity, which were non-significant from 2 to 6% concentrations (P>0.05) and significant from 8 to 10% (P<0.05). The ethanolic root extract displayed maximum activity [106.20±0.84% standard deviation (SD)] at the highest concentration (10%). The preliminary phytochemical screening of the aqueous extract of *D. stramonium* revealed the presence of flavonoids, tannin and alkaloid, but saponin was absent. The aqueous root extract of the plant also exhibited potent antioxidant potential (71.00%) at 2% concentration. The antioxidant activity of the aqueous root extract of the plant closely approximated the antioxidant activity of the ethanolic root extract of the plant (71.00 and 71.40%, at different concentrations, 2 and 5%, respectively). The aqueous root extract of the plant showed good *in vitro* nitric oxide radical scavenging activity (67.80%). The absolute and 70% acetone root extracts of the plant were dull nitric oxide scavengers (33.38 and 26.00%, respectively). The total flavonoid and phenolic contents of ethanolic root extract of *D. stramonium* showed non-significant increases as the concentration increased (P>0.05). The ethanolic root extract failed chelate ion at the highest concentration (10%) (-20.0% activity).

Key words: Phytotoxicology, clinical toxicology, iron-overload, clinical medicine, natural product.

INTRODUCTION

Polyphenols and flavonoids are used for the prevention and cure of various diseases which is mainly associated with free radicals (Havesteen, 1983). *Datura stramonium* belongs to the family Solanaceae (Oseni et al., 2011). The plant is a drug of abuse (Aroulou et al., 2003). The eating and chewing of the plant is a suicide attempt (Monteriol et al., 2007). It is more commonly called the jimson weed or thorn apple (Abdollahi et al., 2003). It is a wild growing flowering plant (Abdollahi et al., 2003). The plant contains atropine alkaloids such as scopolamine, hyosciamine and atropine primarily in the seed, flower (Preissel and Hans-George, 2002). The seed oil of the plant is a good source of protein and nutritionally valuable minerals (Oseni et al., 2011).

The seed possessed hallucinogenic and euphoric effects (Ertekin et al., 2005). It contains three main toxic alkaloids atropine, scopolamine and hyosciamine (Urich et al., 1982). The foliage and the seed of the plant are particularly toxic (De Fratels, 2005). The seed extract induced centrolobular necrosis and dilated central vein in rats (Bouzidi et al., 2011).

The seed extract of the plant possessed agglutination

effect on erythrocytes from several species, and is non-specific with regard to human ABO blood group (Kalpatrict et al., 1978). The aqueous leaf extracts enhanced cytotoxicity on human cancer cells via oxidative stress (Ahmad et al., 2009). The seed extract possessed analgesic effect against acute and chronic pain (Khalili and Atyabi, 2004). Traditionally, the seed extract is used in the treatment of toothache and fever (Sandoval, 1998). Exposure to the smoked extract of the leaf of the plant has deleterious effects on the cytoarchitecture of liver, heart, kidneys and testes in animals (Adekomi et al., 2011). The plant is used as a phytoremediator of explosives and to clear 2,4 ,6- trinitrotoluene via nitro reduction in municipal waste sites (Lucero et al., 1999). Traditionally, smoked leaves of the plant are used as an anti-spasmodic in the treatment of asthma (Foster and Duke, 1990). The root extract mixed with latex of Calotroprocera is used to cure scorpion sting and snake bite (Jain et al., 2011; Najafabadi and Atyabi, 2004).

The methanolic extract, ethanolic and aqueous extracts of the leaves, stem and root showed antibacterial activity (Akharaiyi, 2011; Iranbakhsh et al., 2010). The methanolic extract of the plant showed antioxidant activity (Mishra et al., 2011).

MATERIALS AND METHODS

Collection of plant

The root of *D. stramonium* was collected from the back of Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Oyo State, Nigeria on 4th June, 2011.

Preparation of plant extracts

The plant was washed with distilled water and then cut into pieces. Five different concentrations (2- 10%, w/v) of the root of *D. stramonium* were prepared in 70% ethanol. This was carried out by soaking 2, 4, 6, 8 and 10 g of the root in 100 ml of 70% ethanol in five different containers with lid for 30 min. Each mixture was filtered with Whatman filter paper. Each filtrate was used for biochemical assays of interest.

