

## Nutritional evaluation and antioxidant activity of a lesser - known wild edible fruit *Tristemma hirtum* P. Beauv.

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### Abstract

*Tristemma hirtum* is a lesser known wild edible fruit found in Nigeria. This work aimed at investigating the nutrient composition, antioxidant and acute toxicity of *T. hirtum* fruit extract using standard methods. The fruit extract was high in carbohydrate and protein. Na and Mg were the most abundant macronutrient while Zn, Cu and Mn could supply approximately 41% of the RDA. Also, the extract was richer in vitamin A than vitamin C. Phytochemical investigation revealed an abundance of terpenoids, steroids, phenols, flavonoids, tannins, with antinutrients far below the lethal dose. The antioxidant assays revealed that, the extract exhibited strong DPPH radical scavenging and Fe<sup>2+</sup> chelating activities in *in-vitro*; as well as a dose-dependent decrease in malondialdehyde (MDA) and an increase in superoxide dismutase (SOD) serum levels in *in-vivo*. LD<sub>50</sub> (i.p mice) of the extract showed it was not toxic even at 5000mg/kg. Increased production and consumption of *T. hirtum* fruit may significantly promote good health due to its rich content of beneficial nutrients and phytochemicals as well as contribute to the prevention of diseases associated with oxidative stress.

**Keywords:** *T. hirtum*, wild edible fruit, nutrients, phytocompounds, antioxidant activity, toxicity.

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### 1. Introduction

In developing nations like Nigeria, wild edible plants (WEPs) are exploited as sources of food despite primary reliance of most societies on staple crop plants. Although WEPs represent a minor contribution to family meals, they are potentially important nutrient and cultural resources for local people around the world. (Ermias *et al.* 2011). They often contain higher amount of nutrients, especially those not cultivated for generations (Martins *et al.* 2011). Also, they have great potential as high-value nutraceuticals and sources of bioactive compounds for dietary supplements or functional foods. Therefore, WEPs are important to investigate not only for their nutritive value, but also as potential sources of therapeutic agents that may be active against a wide range of human diseases (Oliveira *et al.*, 2009; Garcia *et al.* 2004). One of such WEP is *Tristemma hirtum*.

*T. hirtum* is a member of the family Melastomataceae; one of the largest families of flowering plants, with less than 200 genera and 4500 species. Members of this family are easily recognized through their leaves featuring pairs of primary lateral veins that run in parallel, converging at the base and leaf apex. They vary from a few metres tall, to woody creepers, to shrubs and are abundant in in tropical and sub-tropical regions of the world. Many species of this family have been reported to exhibit antimicrobial, cytotoxic, antioxidant, hemostatic, anti-inflammatory, antihypertensive, etc. activities in traditional medicine (Kenfack *et al.* 2018; Ukwubile, 2017; Zabidi *et al.* 2012; Balamurugan *et al.* 2013). Phytocompounds such as flavonoids, polyphenols, steroids, alkaloids, fatty acids, etc. have been reported for various species of this family (Yoshida *et al.* 1994; Isaza *et al.* 2001; Calderon *et al.* 2002).

*T. hirtum* (local names: Apiko in Yoruba, Orunchi in Igbo, Eyop inuen in Efik/Ibibio) is an annual plant that grows up to 1.25m in height, with lilac flowers that develop to baby pink small round fruits. In Nigeria and other parts of West Africa, the leaves are used traditionally as a remedy for menstrual pains, skin diseases, headache and pulmonary blood purification. Moreso, a decoction of the leaf extract, is used as antimicrobial, anti-dermatophytic and analgesic (Par Rosine, 2014; Nguenang *et al.* 2018). Also, flavonol glycosides, quercetin, and terpenoid acids have been isolated from the methanol extract of its aerial parts (Kenfack, 2018). The fruit is sweet and is consumed by locals or may be used for animal feed (Burkill, 1985) Work done on this rare species is scanty. However, to the

best of our knowledge, there are no scientific studies from the fruit of this plant. Therefore, we report in this investigation, the mineral content, nutrient and anti-nutrient levels; and antioxidant potentials of the methanol fruit extract of *T. hirtum*.

