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Evaluation of the antioxidant and anti-inflammatory properties of the hydroethanolic extract of the leaves of *Blighia sapida* KD Koenig

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ABSTRACT

Blighia sapida is widely used in traditional medicine for the treatment of several diseases in Togo. This study aimed to assess the antioxidant and anti-inflammatory properties of *Blighia sapida*. To this end, a phytochemical screening was carried out, followed by an assessment of DPPH, total antioxidant capacity, *ex vivo* lipoperoxidation tests on *Sprague Dawley* rats, and an evaluation of anti-inflammatory activity by the egg albumin denaturation test. The leaves extract scavenged the DPPH free radical with an IC_{50} of 172 ± 0.577µg/mL and its total antioxidant capacity value was 40.174 ± 0.638 mg Eq AA/g. In the *ex vivo* lipoperoxidation test on the kidney and liver, the extract showed a significant inhibitory effect. Phytochemical tests revealed the presence of tannins, flavonoids, alkaloids, saponosides, and carbohydrate compounds. The present results showed that *Blighia sapida* leaves possess antioxidant and anti-inflammatory properties which may be linked to the bioactive compounds they contain, suggesting their use to prevent oxidative and inflammatory processes.

Keywords: *Blighia sapida,* antioxidant, anti-inflammatory.

INTRODUCTION

Inflammation is the body's defense response to injury, tissue ischemia, autoimmune reactions, or infectious agents, which can be acute or chronic (Laveti et al., 2013). Inflammation is generally beneficial to the body when acute, but harmful when chronic. Chronic inflammation can arise from acute inflammation and act in a self-sustaining way, leading to chronic disease (Park et al., 2014; Lee and Surh, 2012). Oxidative stress, that results from an imbalance between the production of free radicals and the body's antioxidant defense system. It can be caused by an increase in the production of free radicals, or a reduction in the body's ability to fight them (Moniczewski et al., 2015; Pisoschi and Pop, 2015). Oxidative stress is responsible for the degradation of biomolecules (proteins, lipids, and nucleic acids) leading to cell death and physiological disorders including inflammation (Subedi et al., 2014). Oxidative stress and inflammatory disorders are implicated in the development of several diseases such as atherosclerosis, diabetes mellitus, cancer, and central nervous system

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disorders (Allegra, 2019; Sinmisola et al., 2019; Wu et al., 2020). Studies are continously being carried out to identify more antioxidant and anti-inflammatory molecules with fewer side effects and greater accessibility for populations (Kpemissi et al., 2023).

The use of medicinal plants in the treatment of diseases is a very ancient practice, which plays an important role in drug development (Junchi et al., 2022). These plants are sources of secondary metabolites (Asha et al., 2017), which act as defensive agents and can help humans combat several diseases. Particular attention is paid to medicinal plants with antioxidant and anti-inflammatory properties (Singh et al., 2016; Chauhan et al., 2022), as they are thought to be beneficial in treating various pathologies.

Blighia sapida KD Koenig is a plant belonging to the Sapindaceae family which grows naturally in West Africa. It is cultivated as a fruit tree and as an ornamental shade tree (Ekué et al., 2010). *Blighia sapida* is widely used in traditional medicine; its various parts are used in the treatment of diseases including hypertension and diabetes mellitus (Abolaji et al., 2007; Nabede et al., 2022). In Togo, the leaves of *Blighia sapida* are used for cancer treatment (Kola et al., 2020). Previous works on this plant revealed the antioxidant (Sonibare et al., 2011), anti-inflammatory (Adekola et al., 2022), and analgesic (Olayinka et al., 2021) activities of the stem bark. Little scientific information is available on the pharmacological properties of the leaves of this plant end this preliminary study aimed to assess the antioxidant and anti-inflammatory activity of *Blighia sapida* leaves, since they are used in the treatment of pathologies linked to these disorders in Togo.

MATERIALS AND METHODS

Reagents

Gallic acid, 2,2 Diphenyl-1-picrylhydrazyl, aluminum chloride, Folin-Ciocalteu reagent, Polyvinylpolypyrrolidone, ferric chloride ($FeCl_3$), ammonium molybdate, sulfuric acid, sodium carbonate, rutin, Tris-HCl, malondialdehyde (MDA), and concentrated hydrochloric acid were obtained from Sigma-Aldrich. All other chemicals and reagents were analytical grade.

Plant material

Blighia sapida leaves were harvested in june 2022 in Lomé in the Maritime Region of Togo. The plant material was identified and authenticated in the herbarium of the Botany Department of the Faculty of Sciences of the University of Lomé in Togo, where a voucher specimen was deposited in the herbarium under the number TG14569. The leaves were then dried, finely ground, and kept away from light and humidity for later analyses.

