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Full Length Research Paper

Chemical constituents and antioxidant activity of *Alstonia boonei*

Afolabi C. Akinmoladun[1]*, E. O. Ibukun[1], Emmanuel Afor [1], B. L. Akinrinlola[1], T. R. Onibon[1], A. O. Akinboboye[1], E. M. Obuotor [2] and E. O. Farombi[3]

[1]Department of Biochemistry, Federal University of Technology, Akure, Nigeria.
[2]Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.
[3]Drug Metabolism and Toxicology Unit, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

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The chemical composition and some antioxidant indices of *Alstonia boonei* stem-bark extract were evaluated. *A. boonei* was found to contain important minerals like calcium, phosphorus, iron, sodium, potassium, and magnesium. Alkaloids, tannins, saponins, flavonoids and cardiac glycosides were among the phytochemicals detected together with the important vitamin, ascorbic acid. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, total phenolic content and reducing power were 41.58 ± 1.43 %, 2.09 ± 0.04 mg/g gallic acid equivalent and 0.32 ± 0.01 respectively. Against the backdrop of the many medicinal uses of the plant, the results of the present work indicate that phytochemicals, other than phenolics, the mineral elements and vitamin C may be the critical factors in the medicinal effects of *A. boonei.*

Key words: *Alstonia boonei,* antioxidants, minerals, phytochemicals, medicinal effects.

INTRODUCTION

Plants need minerals for healthy growth and to build up the active ingredients responsible for their pharmacological properties and antioxidant activity. They obtain these minerals from the soil. Animals also need a regular intake of minerals and vitamins for good health and maximum productivity. They obtain most of these nutrients and minerals from plants through their food. Where the food does not contain sufficient minerals and vitamins, deficiency diseases develop (FAO/WHO, 1998).

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process (Aruoma, 2003; Dasgupta and De, 2004; Coruh et al., 2007).

The therapeutic effects of several plants and vegetables, which are used in traditional medicine, are usually attributed to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant- based antioxidants are now preferred to the synthetic ones because of safety concerns (Grice, 1986; Wichi, 1988; Sherwin, 1990). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Jayaprakasha et al., 2001; Gulcin et al., 2002). A profile of the chemical composition of a plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potential.

Alstonia boonei de Wild (Apocynaceae) is a medicinal plant that is widely used across Africa for various ailments. The stem bark of *A. boonei* has been reported to possess anti-inflammatory, analgesic and antipyretic activities (Olajide et al., 2000). The stem bark is commonly used in malaria, and it is listed in the African Pharmacopoeia as an antimalaria drug. An infusion of the

*Corresponding author. E-mail: akinmoladunfc@yahoo.com. Phone: +234 803444 5893.

bark is used as antivenom for snake bites. It is also used in treating painful micturation and rheumatic conditions (Ojewole, 1984; Asuzu and Anaga, 1991). An infusion of the root and stem bark is taken as a remedy for asthma. A liquid made from the stem bark and leaves is drunk to treat impotence. In Ghana, it is given to assuage toothache and, after child delivery, to aid in expelling the placenta. In Cote d'Ivoire and Burkina Faso, it is applied topically to reduce oedema and to clear suppurant sores and exposed fractures. In Nigeria, it is used for ulcers and in Cameroon and Liberia as remedy for snake bite and arrow poison.

The present work was designed to investigate the phytochemical, mineral and vitamin composition and the antioxidant potential of the stem bark extract of *A. boonei* in order to gain an insight into the molecular basis for some of its therapeutic properties and folkloric use.

MATERIALS AND METHODS

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, gallic acid, ascorbic acid and Folin-Ciocalteau reagent were obtained from Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Plant material and extraction

The stem bark of *A. boonei* was collected from a farmland in Akure, South-Western Nigeria and was identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The stem bark was dried under active ventilation at room temperature, packed in paper bags and stored. The dried stem bark was milled. The pulverized sample was weighed (200 g) and extracted in 500 ml of 80% methanol by maceration for 72 h. The methanolic extract was concentrated in a rotary evaporator, lyophilized and thereafter preserved for further use. In the same vein, part of the pulverized sample was extracted with water for the purpose of comparison of the phytochemical constituents with that of the methanolic extract.