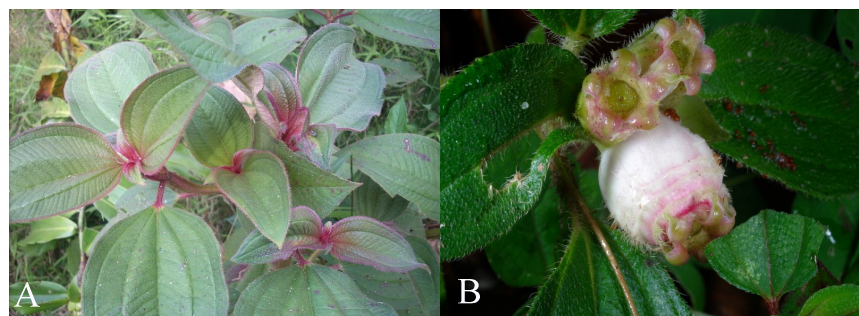


Fig1. *T. hirtum* plant (A) and fruit (B).

## 2. Materials and Methods

### 2.1 Sample collection and preparation

Fresh and mature fruits of *T. hirtum* were collected from marshy forest in Abak Local Government Area, Akwa Ibom State, Nigeria in November, 2016. The plant was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, where a voucher specimen was deposited. The fruits were washed, cut open and shade-dried for four days. The dried sample was pulverized to uniform mixture using blender and stored in an airtight container.

### 2.2 Sample extraction

The dried and pulverized fruit of *T. hirtum* (673.84g) was subjected to cold extraction using absolute methanol for 48hours, then filtered with Whatman No.1 filter paper. The filtrate was concentrated to dryness in a hot-air oven at 40 °C to obtain the methanolic extract (Thf). The extract was weighed, placed in a well-labelled bottle and stored in a refrigerator at 4 °C for future use.

### 2.3 Determination of percentage yield of extract

The yield of the fruit extract (dry weight) was calculated using:

$$\text{Percentage yield (\%)} = \{(W_1 \times 100)/W_2\}$$

where  $W_1$  is the mass of the extract after extraction and  $W_2$  is the mass of the dried fruit sample.

### 2.4 Qualitative phytochemical screening

Qualitative phytochemical screening was carried out using standard methods (Harbourne, 1973; Sofowora, 1984; Trease and Evans, 2002).

### 2.5 Proximate composition analysis

The methods of AOAC (2006), were used to determine the proximate composition of *T. hirtum* fruit extract.

### 2.6 Determination of vitamin content

Vitamin A and C were determined using the AOAC method (1990).

### 2.7 Mineral content evaluation

Mineral contents in *T. hirtum* fruit extract was analysed using an atomic absorption spectrophotometer (AAS Unicam 919) according to the method of Nanda *et al.* (2003) with some modification. Briefly, 1g of sample was ashed in a furnace at 500°C – 700°C for 2 hours, cooled, and dissolved in 1% HCl (5mL) in a 50mL beaker. An aliquot of this solution was used for the determination of iron, zinc, sodium, calcium, manganese, magnesium, potassium and copper.

### 2.8 Anti-nutrients analysis

Quantitative determination of anti-nutrients in *T. hirtum* fruit extract was carried out. Phytic acid was evaluated by the colorimetric method of Harbourne (1973). Total tannin was determined according to the method of Van-

Burden and Robbinson (1981). The method of Eling *et al.* (2012) was used to determine total cyanide. Total saponin was evaluated according to the method of Obdoni and Ochuko (2001).

### 2.9 Evaluation of in-vitro antioxidant activity

For *in-vitro* antioxidant activity, the DPPH radical scavenging activity, iron chelating activity and contents of total phenolics and flavonoids were assayed. The DPPH radical scavenging activity of the fruit extract was determined using the method of Blois (1985) at 517nm. BHA and ascorbic acid were used as the positive controls. Percentage scavenging activity (or inhibition) was calculated using the following equation:

$$\% \text{ DPPH scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Concentration of the sample giving 50% inhibition of DPPH (IC<sub>50</sub>) was obtained from the graph of percentage inhibition versus concentration of the sample (Guangrong *et al.* 2008). Iron chelating activity was evaluated based on the method of Dinis *et al.* (1994), and the ferrous ion monitored by measuring the formation of the red ferrozine-Fe<sup>2+</sup> complex at 562nm. The iron chelating activity was calculated using the equation:

$$\% \text{ Iron chelating activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100. \text{ EDTA was used as a positive control.}$$

Total phenolics was determined with the Folin-Ciocalteu reagent using the method of Meda *et al.* (2005) and expressed as milligrams gallic acid equivalent per gram of extract (mgGAE/g). Flavonoid content was determined using the method of Meda *et al.* (2005) and expressed as mg quercetin equivalent per gram of extract (mgQE/g).

