Antioxidant Activity and Mineral Elements Profiles of Isoberlinia Doka
Leaves from Nigeria

I.E. Abdulkadir, A.B. Aliyu, M.A. Ibrahim, S.B.D. Audu and A.O. Oyewale

Abstract: Antioxidants activity of methanol leaves extract of Isoberlinia doka was evaluated using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging and reducing power assay. Total phenolics content, mineral element composition and preliminary phytochemical analyses were also carried out. The extract was found to have different levels of antioxidant capacity in the two models tested. The result of DPPH radical scavenging exhibits strong antioxidant activity of the extract with 93.25% at higher concentration. The reducing ability of the extract (0.106± 0.002 nm) was found to be higher than that of Gallic and ascorbic acids and the total phenolics content of 316 mg/100g Gallic acid equivalent (GAE) may supports the significant antioxidant activity of the extract. Some essential elements linked to antioxidants such as Fe (283±11 mg/100g) and Zn (66±1 mg/100g) are found in remarkable quantities. Macro minerals such as Ca (1333±164 mg/100g), K (1022±21 mg/100g) and P (1031±112 mg/100g) are found abundantly present. The results obtained in this study indicates that the methanol leaves extract of the plant has promising antioxidant potentials which may also enhance the therapeutic properties of the plant against diseases caused by free radicals.

Key words: Isoberlinia doka, mineral elements, phytochemical analysis, antioxidant activity.

INTRODUCTION

Substantial literature evidence has implicated the role of reactive oxygen species (ROS) in the etiology of quite a number of human diseases (Halliwell and Cross, 1994; Bandyopadhyay et al., 1999; Forbes et al., 2008). Although the ROS are normally produced in living organisms with a well recognized beneficial role at low or moderate concentrations to help the normal body processes such as regulation of signal transduction, gene expression, activation of receptors and nuclear transduction factors (Valko et al., 2006); but they equally have potentials to damage biological molecules such as lipids, proteins, polysaccharides and DNA (Valko et al., 2006) through an oxidative attack on such molecules. This usually occurs when the animal endogenous defense enzymes (glutathione peroxidase (GST-Px), superoxides dismutase (SOD) and catalase (CAT)) whose role is to neutralize any deleterious effects of the ROS become overwhelmed due to metabolic imbalance that leads to an overdrive of the mitochondrial electron transport chain, exposure to environmental pollutants, cigarette smoke, UV rays, some parasitic infections among others. This situation results to an imbalance between the endogenous anti-oxidative system and the oxidant ROS referred to as oxidative stress (Vaziri, 2008). The consequences of this imbalance have been linked to inflammation and hypertension (Vazini, 2004, 2006), complications in diabetes mellitus (Moussa, 2008), carcinogenesis (Hemmani and Parihar, 1998; Pillai and Pillai, 2002) and indeed most other metabolic diseases.

Vegetables and plants consumed as food or medicines are widely accepted to provide new sources of antioxidants because of their potential biological and pharmacological activities. This is because compelling literature reports suggest the protective effects of phytochemicals (polyphenolics, proanthocyanidins or bioflavonoids) and vitamins (vitamins A,C and E) against cardiovascular diseases (Duthie et al., 2000), Alzheimer disease (Engelhart et al., 2002), cancer (Park and Pezzuto, 2002; Saleem et al., 2003) and trypanosomes infections (Umar et al., 2008). This is indicative of the crucial role antioxidant substances could play in the development of newer generation of chemotherapeutic agents that could be used to treat such diseases. In recent times, research activities on antioxidants from plants sources have attracted a wide range of interest across the world. The focus has always been evaluating antioxidant capacity of medicinal plants whose traditional remedies are linked to disease conditions associated with oxidative stress. This development has tremendously improved our understanding of the potential roles of medicinal plants in disease prevention and drug discovery.

Isoberlinia doka is a tree native to northern Nigeria and known as doka (Hausa). It grows to about 20 m high, with spreading crown and thrives in both temperate and tropical areas. The tree has been used by traditional medical practitioners for the treatment of diabetes, ulcer, wounds and cough (Personal communication). Phytochemical screening of the stem bark extract revealed the presence of saponins, flavonoids, alkaloids and volatile oils; the extract was found to exhibits antibacterial activity against Bacillus subtilis (Kubmarawa et al., 2007). In our continuous investigation on the phytochemical and pharmacological
properties of medicinal plants belonging to the Nigerian flora, we evaluated the antioxidant properties of methanol leave extract of *Isoberlinia doka* with a view to assess its potentials as natural antioxidant as well as to understand the scientific basis of its therapeutic properties in traditional medicine.