Biological material

Organs such as liver and kidneys from Sprague Dawley rats from the animal house of the Department of Animal Physiology at the University of Lomé were used in this study. The rats (male and female) were selected according to age (8 to 10 weeks) and weight (150 to 180 g). Breeding was carried out at the Animal Physiology Department of the University of Lomé. Experimental animals were kept at room temperature, 27±2°C following 12 hours/12 hours' light/dark cycle, with free access to drinking water and food. All tests using rats, blood and eggs were performed with the approval of the ethics committee of the Department of Animal Physiology of the University of Lomé, a branch of the ethics committee for the control and supervision of animal experiments and the use of blood, Ref n◦ 007/2020 / BC-BPA / FDS-UL.

Preparation of plant material

The weight 800 g of the leaf powder was macerated in 8 L of a 50:50 water-ethanol mixture for 72 hours. After filtration of the mixture obtained, the macerate was evaporated under vacuum using a rotary evaporator (Buchi R100) at 45°C. The dry extract was collected and stored at 4°C for further analysis.

Phytochemical screening

The main chemical groups such as alkaloids, flavonoids, tannins, saponosides, phenolic compounds, and reducing compounds

were sought in the extract by staining tests described by Harborne (1998).

Dosage of total phenols and tannins

The phenol and total tannin contents of the extracts were determined respectively by the Folin-Ciocalteu method and the tannin fixation method (Motto et al., 2021).

Total phenols

The volume 200 µL of the Folin-Ciocalteu reagent (10%) was added either to 200 µL of the gallic acid solution (25-100µg/mL) or the extract (1mg/mL). After 30 min of incubation at ambient temperature, 800 µL of a sodium carbonate solution (700 mM) was added. The absorbance of each sample was read at 735 nm after 2 h of incubation. The absorbance of the extract was related to the standard curve of gallic acid to determine the amount of total phenols they contain. This quantity was expressed in mg of gallic acid equivalent per gram of extract.

Total tannins

For the dosage of the tannins, the same test was repeated with the extract previously treated with Polyvinylpolypyrrolidone (PVPP). Five hundred (500) µL of the extract was first added to 10 mg of PVPP and the mixture was incubated on ice for 30 minutes. The supernatant obtained was treated in the same way. After centrifugation, the phenol assay was carried out on the last supernatant. The difference between the absorbance obtained during the determination of total phenol and this second determination was calibrated with the gallic acid curve to express the tannin content.

Assay of total flavonoids

The total flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method. To 2 mL of extract (1 mg/mL) or of rutin prepared at 0, 5, 25, 50, 100, 200 µg/ml, were added: 2 mL of aluminum chloride (20 mg/ml) and 6 mL sodium acetate (50 mg/mL). The blank was prepared with 2 mL of ethanol instead of the sample. The optical density was read at 440 nm after 150 minutes of incubation (Motto et *al*., 2021).

Antioxidant activity

DPPH test

The capacity of the extract to trap the DPPH radical was evaluated according to the method described by Kpoyizoun et al. (2019). Briefly, 1.5 mL of methanolic solution of DPPH (100 µmol/L) was brought into contact with 0.25 mL of methanolic solution of extract (62.5 - 500 µg/mL) or ascorbic acid used as standard. The control consists of a mixture of 1.5 ml of DPPH + 0.25 mL of methanol. After 10 min of incubation, the optical density was measured with a spectrophotometer at 517 nm. The DPPH radical scavenging effect of the extract and the standard is expressed as percentage inhibition (PI) determined using the following formula:

$PI = [(A_0 - A_1) / A_0] \times 100$; A_0: control absorbance, A_1: absorbance of each sample of extract or ascorbic acid.

Total antioxidant capacity

This test is based on the reduction of the molybdenum ion Mo (+6) to Mo (+5) by antioxidants in an acid environment, leading to the formation of the green Phosphate-Mo (+5) complex (Prieto et al., 1999). To 3 mL of the prepared reagent (0.6 M sulfuric acid; 28 mM sodium phosphate and 4 mM ammonium molybdate), 0.3 mL of methanolic extract solution (1 mg/mL) or ascorbic acid different concentrations of 0 - 250 µg/mL was added. The whole was incubated at 95°C for 90 min. The absorbance of the reaction medium was read at 695 nm against a blank (methanol). The antioxidant activity was expressed in mg equivalent of ascorbic acid/g of extract.

Evaluation of ex-vivo antioxidant activity on liver and kidney tissues

The inhibitory effect of the lipid peroxidation induced by the $FeCl_2$ -Ascorbic acid mixture in the liver and the kidneys was investigated. Sprague Dawley rat liver and kidney tissues were removed and ground with 150 mM KCl Tris-HCl buffer (pH 7.4) and homogenates were obtained.