### 2.10 Animals for acute toxicity test

Albino mice were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The mice were kept in the Animal House in well ventilated aluminium cages at room temperature. The animals were provided with commercial rodent pellet and water *ad libitum* for one week before the commencement of the experiment. All experiments were performed according to the guide for the care and use of laboratory animals (DHHS, 1985).

### 2.11 Determination of acute toxicity (LD<sub>50</sub>) of extract

The acute toxicity (LD<sub>50</sub>) of *T. hirtum* fruit extract was performed intraperitoneally following the method of Lorke (1983). This involved the administration of different doses of the extract to groups of three mice each. Behavioural changes and signs of toxicity were observed up to 24 hours. The extract was found to be non-toxic even at doses of 5000 mg/kg body weight, i.e, no mortality was recorded. Hence the LD<sub>50</sub> was estimated at 5000 mg/kg.

### 2.12 Experimental animals for in-vivo antioxidant test

Twenty male albino wistar rats were randomly divided into four groups of five animals each: Group 1 served as the control and received 10 ml of distilled water daily. Group 2 received 500 mg/kg of the *T. hirtum* fruit extract daily. Group 3 received 1000 mg/kg of the *T. hirtum* fruit extract daily and group 4 received 1500 mg/kg of the *T. hirtum* fruit extract daily. The animals were dosed daily for 14 days and observed for changes and other signs of toxicity and death throughout the period of study. Twenty-four hours after the last treatment, blood obtained through direct cardiac puncture was used to assay for *in-vivo* antioxidant activity.

### 2.13 Serum preparation

Blood used for serum preparation was collected via direct heart puncture with a needle attached to a 5 mL syringe, following mild chloroform anaesthesia of the rats. The serum was prepared by allowing the blood to clot for 1hour and then centrifuged at 4000rpm for 15 minutes to harvest the serum (Meite *et al.* 2014).

### 2.14 Determination of in- vivo antioxidant assay

Lipid peroxidation and superoxide dismutase assays were used for *in-vivo* antioxidant assay of the fruit extract. For lipid peroxidation assay, levels of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production were measured in serum according to Draper and Hadley (1990). Briefly, the serum (50 µL) was deproteinized by adding 1 mL of 14% trichloroacetic acid and 1 mL 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 minutes and then cooled on ice for 5 minutes. After centrifugation at 2000 rpm for 10 minutes, the absorbance of the coloured product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the following equation.

$$A = \Sigma CL,$$

where A = absorbance,  $\Sigma$  = molar coefficient of malondialdehyde ( $1.56 \times 10^4$  mol/L/cm), C = concentration and L = path length. The result was expressed in mol/mg of protein.

Superoxide dismutase activity was assayed according to the method of Sun *et al.* (1998). To the reaction mixture containing 0.1 ml phenazine methosulfate and 1.2 ml sodium pyrophosphate buffer, 0.3 ml of the supernatant after centrifugation of homogenate was added. Enzyme reaction was initiated by adding 0.2 ml NADH (reduced nicotinamide adenine dinucleotide) and stopped after 1 minute by adding 1 ml glacial acetic acid. The concentration of chromogens formed was measured by recording colour intensity at 560 nm. Results were expressed in unit/ml.

### 2.15 Statistical Analysis

Determinations were carried out in triplicate. SPSS Statistics 21 was used for all statistical analysis.

## 3.0 Results and Discussion.

Proximate composition of *T. hirtum* fruit (Thf) extract is given in Table 1. The fruit is rich in energy, carbohydrate and protein. The high content of carbohydrate and protein in this fruit suggest that it can serve as a complementary source of energy and protein for humans, particularly the rural dwellers. Lower carbohydrate and protein contents have been reported for *H. gabonii*, *C. maxima* juice and indigenous fruits and vegetables from temperate region of Indian Himalayas (Bouba *et al.* 2012; Ani and Abel, 2018; Gani *et al.* 2018), while higher values have been reported for legumes (Gani *et al.* 2018).

