**Sapium ellipticum** (Hochst.) Pax Leaf Extract: 
*In-vitro* Antioxidant Activities and Lethal Dose (LD$_{50}$) Determination in Wistar Rats

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**Authors’ contributions**

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/BJMMR/2016/8253

Editor(s):  
(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.  
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Complete Peer review History: [http://www.sciencedomain.org/review-history/16380](http://www.sciencedomain.org/review-history/16380)

Received 6th December 2013  
Accepted 7th April 2014  
Published 29th September 2016

**ABSTRACT**

Prior to this investigation, the LD$_{50}$ and antioxidant activities of *Sapium ellipticum* leaf extract have not been reported, hence; the focus of this study. The LD$_{50}$ of the extract was determined through three routes of administration using the method of Lorke. The Intraperitoneal (i.p) and intramuscular (i.m) LD$_{50}$ values were determined as 979.80 and 1,341.60 mg/kg body weight (BW) respectively. Oral administration of the extract (to a dose of 45,000 mg/kg BW) did not cause any negative behavioral changes in the animals, and no mortality was recorded within and after 24h of the experiment. The antioxidant properties of the extract was assessed *in vitro* in terms of its free radical scavenging, metal chelating and reducing power activities as well as its ability to inhibit the formation of malondialdehyde (MDA), an index of lipid peroxidation. The total phenolic content of the extract was determined as 74.23±3.12 mg GAE/g. Data obtained indicate that the extract exhibited appreciable free radical scavenging activity (IC$_{50} = 0.128$ mgmL$^{-1}$) and strong reducing power activity.

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power in comparison with butylated hydroxyl toluene (BHT, $IC_{50} = 0.118 \text{ mg mL}^{-1}$) and ascorbic acid ($IC_{50} = 0.120 \text{ mg mL}^{-1}$) used as reference antioxidants. The inhibition of linoleic acid induced-lipid peroxidation by the extract was comparable to that of BHT and greater than that elicited by ascorbic acid. In terms of metal chelating activity, ethylene diamine tetraacetic acid (EDTA) used as positive control elicited a significantly ($p \leq 0.05$) higher activity than the extract at all concentrations used. Findings from this study credit *Sapium ellipticum* ethanol leaf extract with significant antioxidant properties. The plant material may therefore be of immense relevance in combating oxidative stress and its related ailments.

**Keywords:** *Sapium ellipticum*; lethal dose; antioxidant; Wistar rats.

### 1. INTRODUCTION

Free radicals or reactive oxygen species (ROS) induced-oxidative stress has been widely implicated in the aetiology and progression of several disease conditions [1-4]. Normally, reactive species exist in all aerobic cells in balance with cellular antioxidants [5]. Oxidative stress occurs when the production of ROS exceeds the level the body’s natural antioxidant defense mechanisms can cope with; causing damage to cell membranes and molecules such as DNA, proteins and lipids [6-8]. Interestingly, many plants known to possess antioxidant properties have been proposed in the prophylactic and curative treatments of different pathologies induced by oxidative stress. This is probably consequent upon their ability to scavenge or mop up free radicals amidst other protective properties such as metal chelating and reducing power activities.

The antioxidant properties of *Sapium ellipticum* stem bark extract has earlier been reported by Adesegun et al. [9]. Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines indicated that *S. ellipticum* leaf extract expressed the highest cytotoxic activity among other plants with anticancer potential [10]. *S. ellipticum* (Hochst.) pax (family *Euphorbiaceae*) is commonly referred to as jumping seed tree. The plant is common on the outskirts of evergreen forest and in wooded ravines. It is widely distributed in Eastern Africa and tropical Africa. In Western part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly known as *aloko-agbo*. According to Burkill [11-13], a number of pharmacological effects have been traditionally associated with the plant: A preparation of dried leaves of *S. ellipticum* is applied to wounds in Tanzania, the leaf preparation is also used for sore-eyes and abdominal swelling. In Central Africa, the decoction of the stem bark is used as purgative in Congo and for treatment of eczema. Interestingly, it is believed to be a cure for stammering in Zaire. A root-concoction is prepared as a fomentation in East Africa for enlarged spleen in babies and is taken by adults for malaria. In Tanganyika, a leaf-preparation is used to relieve pains in the head, chest, shoulders and back. Surprisingly, extremely few investigations have been conducted on the plant. The focus of this present study was to evaluate the antioxidant status of the plant’s leaf extract and prescribe a safe dose range for its use through the knowledge of its lethal dose vis-a-viz different routes of administration.