Phytochemical screening

Chemical tests were carried out on the aqueous and methanolic extracts for the qualitative determination of phytochemical constituents using standard procedures as described by Harborne (1973), Trease and Evans (1985) and Sofowora (1993).

Determination of mineral content

The levels of Na and K were determined using flame photometry. The standard solutions of 100 mg/ml of Na and K were prepared from NaCl and KCl salt. Working standard of 0, 2, 4, 6, 8 and 10 mg/l were prepared from the standard solution by serial dilution. Each standard was aspirated into the flame photometer (Jenway FP9) and its emission recorded to prepare a standard curve. The prepared sample solutions for each extract were also aspirated into the flame photometer and their emission recorded. The Na and K concentrations were calculated from the standard curve.

The levels of Ca, Mg were determined by titrimetry. Five drops of 2% KCN followed by 5 drops of hydroxyl ammonium chloride were added to 10 ml of sample solution. Ammonium buffer was then added to raise the pH to 11.3 followed by the addition of 3 drops of Eriochrome black T indicator. The solution obtained was titrated against 0.01 M EDTA until a blue colour was obtained. The Ca/Mg concentration was calculated from the titre value. Phosphorus determination was carried out using the vanadomolybdate spectrophotometric method (Jekabsone et al., 1997).

The determination of the levels of Fe, Zn, Mn, Cu, and Co was carried out using atomic absorption spectrophotometry (Perkin-Elmer, 1982).

Determination of vitamin composition

The vitamin content of the plant sample was determined by high performance liquid chromatography (Varian model) at a wavelength of 270 nm. The mobile phase consisted of the solvent and methanol (3:1). The solvent was prepared by dissolving 1 g of hexane sulphonic acid sodium salt in 750 ml distilled water and then adding 3 ml of acetic acid. The solution was made up to 1 litre with distilled water. The solvent was used to prepare 0.25 mg/l vitamin standards which were loaded and injected into the column to obtain the area count of each vitamin. A solution of the plant sample was also prepared, loaded and injected into the column to obtain the area count of the vitamin present in the plant sample.

DPPH radical scavenging activity

This was carried out according to the DPPH spectrophotometric method of Mensor et al. (2001). The concentration of extract and standards (galllic acid and ascorbic acid) used was 300 µg/ml. One ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standards and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formula:

$$AA\% = 100 - [(Abs\ sample/Abs\ control) \times 100]$$

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank. One ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solutions of ascorbic acid and gallic acid served as positive controls.

Total phenolic content

Total phenolic content was determined using Folin – Ciocalteu reagent as previously described (McDonald et al., 2001). Total phenol value was obtained from the regression equation: y = 0.0055x + 0.1139 and expressed as mg/g gallic acid equivalent using the formula:

$$C = \frac{c.V}{M}$$

Where C = total content of phenolic compounds in mg/g gallic acid equivalent, c = the concentration of gallic acid (mg/ml) established from the calibration curve, V = volume of extract, and m = the weight of pure plant methanolic extract (g).

Reductive potential

This was determined according to the method of Oyaizu (1986). The extract (150 µg/ml) was mixed with phosphate buffer and pota-

Table 1. Phytochemicals in methanolic and aqueous stem bark extracts of *A. boonei*.

Phytochemicals	Methanolic extract	Aqueous extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Phlobatannins	-	-
Anthraquinones	-	-
Steroids	+	+
Terpenoids	+	+
Flavonoids	+	+
Cardiac glycosides		
with steroidal ring	+	+
with deoxy – sugar	+	+

- Absent + = Present

Table 2. DPPH radical scavenging activity of *A. boonei* stem-bark extract and standards.