**Table 1: Proximate composition of *T. hirtum* fruit extract**

Parameter	Thf extract*
Moisture (%)	11.82 ± 0.08
Ash Content	4.18 ± 0.02
Fibre	3.24 ± 0.03
Protein	10.79 ± 0.27
Crude Fat	2.44 ± 0.14
Carbohydrate	67.53 ± 0.14
Caloric value (kcal)	335.72 ± 3.83
Yield (%)	16.30 ± 0.00

\* mean of triplicate determinations

Moisture content of the fruit extract was 11.82%. Moisture content affects the stability and quality of food during storage. Our result is similar to *D. microcarpum* fruit (Florence *et al.* 2014), but higher than *S. zenkeri* and *G. gabonii* fruits (Bouba *et al.* 2012); however, since it is less than the 15% maximum moisture required as safe storage limit for food materials, the fruit can be stored for a reasonable period with minimal deterioration (Sena *et al.* 1998). Fats add to palatability of foods through absorption and retention of its flavours. Crude fat of *T. hirtum* fruit extract was low (2.44%) compared to beniseed, soyabean, cashewnut, etc (Njoku *et al.* 2010), but comparable to *D. microcarpum* (Florence *et al.* 2014). Excess fat consumption has been implicated in certain cardiovascular disorders such as atherosclerosis, cancer, and aging, whereas a diet providing 1–2% of its caloric energy as fat is said to be sufficient to humans (Aruah *et al.* 2011). Crude fibre (3.24%) was lower than fruits from the Indian Himalayas, but had a higher ash content, suggesting a higher content of mineral elements.

Minerals are required in trace amounts for the proper functioning of the body. Mineral content of *T. hirtum* fruit extract is given in Table 2. Amongst the macro nutrients, Na and Mg are the most abundant. These contents suggest that the fruit extract is a good source of these elements as it could supply 24.8% and 45.9% of the recommended dietary allowance of Na and Mg respectively. In comparison with other works, our content of macronutrients was higher than *Citrus maxima* juice and peel extract (Ani and Abel, 2018). Macro elements such as Na, Mg, Ca and K play important cellular processes such as enzyme activation, blood clotting, maintenance of body fluid, nerve impulse balance and other biochemical and physiological processes within the body (Schrauzer, 2000). Micro nutrients such as Zn, Cu, Mn and Fe were also present in *T. hirtum* fruit extract, though in lesser amounts. Lower values were reported for Indian Himalayas fruits, while higher content of Fe, Zn and Co have been reported for *Cucurbita pepo* L seed extracts (Bouba *et al.* 2012; Elinge *et al.* 2012). Generally, content of micronutrient in *T. hirtum* fruit extract indicate that the fruit can provide  $\approx$  41.7% of recommended dietary allowance of Zn, Cu and Mn, which are needed for optimal functioning of many biochemical and physiological processes in the body.

**Table 2. Levels of minerals and vitamins in *T. hirtum* fruit extract**

Parameter	Thf extract* (mg/100g)	Recommended dietary intake	
		RDA <sup>a</sup> (mg)	UL <sup>a</sup> (mg)
Sodium	373.41 ± 2.16	1500	2300
Magnesium	193.87 ± 1.03	420	350
Calcium	68.18 ± 0.62	1,300	2500
Potassium	26.49 ± 0.23	4,700	not established
Zinc	4.59 ± 0.01	11	40
Copper	2.10 ± 0.01	0.9	10
Manganese	1.32 ± 0.02	2.3	11
Iron	0.20 ± 0.01	18	45
Vitamin A	11.70 ± 0.52	0.9	60
Vitamin C	2.46 ± 0.01	90	2000

\* mean of triplicate determinations; RDA= recommended dietary intake for adults; UL= upper limits for adults;  
<sup>a</sup> = (WHO, 2016).

Vitamin A (11.70mg/100g) in the fruit extract was higher than vitamin C (2.46mg/100g) as presented in Table 2. Vitamins play different roles in promoting health. Vitamin A is implicated in promoting resistance to infections, aid eye, nail and hair health, has antioxidant properties as well as delay ageing, while vitamin C has antioxidant properties, promotes healthy teeth, gums, joints, bones and aid in blood purification (Ogundola *et al.* 2018). Vitamin C content of *T. hirtum* fruit is lower than *Detarium microcarpum* fruit (55.1mg/100g) and *Gynochthodes umbrellata* fruit (25mg/100g fw) (Florence *et al.* 2014; Sudhakaran and Nair, 2016), while vitamin A in the fruit extract is higher than *G. umbellata* (1.29mg/100g fw) but lower than *Solanum verbascifolium* (371.72mg/100g) (Sudhakaran and Nair, 2016; Sam *et al.* 2012). However, the high level of vitamin A indicates that *T. hirtum* fruit is an excellent natural source of this vitamin and can supply 100% of the recommended dietary allowance.