### 2. MATERIALS AND METHODS

#### 2.1 Collection of *Sapium ellipticum* and Preparation of Leaf Extracts

Fresh *S. ellipticum* leaves were harvested in the month of December 2012 from a forest in a suburb of Ibadan, Southwest of Nigeria. The taxonomical authentication was done by a botanist (Mr.Odewo, T.K) at the Lagos University Herbarium (LUH), Nigeria, where herbarium specimen with voucher number LUH 5423 was deposited. The plant material was freed of extraneous materials; air dried at room temperature and was milled into a fine powder with a milling machine. Fifty grams of the dried powdery sample was macerated in 500mL of the extracting solvent (absolute ethanol) at room temperature. The mixture was allowed to stand for 72 h and stirred intermittently to facilitate extraction. Sieving of the mixture was achieved with a muslin cloth of mesh size, 42. The resulting volume on sieving was reduced with a rotary evaporator. Final solvent elimination and drying was done using a water bath at 40°C. The crude extracts were stored in sterile screwed (air-tight) bottles and aliquots were taken when required.
2.2 The Animals-collection and Management

Male and female albino rats of the Wister strain (100 to 120 g) were used for lethal dose determination. They were purchased from the animal breeding unit of Institute for Advance Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. Ethical approval number (LCUEC 107) was obtained from the local institution ethical committee. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science and published by the National Institute of Health [14]. The animals were handled humanely, kept in a plastic suspended cage placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets and served water ad libitum and subjected to natural photoperiod of 12 h light and 12 h dark cycle. The animals were allowed two weeks of acclimatization before the commencement of the study.

2.3 Lethal Dose Determination (Acute Toxicity Test)

Lethality studies to determine the 50% lethal dose (LD$_{50}$) of the extract was performed according to the procedure described by Lorke [15]. It was assessed through three routes of administration {Intraperitoneal (i.p), intramuscular (i.m) and oral (p.o)}. For each of i.p and i.m determinations, forty rats (both sexes) were randomly assigned to ten (10) groups, with each group having four (4) animals. They were respectively treated with 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 mg/kg body weight of the extract in saline. The animals were then returned to their respective cages, allowed free access to food and drinking water. They were thereafter monitored for clinical signs, symptoms and mortality within 24h of the experiment.

In the oral LD$_{50}$ determination, three different sets of ten (10) animals equally assigned to 5 groups were respectively treated with doses of 15,000, 20,000, 30,000, 40,000 and 45,000 mg/kg BW of the extract in saline. They were closely observed for negative behavioral changes and mortality within 24h of the experiment. The lethal dose of the extract through the different routes was calculated using the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}.$$  

Where $D_0$ = Maximum dose that produce 0% mortality, $D_{100}$ = Minimum dose that produce 100% mortality.

2.4 Antioxidant Assays

2.4.1 DPPH radical scavenging activity

The free radical scavenging activity of extract was measured from the bleaching of the purple-coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of Burits and Bucar [16]. One milliliter of various concentrations (0.025-0.25 mgmL$^{-1}$) of the extract in ethanol was added to 4 mL of 0.004% DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was then read at against a blank at 520 nm using spectrophotometer. Ascorbic acid and BHT were used as positive controls and deionized water in place of extract in addition to other reagents was used as blank. The decrease in absorbance of the reaction mixture indicated higher free radical scavenging activity. Percentage inhibition of free radical was calculated using the formula % inhibition = $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$, where $A_{\text{blank}}$ is the absorbance of the control reaction (containing all reagents except the test extract) and $A_{\text{sample}}$ is the absorbance of the test material. Extract concentration providing 50% inhibition (IC$_{50}$) was calculated from the plot of percentage inhibition against extract concentration. Tests were carried out in triplicate.

2.4.2 Fe$^{2+}$-chelating ability

The Fe$^{2+}$-chelating ability of the extract with EDTA used as positive control was assessed using the method described by Decker and Welch [17]. The extract (1 mL, 0.1-10.0 mg mL$^{-1}$) was diluted to 20% of the original concentration with water, mixed with FeCl$_2$ (0.1 mL, 2.0 mM) and after 30 min, ferrozine (0.2 mL, 5 mM) was added. The resulting mixture was shaken
vigorously and left to stand for 10 min at room
temperature. The absorbance of the resulting
solution was measured at 562 nm. Decreased
absorbance of the reaction mixture indicates
higher Fe²⁺-chelating ability. The percentage of
inhibition of ferrozine-Fe²⁺ complex formation
was calculated from the absorbance ratio to that
of the blank without any sample. All
determinations were carried out in triplicate.

