

Proximate composition and evaluation of some antioxidant properties of the fresh fruits of *Harungana madagascariensis* Lam. Ex Poir. (Hypericaceae)

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ABSTRACT: This study reports the proximate composition and some antioxidant properties: free radical scavenging (FRS), total phenolics content (TPC) and total flavonoids content (TFC) of the under-utilized fresh fruits of *Harungana madagascariensis*, a plant widely used in folklore medicine. The proximate compositions were determined following the AOAC method. The antioxidant properties FRS, TPC and TFC were respectively evaluated *in vitro* using the standard diphenyl picryl hydrazine (DPPH), Folin ciocateau and Aluminium chloride spectrophotometric assays. The proximate compositions: water content (13.771 g/100g fresh fruit), dry matter (82.229 g/100g fresh fruit), ash (3.087 g/100g fresh fruit), crude fat (42.011 g/100g fresh fruit), crude protein (6.489 g/100g fresh fruit), crude fibre (0.396 g/100g fresh fruit), total carbohydrate (34.246 g/100g fresh fruit) and Energy value (541.036 Kcal/100 g fresh fruit) were obtained from the fruit of *H. madagascariensis*. For the antioxidant properties, a TPC (6.758 mg tannic acid equivalent per g fresh fruit), TFC (0.055 mg quercetin equivalent/g fresh fruit) and FRS ($IC_{50} = 0.05$ mg/mL) were obtained. This study is reporting for the first time the proximate composition of the fruits of *H. madagascariensis* a plant widely used in ethno-medicine in addition to its antioxidant potentials and recommends it as a good source of functional food.

Keywords: *Harungana madagascariensis*, under-utilized fruits, proximate composition, functional foods.

INTRODUCTION

Harungana madagascariensis is a monotypic medium to tall tree species commonly called dragon's blood tree. It is used in folkloric treatment of jaundice, diarrhea, dysentery, typhoid fever, constipation, liver problems, anaemia and malaria with several scientific reports validating the folkloric use of the leaves and stem bark of this plant in the treatment of anaemia (Ntie-Kang et al., 2013; Biapa et al., 2013), diabetes (Agnaniet et al., 2016), oxidative stress related diseases (Antia et al., 2015), bacterial infections (Moulari et al., 2006; Kengni et al., 2013), diarrhoea and gastro-intestinal disorders (Mba et al., 2017; Malan et al., 2015), and malaria (Biapa et al., 2013) documented. Report on the use of the fruits in traditional diet is also documented (Ruffo et al., 2002). Whereas the nutritional composition of the stem bark (Antia et al., 2015) as well as

the phytochemistry (Moulari et al., 2006) and biological activities (Moulari et al., 2006; Kengni et al., 2013; Mba et al., 2017; Malan et al., 2015) of the roots, leaves and stems bark of *Harungana madagascariensis* have been investigated extensively, similar report of same for the fruits are few except for our earlier reports on: its antibacterial (Afiero et al., 2012), trace elemental and anti-anaemic activity (Shorinwa et al., 2018), the phytochemistry and acute toxicity (Afiero et al., 2017), and nitric oxide scavenging activity (Afiero and Afiero, 2017) of the fruit's oil. As a follow-up to these earlier reports on the fruits (Afiero et al., 2012; Shorinwa et al., 2018; Afiero et al., 2017; Afiero and Afiero, 2017), this present study reports for the first time, the proximate composition of the fruits of *H. madagascariensis*, a plant

widely used in ethno-medicine. It also reports some of its antioxidant properties which could be exploited in the development of functional foods that could be used in the management of diseases with oxidative stress related complications thus justifying related reported medicinal utilities (Antia et al., 2015). Flavonoids and other classes of phenolic compounds are secondary plant metabolites known to have good antioxidant properties (Cook and Samman 1996; Kumar and Pandey, 2013; Leopoldini et al., 2006; Rice-Evans et al., 1995).

MATERIALS AND METHODS

The fresh fruits of *Harungana madagascariensis* were collected from the forest adjoining the University of Port Harcourt, Nigeria and authenticated at the Herbarium Unit of the Plant Science and Biotechnology Department of the same university with voucher number: UPH/P/080; UPH/V/1,219. Reagents and solvents used were of analytical grade and include: methanol, ethanol, folin ciocalteau reagent, 10% aluminium chloride, tannic acid, quercetin, ascorbic acid, sodium carbonate, sodium hydroxide and diphenyl picryl hydrazine (DPPH). Equipment used include: rotary evaporator and UV-visible spectrophotometer (Novel series N4).