Another 5% extract (w/v) of the root of the plant was prepared by soaking 5 g of the root of the plant in 100 ml of each of the solvents used (water, 70% ethanol, absolute acetone and 70% acetone). The mixture was filtered using Whatman filter paper. The filtrate obtained was used for assays of interest.

Chemicals and reagents

The phenanthroline used was a product of British Drug House (BDH), UK. Folin-Ciocalteu reagent used was a product of Merck, Germany. The stable free radical utilized (2,2-diphenyl -1-picrylhydrazyl (DPPH)) was a product of Sigma-Aldrich, USA.

In vitro assays

In vitro Fe^{2+} *chelating ability assay*

The *in vitro* Fe^{2+} chelating ability of the plant extract was assayed according to the modification of Minnoti and Aust (1987). Briefly, 200 µl of sample was mixed with 150 µl ($FeSO_4$, 500 µM) (freshly prepared), 168 µl of Tris HCl (0.1 M, pH 7.4) and 218 µl of saline (NaCl, 0.9% w/v). The mixture was incubated for 5 min, after which 13 µl aqueous phenanthroline (0.25%, w/v aqueous) was added. The absorbance of the solution was read at 510 nm with distilled water as blank on a spectrophotometer. The *in vitro* of Fe^{2+} chelating ability of the sample was calculated by using the following formula:

$$\text{Chelating ability (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control (reaction mixture in the absence of sample) ($FeCl_3$ alone); A_{sample} is the absorbance of sample.

In vitro nitric oxide radical scavenging potential assay

The *in vitro* nitric oxide scavenging activity was estimated according to the method of Marcocci et al (1994). To 1 ml sample, 1 ml of sodium nitroprusside (10 mM, aqueous) and 1 ml buffer (sodium phosphate buffer, 0.2 M) were added. The mixture was incubated at room temperature for 150 min (2 h 30 min) followed by the addition of 01 ml Griess reagent. The absorbance of the pink colour solution was read at 540 nm on a spectrophotometer. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-naphthyl ethylene diamine dihydrochloride (NED) was measured spectrophotometrically at 540 nm.

The *in vitro* nitric oxide scavenging activity of the sample was calculated by using the following formula:

$$\text{Nitric oxide scavenging activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

In vitro antioxidant activity (DPPH based) assay

The *in vitro* antioxidant activity of the sample was quantitated according to the traditional method of Blois (1958). To 1 ml of plant extract, 1 ml of methanolic solution of DPPH (0.2 mM) was added. The mixture was incubated in the dark for 30 min. The absorbance of the yellow colour solution was read at 517 nm on a spectrophotometer using distilled as blank. This spectrophotometric assay uses the stable radical DPPH as a reagent. The purple colour of the methanolic DPPH which resembles the colour of $KMnO_4$ was changed to yellow colour in the presence of hydrogen or electron donating antioxidant, giving diphenyl picryl hydrazine as a product. DPPH reacts with reducing agents and then electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (Blois, 1958).

$$\text{DPPH scavenged (\%)} = (A_{DPPH} - A_{sample})/A_{DPPH} \times 100$$

A_{DPPH} is the absorbance of methanolic solution of 2,2-diphenyl-1-picryl hydrazyl; A_{sample} is the absorbance of sample.

Total phenolic content assay

The phenolic content of the sample was determined according to the method of Taga et al. (1984). To 0.1 ml of sample, 2 ml of sodium carbonate solution (0.2% w/v) was added, followed by the addition of 0.1 ml of Folin-Ciocalteu reagent (10%, v/v). The mixture was incubated for 10 min. The absorbance of the blue colour solution was read at 480 nm. This is based on chemical reduction of tungsten and molybdenum oxides affording a blue colour solution, which was measured spectrophotometrically. The of total phenolic

Table 1. Changes in levels of phenolics, flavonoids, antioxidant and chelating abilities of 70% root ethanolic extract of Siam weed (*Datura stramonium*).

