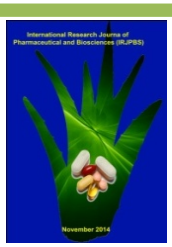
RESEARCH ARTICLE

International Research Journal of Pharmaceutical and Biosciences

Pri -ISSN: 2394 - 5826
http://www.irjpbs.com e-ISSN: 2394 - 5834



In-vitro Antioxidant potentials, Phytochemical and Proximate Analyses of *Borassus aethiopium* and *Vitex doniana* Fruits Extracts.

Ibrahim Sani*, Fatima Bello and Amina Sulaiman.

Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria.

Article info

Article history:
Received 19 JAN 2016
Accepted 31 JAN 2016

***Corresponding author:**
isani76@gmail.com

Abstract

This research work was conducted in order to reveal the medicinal and nutritional benefits of *Borassus aethiopium* and *Vitex doniana* fruits. *In vitro* antioxidant potentials as well as phytochemical and proximate analyses were carried out. Fresh fruits were used for the proximate analysis while their aqueous and ethanolic extracts were used for the phytochemical and antioxidant potential screening. All the analyses were conducted using standard methods. The results of the antioxidant potentials of the ethanolic and aqueous extracts of *Borassus aethiopium* were (35.20 ± 18.75%) and (39.81 ± 18.06%) respectively. While, those of *Vitex doniana* were (18.25 ± 8.18%) and (19.35 ± 11.61%) respectively. The phytochemical screening shows the presence of alkaloids, saponins, terpernoids, and flavonoids in both *Vitex doniana* and *Borassus aethiopium*. Tannin is however found only in *Vitex doniana.* The Proximate analyses revealed that the *Vitex doniana* and *Borassus aethiopium* fruits have moisture content of 30.00 ± 7.07% and 65.00 ± 3.54%, protein content of 03.24 ± 0.13% and 03.94 ± 0.13%, ash content of 03.33 ± 0.78% and 05.30 ± 0.82%, lipid content of 02.67 ± 1.11% and 01.19 ± 0.54%, fibre 02.10 ± 0.53% and 16.70 ± 3.83%, then, carbohydrate content of 58.56 ± 5.49% and 07.87 ± 0.04% respectively. *Borassus aethiopium* shows greater antioxidant potential in both aqueous and ethanolic extracts compared to that of *Vitex doniana*. The antioxidant, phytochemicals and proximate analyses of these fruits have shown that, both of them have medicinal and nutritional benefits.

Keywords: Proximate analysis; Phytochemicals; Antioxidant potential; *Borassus aethiopium*; *Vitex doniana.*

INTRODUCTION

Antioxidants are substances that may protect cells against effects of free radicals. Free radicals are molecules produced when body breaks down food or by environmental exposures like tobacco smoke and radiations [1]. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from many sources which oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant compounds like flavonoids scavenge free radicals such as peroxide thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Borassus aethiopium is a wild plant with edible fruit that belongs to the family Arecaceae. It is called *Giginya* in Hausa Language. The plant is distributed around West African regions in countries like Benin, Burkina Faso, Cameroon, Chad, Ethiopia, Kenya, Niger, Nigeria, and Senegal. They are found across sub-Saharan Africa [2].

Almost all the parts of *Borassus aethiopium* are used medicinally for the treatment of abdominal pain, respiratory and inflammatory diseases [3].

Vitex doniana is a deciduous tree which also contains fruits that are edible. The ripe black fruit pulp is eaten raw. It has a sweet taste, and known as *Dinya* in Hausa Language.

Vitex doniana fruit is used especially in the treatment of some disorders, such as rheumatism, hypertension, cancer, and inflammatory diseases. Dried and fresh fruits are eaten against diarrhoea, and as a remedy against lack of vitamin A and B [4].

MATERIALS AND METHODS

Sample collection and Identification

The fresh ripped fruits of *Vitex doniana* were collected from garden in Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. The fruits of *Borassus aethiopium* were purchased from a market within Aliero town, Kebbi State, Nigeria. The samples were taken to the Department of Biological Sciences in the same University for authentication, and then used for all the analyses.