Proximate analysis of *H. madagascariensis* fruits

Proximate analysis was done using standard procedures (James, 1995; AOAC, 2000). All analyses were done in triplicate using standard procedures and the mean values \pm standard deviation recorded. Briefly, the dry matter and water content determination was done using the loss on drying technique. Total ash content determination was done by incinerating the dried sample in a muffle furnace. Crude protein was determined using the Kjeldahl nitrogen determination method with 6.25 used as the conversion factor. Crude fat content was evaluated following the method of exhaustive soxhlet extraction with the non-polar organic solvent - petroleum ether (boiling point = 40 to 60°C). The acid and alkaline digestion method was used for crude fibre determination while total carbohydrate content was evaluated by difference with the content of fat (F), crude protein (Cp), crude fibre C_F and ash (A) subtracted from 100. Calorific or energy content was obtained by multiplying the content of crude protein (Cp), fat (F) and available carbohydrate (C) with their respective appropriate at water factor of 4, 9 and 4/Kcal/100g wt of dried sample and adding up to obtain the calorific value.

Determination of the total phenolic content

This was determined by the Folin-Ciocalteau method (Miliauskas et al., 2004) with modification and the total

phenolic content expressed as milligram tannic acid equivalent per gram of fresh fruit. Briefly, a 1 mg/ml tannic acid stock solution was prepared in methanol. 0.05, 0.10, 0.20, 0.40 and 0.60 mL of this stock solution were separately transferred into their respective 10 ml volumetric flask labeled R1 to R5. To the content of each of these volumetric flasks (R1 to R5) was separately added 800 μ L of Folin-Ciocalteau reagent followed by 2 mL of aqueous sodium carbonate (75 g/L) and the volume adjusted to 10 mL mark with distilled water. This was thereafter left to incubate in the dark for 2 hours for complete reaction. The absorbance of each of the reaction solutions in R1 to R5 was taken at 765 nm and the calibration curve obtained from a plot of absorbance against concentration using Microsoft Excel 2007 package and the regression equation noted. For the *H. madagascariensis* fresh fruits, 1.008 g of the fresh fruit was exhaustively extracted with methanol and the volume of the methanol filtrate adjusted to 50 ml in a 50 ml volumetric flask. A 0.5 ml aliquot of this resulting solution was then transferred to a 10 mL volumetric flask followed by the addition of 0.8 mL of Folin-Ciocalteau reagent, 2 mL of aqueous sodium carbonate (75 g/L) and the volume adjusted to 10 mL mark with distilled water. This was thereafter left to incubate in the dark for 2 hours for complete reaction. The absorbance of the reaction solutions was taken at 765 nm and the total phenolic content (TPC) calculated from the regression equation $y = 64.958x - 0.0191$ obtained from the standard tannic acid calibration curve. A separate solution containing 0.8 mL of Folin-Ciocalteau reagent and 2 mL of aqueous sodium carbonate (75 g/L) with the volume adjusted to 10 mL mark using distilled water was used as the blank solution. Determination were performed in triplicate. Total phenolic content (TPC) in the *H. madagascariensis* fresh fruits as tannic acid equivalents (TAE) was calculated using the formula:

$$\text{TPC} = (C \times \text{DF}) / w$$

Where TPC is the total phenolic content in mg TAE/g of the fresh fruit, C is the concentration (mg/mL) of tannic acid calculated from the regression equation, DF is the test sample dilution factor (1000), w is the weight (g) of the fresh *H. madagascariensis* fruits extracted.

