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Original article

In-vitro antioxidant and anti-inflammatory activities of ethanol stem-bark extract of *Blighia sapida* K.D. Koenig

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ABSTRACT

Blighia sapida (*B. sapida*) K.D. Koenig (Family Sapindaceae) is a branchless straight bole approximately 15 m in length. The study evaluated the antioxidant and anti-inflammatory activities of ethanol extract and fractions of *B. sapida* stem-bark using in vitro methods. Ethanol extract and its fractions were investigated for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), and quantitative phenolic and flavonoid contents. Anti-inflammatory activity was evaluated using albumin denaturation and membrane stabilization assays. The extract and its fractions exhibited radical scavenging and anti-inflammatory properties. The ethyl acetate fraction possessed maximum phenolic and flavonoid contents (136.67 ± 1.55 gallic acid equivalent mg/g and 75.76 ± 4.03 quercetin equivalent mg/g, respectively). Antioxidant studies revealed that the ethyl acetate fraction displayed superior activity with an IC_{50} = 0.09 ± 0.03 mg/mL DPPH, and values of 146.96 ± 3.81 ascorbic acid equivalent (AAE) mg/g and 359.20 ± 4.98 AAE mg/g for FRAP and TAC, respectively. Furthermore, the anti-inflammatory activity was revealed by inhibition of heat-induced albumin denaturation and red blood cell membrane stabilization at concentrations of 200–1000 μg/mL and 50–250 μg/mL, respectively. The ethanol extract and fractions exhibited antioxidant and anti-inflammatory activities, with ethyl acetate fraction showing superior activity, which could be attributed to secondary metabolites, mainly phenolic compounds. Overall, the antioxidant and anti-inflammatory activities of *B. sapida* can be exploited by ethnomedicinal users.

1. Introduction

Inflammation is a protective response that eliminates the initial cause of cell injury, weakening, destroying, or neutralizing harmful agents, along with the removal of damaged tissue, and new tissue generation. Inflammation is defined as a hosts' defense response to injury, tissue ischemia, autoimmune reactions, or infectious agents. Signs and symptoms can be prolonged for days or weeks, for instance, in bronchitis, pharyngitis, appendicitis, and dermatitis. Chronic inflammation is detrimental to the system as it represents inflammatory reactions occurring for an extended period [1]. Unabated inflammation can cause chronic diseases such as cancer, diabetes, asthma, heart attack, and arthritis [1]. Inflammation has been described as a response activated by harmful stimuli and other injurious conditions. Moreover, the relationship between oxidative stress induced by free radicals and the inflammatory response has been reported by numerous studies [2]. It is postulated that free radicals and other reactive species generated in living organisms are involved in diseases such as tumors, hepatitis, liver injury, and immunodeficiency disorders [3].

Blighia sapida (*B. sapida*) is a member of the Sapindaceae family. It is generally known as Ackee in Nigeria but specifically called Gwanja Kusa, Isin, and Okpu by the Hausa, Yoruba, and Igbo, respectively, all ethnic tribes in Nigeria. *B. sapida* is an evergreen tree approximately 33 to 40 ft (8–24 m) in height, with a dense crown and straight branches. Its bark is greyish in color and nearly smooth in appearance [4,5]. Different parts of the plant, including the oil from the seed, have been evaluated for their various

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activities. However, to the best of our knowledge, no study had been carried out on the antioxidant and anti-inflammatory potentials of ethanol stem-bark extract of *B. sapida*. Therefore, the aim of this study was to investigate the antioxidant and anti-inflammatory potentials of ethanol extract and fractions of *B. sapida* stem-bark using in vitro methods.

2. Materials and methods

2.1. Reagents

Gallic acid (GA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), phenol reagent, iron III chloride, quercetin (Q), Follin Ciocalteu's reagent, trisodium citrate, and ascorbic acid were obtained from Sigma Fine Chemicals Limited (Uppsala, Sweden) and British Drug House (BDH) Chemicals Limited (London, UK). All other reagents used in the study were of analytical grade and were obtained from reputable sources.

2.2. Plant materials

Fresh *B. sapida* stem-bark was collected from Sekona-Ede Road, Osun State, Nigeria. The plant material was identified and authenticated at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.3. Preparation of plant material

The fresh stem-bark of *B. sapida* was prepared, and pulverized material (1 kg) was macerated in (70 %, *V/V*) ethanol/water for 72 h at room temperature, according to the process described by Adekola et al. [6]. The resulting suspension was filtered, the filtrate was sieved using white cotton gauze, followed by filtration using filter paper, and concentration using a rotary evaporator at 40 °C to obtain the ethanol extract (EE). The extract was weighed, labeled, and stored in a desiccator until further analysis.

