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Original Article

Phytochemical and *in vitro* antioxidant properties of the methanolic extract of fruits of *Blighia sapida, Vitellaria paradoxa* and *Vitex doniana*

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Abstract
The Methanolic extract of fruits of *Blighia sapida, Vitex doniana and Vitellaria paradoxa* were screened for their phytochemical constituents and in vitro antioxidant properties using standard procedures. Results revealed the presence of alkaloids, tannins, saponins, flavoniods and phenols in all the fruits studied. Quantitative phytochemical determination of the fruits showed that tannin content of the extract ranged between 1.04 ± 0.05 mg/g and 1.44 ± 0.05 mg/g, saponins between 2.3 ± 0.05 mg/g and 7 ± 0.04 mg/g, total phenols between 55.6 ± 0.05-96.4 mg/g, and total flavonoid content between 20.8 ± 0.05 mg/g and 72.8 mg/g. All the fruits studied showed antioxidant activity in a dose dependent manner. *Blighia sapida* extract showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPHH) free radical scavenging activity (78 ± 0.01%) and reductive potential compared to other extracts. *Vitellaria paradoxia* showed the highest capacity to inhibit lipid peroxidation (72%) than other fruit extracts while *Vitex doniana* had the lowest effect in inhibition of lipid peroxidation (60.7 ± 0.05%). This study showed that methanolic extracts of fruits of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana* could be a source of antioxidants.

INTRODUCTION

Several varieties of fruits are either consumed for their nutrients or for their medicinal values in different parts of the world. They are known to be rich in vitamins, minerals and antioxidants. Research is shifting to antioxidants of plants and vegetable origin as they have been shown to help in reducing the incidence of degenerative diseases such as diabetes, cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the aging process [1, 2]. Antioxidants are substances which when present at low concentration are capable of preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species (ROS). These ROS include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals such as hydrogen peroxide, hypochlorous acid, *etc*. Antioxidants scavenge radicals by inhibiting the initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen [3]. The most abundant antioxidants in fruits are polyphenols, Vitamin C, and E while carotenoids are present to a lesser extent in some fruits. These polyphenols mostly flavonoids, are present mainly in their ester and glycoside forms [4].

Blighia sapida is a tropical food plant with a dense crown that belongs to the family of Sapindaceae. The fruit is popurlarly known as “ackee” (English), “okpu” (Ibo: Eastern Nigeria), “isin” (Yoruba: Western Nigeria), “gwanju kusa” (Hausa, Northern Nigeria) and “yila”(Nupe: North Central Nigeria). The fruit of the tree, splits to expose a freshly cream colored pulp (aril) attached to a shiny black oblong seed [5]. The oily aril is the edible portion and is consumed either fresh or cooked as vegetable [6]. In Jamaica, they are often cooked with codfish, onions and tomatoes [7]. *B.sapida* has also been reported to have several ethnomedicinal

uses; for instance the pulp and leafy juice are used as eye drops in ophthalmic lesions and conjuntivitis [8]. Various preparations and extracts have been made for the treatment of diseases such as dysentery, epilepsy, yellow fever and diabetes [9].

V*itellaria paradoxa* (previously *Butyrospermum parkii*) belongs to the Sapotaceae family indigenous to Africa and occurs in Mali, Cameroon, Congo, Cote d'Ivoire, Ghana, Guinea, Togo, Nigeria, Senegal, Sudan, Burkina Faso and Uganda. The plant species (*Vitellaria*) can easily be distinguished by its very long leaf stalks, more widely spaced nerves and abundant white latex when slashed [10]. The shea fruit consists of a thin, tart, nutritious pulp that surrounds a relatively large, oil-rich seed from which shea butter is extracted. The fruits are eaten for its nutritive and medicinal value in various part of Africa. Shea butter obtained from the seed of shea tree has been marketed as a skin and hair moisturizer and as a treatment for a variety of skin conditions including acne, burns, chapped lips, dry skin, eczema, psoriasis, scars, stretch marks, and wrinkles. It has also been used as a cream to relieve arthritis and rheumatism and to heal bruises and muscle soreness. Based on human study, shea butter may be effective for relief of nasal congestion, lowering cholesterol levels, and for blood thinning [11].