The reaction mixture was composed of 500 µL of homogenate from each organ, 200 µL of Tris-HCl buffer (pH 7.4), 100 µL of 0.1 mM ascorbic acid, 100 µL of 4 mM $FeCl_2$, and 100 µL of various concentrations of extract or standard. This mixture was incubated at 37°C for 1 hour. At the end of the incubation, the level of malondialdehyde was determined in each sample as follows. To 1.3 mL of phenylindole (10.3 mM) activated (by addition of $FeCl_2$ 32 µM in the proportions 3:1), are added respectively 500 µL of a sample of pre-treated homogenates, and 300 µL of HCl 37 %. The mixtures were incubated at 45°C for 1 h then centrifuged at 3000 rpm for 10 min and the absorbance was measured by spectrophotometry at 586 nm. A standard curve of 1,1,3,3-tetra-methoxy propane was also performed for the quantification of MDA (Kpemissi et al., 2019).

Inhibition of albumin denaturation

Blighia sapida extract's ability to inhibit albumin denaturation was assessed using the method described by Saleem et al. (2020).

The reaction mixture (5 mL) consisted of 0.2 mL of fresh chicken egg albumin, 2.8 mL of PBS (pH 6.4 mL), and 2 mL of plant extract or Diclofenac sodium at different concentrations (0 to 1000 µg/mL). Samples were incubated at 37°C for 25 min and heated to 70°C for 5 min. After cooling, the absorbance of each sample was measured at 660 nm. The percentage inhibition of protein denaturation was determined using the formula $[(A_0 - A_1) / A_0] \times 100$; A_0 is the absorbance of the control (without extract) and $A_{1\,is}$ the absorbance in the presence of the extract or the reference.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 6.02 software. The results were expressed as Mean ± standard error of the mean. Statistical differences among treatments were tested with One-way ANOVA, followed by Tukey's post hoc test for multiple comparison tests at a significant level of $p \leq 0.05$.

RESULTS

Phytochemical screening

The major phytochemical groups found in the extract of *B. sapida* leaves are presented in the table below (Table 1). The hydroethanolic extract of *B. sapida* revealed major metabolites.

Contents of total phenols, tannins, and flavonoids

The extract's contents of total phenols, tannins, and flavonoids estimated by the Folin-Ciocalteu and aluminum chloride methods is reported in Table 2.

Antioxidant effect of *Blighia sapida* leaf extract

DPPH radical reduction test

The DPPH radical scavenging activity of the extract is compared to the reference (ascorbic acid). The IC_{50} values of the extract and the reference are presented in the table below (Table 3).

Total antioxidant capacity

The total antioxidant capacity of the extract is **40.174 ± 0.638** mg ascorbic acid equivalent per gram of extract. This value is expressed as mean ±ESM.

Inhibition of $FeCl_2$ -ascorbic acid-induced peroxidation on organ homogenates

Incubation of liver and kidney homogenates of Sprague Dawley rats with $FeCl_2$ -ascorbic acid resulted in a significant increase in MDA levels. Rutin and the extract at its different concentrations significantly ($P < 0.001$) reduced this MDA level (Figure 1).

***In vitro* anti-inflammatory activity of *Blighia sapida* leaf extract**

The ability of the extract to inhibit albumin denaturation of the egg was investigated. The table below shows the IC_{50} of the extract compared to Diclofenac used as a reference (Table 4).

Table 1: Phytochemical groups present in the hydroethanolic extract of *B. sapida*.

Phytochemical groups	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Saponosides	+
Phenolic compounds	+
Carbohydrates	+

+: present

Table 2: Total phenol, tannin, and flavonoids contents of *Blighia sapida* extract.

Total phenols (mg Eq GA/g)	**Tannins** (mg Eq GA/g)	**Flavonoids** (mg Eq R/g)
37.726 ± 0.550	32.368 ± 0.489	148.427 ± 4.295

Values are expressed as mean ± in SEM. mg Eq GA/g: mg equivalent of gallic acid/g of dry extract; mg Eq R/g: mg rutin equivalent/g of extract.

Table 3: IC_{50} of ascorbic acid and extract showing their DPPH scavenging activity.