Determination of the total flavonoid content

This was determined by the AlCl₃ colorimetric method (Kumaran and Karunakaran, 2007) with modification and the total flavonoid content (TFC) expressed as milligram quercetin equivalent per gram of fresh fruit. Briefly, a 0.5 mg/ml reference quercetin stock solution was prepared in methanol. 0.10, 0.20, 0.40, 0.60, 0.8 and 1.0 mL of this stock solution were separately transferred into their respective 10 ml volumetric flask labeled Q1 to Q6. To the

content of each of these volumetric flasks (Q1 to Q6) was separately added 0.5 mL of 10% AlCl_3 and the volume adjusted to 10 mL mark with methanol. This was thereafter left to incubate in the dark for 30 minutes for complete reaction. The absorbance of each of the reaction solutions in Q1 to Q6 was taken at 430 nm and the calibration curve obtained from a plot of absorbance against concentration using Microsoft Excel 2007 package and the regression equation noted. For the *H. madagascariensis* fresh fruits, 1.008 g of the fresh fruit was exhaustively extracted with methanol and the volume of the methanol filtrate adjusted to 50 ml in a 50 ml volumetric flask. A 0.5 ml aliquot of this resulting solution was then transferred to a 10 mL volumetric flask followed by the addition of 0.5 mL of 10% AlCl_3 and the volume adjusted to 10 mL mark with methanol. This was thereafter left to incubate in the dark for 30 minutes for complete reaction. The absorbance of the reaction solution was then taken at 430 nm and the total flavonoid content (TFC) calculated from the regression equation $y = 63.7x - 0.0495$ obtained from the standard quercetin calibration curve. Methanol was used as the blank solution. Determination were performed in duplicate. Total flavonoid content (TFC) in the *H. madagascariensis* fresh fruits as quercetin equivalents (QE) was calculated using the formula:

$$\text{TFC} = (\text{C} \times \text{DF})/w'$$

Where TFC is the total flavonoid content in mg QE/g of the fresh fruit, C is the concentration (mg/mL) of quercetin calculated from the regression equation, DF is the test sample dilution factor (1000) and w is the weight (g) of the fresh *H. madagascariensis* fruits extracted.

Free radical scavenging assay

This was done using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay (Molyneux, 2004), with suitable modifications. Briefly, dried ethanol extract of the fresh fruits of *H. madagascariensis* was obtained and separate 0.40, 0.32, 0.24, 0.16, 0.08, 0.04 and 0.02 mg/mL solution of this extract in methanol were prepared. 2 ml of each preparation was mixed with equal volume of 5% DPPH in methanol. After 30 minutes of incubation in the dark at room temperature, absorbance was measured at 517 nm. The absorbance of a control sample containing all the reagents except the extract was also taken. Methanol was used as the reagent blank. The percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where: A_c = absorbance of control and A_s = Absorbance of sample.

As a reference standard for comparison, ascorbic acid at

concentration 0.080-0.005 mg/ml was similarly evaluated. The half maximal inhibitory concentration (IC_{50}) was determined by regression curve of a plot of % DPPH inhibition against concentration.

RESULTS AND DISCUSSION

The results of the proximate composition are as stated in Table 1. This showed that the fruits of *Harungana madagascariensis* are a good source of vegetable oil, carbohydrate, protein and essential mineral element but low in dietary fibre. Although the high fats and oil content value of 42.01 g/100 g fresh fruit (48.7 g/100g dried matter) would have suggests risk to cardiovascular health, this concern should not be since earlier report on the physico-chemical analysis of the fruits oil (Afieroho et al., 2017) showed that it is high in poly unsaturated fatty acids like linoleic acid and its esters among others which are of health benefits. The *H. madagascariensis* fruits could be considered as a carbohydrate-rich fruit considering the high carbohydrate content (Table 1) compared to those reported in literature for some common oil-rich fruits like wild olive (Chopra and Kanwar, 1980). Carbohydrates are energy giving nutrient and this has translated to the high calorific value (Table 1) calculated for the fruits. The crude protein content 6.49 g/100 g (7.53 g/100 g dried matter) of the *H. madagascariensis* fresh fruits is relatively high compared to similar report for some low protein fruits and vegetables (Chopra and Kanwar, 1980), and comparable to that reported in literature for some edible cereals though low when compared to protein-rich vegetables (Gulfranz et al., 2009) and legumes (Hussain et al., 2009). Proteins are food nutrients required for growth and repair of body tissues. Compared to common edible fruits and vegetables, the obtained water content is low translating to a high dry matter content (86.23 g/100 g fresh fruit). Thus, the *H. madagascariensis* fruits are less prone to post-harvest spoilage due to microbial attack. High water content in foods is a pre-disposing factor for microbial growth and by extension post-harvest spoilage and loss. The ash content value 3.09 g/100 g fresh fruit (3.58 g/100 g dried matter) explained the earlier high content of essential mineral elements reported in an earlier study (Shorinwa et al., 2018). The low crude fibre content is an indication that the fruits are not good source of dietary fibre. Dietary fibres are known to have health-promoting properties as they are associated with reduced risk of obesity and cardiovascular ailments (Ajah and Madubuike, 1997).