Extract/standard	Abs_{518} nm	% AA
A. boonei	0.71 ± 0.02	41.58 ± 1.43*
Gallic acid	0.10 ± 0.01	92.05 ± 3.16#
Ascorbic acid	0.08 ± 0.00	93.50 ± 3.21#

Results are presented as mean ± SEM.
*Significantly different from gallic acid and ascorbic acid (P<0.001).
Not significantly different (P>0.05).

ssium ferricyanide. The mixture was incubated at 50˚C for 20 min. Tricloroacetic acid (10%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicates higher reductive potential.

Statistical analysis

Data were expressed as mean ± SEM. A one – way analysis of variance was used to analyze data. P<0.5 represented significant difference between means (Duncan's multiple range test).

RESULTS AND DISCUSSION

Yield of extract

Yield of methanolic extract of *A. boonei* following removal of solvent and dry freezing was 3.9%.

Phytochemicals

Tables 1 and 2 show the result of the phytochemical screening of the methanolic and aqueous stem bark extracts of *A. boonei*. The phytochemicals present in the methanolic and aqueous extracts were identical. In traditional usage, decoction or infusions of herbs are usually made with either alcohol or water as the solvent. At times, marked differences exist between the phytoche-mical profile of alcoholic and aqueous extracts. In the case of *A. boonei*, the aqueous extract is recommended because no vital phytochemical seemed to be left out and also because of probable unwanted effects that alcohol, which is another drug on its own, may produce.

Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Flavonoids also lower the risk of heart diseases. Saponins are capable of neutralizing some enzymes in the intestine that can become harmful, building the immune system and promoting wound healing. Alkaloids have been documented to possess analgesic, antispasmodic and bactericidal effects. Tannins hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). Cardiac steroids are widely used in the treatment of congestive heart failure. They help in increasing the force of contraction of the heart (positive inotropic activity) in heart failure patients. The presence of these phytochemicals supports the medicinal use of *A. boonei*.

Mineral composition

Figures 1 and 2 show the micro- and macro-elements present in the studied extract. All five macroelements investigated were found in the extract with phosphorous occurring in the largest amount and Mg the least. All the macro-elements in *A. boonei* extract have vital roles to play in the metabolism of living organisms especially man. Ca salts provide rigidity to the skeleton and calcium ion plays a role in many if not most, metabolic processes. Many neuromuscular and other cellular functions depend on the maintenance of the ionized calcium concentration in the extracellular fluid. Calcium fluxes are important mediators of hormonal effects on target organs through several intracellular signaling pathways (FAO/WHO, 1998). Phosphorous is also important in bone formation and many essential metabolic activities in the body for example phosphorylation reactions. Hypophosphatemia has been adduced as a cause of failed weaning. Soft tissue magnesium functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissue and cell membranes. Mg plays an important role in the metabolism of calcium (Al-Ghamdi et al., 1994). Among the micronutrients, only iron was detected. Iron deficiency leads to reduction of physical working capacity in animals and man (Scrimshaw, 1984) and the impairment of immunologic response and phagocytic action of neutrophil leukocytes. Iron has several essential roles in the body such as in oxygen

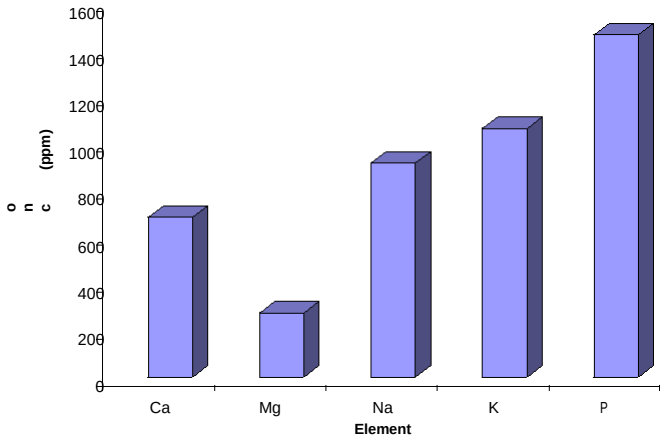

Figure 1. Macroelement content of *A. boonei* extract.