	Ascorbic acid	***Blighia sapida***
IC_{50} (µg/mL)	27±0.115	172 ± 0.577

IC_{50}: Inhibitory concentration 50, expressed as mean ± SEM

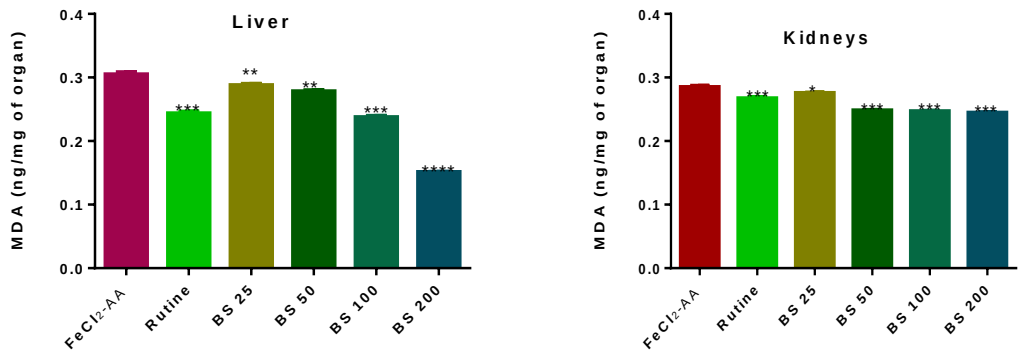

Figure 1: Effect of *B. sapida* extract on lipoperoxidation induced by $FeCl_2$ -AA in the liver and kidneys of Sprague Dawley rats. The results are expressed as mean ± SEM. $^{***}p < 0.001$; $^{**}p < 0.01$ and $^{*}p < 0.05$ (compared to the Positive Control).

Table 4: IC_{50} showing the anti-inflammatory effect of *B. sapida* extract.

	Blighia sapida	**Diclofenac**
IC_{50} (µg/mL)	225.5 ± 0.50	66.5 ± 0.289

Values are expressed as mean ± SEM.

DISCUSSION

The present study highlighted the antioxidant and anti-inflammatory properties of the hydroethanolic extract of *B. sapida* leaves.

Inflammation involves the denaturation of proteins during attacks on the organism by various agents that may be microbial, toxic, physical, or chemical (Yang et al., 2020). It is a process in which proteins lose their tertiary and secondary structure due to stress or an external compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat (Kola et al., 2022; Saleem et al., 2020). This leads to the production of self-antigens, exacerbating the inflammatory response (Chandra et al., 2012). Thus, studies have shown that any plant component with the ability to inhibit protein denaturation could contribute significantly to countering inflammation (Dadoriya et al., 2020). In the present study, the extract prevented heat-induced protein denaturation at different concentrations and could therefore be a potential anti-inflammatory agent.

Free radicals are involved in the pathophysiology of diseases with an inflammatory component. The antioxidant evaluation of *B. sapida* leaves was carried out by the DPPH, the TAC, and the MDA assay. The results show that the extract scavenged the DPPH radical in a dose-dependent manner. DPPH is a stable free radical that can accept an electron or a hydrogen radical to become a stable molecule. They react with various electron donor molecules (reducing agents or antioxidants) and when the electrons are paired, discoloration is observed (Kpemissi et al., 2019). Any substance capable of scavenging free radicals has antioxidant activity. The total antioxidant capacity (TAC) based on molybdenum (VI) reduction to molybdenum (V) in the presence of a reducing agent (antioxidant) (Chohra et al., 2020) confirms the antioxidant activity of the extract. This is further supported by the extract's inhibitory effect on $FeCl_2$-AA-induced lipid peroxidation in organ homogenates, reflected by a decrease in MDA. $FeCl_2$-AA induces lipoperoxidation via the free radicals which oxidize the unsaturated lipids of the cell membrane (Kpemissi et al., 2016; Tanas et al., 2010). The results then indicate that the extract could intervene in kidney and liver protection through its antioxidant activity. The present results corroborate those reported by Adekola et al. (2022) on the antioxidant and anti-inflammatory effects of *B. sapida* stem bark. The activity of the extract would probably be due to the presence of phenolic compounds which are known as substances having anti-inflammatory and antioxidant effects (Heim et al., 2002; Hannebelle et al., 2004).

The phytochemical screening revealed the presence of phenols, tannins and flavonoids, saponosides, alkaloids, and carbohydrates. Total phenols, tannins, and flavonoids were quantified as they are known as anti-inflammatory and antioxidant compounds through their redox property which can play an important role in several diseases (Yessoufou et al., 2013). These compounds would be involved in the effects mentioned above.

Conclusion

The results obtained in this study show that the hydroethanolic extract of *Blighia sapida* leaves possesses an anti-free radical effect, antioxidant capacity, and protein denaturation inhibitory activity. These effects could be attributed to the phenolic compounds found in the extract. This preliminary study justifies the use of the plant in traditional medicine and makes *B. sapida* leaves a potential anti-inflammatory candidate that could contribute to the treatment of several pathologies.

COMPETING INTERESTS

The authors declare that they have no competing interests on this work.

AUTHORS' CONTRIBUTIONS

Conceptualization, Data curation and formal analysis : AD, WT, KM; Supervision : PK, KM, KEG ; Analysis, interpretation and writing : WT, PKK, AD; Review and final revision approval : all authors.

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