Concentration (%, w/v)	Phenolic content (mg/ml)	Flavonoid content (mg/ml)	Chelating ability (%)	Antioxidant activity (%)
2	12.20 ± 0.10	2.00 ± 0.10	70.00 ± 1.23	71.41 ± 0.89
4	12.80 ± 0.12	2.60 ± 0.07	40.00 ± 1.23	74.00 ± 1.0
6	13.80 ± 0.14	3.20 ± 0.19	20.00±1.00	87.80 ± 0.84
8	14.60 ± 0.07	4.80 ± 0.27	25.00±0.71	90.40 ± 0.55
10	15.20 ± 0.12	5.40 ± 0.20	-20.00± 1.22	106.20 ± 0.84

Table 2. Changes in the levels of antioxidant and nitric oxide scavenging activities and flavonoid content in 5% aqueous, absolute and 70% acetone extracts of *Datura stramonium* extracts.

Solvent	Flavonoid content (mg/ml)	Antioxidant activity (%)	Nitric oxide radical Scavenging (%)
Water	15.20 ± 1.10	71.00 ± 1.00	67.80 ± 0.84
Absolute acetone	5.20 ± 1.10	68.40 ± 1.14	33.38 ± 0.91
70% acetone	4.40 ± 0.89	70.40 ± 0.89	26.00 ± 0.71

concentration (mg/ml) in the extract was extrapolated from pyrocatechol calibration curve.

Total flavonoid content assay

The flavonoid content of the sample was determined according to the method of Lamaison and Carnet (1990). To 0.5 ml sample, 0.5 ml of 70% $AlCl_3.6H_20$ (2%) was added and the mixture incubated for 10 min. The absorbance of the yellow colour solution was read at 430 nm after 10 min on a spectrophotometer using distilled water as blank. The total flavonoid concentration (mg/ml) of the extract was obtained from a calibration curve using quercetin as a standard flavonoid. In this colorimetric assay, the $AlCl_3$ acid stable complexes with the 4-keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols also form acid labile complexes with the ortho-dihyroxyl groups in the A or B-ring of flavonoids.

RESULTS

The ethanolic root extract of the plant showed potent chelating ability (70.0% activity) at the lowest concentration (2%). The ethanolic extract of the plant displayed excellent antioxidant activity (106.20% at the highest concentration, 10%). The *in vitro* chelating potential ranged from -20.0 to 70.0% (Table 1). The total flavonoid and phenolic contents of the ethanolic root extracts showed non-significant increases as the concentration of the plant extract increased (P>0.05). The maximum total phenolic concentration of the ethanolic root extract was 15.20 mg/ml. At the highest concentration, the ethanolic root extract of *D. stramonium* failed to chelate ion *in vitro*. The antioxidant activity of the aqueous root extract of the plant closely approximated the antioxidant activity of the ethanolic root extract of the plant (71.00 and 71.40%, at different concentrations, 2 and 5%, respectively) (Tables 1 and 2).

The order of *in vitro* nitric oxide, radical scavenging activity of *D. stramonium* selected was investigated: water > absolute acetone > 70 acetone. The *in vitro* nitric oxide radical scavenging activity of the aqueous of the plant was significantly higher than that of 70% acetone extract (P<0.001). The 70% and absolute root extracts of *D. stramonium* were weak *in vitro* nitric oxide scavengers (26.0 and 33.38% nitric oxide scavenging potential, respectively). The order of flavonoid content in selected solvents (water, absolute and 70% acetone) was: water > absolute acetone > 70% acetone acetone. The *in vitro* nitric oxide scavenging activity of absolute ethanolic extract of the plant was higher than the 70% acetone extract, but the difference was not significant (P>0.05). In addition, the aqueous, absolute and 70% acetone extracts of the plant demonstrated potent antioxidant activity. The antioxidant activity of the aqueous extract of the plant and the 70% acetone extract was in the region of 70%. The values of *in vitro* antioxidant potentials of the absolute and 70% acetone extracts were significantly higher than *in vitro* nitric oxide radical scavenging in the two solvents (P<0.001).

Phytochemical screening revealed that saponin was absent in the aqueous root extract of the plant. Flavonoid, tannin and alkaloids were detected in the aqueous extract of the plant (Table 3).

DISCUSSION

Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals (Flora and Pachauri, 2010). Metals with normal concentration have essential roles in body metabolism; however, in higher concentration

Table 3. Preliminary screening of bioactive constituents of aqueous root extract of *D. stramonium*.