Vitex doniana (Verbernaceae) called "oori-nla" in Yoruba, is widely spread in the Southwestern Nigeria as a perennial tree. The fruits are black, edible, sweet and mealy. It is frequently eaten as a snack and sold in local markets In Nigeria. A decoction of the chopped stem bark part of *V.doniana* is taken orally for treatment of gastroenteritis. It is administered for ailments including diarrhea and dysentery. It is also taken to improve fertility generally [12] and the juice may be squeezed into the eyes to treat eye troubles. It is also used in the treatment of liver disease and jaundice [13].

In recent times, various researches are focused on sourcing for antioxidants from natural origin which will be more effective, safer and cheaper than synthetic antioxidants. In this study it was aimed to investigate the phytochemical and *in vitro* antioxidant properties of the methanolic extracts of fruits of *Blighia sapida, Vitellaria paradoxa* and *Vitex doniana*.

MATERIALS AND METHODS

Plant collection

Fresh fruits of *Blighia sapida, Vitellaria paradoxa and Vitex doniana* were collected from Garatu and Kaffinkoro Villages, Niger state, Nigeria between March and June. They were identified at the Department of Biological Science of Federal University of Technology, Minna, Niger State, Nigeria.

Sample preparation

Fresh fruits of *B.sapida, V.doniana and V.paradoxa* were removed from the pod and separated from the seed. They were blended into a paste and then 50 g of the sample was weighed into a round bottom flask to which 400 ml of methanol was added. A reflux condenser was then used to extract crude compounds at 64ºC for 2 h after which it was filtered .The filtrate was evaporated using a rotary evaporator and concentrated further using water bath. The extract was collected and stored in the freezer until required for use.

Qualitative phytochemical screening

The extracts were screened for phytochemical properties using standard methods [14].

Quantitative determination of phytochemicals

-Total phenols were quantified according to the methods described by Edeoga *et al* [15]. Briefly, 1 g of each extract was defatted with 100 ml of methanol using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components for 15 min. Then 5 ml of the extract was pipetted into a 50-ml flask; 10 ml of distilled water, 2 ml ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added. The sample was then made up to mark and left to react for 30 min for color development. This mixture was then measured at 765 nm using a spectrophotometer (754 UV-Vis, All Pro Corporation, Qingdao, PR China). The total phenol content was expressed in terms of gallic acid equivalent (mg/g).

-Total flavonoids were determined using aluminum chloride colorimetric method [16]. Each plant extracts (0.5 ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M Sodium acetate and 2.8 ml of distilled water. The mixture was allowed to stand at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer (Waltham, MA, USA) UV-visible spectrophotometer. The calibration curve was prepared by using quercetin solutions at concentrations of 12.5 to 100 µg/ml in methanol.

-Saponins; exactly 0.5 g of the extract was added to 20 ml of 1NHCl and was boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. Five milliliter of acetone-ethanol (1:1) was added to the residue and 0.4 ml of each was taken into 3 different test tubes. Ferrous sulphate reagent (6 ml) was added to each followed by 2 ml of concentrated sulphuric acid (H_2SO_4). It was thoroughly mixed after 10 min and the absorbance was taken at 490 nm [17]. Saponin content was expressed in terms of saponin standard equivalent.

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-Tannins; the sample(0.2 g) was measured into a 50 ml beaker in which 20 ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80ºC for 1 h. It was shaken thoroughly to ensure a uniform mixing .The extract was quantitatively filtered using a double layered Whatman No.41 filter paper into a 100-ml flask; then 20 ml of water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water, mixed well and allowed to stand for 20 min. A bluish-green color was developed. The absorbances of tannin acid standard solution as well as sample were read after color development using a spectrophotometer at wave length of 760 nm [18].

***In vitro* antioxidant determination**

-DPPH radical scavenging activity was determined according to the method described by Mensor *et al* [19]. Sample stock solution (1 mg/ml) was diluted to final concentrations of 250, 125, 50, 10,5 µg/ml in methanol. One milliliter of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbances (Abs) of the resulting mixture was measured at 518 nm and converted to percentage antioxidants activity (AA %) using the following formula:

$$\mathbf{AA\% = 100 - [(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}}$$

Methanol (1 ml) plus extract solution (2.5 ml) was used as a negative control. Solution of gallic acid served as positive control.