Qualitative phytochemical composition of the fruit extract indicated high abundance of tannins, saponins, steroids, phenols and terpenoids (Table 3).

**Table 3: Phytochemical composition of *T. hirtum* fruit extract**

Test	Thf extract
Alkaloids	+
Saponins	+++
Terpenoids	+++
Cardiac glycosides	++
Carotenoids	++
Phenols	+++
Carbohydrates	++
Steroids	+++
Flavonoids	+++
Tannins	+++
Cyanide	+

+ = trace; ++ = moderately present; +++ = abundantly present;

Similar results have been reported for fruits of *Canarium schweifurthii* and *Cucumis sativus* (Shaba *et al.* 2013; Agatemor *et al.* 2018). Phytochemicals exhibit beneficial biological activities to humans including free radical scavenging, hypocholesterolemic, anti-inflammatory, anti-microbial, antiviral, analgesic effects, immune system stimulation, etc (Agatemor *et al.* 2018). Our result suggests that *T. hirtum* fruit is a potent source of beneficial phytochemicals that can enhance health.

Content of antinutrients in the fruit extract was low (Table 4). Antinutrients may inhibit the intake, absorption and utilization of nutrients within the body (Ani and Abel, 2018).



**Table 4: Antinutrient composition of *T. hirtum* fruit extract**

Antinutrient composition		Limit
Antinutrient	Thf extract*	Lethal dose
Tannin	7.11 ± 0.02	16.00
Cyanide	0.69 ± 0.02	36 .00
Phytate	7.94 ± 0.01	22.10
Saponin	16.41 ± 1.76	48.50

\* expressed in mg/100g, except cyanide (expressed in mg/kg) and saponins (expressed in %).

Tannin (7.11mg/100g) was lower in our fruit extract than *C. maxima* fruit juice (36.99mg/100g), *S. birrea* fruit juice (2,744mg/100), apple fruit juice (8.50mg/100g), but higher than banana fruit juice (3.40mg/100g) (Ani and Abel, 2018; Hassan *et al.* 2010). The antinutritive properties of tannins may be attributed to their ability to impair digestion of nutrients, thereby preventing the body from utilizing bioavailable substances. In addition, they bind and shrink proteins and deactivate digestive enzymes (Salunkhe *et al.* 1990). However, the tannin content of *H. hirtum* fruit was lower than the recommended lethal dose. Cyanide content of the fruit was low, suggesting that cyanide toxicity following consumption of *T. hirtum* fruit is unlikely. Higher values have been reported for *Dialium guineense* (2.16mgHCN/kg), *Persia americana* (4.87mgHCN/kg), *Chrysophyllum albidum* (6.07mgHCN/kg) and *Primus malus* (3.39mgHCN/kg) (Uhegbu *et al.* 2011). Like cyanide, levels of phytate and saponins were also low in the fruit extract, and were below the lethal dose. Overall, the antinutrient levels obtained in this study is less likely to produce any adverse effect associated with the consumption of this fruit.

The bioavailability of important minerals may be predicted based on their antinutrient to nutrient molar ratio, as depicted in Table 3. Our result show that all the antinutrient to nutrient molar ratios

**Table 5: Antinutrient to nutrient molar ratio**

Molar ratio	Thf extract (mol/kg)	Critical value
[Phytate]/[Zinc]	0.170	15
[Calcium][Phytate]/[Zinc]	0.290	0.50
[Phytate]/[Iron]	3.400	1
[Phytate]/[Calcium]	0.007	0.24

were below the critical value except [phytate]/[iron], suggesting the availability of zinc and calcium. However, the bioavailability of iron may be affected by phytate content. This could be overcome by processing.

Plant phenolics are a large group of natural compounds common in the plant kingdom with remarkable biochemical properties, including antioxidant properties. Total phenolic content (Table 6) of *T. hirtum* fruit extract (6.21mgGAE/g) was higher than fruits such as *B. lycium* (1.05mgGAE/g), *R. brunonii* (0.97mgGAE/g) and banana (0.90mgGAE/Gg) but lower than pineapple methanol extract (51.1mgGAE/g) (Shan *et al.*, 2019; Hossain and Rahman, 2011).

