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Phytochemical Screening and *in Vitro* Antioxidant Activity of Aqueous Extract of *Blighia Sapida* Stem Bark

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Keywords: *blighia sapida, phytochemicals, antioxidant, metabolites.*

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Phytochemical Screening and *in Vitro* Antioxidant Activity of Aqueous Extract of *Blighia Sapida* Stem Bark

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Abstract- ***Blighia sapida*** **is a plant belonging to the family of sapindaceae. In this study we aimed to carry the phytochemical screening of aqueous extract of** ***Blighia sapida*** **stem bark and evaluate its** ***in vitro*** **antioxidant activity. It was found that the aqueous extract of** ***Blighia sapida*** **stem bark showed the presence of tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also contains trace elements zinc and selenium. Furthermore it showed some scavenging activity but not as such could be compared with the various standards used except for nitric oxide scavenging activity. These are indications that the aqueous extract of*** **Blighia sapida** **contains some physiologically significant secondary metabolites and also possesses appreciable** ***in vitro*** **antioxidant activity.**

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I. Introduction

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. In fact, phytochemicals (or antioxidants) such as phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit, or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction.

Blighia sapida is a plant belonging to the family of Sapindaceae. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). It is an evergreen tree of about 33 to 40ft (10-12m) with a dense crown of spreading branches. It is rather hand usually with a short trunk to 6ft (1.8m) in circumference. Its bark is grey and nearly smooth. The leaves are compound with three to five pairs of oblong, ovate-oblong, or elliptical leaflets 1.5-3.0cm long. The seed of the fruit is not edible, whereas the fleshy aril is edible. The fruit is known to contain saponins, which are hemolytic (Aderinola *et al.*, 2007).

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Most of the earlier studies on *Blighia sapida* have been on the nutritional qualities of the root (Abolaji *et al*, 2007) and the leaves as a dry season feed resource for West African dwarf goats in the Northern savanna zone of Nigeria (Aderinola *et al*, 2007). The repellant potential of the fruit part components against stored-product insect pests (Khan and Gumbs, 2003) as well as neutropenia and thrombocytopenia effects of the aqueous and lipid extracts of the unripe fruit have been investigated in mice (Gardiner *et al*, 1996). More recently, the physicochemical properties of the oil from the fruit of the species and toxicological evaluation of the oil – based diet in Wister rats have been investigated (Oladiji *et al*, 2009).

Tree bark is an important component of African traditional medicine as herbal medicine is still the main source of health care for the majority of Africans and in particular, Nigerians.There has been increasing demand for the use of plant products with therapeutic activity. The high cost, availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs or drugs from other animal sources are some of the factors leading to a strong preference for drugs of plants origin.

This study is thus aimed at investigating the phytochemicals and *in vitro* antioxidant activity of the readily available *Blighia sapida* stem bark.

II. Materials and Methods

Chemicals: All chemicals used were of analytical grade and items are products of BDH and Sigma Chemical Ltd., UK and Roche Diagnostic, Germany.

Sourcing for the Tree Bark of Blighia sapida: A sizeable quantity of the tree bark of *Blighia sapida* was obtained from the compound of the Federal Polytechnic, Ado Ekiti, Nigeria.

Identification of Plant: The fruits and leaves of *Blighia sapida* plant were obtained from the compound of the Federal polytechnic, Ado Ekiti, Ekiti State, Nigeria and were used for the purpose of authentication of the identity of the plant at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The voucher number of identification is UIH624.

Processing of sample and preparation of extract: The sample obtained was air-dried at room temperature for

fifty-six (56) days until a constant weight was obtained. The air-dried tree bark of *Blighia sapida* was pulverized. 100g of the pulverized sample was extracted with 800ml of distilled water for seventy-two (72) hours in an extractor. The aqueous extract was obtained by filtering with Whatman filter paper and subsequently freeze-dried in Armfield freeze-drier for ten (10) days.

Qualitative Phytochemical Analysis: Chemical tests were carried out on the aqueous extract using modified standard procedures to identify the constituents as described by Sofowora(1993), Trease and Evans(1989) and Harborne(1973).

Quantitative Determination of Phytochemicals

Determination of Tannin: The tannin content of the extract was determined by using the modified procedure of Makkar (1994).

Determination of Saponin: The method used was that described by Obadoni and Ochuko (2001).

Determination of Flavonoid: The method of Boham and Kocipal-Abyazam (1994) was used.

Determination of Alkaloid: The total alkaloid content of the extract was determined using the method described by Harborne (1973).

Determination of Total Phenols: The total phenolic content was determined using the method described by Singleton and Rossi(1965) using Folin-Ciocalteu's phenol reagent.

Determination of Zinc and Selenium: The level of zinc and selenium were determined by the method described by AOAC (2006).

In vitro Antioxidant Assay

DPPH free radical scavenging assay: The hydrogen or radical scavenging properties of the extract was determined using the stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl hydrate) according to the method proposed and described by Blois (1958).