Phytochemical	Solvent (Distilled water)
Flavonoid (Shibata's test)	+
Tannin	+ +
Alkaloid (Meyer's test)	+ +
Saponin (Frothing test)	-

$^{+}$Indicates slightly positive, while $^{++}$Indicates strongly positive.

concentration they can induce severe toxicity (Mirzaei and Khatami, 2013). A sample high in polyphenol might not chelate metal ions if the polyphenol present did not possess suitable groups that could chelate the metal cations (Hider et al., 2001). Bidentate ligands are more powerful scavengers of metal cations than monodentate ligands (Hider et al., 2001). Chelating agents are effective as secondary antioxidants (Gordon 1990).

The lowest concentration of the 70% root ethanolic extract of Siam weed (*D. stramonium*) (2%) displayed potent chelating ability *in vitro*. This is the first report of *the in vitro* discovery highly chelatable extract of the plant in 70% ethanol. The value obtained in the present study was low when compared with the maximum chelating ability of hydroalcohol extract of *Coriander sativum* (90%) previously reported (Mirzae and Khatami, 2013). Our experimental value for chelating ability of *D. stramonium* extract *in vitro* state (70%) was the same to that of chelating ability of aqueous stem extract of *Euphorbia macrolada* (70%, at 1 mg/ml) reported by Farhan et al. (2013). In the present study, the ethanolic root extract of *D. stramonium* showed concentration dependent decrease in chelating ability as the concentration of the extract increased, which was consistent with research work on chelating ability of *Bauchinia purpurea* stem bark extracts with ethyl acetate, petroleum ether and methanol as extraction solvents (Chaudhari and Nagar, 2013).

It is known that the ability of phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups (Van-Acker et al., 1996). Phytochemical screening of the aqueous extract of *D. stramonium* in our present work revealed the presence of flavonoids and tannins, which was consistent with some phytoconstituents of the ethanolic leaf extract of *Phyllanthus amarusschonn* earlier reported (Ujwala et al., 2012). The phytochemicals evaluations of plants which have suitable use in folkore have often resulted in the iso-lation of principles with remarkable bioactivities (Afolabi et al., 2007). Flavonoids are known to exhibit antioxidant properties through chelating with transition metals, pri-marily Fe^{2+}, Fe^{3+} and Cu^{2+}, which participate in reactions generating free radicals (Malesev and Kuntic, 2007). Flavonoids constitute a large group of polyphenolic compounds with antioxidant properties which are over-whelmingly exerted through direct free radical scavenging (Malesev and Kuntic, 2007). Antioxidant activity of phenolic compounds is due to their tendency to chelate metals (Michalak et al., 2006).

Plant extracts offer promising sources of natural antioxidants (Tupe et al., 2003). The *in vitro* antioxidant activity of the ethanolic carrot (*Daucus carota*) root crude extract (27.5%) (Chatatikun and Chiabchalard, 2013) was lower than the maximum antioxidant activity of the root ethanolic extract (106.20%) observed in the present study at the highest concentration of the plant (10%). The phenolic compounds may contribute directly to antioxidative action (Awika et al., 2003). Rutin is a standard flavonoid and exhibited *in vitro* antioxidant activity (69.83%) at 1000 µg/ml (Kumar et al., 2010). However, the antioxidant activity of root ethanolic extract *D. stramonium* was obtained in this work (106.20%) at 10% concentration greater than that of rutin. It has been reported that radical scavenging action is dependent on both the reactivity and concentration of the antioxidant (Resat et al., 2007). The antioxidant activity of an antioxidant compound has been attributed to various mechanisms among which are the binding of transition metal ion catalysts, prevention of hydrogen abstraction and radical scavenging (Gulcin et al., 2005). DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants (Duh et al., 1999).

The reduction of nitric oxide with phenolic groups present in antioxidant may serve to attenuate the concentration of nitric oxide (Wilcox and Janzen, 1993). Nitric oxide radical scavenging capability of ascorbic acid *in vitro* state (95%, at 3 or 5 mg/ml) reported elsewhere (Chai et al., 2013) was greater than our experimental values (67.80, 33.38 and 26% for water, absolute acetone and 70% acetone, respectively).

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