2.4. Fractionation of ethanol extract

The EE was partitioned with solvents of varying polarities as described by Apalowo et al. [7]. The extract (30 g) was suspended in distilled water (200 mL), allowed to completely dissolve, thoroughly shaken, and filtered using a filter paper (Whatman No. 1). The filtrate was partitioned sequentially with 400 mL of each solvent (ethyl acetate, and butanol) using a separating funnel. The mixture was thoroughly shaken, allowed to separate into layers, and separated. Fractions of the different solvents were separately concentrated using the rotary evaporator, while the volume of the aqueous fraction was only reduced before lyophilization. Three fractions, namely, the ethyl acetate fraction (EAF), butanol fraction (BF), and aqueous fraction (AqF), were obtained and stored in desiccators until further use.

2.5. Determination of total phenolics and flavonoid contents

B. sapida EE/fractions were quantitatively evaluated for total phenol and flavonoid contents using the phenol reagent procedure as described by Boussoualim et al. [8] and Benariba et al. [9], respectively.

2.6. DPPH radical scavenging activity

The radical scavenging activity of the extract/fractions was determined as described by Aouachria et al. [10]. DPPH radical scavenging activity was estimated using the following equation:

$$\text{Scavenging activity } (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

The half-maximal inhibitory concentration (IC_{50}) was then obtained from a linear regression plot of percentage inhibition against the extract concentration.

2.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP of the EE/fractions of *B. sapida* was investigated spectrophotometrically, as reported by Amri et al. [11]. The assay was based on the reduction of colorless ferric-tripyridyltriazine to its blue ferrous-colored form due to electrons donation by antioxidants. The FRAP was calculated and expressed as ascorbic acid equivalent (AAE) per fresh weight (AAE mg/g).

2.8. Evaluation of total antioxidant capacity (TAC)

The antioxidant capacity of the EE/fractions was examined as described by Jahan et al. [12]. A typical blank solution was prepared to contain 1000 μL of reagent solution and an appropriate methanol volume. The capacity was evaluated as AAE mg/g.

2.9. Determination of anti-inflammatory studies

The in vitro anti-inflammatory activities of the extract and its fraction were estimated by the membrane-stabilizing activity and inhibition of albumin denaturation.

2.9.1. Membrane stabilizing activity

The membrane stabilizing activity was performed as described by Oyedapo et al. [13] using 2% (*V/V*) bovine erythrocyte and diclofenac (as standard drug). The percentage of membrane stability was estimated using the following expression:

$$\text{Percentage of membrane stability} = \left(100 - \frac{A_{\text{test drug}} - A_{\text{drug control}}}{A_{\text{blood control}}}\right) \times 100\%$$

Where blood control represents 100% lyses or zero percent stability.

2.9.2. Albumin denaturation inhibition

The ability of the EE/fractions of *B. sapida* to inhibit albumin denaturation was evaluated according to the method described by Sakat et al. [14]. The percentage inhibition of albumin denaturation was calculated using the following equation:

$$\text{Percentage inhibition of albumin denaturation} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100\%$$

2.10. Statistical analysis

All the measurements were performed in triplicates, and the results are expressed as the mean ± standard error of mean (SEM) using Graphpad Prism 5.0 (Graphpad software, Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was performed, and a *P* value of less than 0.05 was deemed significantly different. Linear regression analysis was used to calculate IC_{50} value.

3. Results

3.1. Total phenolic and flavonoid contents

As presented in Table 1, the EE, EAF, BF, and AqF of *B. sapida* were estimated for total phenolics using GA as the standard, with total flavonoids estimated with a Q equivalent. Both the plant EE and the fractions revealed the presence of total phenolics and flavonoids, with EAF showing the highest contents.

3.2. DPPH radical scavenging activity

DPPH is a stable nitrogen-based free radical that is violet in color and turns to yellow after reduction by either hydrogen or electron transfer. As presented in Table 2, IC_{50} values of EE, EAF, BF, AqF, and ascorbic acid (standard drug) were 0.91 ± 0.04, 0.09 ± 0.03, 1.20 ± 0.07, 0.49 ± 0.05, and 0.23 ± 0.00 mg/mL DPPH. The plant EE and its fractions showed DPPH radical scavenging activity in a concentration-dependent manner, with EAF demonstrating the highest activity. The order of DPPH radical scavenging activity was as follows: EAF > AqF > EE > BF.