-Inhibition of lipid peroxidation was determined by a modified thiobarbituric acid reactive substances (TBARS) assay used to measure the lipid peroxide formed using egg yolk homogenate as lipid rich media [20]. Egg yolk homogenate (0.5 ml, 10% v/v) and 0.1 ml of each extract was added to a test tube and made up to 1 ml with distilled water. Exactly 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 20% TCA was added and the resulting mixtures were vortexed and then heated at 95°C for 60 min. After cooling, 5 ml of butan-1-ol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic layer was measured at 532 nm. Percentage inhibition of lipid peroxide formed by the extracts was calculated as follows:

$$\mathbf{(1 - E / C) \times 100}$$

-C; the absorbance value of the fully oxidized control

-E; absorbance in the presence of extract

-Reductive potential was determined according to the method of Oyaizu [21]. Different concentration of the methanolic extract of fruits (20, 40, 60, 80, 100 µg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. Tricholoroacetic acid (2.5 ml, 10%) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.5 ml, 0.1%,) and the absorbance was measured at 700 nm in a spectrophometer. Ascorbic acid was used as standard.

Stastical analysis

All values were expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) and individual comparisons of the group mean values was done using Duncan test.

RESULTS

Qualitative phytochemicals

The results of phytochemical screening of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana* is shown in Table 1. *Vitex doniana* fruit extract was shown to contain tannin in high concentration, saponins, cardiac glycosides, steroids, anthraquinone, terpenes, phenols and anthranoids in moderate concentration and flavonoids in low concentration. Cardiac glycosides, steroids and terpenes were shown to be highly present while phenol, flavonoids, anthranoids, and anthraquinones were in moderate concentration and alkaloid, saponins and tannin were in very low amount in *Blighia sapida* fruit extract. *Vitellaria paradoxa* fruit extract was observed to contain saponins in very high amount while tannins, flavonoids, cardiac glycosides, steroids, terpenes and phenols were moderately present.

Quantitative phytochemicals

The result of the quantitative phytochemicals of the methanolic extract of fruits of *Blighia sapida*,*Vitellaria paradoxa* and *Vitex doniana* is shown in Table 2. The fruits extract showed that tannin contents of the extract ranges between 1.04 ± 0.05 and 1.44 ± 0.05 mg/g, saponins between 2.3 ± 0.05 and 7 ± 0.04 mg/g, total phenols between 55.6 ± 0.05 and 96.4 ± 0.01 mg/g, and total flavonoid contents between 20.8 ± 0.05 and 72.8 ± 0.05mg/g. Methanolic extract of *Vitex doniana* and *Blighia sapida* showed very high total phenol content while *Blighia sapida* had the highest total flavonoid content. Phenol was observed to be the most abundant phytochemical for *Blighia sapida* and *Vitex doniana* while flavonoid was shown to be in highest concentration in *Vitellaria paradoxa.*

***In vitro* antioxidants**

-DPPH radical scavenging activity: Fig.1 shows that all the fruits extract scavenge DPPH radical in a dose dependent manner with *Blighia sapida* fruit having the highest DPPH scavenging activity at all concentrations when compared to other fruits.

Table 1: Phytochemical constituents of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*

Phytochemicals	*Vitex doniana*	*Blighia sapida*	*Vitellaria paradoxa*
Alkaloid	- - -	+ - -	- - -
Saponin	+ + -	+ - -	+ + +
Tannin	+ + +	+ - -	+ + -
Flavonoid	+ - -	+ + -	+ + -
Cardiac glycoside	+ + -	+ + +	+ + -
Steroid	+ + -	+ + +	+ + -
Anthraquinone	+ + -	+ + -	- - -
Terpene	+ + -	+ + +	+ + -
Phenol	+ + -	+ + -	+ + -
Anthranoid	+ + -	+ + -	- - -
Phlobatannin	- - -	+ - -	- - -

KEY: **- - -** absent, **+ - -** faintly present, **+ + -** moderately present, **+ + +** highly present

Table 2. Quantitative phytochemicals of methanolic extracts of the methanolic extract of fruits *Blighia sapida*,*Vitellaria paradoxa* and *Vitex doniana* (mean ± SD)

Phytochemicals	*Blighia sapida* (mg/g)	*Vitellaria paradoxa* (mg/g)	*Vitex doniana* (mg/g)
Tanin	1.04 ± 0.07	1.2 ± 0.01	1.44 ± 0.05
Saponin	2.36 ± 0.01	7 ± 0.05	6.48 ± 0.1
Phenol	91.8 ± 0.03	55.6 ± 0.03	96.4 ± 0.01
Flavonoid	72.8 ± 0.05	64.8 ± 0.01	20.8 ± 0.05

