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Phytochemical Screening and in vitro Antioxidant Activity of Hydro-Ethanolic 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 out the phytochemical screening of hydro-ethanolic (15%) extract of Blighia sapida stem bark and evaluate its in vitro antioxidant activity. It was found that the hydro-ethanolic extract of Blighia sapida stem bark showed the presence of 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.
Keywords: *Blighia sapida,* phytochemicals, antioxidant, trace elements
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1. 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 (Larson 1988; Hall & Cuppett 1997) 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 native to Western Tropical Africa and was introduced into Jamaica in the late 18th century. It has spread to other parts of tropical America but it is still more widely known in Jamaica than elsewhere. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). The fruit is known to contain saponins, which are hemolytic (Aderinola *et ai.* 2007). 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) and 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, Nigeria, 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.

2. Materials and Methods

2.1 *Chemicals*: All chemicals used were of analytical grade and items are products of BDH and Sigma Chemical Ltd., UK and Accu-chek ® Advantage, Roche Diagnostic, Germany.

2.2 *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.

2.3 *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.

2.4 *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 15% ethanol (hydro-ethanol) solution for seventy-two (72) hours in an extractor. The hydro-ethanolic extract was obtained by filtering with Whatman filter paper and subsequently freeze-dried in Armfield freeze-drier.

2.5.*Qualitativ.e Phytochemical Analysis*

Chemical tests were carried out on the hydro-ethanolic extract using modified standard procedures to identify the constituents as described by Sofowora (1993), Trease & Evans (1989) and Harborne (1973).

2.6.*Quantitative Determination of Phytochemicals*

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

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

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

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

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

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

2.7.*In vitro Antioxidant Assay*

2.7.1 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).

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

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

2.7.4. 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).

2.7.5. 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 & Strain 1999).

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

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

3.0 Results

3.1 Phytochemical screening of extract

The result of phytochemical screening of hydro-ethanolic extract of *Blighia sapida* stem bark shows the presence of saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also shows the presence of the trace elements zinc and selenium (Tables 1 and 2).

Table 1: Qualitative analysis of the phytochemicals of the hydro-ethanolic extract of *Blighia sapida* stem bark

Species	Extract Type	TNN	SPN	FLV	STR	TPN	AKD	PHN
Blighia sapida	Hydro-ethanolic	-	+	+	+	+	+	+

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 tannin, saponin, flavonoid, alkaloid, phenols, ascorbic acid, zinc and selenium in hydro- ethanolic extract of *Blighia sapida* tree bark.

Species	Extract type	Tannin (%)	Saponin (%)	Flavonoid (%)	Alkaloid (%)	Phenols (%)	Ascorbic Acid (mg/100g)	Zinc (ppm)	Selenium (ppm)
Blighia sapida	Hydro-ethanolic	0.00	18.30 ±1.20	16.70 ±0.02	21.70 ±3.60	9.87 ±0.98	21.30 ±4.25	16.10 ±0.02	<1.0

Values are mean ± S.E.M of five replications of the tested extract.

3.2 *In vitro* antioxidant activity

In vitro antioxidant studies revealed that hydro-ethanolic extract of *Blighia sapida* stem bark showed some scavenging activity by total antioxidant capacity (TAC) with the IC_{50} value of 18.80±0.89µg/ml, ferric reducing antioxidant property with the IC_{50} value of 7.03±0.38 µg/ml, DPPH radical scavenging activity with the IC_{50} value of 2,059.00±107.86 µg/ml, total phenol of 9.87±0.98 µg/ml GAE, hydrogen peroxide radical decomposition activity with the IC_{50} value of 6,683±791.80 µg/ml, hydroxyl radical scavenging activity with IC_{50} value of 5,971.00±938.80 µg/ml and nitric oxide scavenging activity with IC_{50} value of 482.37±20.60 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 3: *In vitro* antioxidant activity of *Blighia sapida* stem bark hydro-ethanolic extract

	Parameters						
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)							
Hydro-thanolic	18.80 ± 0.89	7.03 ± 0.38	2059.00 ± 107.86	9.87 ± 0.98	6683.00 ± 791.80	5971.00 ± 938.80	482.37 ± 20.60
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 of five determinations ± S.E.M of tested extract.

4.Discussion

Antioxidants such as phenolic compounds (tocopherols flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Larson 1988; Hall & Cuppett 1997) 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).The result obtained in this study is also in agreement with those published by Amira & Oloyede (2017) in which they studied the secondary metabolites present in the aqueous extract of *Blighia sapida* stem bark. Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Del-Rio *et al.* 1977; Salah *et al*. 1995; 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 & Cullen 2009).

In vitro antioxidant studies revealed that hydro-ethanolic 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.

5.Conclusion

A major finding of the study is that hydro-ethanolic extract of *Blighia sapida* stem bark showed the presence of metabolites such as 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 hydro-ethanolic extract showed some *in vitro* antioxidant 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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