Sample Preparation

The fruits were washed in a running tap water and stored in a refrigerator at 4^0C before the commencement of the work. Some were used for the determination of moisture content and the rest were air dried to a constant weight. The dried mesocarps (fleshy part) of the

fruits were powdered using an electric blender with steel blades and stored in screw capped containers at room temperature before use.

Preparation of Extracts

One hundred gram (100g) of each dried sample was weighed and soaked in 250 mL of ethanol and distilled water. The mixtures were then kept at room temperature for 24 h, and then filtered twice; initially with a muslin cloth and later with a Whatman filter paper No.1. The filtrates were evaporated to dryness at 45^0C using rotary evaporator. The dried residues were used for the antioxidant and phytochemical analyses.

Proximate Analysis

The methods described by AOAC [5] were used.

Moisture Content

A clean crucible was weighed as W_1. 5g of the sample was added into the crucible and weighed as W_2. It was then allowed to dry in hot air oven for 24 h at 100^0C and Cooled in a desiccator and then weighed as W_3. The dried sample was return into the oven for further 24 h to make sure drying is completed. The percentage moisture was calculated using Eq-1.

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \text{ X } 100 \quad \text{.....Eq-1}$$

Ash Content

The crucible was weighed as W_1. A 5g of the sample was added into the crucible and weighed as W_2, the crucible containing the sample was placed in muffle furnace at 500^0C for 6 h for ashing. Then cooled in a desiccator and weighed again as W_3. Percentage ash was calculated using Eq-2.

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \text{ X } 100 \quad \text{.....Eq-2}$$

Determination of Crude fiber

A 2g of the sample was weighed into 1 L conical flask as W_1, 200 mL of boiling 1.25% H_2SO_4 and 200 mL of distilled water were added gently and boiled for 30 minutes using cooling finger to maintain a constant volume. A muslin cloth was used to filter the mixture and distilled water was used in rinsing. Spatula was used to scrape the residue back into the flask, 200 mL of boiling 1.25% NaOH was added to boil for another 30 minutes using a cooling finger to maintained a constant temperature. A poplin cloth was used in filtering, the residue was rinsed thoroughly with hot distilled water, and rinsed once with 10% HCl and twice with ethanol. Petroleum ether was finally used to rinse three times. It was then allowed to dry overnight in an oven at 105^0C, and then cooled in a desiccator and weighed

as W_2. It was kept in the muffle furnace to ash at 550^0C for 90 minutes, then cooled and weighed as W_3. %fibre was calculated using Eq-3.

$$\% \text{ Fibre} = \frac{W_2 - W_3}{W_1} \text{ X } 100 \quad \text{.....Eq-3}$$

Determination of Crude Fat

A 250 mL extraction flask was dried in an oven at 105^0C and then cooled weighed. Then, 5g of the ground sample was weighed into a labeled porous thimble then its mouth covered with white clean cotton wool. A 200 mL of petroleum ether was added into the extraction flask. The covered porous thimble was placed into the condenser and the apparatus was assembled for subsequent extraction which continued for 6 h. The porous thimble was removed and the extraction flask was placed on a water bath to make it free from the petroleum ether. The extraction flask containing the oil was dried in an oven for 1 h then cooled in a desiccator and weighed.

The percentage fat was calculated as follows:

Weight of empty porous thimble = W_0, Weight of thimble + Ground Sample = W_1, Weight of ground Sample = W_1 - W_0, Weight of empty extraction flask = W_2, Weight of extraction flask + oil = W_3

$$\% \text{ Fat} = \frac{W_3 - W_2}{W_1 - W_0} \text{ X } 100 \quad \text{.....Eq-4}$$

Determination of Crude Protein

A 0.5g of sample was weighed into a dry 500 mL macro-kjeldahl flask then 20 mL of distilled water was added. The flask was swirled for few minute and allowed to stand for 30 minute, then, 1 tablet of mercury catalyst was added together with 30 mL of conc. H_2SO_4 using an automated pipette. The flask was cautiously heated with low heat on the digestion stand until a clear digest was obtained, then boiled for 5 h. The flask was allowed to cool and then 100 mL of distilled water was slowly added to it. A 10 mL of the digest was transferred into another clean macro-kjeldahl flask (750 mL). The remaining residue was then washed with 50 mL distilled water 4 times and aliquot transferred into the flask. A 20 mL of H_3BO_3 indicator solution was added into a 250 mL Erlenmeyer flask which was then placed under the distillation apparatus. The 750 mL kjeldahl flask was then attached to the distillation apparatus, then, 150 mL of 10N NaOH was poured into it then kept under control by allowing sufficient cold water to flow through. A 40 mL of the distillate was collected and then distillation stops. The NH_4OH in the distillate was determined by titrating with 0.01N standard HCl. The percentage Nitrogen was calculated using the following expression:

$$\text{Nitrogen } (\%) = \frac{\text{TV} \times \text{NA} \times 0.014 \times \text{DF}}{\text{Volume of aliquot} \times \text{weight of sample}} \text{ X } 100 \quad \text{.....Eq-5}$$

NA = Normality of acid used (0.01N), TV = Titer Value, DF = Dilution Factor, Volume of aliquot = 10 mL

Carbohydrate Content

The total carbohydrate was determined by subtracting the summation of percentage moisture, ash, crude protein, crude fibre and lipid from 100.

Qualitative Phytochemical Screening

Reported standard methods of analyses [6-10] were used.

Test for Flavonoids

A 2 mL of 10% sodium hydroxide was added to 2 mL of the extract in a test tube. A yellow colour was formed which turned colourless upon addition of 2 mL of dilute hydrochloric acid indicating a positive result.

Test for Phenols

Two milliliter (2 mL) of the extract was mixed with few drop of 10% ferric chloride solution. The formation of greenish blue or violet or blue-black colouration was an indication of a positive result.

Test for Tannins

Five (5) drops of 0.1% ferric chloride was added to 2 mL of the extract. Formation of a brownish green or blue-black colouration indicates a positive result.

Test for Saponin

A 2 mL extract was diluted with 2 mL distilled water. The mixture was agitated in a test tube for 5 min. Appearance of about 1mm layer of foam indicates a positive result.

Test for Phlobatannins

A 2 mL extract was boiled with 1% aqueous hydrochloride. Deposition of a red precipitate indicates a positive result.

Test for Alkaloids

To 2 mL of the extract, 2 mL of 10% HCl was added. To the acidic medium, 2 mL of Meyer's reagent was added. Formation of an orange precipitate indicates a positive result.

Test for Terpenoids

A 2 mL extract was mixed with 2 mL of chloroform and 1 mL of concentrated sulphuric acid was carefully added to form a layer. Formation of a reddish brown interphase between two clear layers indicates a positive result.

Test for Glycosides

To 2 mL of acetic acid, 2 mL of the extract was added. The mixture was cooled in cold water bath, and then 2 mL of concentrated H_2SO_4 was added. Colour development from blue to bluish green indicates the presence of glycosides.

Test for Anthraquinones

A 2 mL extract was boiled with 5 mL of 10% HCl for 3 minutes, then, 5 mL of chloroform was added followed by further addition of 5 drops of 10% ammonia. A rose pink colouration indicates a positive result.

Determination of Antioxidant Potential

The ferric reducing power of extract was determined by using potassium ferricyanide-ferric chloride method [11]. A 2 mL extract was added to 2.5 mL of potassium ferricyanide and the mixture was incubated at 50°C for 20 minutes. Then, 2.5 mL of trichloroacetic acid was added to the mixture then centrifuged at 650 × g for 10 minutes. To 2.5 mL of the supernatant, 2.5 mL of distilled water and 0.5 mL ferric chloride were added. The absorbance was read at 700 nm spectrophotometrically. Greater reducing capacity is indicated by higher absorbance and is calculated using Eq-6.

$$\text{Reducing power} = \frac{\text{AM}}{\text{AC}} \times 100 \quad \text{.....Eq-6}$$

AM = Absorbance of reaction mixture, AC = Absorbance of control mixture (distilled water).

RESULTS

The results of the phytochemical screening are presented in Table 1, while those of the antioxidant potential and proximate analyses are presented in Table 2 and 3 respectively.