3.3. Ferric reducing antioxidant power and total antioxidant capacity

The antioxidant power of the EE and its fractions was measured and evaluated based on the reduction of Fe (III) to Fe (II). The values are expressed in AAE mg/g for EE, EAF, BF, and AqF, with the EAF displaying the highest activity. Total antioxidant capacity (TAC) revealed a concentration-dependent change in the antioxidant capacity of the EE and its fractions. The values are expressed in AAE mg/g, with the EAF showing the highest TAC (Table 3).

3.4. Membrane stability

For the EE and fractions of *B. sapida*, the potential to stabilize the red blood cell (RBC) membrane subjected to both heat- and hypotonic-induced hemolysis was examined to clarify the underlying anti-inflammatory mechanism. At concentrations of 50–250 μg/mL, the EE and prepared fractions protected RBCs against hemolysis, as shown in Fig. 1**.** The EAF showed maximum inhibition. Diclofenac was used as the standard drug.

Table 1
Total phenolic and flavonoid contents of EE and fractions of *B. sapida*.

Constituents	Total phenolic (GAE mg/g)	Total flavonoid (QE mg/g)
EE	67.68 ± 5.13	23.29 ± 0.23
EAF	136.67 ± 1.55	75.76 ± 4.03
BF	64.61 ± 0.58	32.53 ± 1.91
AqF	65.82 ± 4.05	21.23 ± 4.03

Each value represents the mean ± SEM of three replicates. GAE: gallic acid equivalent; QE: quercetin equivalent; EE: ethanol extract; EAF: ethyl acetate fraction; BF: butanol fraction; AqF: aqueous fraction.

3.5. Inhibition of albumin denaturation

The mechanism of action of the EE and fractions of *B. sapida* (200–1000 μg/mL) was evaluated based on the capacity to inhibit albumin denaturation; indomethacin was used as the standard drug. EAF showed the highest inhibitory activity on albumin denaturation at various concentrations, as shown in Fig. 2.

4. Discussion

In the present study, the antioxidant and anti-inflammatory effects of *B. sapida* stem-bark EE and its fractions were evaluated. Our findings revealed that both the EE and fractions were composed of phenolic compounds. These compounds showed radical scavenging activities, afforded the RBC membrane protection against heat and hypotonicity-induced hemolysis, and inhibited albumin denaturation. As the primary natural antioxidants [15], phenolic compounds are responsible for the phenolic content, radical scavenging, and antioxidant activities displayed by the *B. sapida* EE and its fractions. Emmanuel et al. [16] have reported the presence of phytochemical constituents in the seed and seed oil of *B. sapida*. Apart from the benefits to the plant itself, these metabolites dictate the nutritional value, color, taste, and fragrance and are responsible for observed pharmacological properties, including antimicrobial, antihypertensive, cholesterol-lowering, and anti-inflammatory effects [17]. In the present study, EAF demonstrated the highest content of total phenolics (136.67 ± 1.55 GAE mg/g) and flavonoids (75.76 ± 4.03 QE mg/g) when compared with BF, AqF, and EE, in the order EAF > EE > AqF > BF for phenolics and EAF > BF > EE > AqF for flavonoids; this demonstrates the efficacy of ethyl acetate to extract phenolics and flavonoids better than other solvents employed. Senhaji et al. [18] have reported that the EAF of aerial parts of *Anabasis aretioïdes* also demonstrates the highest phenolic content among other aqueous and organic solvents used for extraction.

DPPH, a stable free radical, violet in color, turns to yellow after reduction owing to either hydrogen or electron transfer. Substances that possess this activity can be termed as antioxidants and, therefore, free radical scavengers [19]. In the present study, *B. sapida* EE and its fraction scavenged DPPH free radicals in a dose-dependent manner, as the concentration of extracts increased the DPPH radical scavenging activity. Ethyl acetate had the lowest IC_{50} value when compared with ascorbic acid (Table 2). Hence, the extract of *B. sapida* may serve as an important source of free radical scavengers that support its efficacy in managing different ailments. Moreover, the findings observed in the present study support the review of Sinmisola et al. [3], which revealed that the EAF of *B. sapida* pods and seeds display potent DPPH antioxidant activities and high total phenolic and flavonoid contents. The presence of secondary metabolites such as flavonoids and phenols in the extract may be responsible for the observed radical scavenging activity, which indicates the antioxidant potential of the extract. Furthermore, the study corroborates the findings of Ojo et al. [20],

Table 2
DPPH radical scavenging activity of the EE and fractions of *B. sapida*.