**MATERIALS AND METHODS**

**Chemicals and Reagents:**
Deionized water, Folin Ciocalteu reagent (Fluka, UK), Gallic acid (Fluka, UK), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Trichloroacetic acid (Sigma-Aldrich Co), anhydrous ferric acid, potassium ferricyanide, anhydrous sodium carbonate, ascorbic acid and other chemicals used were of analytical grade BDH chemical laboratory (England, UK).

**Plant Materials:**
The leaves of *Isoberlinia doka* were collected from Zaria on 21st August, 2010. It was authenticated by Mr U.S. Gallah of the herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimen number (90013) was deposited there. It was air-dried for two weeks and then pulverized using a mortar and pestle. The powdered plant sample was then sieved and stored in an air tight container until further use.

**Extraction:**
Pulverized leaves of plant sample (400g) were extracted exhaustively with 1.5L of methanol using cold extraction. The extract was filtered using Whatman filter no.2 and concentrated on a Büchi rotary evaporator (Büchi Rota vapor R-124) at 45°C which afforded crude extract (53.44g) referred to as *Isoberlinia doka* methanol extract (IDME), with a percent recovery of 13.36%.

**Phytochemical Screening:**
Phytochemical screening was carried out to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and anthraquinones, using standard phytochemical methods as described by Sofowora (1993).

**Total Phenolics Content:**
The total phenolics content was determined using the methods of McDonald *et al.*, (2001) with slight modifications. A calibration curve was produced by mixing ethanolic solution of Gallic acid (1ml; 0.02-0.2 mg/ml) with 5ml Folin-Ciocalteau reagent (diluted tenfold) and sodium carbonate (4ml, 0.7M). Absorbance was measured at 765nm and a standard curve was drawn. 1ml of IDME (5g/L) was also mixed with the reagents above and after 30 minutes, the absorbance was measured to determine the total phenolics contents. All determinations were carried out in triplicates. The total phenolics content in Gallic acid equivalent (GAE) was calculated by the formula: $T=C \times V/M$. Where $T =$ total phenolics content, mg/g of IDME in GAE; $C =$ concentration of Gallic acid established from the calibrat ion curve in mg/ml; $V =$ volume of extract in ml; $M =$ weight of IDME in g.

**Reducing Power Assay:**
This was determined according to the method of Oyaizu (1986). Sample (IDME) solution or standards (100µg/ml) was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Trichloroacetic acid (10%, 2.5ml) was added to the mixture. A portion of the resulting mixture was mixed with FeCl₃ (0.1%, 0.5ml) and the absorbance was measured at 700 nm in a spectrophotometer (Jenway, 6025). Increase in absorbance of the reaction mixture indicates reducing potential.

**Evaluation of Free Radical Scavenging Action:**
The determination of the free radical scavenging activity of IDME was carried out using the DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) assay as described by Mensor *et al.*, (2001) with a slight modification. Various concentrations of 10, 25, 50, 125 and 250 µg/ml of IDME in methanol were prepared. 1ml of a 0.1mM of DPPH in methanol was added to 2.5ml of IDME or standards and allowed to stand at room temperature in a dark chamber for 30min. The change in colour from deep violet to light yellow was measured at 518nm with a spectrophotometer (Jenway, 6025). The decrease in absorbance was then converted to percentage antioxidant activity (%AA) using the formula: $%AA = 100 - \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}$. Blank = methanol (1ml) plus sample solution (2ml), Negative control = DPPH solution (1.0ml, 0.25mM) plus methanol (2.0ml), ascorbic and Gallic acid were used as standards.
**Mineral Content Analysis:**

The major elements, comprising calcium and magnesium and trace elements (iron, manganese, copper, cobalt, chromium, cadmium, lead and zinc) were determined according to the method of Shahidi et al. (1999) with slight modification. The sieved powdered sample (2 g) was subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/H_2O_2 (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in a crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in the crucible was transferred into a 100 ml volumetric flask by filtration through a whatman filter paper and the volume was made to mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). Concentration of each element was calculated on percentage of dry matter.

**Statistical Analysis:**

All experiments were carried out in triplicates. The results are given as mean ± standard deviation (SD). Student’s t-test was used for comparison between two means. A difference was considered statistically significant if p ≤ 0.05.

**RESULTS AND DISCUSSIONS**

The results of phytochemical screening of methanol leaves extract of *Isoberlinia doka* revealed the presence of alkaloids, flavonoids, saponins, tannins, triterpenes and cardiac glycosides, but anthraquinones were absent. The total phenolics content was found to be 316mg/100g Gallic acid equivalent (Table 1). The IDME demonstrated a powerful ferrous reducing power that is statistically similar to ascorbic acid and significantly higher than Gallic acid (Figure 1). Figure 2 presents the free radical scavenging activity (%) of the extract. A dose dependent DPPH radical scavenging activity was observed. Furthermore, no significance difference (P>0.05) between IDME and ascorbic acid and or Gallic acid was recorded at all the concentrations tested. Mineral elements content analysis showed the presence of calcium (1333±164 mg/100g), phosphorus (1031±112 mg/100g) potassium (1022±21 mg/100g) and sodium (905±4 mg/100g). Trace elements such as iron (283±11 mg/100g) and zinc (66±1 mg/100g) were also found. Manganese was however not detected (Figure 3).