From the results of the evaluation of the antioxidant properties (Table 2 and Figure 1), a TPC (6.758 mg tannic acid equivalent per g fresh fruit), TFC (0.055 mg quercetin equivalent/g fresh fruit) and FRS ($\text{IC}_{50} = 0.05$ mg/mL) were obtained. This quantitative phytochemical data (TPC and TFC) are in tandem with earlier reports on the qualitative phytochemical analysis of the fruits of *H. Madagascariensis* which showed that phenolics compounds are present in

Table 1. Proximate composition of *Harungana madagascariensis* fruits.

Proximate parameter	g/100 g matured fresh fruits	g/100 g dried fruits matter
Water	13.771±0.305	NA
Dry matter	86.229 ±0.305	NA
Total ash	3.087±0.104	3.580±0.131
Crude fat	42.011±0.421	48.719±0.350
Crude fibre	0.396±0.026	0.459±0.029
Crude protein	6.489±0.173	7.525±0.175
Carbohydrate content	34.246±0.304	39.717±0.446
Energy (Kcal/100g)	541.036±3.334*	627.439±1.772*

*Expressed in Kcal/100 g and not g/100 g).

Table 2. Some Antioxidant indices of *Harungana madagascariensis* fresh fruits.

Antioxidant indices	Potency
Total phenolic Content	6.758 mg TAE/g fresh fruit
Total flavonoid content	0.055 mg QE/g fresh fruit
Free Radical scavenging activity (IC ₅₀)	0.050 mg/mL*

*The reference ascorbic acid has IC₅₀ = 0.012 mg/mL.