Concentration (mg/mL)	EE	EAF	BF	AqF	Ascorbic acid
0.03125	8.25 ± 1.75	36.46 ± 4.39	11.54 ± 2.00	11.64 ± 2.57	27.24 ± 2.49
0.0625	9.82 ± 1.28	48.25 ± 4.26	13.83 ± 2.43	14.39 ± 3.03	28.89 ± 0.37
0.125	15.79 ± 0.30	58.14 ± 2.21	22.13 ± 1.99	22.57 ± 3.38	49.54 ± 1.11
0.25	24.04 ± 1.33	63.34 ± 2.89	22.78 ± 2.27	37.02 ± 3.62	63.54 ± 1.51
0.5	27.89 ± 2.19	71.98 ± 0.68	31.29 ± 3.23	49.36 ± 3.26	78.35 ± 1.93
IC_{50}	0.91 ± 0.04	0.09 ± 0.03	1.20 ± 0.07	0.49 ± 0.05	0.23 ± 0.00

Each value represents the mean ± SEM of three replicates. IC_{50}: half-maximal inhibitory concentration.

Table 3
Total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP) of the EE and fractions of *B. sapida*.

Constituents	TAC (AAE mg/g)	FRAP (AAE mg/g)
EE	243.79 ± 11.16	20.62 ± 1.43
EAF	359.20 ± 4.98	146.96 ± 3.81
BF	150.63 ± 5.84	31.85 ± 0.93
AqF	258.09 ± 4.64	39.61 ± 1.76

Each value represents the mean ± SEM of three replicates. AAE: ascorbic acid equivalent.

who reported a dose-dependent increase in DPPH scavenging activity in non-insulin-dependent diabetes mellitus. The DPPH scavenging activity of the extract, especially the EAF, can be associated with high phenolic and flavonoid contents, as the antiradical activities of these metabolites have been reported by Kobus-Cisowska et al. [21].

The reductive potential of antioxidants is based on the reduction of Fe^{3+} to Fe^{2+}. The reducing ability of a compound is commonly linked to its electron transfer activity, thus highlighting its potential antioxidant power [22,23]. The change in the reaction mixture color indicates the reducing potential of the *B. sapida* EE and fractions, measured in AAE mg/g. The EE and fractions of *B. sapida* exhibited varying degrees of TAC and FRAP. The FRAP was evident from the reduction of colorless ferric ions to ferrous ions, forming a blue complex measured at 593 nm. It was observed that the extract was capable of donating electrons that reacted with free radicals, converting them into stable products. The TAC was indicated by the reduction of molybdenum (VI) to molybdenum (V), with the formation of the green complex by the EE and its fractions. As observed for the phenolic and flavonoid contents and DPPH scavenging activity, the EAF showed the highest levels of TAC and FRAP among the other fractions and EE. Herein, the results revealed that the EAF is rich in phenolic compounds, well-known reducing agents responsible for the antioxidant capacity of the fraction. The finding regarding the antioxidant activity of phenolics has been reported by Senhaji et al. [18]. Moreover, Amira and Oloyede [24] have observed that an aqueous extract of the *B. sapida* stem-bark significantly improves antioxidant enzyme activities in the kidney and pancreas of diabetic rats during a 21-day experiment.

RBC membrane stabilization has been utilized to investigate the in vitro anti-inflammatory activity of plants, as RBC membrane resembles the lysosomal membrane. Maintenance and stability of the lysosomal membrane remain crucial as lysosomal contents, such as bactericidal enzymes and proteases, are released during inflammation; further exacerbation can be induced by cell membrane rupture, cell disintegration, and loss of cations from the membrane. This results in denaturation and loss of protein

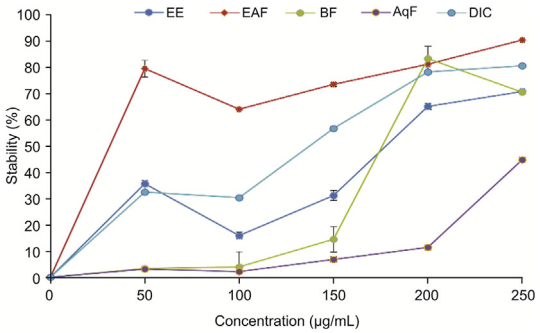

Fig. 1. Effect of various concentrations of *B. sapida* K.D. Koenig EE and its fractions on RBC membrane-stabilizing in response to both heat and hypotonicity-induced hemolysis. EE: ethanol extract; EAF: ethyl acetate fraction; BF: butanol fraction; AqF: aqueous fraction; DIC: diclofenac.