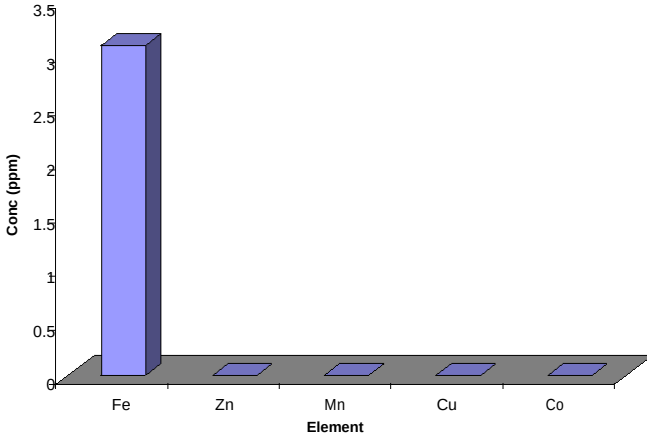

Figure 2: Fe, Zn, Mn, Cu and Co levels in *A. boonei* stembark extract.

transport and oxidative metabolism (Bothwell, 1979). Past and on-going investigation on the medicinal properties of plants point to the fact that their mineral compositions also have a major role to play in their therapeutic effect. Some antioxidant enzymes require metal ions for their activity; other metals have been directly classified as antioxidants.

Vitamins

Five vitamins were investigated – ascorbic acid, thiamine, riboflavin, niacin and pyridoxine. Only vitamin C was detected in the stem-bark extract of A. boonei (0.58 ± 0.00 mg/g plant material). Humans and primates lack the terminal enzyme in the biosynthetic pathway of ascorbic acid, L- gulonolactone oxidase. Where there is insufficient vitamin C in the diet, humans suffer from the potentially lethal disease scurvy (Stewart and Guthrie, 1953). Vitamin C is an important antioxidant. It acts as an electron donor for eight important enzymes in humans (Moncada and Higgs, 1993). Ascorbic acid may protect against the oxidative damage of light in the eye (Koskela et al, 1989) and may also play an important role in sperm maturation (Hornig, 1975) . It helps in stabilizing various plasma components and has been shown to be an effective scavenger of superoxide radical anion, H_2O_2, the hydroxyl radical, singlet oxygen and reactive nitrogen oxide (Tannenbaum et al, 1991; Weber et al, 1996). The known physiological and medicinal effects of ascorbic acid are in accord with the medicinal properties of *A. boonei.*

Antioxidant activity, reductive potential and total phenol content

The DPPH radical scavenging activity of the stem bark extract of *A. boonei* was low compared to the standards (Table 2). Both the total phenol value (2.09 ± 0.04 mg/g gallic acid equivalent) and the reductive potential (0.32 ± 0.01) were also low.

Conclusion

The medicinal effects of plants are often attributed to the antioxidant activity of the phytochemical constituents, mainly the phenolics (Thabrew, 1998). The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, metal chelators and free radical quenchers (Rice- Evans et al., 1996). Plants having significant medicinal values have often been found to be rich in phenolics and to have high antio-xidant potentials. It was expected that *A. boonei*, with its many medicinal uses, would have a significant amount of phenolics and possess a high antioxidant potential. However, the results of the present study indicated that the opposite was the case. The antioxidant indices evaluated showed low values for the plant. This presupposes that other classes of phytochemicals, for example the alkaloids, may be the major players in the medicinal and therapeutic value of *A. boonei*. A synergistic relationship amongst phytochemicals has been adduced to be responsible for the overall beneficial effect derivable from plants (Liu, 2004). Tests are not yet available to measure this synergy. The synergy of phytochemicals may make-up for the apparent low values for individual classes of phytochemicals. The important minerals and vitamin found in the plant might also be major contributors to the medicinal value of the plant. Mineral elements may have more roles to play, than presently acknowledged, in the synergy of phytochemicals for the health benefit of man.

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