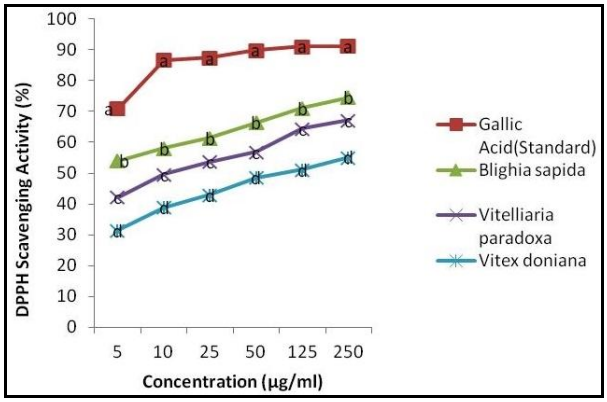

Figure 1. DPPH Scavenging activity of methanolic extracts of fruits of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*. Values with same letters are not significantly different at P ≥ 0.05, different letters are significantly different at P < 0.05.

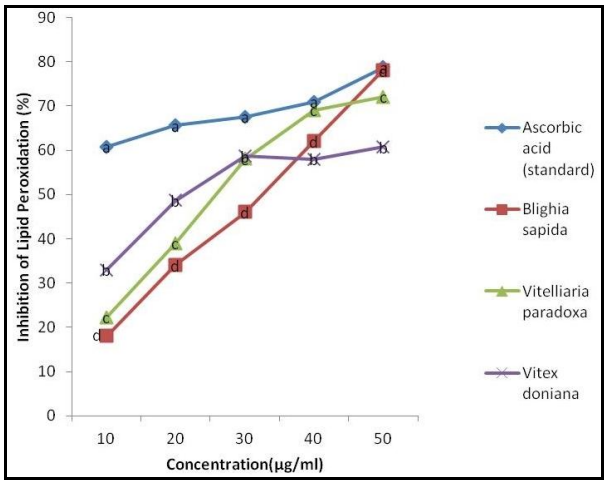

Figure 2. Inhibition of lipid peroxidation of methanolic extract of fruits of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*. Values with same letters are not significantly different at P ≥ 0.05, different letters are significantly different at P < 0.05.

-Inhibition of lipid peroxidation: Fig.2 show that all the fruits extract inhibited lipid peroxidation in a dose pendent manner; however, *Vitellaria paradoxa* and *Blghia sapida* fruit extract showed better inhibition at higher concentrations (40-50 µg).

-Reductive potential: Fig.3 show that *Vitellaria paradoxa* and *Blighia sapida* fruits extract exhbited better reductive potential at high concentration.

DISCUSSION

The result obtained from the qualitative phytochemical screening of methanolic extracts of fruits of *Blighia sapida, Vitellaria paradoxa and Vitex doniana* showed the presence of tannins, saponins, cardiac glycosides, alkaloids, steroids, anthraquinones, terpenes, phenols, and flavonoids (Table 1). Phlobatanin was found to be slightly present only in the methanolic extract of fruits of *Blighia sapida* with complete absence in *Vitex doniana and Vitellaria paradoxa*. This implies that these fruits can be used for several therapeutic purposes. The results of these phytochemicals, especially those of flavonoids and phenolic content, was shown to be higher than that found in some previously studied vegetables and plants [22].

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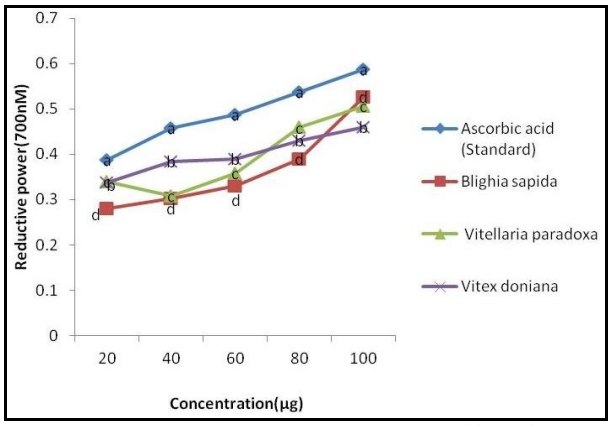

Figure 3. Reductive potential of methanolic extract of fruit of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*. Values with same letters are not significantly different at $P \geq 0.05$, different letters are significantly different at $P < 0.05$.

The medicinal and health promoting properties of these fruits, vegetables may be related to their phytochemical constituents. For instance, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have been shown to have remarkable activity in cancer prevention and treatment [23]. Saponins are known to produce inhibitory effect on inflammation and have hypocholesteremic and anti-diabetic effects [13]. Cardiac glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia [24].