Total flavonoid content (9.90mgQE/g) of *T. hirtum* fruit was higher than *C. maxima* juice (3.77mg/g), but lower than pineapple water extract (39.4mgQE/g) (Ani and Abel, 2018; Hossain and Rahman, 2011). These results suggest that *T. hirtum* fruit extract is a rich natural source of phenolic compounds.

**Table 6: In-vitro antioxidant activity of *T. hirtum* fruit extract**

Parameter	Thf extract (mg/mL) *	Controls	
		Vitamin C	EDTA
DPPH activity <sup>a</sup>	0.06	0.05	-
Iron chelating activity <sup>a</sup>	0.25	-	0.06
Total phenolics (mg GAE/g)	6.21	-	-
Total flavonoids (mg QE/g)	9.90	-	-

\* mean of triplicate determinations; <sup>a</sup> = IC<sub>50</sub> value (mg/mL) is the effective concentration where DPPH radical is scavenged by 50%, ferrous ion is chelated by 50%. IC<sub>50</sub> was obtained using the regression equation.

*In-vitro* antioxidant activity of *T. hirtum* fruit extract was evaluated by measuring the DPPH radical scavenging and iron chelating activity (Fig.1). The DPPH radical scavenging activity of the extract increased in a dose-dependent manner, and ranged from 76% at 0.1mg/mL to 85% at 0.5mg/mL, suggesting a strong antioxidant activity. Observed DPPH activity was similar to reports for *D. indica* (79.51%), *V. grandiflorum* (84.87%), lower

than *J. regia* (93.35%), *A. comosus* (89%), but higher than *O. ferruginea* (61.09%), *G. optiva* (34.87%) and indigenous fruits and vegetables from the Indian Himalayas (10.67% - 77.59%) (Shan *et al.* 2019; Hossain and Rahman, 2011 and Gani *et al.* 2018). IC<sub>50</sub> value (Table 4) was similar to the control (Vitamin C) but higher than *H. polyrhizus* (11.34mg/mL) and *H. undatus* (14.61mg/mL) fruits (Choo and Yong, 2011). This result suggests the ability of the extract to act as a free radical scavenger or hydrogen donor.

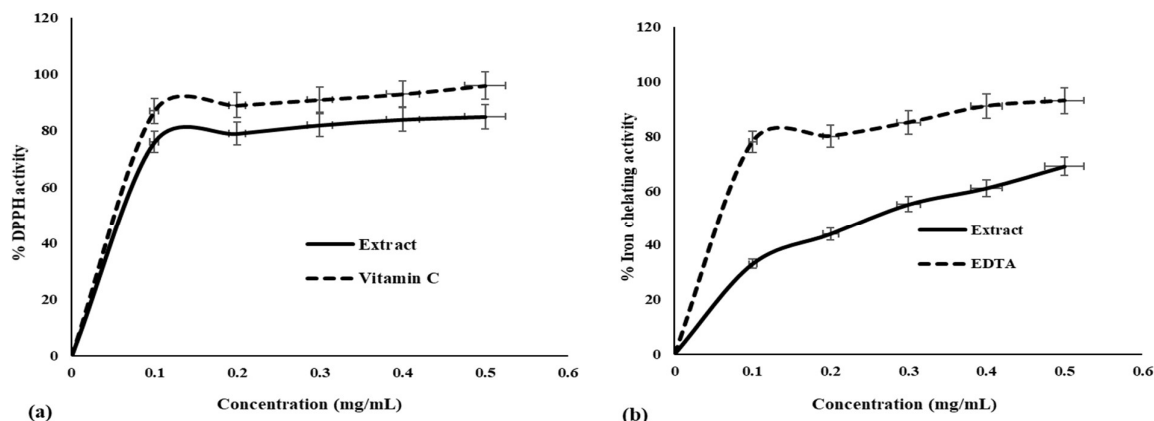


Fig.1: DPPH activity (a) and iron chelating activity (b) of *T. hirtum* fruit extract

Like the DPPH assay, the Fe<sup>2+</sup> chelating ability of the extract increased with increasing concentration of the extract. (Fig. 1). Result indicate that *T. hirtum* fruit extract exhibited potent Fe<sup>2+</sup> chelating ability (IC<sub>50</sub>= 0.25mg/mL), which was inferior to EDTA (Table 4), but higher than *H. undatus* and *H. polyrhizus* fruits (Choo and Yong, 2011). Generally, IC<sub>50</sub> values less than 10mg/mL are considered to be effective in antioxidant properties (Lee *et al.* 2007). The potent Fe<sup>2+</sup> chelating activity of the extract indicates its ability to prevent oxidative damage arising from lipid peroxidation processes such as Fenton type or similar decomposition reactions (Ita and Ndukwe, 2007).