2.4.3 Total phenol estimation

The total phenol content of the extract was
estimated according to the method of Singleton
et al. [18] using Folin-Ciocalteau reagent. The
extract (100 mg mL⁻¹, 1.0 mL) was mixed
thoroughly with 5 mL Folin-Ciocalteau reagent
diluted ten-fold) and after 5 min., 4.0 mL of
sodium carbonate (0.7 M) was added and the
mixture was allowed to stand for 1 h with
intermittent shaking. The absorbance was
measured at 765 nm in a spectrophotometer.
Gallic acid was used as a standard. Serial
dilution of 10 mg/mL of the standard was made
to obtain a calibration curve. Total phenol was
determined from the calibration concentration
curve as gallic acid equivalent (GAE). All
determinations were carried out in triplicate.

2.4.4 Anti-peroxidant assay

The method of Chang et al. [19] was used to
measure the anti-peroxidant activity of the extract, indexed by its ability to inhibit linoleic
acid emulsion induced-lipid peroxidation. The
extract (0.5 mL, 1.0-8.0 mg mL⁻¹) was mixed
with phosphate buffer (2 mL, 0.2 M, pH 7.0) and
linoleic acid emulsion (2.5 mL, 0.56% w/v, pH
7.0). The mixture was then incubated at 60°C in
the dark for 12 h to accelerate oxidation. Ethanol
(4.5 mL, 75%), ammonium thiocyanate solution
(0.2 mL, 4 M), sample solution (0.1 mL) and
ferrous chloride (0.2 mL, 20 mM in HCl) were
mixed in sequence and after 3 min the
absorbance for the red colour was measured at
700 nm. The level of lipid peroxidation inhibition
(%) by the extract was calculated from the
absorbance ratio to that of the blank without any
sample. BHT and Ascorbic acid were used as
standards. All determinations were carried out in
triplicate.

2.4.5 Ferric reducing/antioxidant power
assay (FRAP)

The method of Lai et al. [20] was used to
measure the reducing power of the extract. One
milliliter of various concentrations (0.2-2.0 mg
mL⁻¹) was added to 2.5 mL phosphate buffer (0.2
M, pH 6.6). This was mixed with potassium
ferricyanide (2.5 mL, 1%). The mixture was
incubated at 50°C for 20 min. A portion (2.5 mL)
of trichloroacetic acid (10%) was added to the
mixture and centrifuged at 1000 g for 10 min.
The upper layer of the solution (2.5 mL) was
mixed with distilled water (2.5 mL) and FeCl₃ (0.5
mL, 0.1%) and the absorbance was measured at
700 nm against a blank in the spectrophotometer. Ascorbic acid and BHT were
used as positive controls. Increased absorbance
of the reaction mixture indicated increased
reducing power. All determinations were carried
out in triplicate.

2.5 Statistical Analysis

Data were analyzed by SPPS version 19.0 using
one way ANOVA and subjected to Fisher LSD
post hoc test. Values are presented as mean ±
SEM. Differences between means were
accepted to be significant at p < 0.05.

3. RESULTS AND DISCUSSION

3.1 Lethal Dose Determination

The LD₅₀ values of the investigated S. ellipticum
leaf extract through i.p and i.m routes were
determined as 979.80 and 1,341.60 mg/kg BW
respectively. The values are respectively about
and above 1000 mg/kg BW. These figures
connote substantial degree of safety for the use
of S. ellipticum leaf extract in terms of toxicity
level assessment through these routes.
Interestingly, p.o administration of the extract (to
a dose of 45,000 mg/kg BW) did not cause any
negative behavioral changes in the animals, and
no mortality was recorded within and after 24 h of
the experiment, rather; increased appetite was
observed in the animals. Possibly, the plant
material was poorly absorbed through this route
of administration (p.o) or biotransformation of the
active component of the extract into non or less
toxic metabolites occurred in the gastrointestinal
tract (GIT) of the animals by the action of certain
modifying enzymes. Increase in food intake by
the animals as observed in this study suggests
the presence of a digestive or appetizing agent in
SE leaf extract.