The presence of tannins, saponins, cardiac glycosides, alkaloids, steroids, anthraquinones, terpenes, phenols, and flavonoids obtained in this study showed that these fruits can be harnessed for both nutritional and medicinal purposes. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with sex hormones [25].

Anthraquinones have been reported to possess astringent, purgative, anti-inflammatory, moderate antitumor and bactericidal effects [26]. Alkaloids, the most revered of all the phytochemicals, are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria [13]. Terpenes or terpenoids have anti-hepatoxic properties, thus, helping to prevent liver damage (cirrhosis). In addition, they equally have anti-microbial or anti-septic properties [25].

Phenolic compounds have also been reported to serve as antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and anti-diabetes [27]. It is reported in literature that flavonoids show considerable antioxidant action on human health and fitness and act either through scavenging or chelating processes [28].

Flavonoid rich food has been suggested to limit neurodegenerative diseases [29] and prevent normal or abnormal deteriation in cognitive performance [30]. Thus the methanolic extract of fruits of *Blighia sapida*, *Vitellaria paradoxa and Vitex doniana* may be a good alternative for the treatment of neurodegenerative diseases. The high presence of flavonoids and phenols in the methanolic extract of fruits of *Blighia sapida*, *Vitellaria paradoxa and Vitex doniana* makes them an important source of antioxidants and can be harnessed as food supplement. Also these fruit extracts may serve as a potential source of bioactive compounds in the prevention and treatment of various ailments arising from oxidative stress.

The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for deep purple color. When DPPH accept an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. DPPH scavenging activities of the methanolic extract of fruits of *Blighia sapida*, *Vitellaria paradoxa and Vitex doniana* (Fig.1) showed that all the fruits extracts investigated revealed that all the fruits are DPPH radical scavengers in a dose-dependent manner. However *B*lighia *sapida* extract had a significantly higher percentage of DPPH scavenging activity when compared with *Vitellaria paradoxa* and *Vitex doniana*. The DPPH scavenging activity of these fruits extracts is higher than the DPPH scavenging activity of most commonly consumed vegetables investigated [31]. This significant scavenging ability in the above fruits extract could be attributed to their high content of phenols and flavonoids. Flavonoids and phenolic acids are the most important groups of secondary metabolites and bioactive compounds in plants [2]. Flavonoids play significant role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA [33].

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage [33]. The initiation of peroxidation sequence in membrane or polyunsaturated fatty acids is due to the abstraction of a hydrogen atom from the double bond in the fatty acids [34]. Malondialdehyde (MDA) is the major product of lipid peroxidation and is used to study the lipid peroxidation process. Incubation of egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation. The ability of the methanolic extract of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana* to inhibit the process of lipid peroxidation is shown in Fig.2. Although all the fruit extracts inhibited lipid peroxidation significantly in a dose dependent manner, *Blighia sapida* extract had higher percentage of inhibition compared to *Vitellaria*

paradoxa and *Vitex doniana* at the concentration of 50 µg/ml. At this concentration the inhibition of lipid peroxidation is almost the same with that of the standard ascorbic acid (78.07%).

Reducing power is a novel antioxidant defense mechanism in which electron transfer and hydrogen abstraction are the two mechanisms available to affect this property [35]. The reductive powers of the methanolic extracts of fruits of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana* were assessed based on their ability to reduce Fe(III) to Fe(II) and the results are presented in Fig.3 as ascorbic acid equivalent. The results revealed that the methanolic extracts of fruits of *Blighia sapida* had the highest reducing power than methanolic extract of *Vitellaria paradoxa* and *Vitex doniana* at a concentration of 100 µg/ml. Since the reducing power of compound is related to its electron transfer ability and hence may serve as an indicator of its potential activity, the antioxidant activity of this extract might partially be due to its reducing ability.

From our result fruits with higher flavonoids and phenolic content had better *in vitro* antioxidant properties. This supports earlier reports, correlating the presence of flavonoids and phenolic compounds to antioxidative actions [35]. Therefore the *in vitro* antioxidant properties exhibited by these fruits may be due to the presence of these antioxidant phytochemicals present in them. The present study established the *in vitro* antioxidant potential of crude methanolic extracts of the fruits of *Blighia sapida, Vitellaria paradoxa* and *Vitex doniana*. Further studies are required to clarify the *in vivo* potential of these fruits in the management of human diseases resulting from oxidative stress and this will be the focus of our group's future investigations.

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