In addition, *in-vivo* antioxidant activity of *T. hirtum* fruit extract was evaluated by measuring level of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) as depicted in Fig. 2.

Result indicate that the extract produced a dose dependent decrease in MDA levels in the serum. At 1500mg/kg, serum MDA level of the treated group was lower than the other groups and control. This result suggest that the extract is capable of increasing the activity of glutathione peroxidase and thereby inactivate reactions that could lead to lipid peroxidation (Ugochukwu *et al.* 2013).

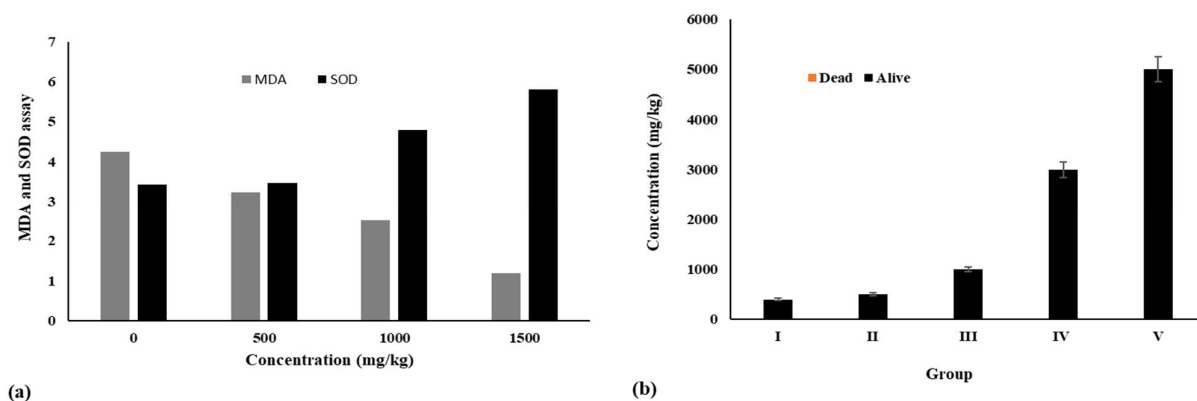


Fig.2: *In-vivo* antioxidant activity (a) and acute toxicity test (b) of *T. hirtum* fruit extract.

Similarly, the extract produced a dose dependent increase in serum levels of superoxide dismutase activity, and this was higher than the control. SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen, and therefore reduces the reaction of superoxide anion with nitric oxide to produce reactive peroxynitrate capable of exacerbating the effect of ROS in biological systems (Onoja *et al.* 2014). Generally, the observed antioxidant activity *in-vitro* and *in-vivo* of *T. hirtum* fruit extract may be attributed to the presence of phenolics, flavonoids, vitamin C, tannins and other phytochemicals in the extract. Polyphenolic compounds such as phenols, flavonoids, condensed tannins, etc have antioxidant properties due to their redox properties and chemical structures. They act as reducing agents, singlet and triplet oxygen quenchers, scavengers of other free radicals and as metal chelators. In addition, vitamin C is a powerful antioxidant present in fruits and vegetables (Gani, 2018). The presence of these phytochemicals indicate that consumption of *T. hirtum* fruit may help ameliorate diseases associated with oxidative stress.

Furthermore, the acute toxicity of the fruit extract was also evaluated (Fig.2). Result indicated that the fruit extract was not toxic even at 5000mg/kg. According to Lorke (1993), toxicity test in which the extract is not lethal up to 5000 mg/kg implies that the plant is not toxic at all, hence, *T. hirtum* fruit is safe for animal and human consumption.

#### 4.0 Conclusion

The present study indicated that *T. hirtum* fruit contained substantial amount of nutrients and phytochemicals while levels of antinutrients were below the lethal dosage. The fruit also showed promising antioxidant potentials in *in-vitro* and *in-vivo* assays. These properties make its consumption useful to humans and animals. However, further studies are necessary to isolate and identify the specific phenolic compounds responsible for the observed antioxidant activity.

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