Table 1: Phytochemical Composition of *Vitex doniana* and *Borassus aethiopium* Fruit Extracts

Phytochemicals	*Vitex doniana*		*Borassus aethiopium*	
	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract
Saponins	++	++	++	+
Tannins	+	+	-	-

Phlobatannins	-	-	-	-
Flavonoids	++	+	++	+
Alkaloids	++	++	++	++
Glycosides	-	-	-	-
Anthraqinones	-	-	-	-
Phenol	-	-	++	+
Terpenoids	++	++	+++	+

+ = Present, ++ = Highly Present, - = Not detected

Table 2: Antioxidant Potentials of *Vitex doniana* and *Borassus aethiopium* Fruit Extracts

Parameter	*Vitex Doniana*		*Borassus Aethiopium*	
	Ethanolic Extract	**Aqueous Extract**	**Ethanolic Extract**	**Aqueous Extract**
Antioxidant Activity (%)	18.25 ± 8.18	19.35 ± 11.61	35.20 ± 18.75	39.81 ± 18.06

Values are presented as mean ± standard deviation of triplicates.

Table 3: Proximate Composition of *Vitex doniana* and *Borassus aethiopium* Fruits

Parameter	***Vitex Doniana***	***Borassus Aethiopium***
Moisture (%)	30.00 ± 7.07	65.00 ± 3.54
Ash (%)	03.33 ± 0.78	05.30 ± 0.82
Crude lipid (%)	02.67 ± 1.11	01.19 ± 0.54
Crude protein (%)	03.24 ± 0.13	03.94 ± 0.13
Crude fibre (%)	02.10 ± 0.53	16.70 ± 3.83
Carbohydrates (%)	58.56 ± 5.49	07.87 ± 0.04

Values are presented as mean ± standard deviation of triplicates.

DISCUSSION

The results of the qualitative phytochemical screening of the aqueous and ethanolic extracts of the fruits as shown in Table-1 indicate the presence of saponins, flavonoids, alkaloids, and terpenoids in all of them. Tannins and phenols are only present in *Vitex doniana* and *Borassus aethiopium* respectively, whereas, anthraquinones, philobatannins and glycosides were absent in both. The presence of these metabolites suggests great potential for the fruits to be sources of useful phytomedicines.

The presence of flavonoids in both fruits extracts indicates their possible application as anti-inflammatory agents as some flavonoids have anti-inflammatory effect on both acute and chronic inflammation, anticancer and anti-allergy [12]. The presence of flavonoids and phenols in the fruits also accounts for their antioxidant properties which have the potentiality to protect cells and other tissues in the body from harmful effects of oxygen radicals [13].

Presence of terpenoids indicates possible use of the fruits as anti-tumor and anti-viral agents as some terpenes are known to be cytotoxic to tumor cells.

Saponins were shown to be present in all the fruits extracts. Fruits containing saponins are believed to have antioxidant, anticancer, anti-inflammatory, and antiviral properties [14]. Saponins help humans to fight pathogenic microorganisms and increase the efficacy of certain vaccines and knock out some kinds of cancer cells, especially those of blood and lung [14]. Saponins serve as innate antibiotics that help in fighting infections and microbial invasion in the body. These compounds are also capable in reducing cholesterol and bile acids level by forming complexes [15]. Saponins have been found to be useful in the management of hypercholesterolaemia [16].

The results of the antioxidant potentials of the fruits as shown in Table-2 indicated that the fruit of *Borassus aethiopium* has higher reducing (antioxidant) potential compared to that of *Vitex doniana*. The assay measures the reducing ability of antioxidant against oxidative effects of reactive oxygen species. Therefore, *Borassus aethiopium* fruit has higher reducing potential than the other fruit, possibly due to the higher additional content of phenolic compounds in the *Borassus aethiopium* fruit extracts. The result for the antioxidant power assay obtained for the *Borassus aethiopium* in this research (39.81%) has no significant difference with that obtained by Sudhakar *et al.*, [17] (46.99%).

The results of the proximate analysis as shown in Table-3 indicated that *Borassus aethiopium* fruit has a fibre content of 16.7 ± 3.83%, which is high compared to that of *Vitex doniana* fruit 02.10 ± 0.53%., suggesting that, the fruit of *Borassus aethiopium* can help effectively to reduce the risk of colon cancer by binding to cancer causing substances in the colon. The ash content of each of the fruit suggests that there is reasonable amount of inorganic minerals in the fruits and this portray the good sources of dietary inorganic minerals.