**Table 1:** Total phenolics and phytochemical screening of methanol extract of *Isoberlinia doka*.

<table>
<thead>
<tr>
<th>Total phenolics</th>
<th>316mg/100g GAE</th>
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<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
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<td>Tannins</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
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<tr>
<td>Saponins</td>
<td>+</td>
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<tr>
<td>Triterpenes</td>
<td>+</td>
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<tr>
<td>Cardiac glycosides</td>
<td>+</td>
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<tr>
<td>Anthraquinones</td>
<td>-</td>
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+= Present; - = absent, GAE= Gallic acid equivalent.

**Fig. 1:** Total reducing power of methanol extract of *Isoberlinia doka* leaves. Data are presented as mean ± SD of triplicate determinations.
Discussion:

*Isoberlinia doka* is a plant native to northern Nigeria and used for numerous medicinal purposes. So far, scientific information on the biological activities of this plant is scanty in the literature. In this study, we reported the *in vitro* antioxidant activity as well as the mineral elements profile of leaves methanolic extract of this plant.

The high total phenolics content and the detection of flavonoids, saponins and tannins in the *I. doka* methanol extract could indicate the involvement of phenolics and these bioactive agents in the bioactivities of this plant and might partly be responsible for the folkloric use of the plant in traditional medicine. Flavonoids have recently been implicated in the anti-venom protease activity of some Nigerian tropical plants (Ibrahim et al., 2011) and the antifungal activity of some saponins has been reported (Khan and Srivastava, 2009).

Antioxidants are radical scavengers which protect the human body from the pathological effects of free radicals. These chemical compounds are found in various processed foods or medicines as preservatives. But the increasing concern about chemical toxicity of the synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has triggered public interest in naturally derived antioxidants from diet, supplements and medicinal plants. The reducing ability of plant extracts may be indicators of their antioxidant potentials (Oyaizu, 1986). The presence of phenolics antioxidants causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form which indicates higher reducing ability. Our results suggest that *I. doka* methanolic extract has a high electron donating capacity as well as strong redox potential and can act as a reducing agent in quenching free radicals. The antioxidant evaluation of plant extracts or pure compounds using DPPH model has been a reliable and reproducible *in vitro* technique reported in the last few years (Burda and Oleszek, 2001; Ara and Nur, 2009). The extract under study demonstrated a superb free radical scavenging activity that is similar to standard antioxidants at all concentration tested. Thus, the powerful antioxidant potentials displayed by this extract, at least in these experimental models, could be linked to the phenolic content and or the phytochemicals detected therein. High antioxidant activity of plant extracts has previously
been attributed to the phenolic compounds (Odagbasoglu et al., 2004) and flavonoids, saponins and tannins (Aliyu et al., 2009).

Mineral elements are important components of biological systems; more than 27% of known enzymes requires minerals, especially divalent cations for enzymatic activity (Mildvan, 1970). Some elements are directly linked to enzymes whose roles are beneficial to cellular protection against the reactive oxygen species (ROS). For instance, zinc is known to regulate the expression in lymphocytes of metallothionein and metallothionein-like proteins that have antioxidant activity (Reilly, 2004). The content of zinc in I. doka (66±1.00 mg/100g) may suggest strong potentials to enhance antioxidant protection in the body. Iron (Fe) is present mainly in the red blood cells as hemoglobin and plays a vital role in oxygen transport due to its ability to exist in two redox states (Minihane and Rimbach, 2002). The importance of iron in maintaining good health has been recognized (Vaughan and Judd, 2003). Calcium is key regulator of many cellular processes including cell signaling and proliferation, metabolism, muscle contraction and bone formation and mineralization (Zarain-Hertzberg, 2011). The presence of these mineral elements could thus indicate that this plant could be useful in the management of diseases where deficiencies of these metal ions are an important mechanism for the disease pathogenesis and progression. It is thus possible that these minerals play a role in the therapeutic properties of this plant as claimed by the traditional practitioners.

In conclusion, considering the results obtained from phytochemical screening, antioxidant activity and mineral elements analysis, the methanol extract of I. doka contains promising antioxidant ingredients and that might be responsible for the therapeutic activity of the plant extract in the treatment of various diseases. Further work to isolate and characterize the organic constituents as well as toxicological studies may enhance the understanding or the scientific basis of its uses in traditional medicine.

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REFERENCES