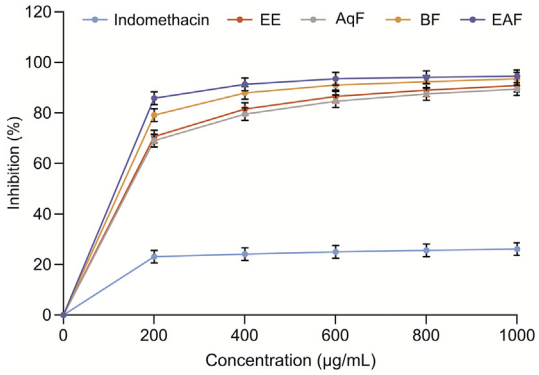

Fig. 2. Inhibition of thermally-induced protein denaturation by *B. sapida* K.D. Koenig EE (crude) and its fractions.

function, which is responsible for the physical integrity and regulation of cell homeostasis in biological membranes [25,26].

The EE and fractions of *B. sapida* stem-bark inhibited heat- and hypotonicity-induced hemolysis at various concentrations by exhibiting dose-dependent stabilization of the RBC membrane. This reinforces that membrane stability is one mechanism underlying the anti-inflammatory activity exhibited by the plant and is most likely to maintain the contents of the lysosomal and RBC membrane during inflammation. The lysosomal constituents include digestive enzymes, which after release within the cell, cause further inflammation and tissue damage [14]. The membrane-stabilizing activity of the extract may be attributed to the interaction of phytoconstituents in the plant extract with membrane components, preventing the release of phospholipase A2 to hydrolyze phospholipids and induce the formation of inflammatory mediators. The EAF exhibited maximum percentage inhibition of hemolytic activities, comparable with that of diclofenac (a standard anti-inflammatory drug). The EE and other fractions also exhibited membrane-stabilizing potential but less than that of the EAF; this property of *B. sapida* corroborates the observation of Musfiq and Ananthi [27]. The effects of *B. sapida* could be mediated via membrane expansion or cell shrinkage and by interacting with the membrane contents.

Protein denaturation has been associated with the inflammation process; the primary mechanism of NSAID action has been attributed to the inhibition of protein denaturation [28]. In the present study, the EE and its fractions effectively prevented albumin denaturation at various concentrations; this indicated that the extract contained secondary metabolites such as phenolic compounds that preserved and maintained the protein structure from denaturation. The ability of the *B. sapida* extract to inhibit protein denaturation may contribute to its membrane-stabilizing activities. The anti-inflammatory activity observed in the extract could be associated with the presence of secondary metabolites such as flavonoids. Notably, flavonoids such as hyperoside, quercitrin, and afzelin have been isolated from the leaves of *Crataegus almaatensis* and reportedly display anti-inflammatory effects [29]. The observed activities of the EAF in the present study could be linked to not only the presence of phenolics and flavonoids in the fraction but also to bioactive compounds such as 2-methoxyl-4-vinyl phenol, 2, 4-bis (1,1-dimethyl ethyl) phenol, *n*-hexadecanoic acid, octadecanoic acid, and pentadecanoic acid, as reported in a study by Adekola et al. [30].

5. Conclusion

The results of the present study indicate that EE and fractions of *B. sapida* possess radical scavenging activity, antioxidant capacity,

and membrane stabilization activities. They also inhibit protein denaturation. These effects could be attributed to the presence of phenolics and flavonoids. The abundance of phytoconstituents in this plant, which may be responsible for its ethnomedicinal use, renders it a promising candidate as an anti-inflammatory drug.

CRediT author statement

Mukaila B. Adekola: Conceptualization, Methodology, Data curation, Writing - Original draft preparation, Visualization, Investigation, Writing - Reviewing and Editing; **Jacob O. Areola:** Supervision, Methodology; **Oladapo F. Fagbohun:** Software, Validation, Writing - Reviewing and Editing; **Funke T. Asaolu:** Data curation, Visualization, Investigation; **Gbenga E. Ogundepo:** Methodology, Software; **Adeniyi O. Fajobi:** Methodology, Software, Validation, Writing - Reviewing and Editing; **Olubunmi O. Babalola:** Supervision, Methodology.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.04.002.

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