Considering the amount of moisture content of the two (2) fruits, especially that of *Borassus aethiopium* which is 65.00 ± 3.54%, preservation for longer period of time cannot be achieved because of high chance of microbial attack when kept at room temperature [18]. *Borassus aethiopium* and *Vitex doniana* have low amount of protein and lipid contents as indicated in Table-3, thus, the fruits cannot contribute significant amount of protein and lipid to the body. For the carbohydrate content, *Vitex doniana* can be a good source of

carbohydrate (having 58.56 ± 5.49%) compared to *Borassus aethiopium* fruit (having 7.87 ± 0.04%), which can eventually be a good source for energy generation.

CONCLUSION

It has been established that, based on the antioxidant activities, phytochemical and proximate compositions of the *Borassus aethiopium* and *Vitex doniana* fruits, they can be useful for both nutritional and medicinal applications, especially *Borassus aethiopium* which has higher antioxidant activity.

REFERENCES

1. Miller, H.E, Rigelhof, F., Marquart L., Prakash, A., and Kanter, M. (2000). Cereal Foods World. *J. of food Sci.* 45(2)**:** 59 - 63.
2. Barot S, Gignoux J, Menaut J.C (1999). Demography of a savanna palm tree: predictions from comprehensive spatial analysis. *J. Ecology* 80: 1987-2005.
3. Cassou, J. D, Depommier and S.J Ouedraogo, (1997). The Ronier palm park (*Borassus aethiopum* Mart.) of Wolokonto in the Southwest of Burkina Faso: Structure, dynamics and interest of the park. *Draft Paper*, pp: 10.
4. Latham, P., (2004). Useful plants of Bas-Congo province, Democratic Republic of the Congo. DFID, London, United Kingdom. Pp. 320.
5. Association of Official Analytical Chemists (A.O.A.C.) (2005). Official Methods of Analysis of the AOAC, 15th Ed., Washington, D.C. Pp 200-210.
6. Trease, G.E. and Evans, W.C. (2002). Pharmacognosy. 15th Ed. Saunders Publishers, London. Pp. 391 – 393.
7. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Lagos-Nigeria: Spectrum books Limited; *Stand. Herbal Med.*, 3: 55-61.
8. Mbatchou, V.C. and Kossono, I. (2012). Aphrodisiac activity of oils from *Anacardium occidentale* L. Seeds. *Phytopharm. – Intern. J. Phytother. Bioact. Nat. Prod.* 2(1): 81–91.
9. Trease, G.E. and Evans, W.C. (1989). Trease and Evans Mexican medical plants. *J. Ethnopharm. Pharmacog*. 13: 222 – 230.
10. Harborne, J.B. (1998). Phytochemistry methods. In: *A guide to modern techniques of plant analysis* 3rd edition. Chapman and Hall publishing. London. United Kingdom.
11. Oyaizu, M. (1986). Studies of products browning reaction; Antioxidant activity of products of browning reaction prepared from glucosamine. *Jap. J. Nut.* 44: 307-315.
12. Ishurd, O., Zgheel, F., Kermagi, A., Flefla, M. and Elmabruk, M. (2004). Antitumor activity of beta-D-glucan from Libyan dates. *J. Med. Food*, 7: 252-255.
13. Ogunleye, D.S. and Ibitoye, S.F. (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. *Trop. J. Pharm. Res.*, 2(2): 239-241.

14. Philips. K.M., Ruggio, D.M and Ashraf-khorassani, M. (2005). Phytosterol composition of nuts seeds commonly consumed in United States. *J. Agric. Food Chem.* 53(24): 9436-9445.
15. Sheren, J. (2011). What are the health benefits of saponins? Chemistry, processing, health benefits. *Jinghua Shi Spring.* Pp 77-91.
16. Hostettmann, K. A. and Marston (1995). Saponins. In: *Chemistry and Pharmacology of Natural Products*. Cambridge: Cambridge University Press p. 377.
17. Sudhakar, K.R., Sandhya S. and Vinod D. Banji (2010). Antioxidant activity of methanolic extracts of *Borassus aethiopium* leaves and roots. *Int. J. of Pharm. and Tech*., 2(3): 67-74.
18. Ojimelukwe P.C., Asumugha V.U. and Omerie G.C. (2005). *Fundamental of Food Science and Nutrition.* BEL'S Books, Nsukka, Nigeria. p.149.