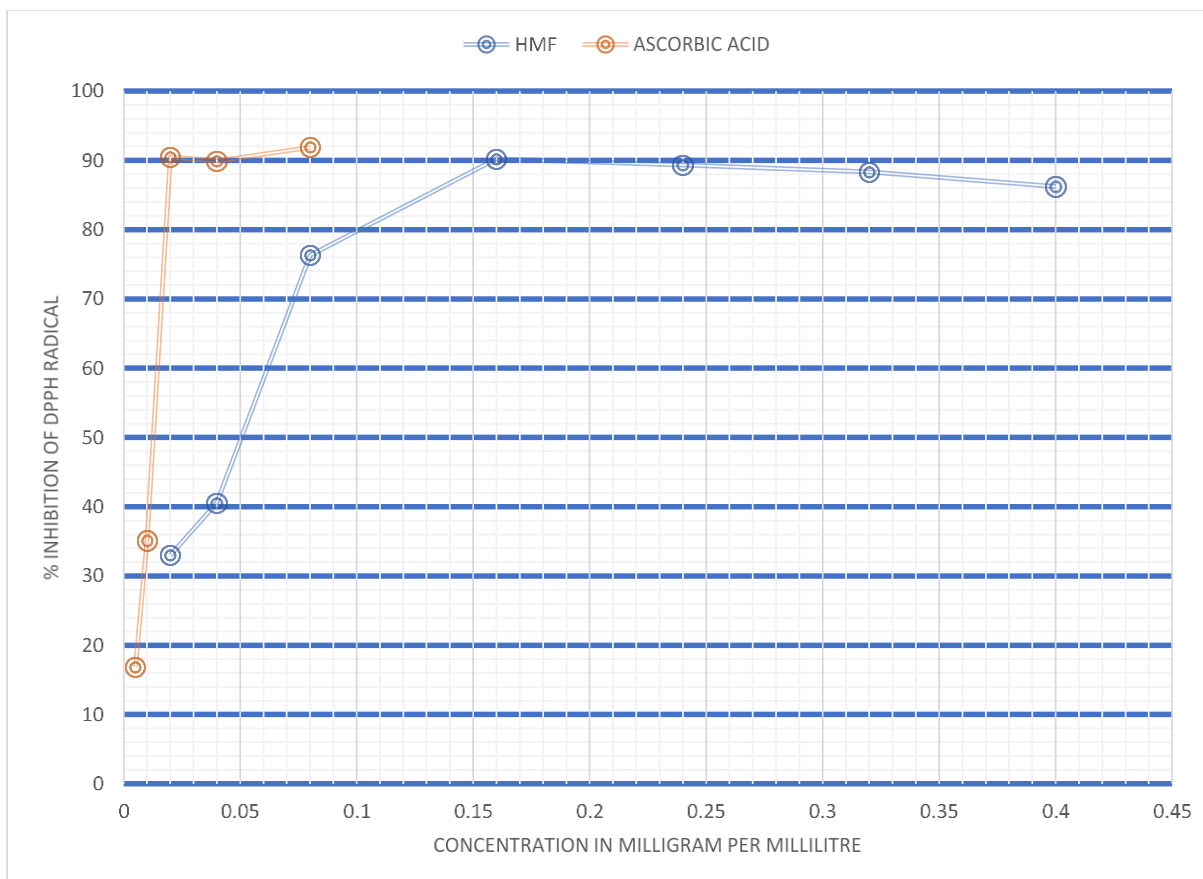


Figure 1. Concentration -response curve for *H. madagascariensis* fruits and the reference antioxidant agent ascorbic acid. Key: HMF = *Harungana madagascariensis* fresh fruits.

this fruit (Afiero et al., 2012; Shorinwa et al., 2018). The total phenolic content assay is a quantitative estimation of the crude content of phenolic compounds in a sample. It encompasses all classes of phenolic compound which include the simple phenolics acids, phenyl propanoids, coumarine, tannins and flavonoids among others unlike the total flavonoids content assay which is specific for only the flavonoids class of phenolic compounds. This underscores the lower TFC value obtained compared to the TPC for the *H. madagascariensis* fresh fruits. The result of the FRS assay showed that the *H. madagascariensis* fruits exhibiting a dose dependent radical scavenging activity at the concentration range of 0.020 to 0.160 mg/mL with a half maximal inhibitory concentration IC₅₀ of 0.05 mg/mL. The IC₅₀ is a measure of the effectiveness of a substance to inhibit a specific biological function. The lower the IC₅₀ value the more potent the inhibitory action of the test substance. Phytochemical compounds which includes flavonoids, are known to have good antioxidant properties (Cook and Samman 1996; Kumar and Pandey, 2013; Leopoldini et al., 2006; Rice-Evans et al., 1995). The presence flavonoids and related phytochemicals in the *H. madagascariensis* fresh fruits therefore offers a plausible rationale for the promising FRS activity though not as active compared to the reference antioxidant agent ascorbic acid (IC₅₀ = 0.012 mg/mL). Similar report on the nitric oxide scavenging activity of the fruits of *H. madagascariensis* have been documented (Afiero and Afiero, 2017). Oxidative stress is clinically associated with the pathophysiology of several diseases like anaemia (Ntie-Kang et al., 2013; Biapa et al., 2013), diabetes (Agnaniet et al., 2016), bacterial infections (Moulari et al., 2006; Kengni et al., 2013), diarrhoea and gastro-intestinal disorders (Mba et al., 2017; Malan et al., 2015), and malaria (Biapa et al., 2013) treated with *H. madagascariensis* in folklore. Uncontrolled generation of radicals in the physiological systems of human is the leading cause of oxidative stress and antioxidants are substances that help in the amelioration of oxidative stress by their quenching action on radicals through the donation of an electron to the radical (Halliwell and Gutteridge, 1984; Sies, 1985). Hence, they are vital in the maintenance of health, well-being, and in the prevention and management of oxidative stress.

CONCLUSION

This work reports for the first time the proximate composition of the fruits of *H. madagascariensis*, a plant widely used in ethno-medicine. It also reports in addition its antioxidant potentials. It revealed the fruits to be a good source of carbohydrates, vegetable oil, proteins and essential mineral elements. Having reported in previous studies the fatty acid composition of the vegetable oil (Afiero et al., 2017) and some individual essential mineral elements (Shorinwa et al., 2018) of the fruits of this

medicinal plant, it could be investigated further for the monosaccharides, amino acids and peptides, essential vitamins and polyphenolics composition to further established its nutraceutical potentials.

CONFLICT OF INTEREST

There is no conflict of interest as regards the publication of this manuscript.

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