3.2 In vitro Antioxidant Assessments

Polyphenolic compounds are strongly associated
with antioxidant capabilities. Compounds with
high phenol content have been found to be good antioxidants [21,22]. This is probably explained by the fact that polyphenols generally have the ability to readily donate electron or hydrogen atom to highly unstable molecules with unpaired electron such as free radicals or reactive oxygen species (ROS). 1, 1-diphenyl-2-picrylhydrazyl (DPPH) model system is often employed as a source of free radicals or unpaired electrons to assess the ability of a test material to function as an antioxidant in vitro [16,19,23]. Findings from this present investigation showed that S. ellipticum leaf extract possesses a total phenolic content of 74.23±3.12 mg GAE/g and elicited appreciable free radical scavenging activity of IC$_{50}$ value (0.128 ± 0.01 mgmL$^{-1}$) comparable to that of BHT (0.118 ± 0.00 mgmL$^{-1}$) and ascorbic acid (0.120 ± 0.01 mgmL$^{-1}$) used as reference antioxidants.

Fig. 1 shows the DPPH radical scavenging effects of the extract, BHT and LAA. The radical scavenging effects increased with increasing concentration in each case. At all concentration studied, there was no significant difference (P≤0.05) in the scavenging activities of the tested materials. The extract remarkably showed significantly greater inhibition of linoleic acid induced-lipid per oxidation in a dose dependent manner, particularly at 5.0 and 7.5 mg/mL than BHT and LAA Fig. 2. The reducing activity on Fe$^{3+}$/ferricyanide complex exercised by the leaf extract followed the same pattern Fig. 3.

In terms of metal chelating activity, ethylene diamine tetraacetic acid (EDTA) used as positive control elicited a significantly (p≤0.05) higher activity than the extract at all concentrations used. The chelating capacity at a concentration of 0.4 mgmL$^{-1}$ was 28.7% compared to 74.2% expressed by EDTA at the same concentration Fig. 4. Higher concentration (0.8 mg/mL) did not cause any significant increase in the chelating ability (31.4%) of the extract. As noted by Adesegun et al. [9] in a previous study on the stem bark extract of the same plant, metal chelation probably contribute very little to the antioxidant relevance of S. ellipticum.

Free radicals or reactive species (RS) effect damage to cell membranes and molecules such as DNA, proteins and lipids through processes involving degradation of fatty acids (per oxidation), removal of electrons (oxidation) and combining with target molecules (formation of adducts) [7,8]. This usually occurs when the endogenous antioxidants (enzymes and molecules) are overwhelmed by excess amount of oxidants or free radicals, a phenomenon describes as oxidative stress. Antioxidants when present even in small concentration but in the right proportion with RS are capable of muffling their actions. Donation of electron or hydrogen atom (reducing activity), scavenging or mopping of free radicals (acceptance of electron), breaking of free radical chains (chelating action) are some of the mechanisms by which antioxidants elicit their protective properties.
Data obtained from this study credit *S. ellipticum* leaf extract with substantial free radical scavenging and reducing activities, as well as lipid per oxidation inhibitory effect comparable to those of BHT and L-Ascorbic acid, well known synthetic and endogenous antioxidants respectively. These chemicals have proven abilities to protect the human body against free radicals and oxidative stress associated injuries. In this regard, findings from this study afford *S. ellipticum* leaf extract significant antioxidant usefulness which may be adduced to arrays of secondary metabolites such as flavonoids and other polyphenols present in the plant. The plant material may therefore be of immense relevance in combating oxidative stress related-derangements. Moreover, comparing the findings of this investigation with the study of Adesegun et al. [9] on the stem bark extract of *S. ellipticum*, suggests that the leaf extract of the plant possess relatively better antioxidant values.

4. CONCLUSION

The investigated extract performed creditably well against renown synthetic and endogenous antioxidants (BHT & L- Ascorbic acid) in most of the *In vitro* antioxidant evaluation model systems employed. Collectively, the results of this study indicate that ethanol leaf extract of *S. ellipticum* is a good source of natural antioxidants and extremely safe for oral consumption. These observations affirm the use of the plant in alternative or traditional medicine across Africa. On the basis of available scientific documentations, this report is apparently the first on the antioxidant properties and LD_{50} status of the leaf extract of *S. ellipticum*. *In vivo* assessment of the extract is currently ongoing in our laboratory. This is necessary because some plant materials with impressive *In vitro* antioxidant relevance fail to replicate the same *In vivo*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication 2010) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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