Hydroxyl radical scavenging assay: Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium (Halliwell *et al.*, 1981).

Hydrogen peroxide decomposition assay: This activity was determined according to a method described by Long *et al.*, (1999).

Nitric oxide (NO) scavenging assay: At physiological pH, nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction (Garratt, 1964).

Ferric reducing antioxidant assay (FRAP): The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method was based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous colour formed owing to the action of electron donation in the presence of antioxidants.

Determination of total antioxidant capacity: The total antioxidant capacity was determined in accordance with the method described by Prieto *et al.*, (1999).

Statistical Analysis: Data were expressed as mean ± S.E.M. of five replicates.

III. Results

Phytochemical screening of extract: The result of phytochemical screening of aqueous extract of *Blighia sapida* stem bark shows the presence of tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also shows the presence of the trace elements zinc and selenium (Table 1 and 2).

In vitro antioxidant activity: In vitro antioxidant studies revealed that aqueous extract of *Blighia sapida* stem bark showed some scavenging activity by total antioxidant capacity (TAC) with the IC_{50} value of 16.63± 0.51μg/ml, DPPH radial scavenging activity with the IC_{50} value of 23,938± 169.28 μg/ml, total phenol of 5.47± 0.93 μg/ml GAE, hydrogen peroxide radical decomposition activity with the IC_{50} value of 5,133.50± 191.70 μg/ml, hydroxyl radical scavenging activity with IC_{50} value of 6904.00± 751.90 μglml and nitric oxide scavenging activity with IC_{50} value of 447.57± 153.28 μg/ml. However, apart from the nitric oxide scavenging activity value that compares with that of the standard (i.e. ascorbic acid), all other values are a lot higher than those of the standards used (Table 3).

Table 1: Qualitative analysis of the phytochemicals of the aqueous extract of *Blighia sapida* stem bark

Specie	Extract Type	TNN	SPN	FLV	STR	TPN	AKD	PHN
Blighia sapida (Stem bark)	Aqueous	+	+	+	+	+	+	+

KEY: + = Presence of constituent
– = Absence of constituent
TNN = Tannin
SPN = Saponin
FLV = Flavonoid
STR = Steroid
TPN = Terpenoid
AKD = Alkaloid
PHN = Phenol

Table 2: Concentrations of some phytochemicals, zinc and selenium in aqueous extract of *Blighia sapida* stem bark.

Species	Extract Type	Tannin	Saponin	Flavonoid	Alkaloid	Phenols	Ascorbic Add(mg/100g)	Zinc (ppm)	Selenium (ppm)
Bllghla saplda	Aqueous	22.0±1.24	21.3±3.30	13.3±1.86	17.3±4.70	5.47±0.93	22.1±6.02	19.6±0.03	<1.0

Table 3: In vitro antioxidant activity of *Blighia sapida* stem bark aqueous extract

EXTRACT TYPE	Total Antioxidant Capacity (TAC)	Ferric reducing antioxidant (FRAP)	DPPH free radical Scavenging	Total Phenol (GAE)	Hydrogen Peroxide	Hydroxyl radical	Nitric oxide
			IC_{50} Values (μg/ml)				
Aqueous	16.63 ± 0.81	9.13 ± 0.51	23938.00 ± 160.28	5.47 ± 0.93	5133.50 ± 191.70	6904.00 ± 751.90	447.57 ± 153.28
			Standards				
Ascorbic acid	–	–	69.19 ± 5.02	–	123.41 ± 4.84	–	264.08 ± 11.07
Butylated Hydroxytoluene (BHT)	–	–	–	–	–	139.73 ± 31.31	–

Values are Mean±S.E.M of five determinations

IV. Discussion

Antioxidants such as phenolic compounds (tocopherols flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions.

In this study, the metabolites shown in Table 1 were known to show biological activity as well as exhibiting physiological activity (Sofowora, 1993). Flavonoids are potent water-soluble antioxidants and free radial scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu*et al.*, 2006). Flavonoids also lower the risk of heart disease. 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 mucuous membrane (Okwu *et al.*, 2006). The presence of these phytochemicals support the medicinal use of *Blighia sapida* (Saidu, 2012).

Zinc and selenium are the trace elements that have been found to be cofactors of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Weydert and Cullen, 2009).

In vitro antioxidant studies revealed that aqueous extract of *Blighia sapida* stem bark only showed a comparative scavenging activity when compared to the standard (i.e. ascorbic acid), by nitric oxide scavenging activity. All other parameters considered in the *in vitro* antioxidant studies did not show promising results when compared with the standards.

V. Conclusion

A major finding of the study is that aqueous extract of *Blighia sapida* stem bark showed the presence of metabolites such as tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It was also found to contain trace elements zinc and selenium. The results also revealed that *Blighia sapida* stem bark aqueous extract showed some *in vitro* antioxidant activity. Ofall the parameters studied, nitric oxide scavenging activity was the only one that showed a somewhat comparable IC_{50} to that of the standard while all others did not compare well with the chosen standards.

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