Chemistry Research Journal, 2021, 6(2):87-93

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Phytoconstituents, Proximate Composition and Antimicrobial Studies on *Dacryodes edulis*

Nwoke Prince Lekpoabari, Marcus Abiye Clement, Nna Prince Joe

Department of Chemistry, Faculty of Natural and Applied sciences, Ignatius Ajuru University of Education, Port Harcourt, Rivers State, Nigeria.
Correspondence email: teeprince2011@gmail.com

Abstract The study was aimed at evaluating phytoconstituents, mineral elements, proximate and antimicrobial activities of seed of *Dacryodes edulis.* Extraction was achieved using n-hexane and ethylacetate. Quantitative, qualitative and elemental analyses followed standard procedures as described by Association of Official Analytical Chemists (AOAC). The results indicated high percentage of protein (16.9%), lipids, (26.3%), and carbohydrates (44.0%). Phytochemicals present were; saponins, phytates, alkaloids, flavonoids and tannins, while elemental analysis showed; iron (120.0 mg/kg), and magnesium (30.65 mg/kg). The antimicrobial assay indicated susceptibility for *V. cholera, E. coli, S. aureus, S. typhi, C. neoformans, R. mucilaginosa, C. glabrata,* and *T. beigelii* at 1000 mg/L concentration, with highest susceptibility for the fungal than the bactericidal pathogens. The minimum inhibitory concentration (MIC) showed best inhibition at 0.001 mg/L, while the turbidity was highest at 0.001mg/L. MBC and MFC revealed that antimicrobial activities of the seeds on the pathogens were bactericidal and fungicidal, which could result in death of cells. The findings from the study justify the use of these seeds in traditional medication in the control of infections and treatment of diseases.

Keywords Antimicrobial, Proximate, Phytochemicals, Bacteria, Virus

Introduction

The impact of plants and their products to human nourishment cannot be exaggerated. In Africa, the demand for fruits is very high, due to its complementary role in diet, where it helps food to attain the status of a balanced diet [1]. Plants in all aspect of human existence have functioned as an important initial material used in the development of drugs [2]. Pharmaceutical floras naturally contain physiologically lively components which have been used in treatment of different illnesses for a long time in trado-medical practices [2]. Several scholars has shown from their works on medicinal plants that comparatively small proportion of fewer than 10% of all the plants on globe is assumed to function as bases of treatment [2]. *Dacryodes edulis*, fruit, a *Burseracea*, is one of such fruits that may well serve the twofold purpose of being a source of nutrition to humans and as well as a starting raw material for industrial drug synthesis, if appropriately coupled [3]. The consumption of the fruit is wide spread in Nigeria especially in the south eastern part of the country. Findings across other parts of the country also showed that the fruit pulp is eaten and the seeds usually thrown away. Khare [4], noted that *D. edulis* seed oil have potential of being used as domestic and industrial oil. The fruit is consumed traditionally in several ways such as raw, boiled or roasted. The pulp can be eaten alone, eaten with roasted maize or with a traditional food called tapioca. It may as well be eaten with bread, where the pulp is applied on the bread as butter. *D. edulis* has many therapeutic

applications. All parts (leaves, bark, stem and root) of the plant have found utility local medicinal practice for the treatment of numerous diseases. *D. edulis,* is commonly known by the following names: Ube (Igbo), Mzembe (Tiv). English names include: African pear, Bush butter tree, Bush fruit tree, Eben tree, Native pear and in French, Safoutie. Various parts of the plant are utilized in the management of a number of diseases in different areas. The seed of the *D. edulis* has not been studied extensively to also check their nutritive and chemical value to ascertain their viability for alternative medicine or as feed for animals or even human consumption. The aim of the study is to investigate the phytoconstituents, mineral elements, proximate content and antimicrobial effects of seed of *Dacryodes eduli.*

Materials and Methods

Sample Collection and Preparation

Dacryodes edulis used in this study were bought from Kaa market in Khana Local Government Area of Rivers State, Nigeria. The seeds were removed from the fruit and then dried in the sun for ten (10) days and milled to fine powder, then stored in an air tight container before further extraction and analysis.

Extraction of Sample

A measured weight of 10g of the powdered sample was put into a glass bottle and 250 ml of ethyl acetate was added and then placed in a microwave oven which was set at low temperature and firstly the bottle was removed after 5 minutes and allowed to cool, the mixture was filtered into a glass container and the residue was allowed to dry. The extracts where evaporated to dryness at a temperature of 33^{o}C.

Qualitative analysis of Phytochemical

Qualitative analysis were done following standard methods [5]. The following phytochemicals were tested; alkaloid, flavonoid, tannins, phytates, and saponins.

Quantitative determination Phytochemical Analysis

Alkaloid, flavonoid, tannins, phytates, and saponins, was determined using standard methods as described by Association of Official Analytical Chemists [6].

Proximate Analysis

The moisture content, percentage ash, carbohydrate, protein, and lipids were determine using standard processes as by Association of Official Analytical Chemists [6].

Determination of Mineral Elements

Two grams of the finely crushed samples was put in a receptacle (crucible) and burnt using a muffle furnace at 550 ^{o}C for 6 hrs. The ash obtained was dissolved in 10 ml of 10 % HNO_3 and gently heated for about 20 minutes and was filtered into sample bottles. The values of the mineral contents was examined in the filtrate by adopting the method of Association of Official Analytical Chemists [7]. Atomic absorption spectrophotometer (AAS) and flame photometer were used to check for the levels of the mineral elements.

Antimicrobial

The pathogenic microbial species (*Vibro cholera, Escherichia coli, Staphylococcus auereus, Salmonella typhi, Cyrptococcuss neoformans, Rhodotonela mucilaginosa, Candida glabrata, and Trishoporon beigelii*) were obtained in bijou bottles from the Microbiological Unit, University of Port Harcourt Teaching Hospital (UPTH), Choba, Port Harcourt, Rivers State, in sterile nutrient agar slants and transported back to the laboratory. In the laboratory, the slants were incubated at 37 ^{o}C for 24 hours to obtain stock cultures, after which these stocks were preserved at 4 ^{o}C prior to further analysis. The different concentrations (1000 mg/L, 100 mg/L, 10 mg/L, 1mg/L, 0.1 mg/L, 0.01 mg/L and 0.001 mg/L) of the extract (n-hexane and ethylacetate) that were used for the antimicrobial screening were

prepared by making ten folds serial dilution of extract in 50% v/v DMSO. The dilutions were then labelled appropriately. The preliminary antimicrobial screening was carried out using the highest concentration (1000 mg/L) of the different extracts to determine whether they are bioactive or not, the disc diffusion method was used for this screen. For this, sterile 6mm disc were inserted into the DMSO solution containing 1000 mg/L concentration of the extract (n-hexane and ethyl acetate) of the seed samples and were dried aseptically. After drying, disks of the different extract were inserted each into different Mueller Hinton ager plates seeded with 0.1 ml 10^8 cells of the different pathogen, and incubated. The bacteria plates were incubated at 37 ^oC for 24 hours while the fungi plates were incubated at 28 ^oC for 72 hours. After incubation, zones of inhibition were measured in millimetre (mm) for each extract disc and for each pathogens. The minimum inhibitory concentration (MIC) was done following the bruth dilution method described by Nna *et al.,* [8]. The minimum bacteria concentration (MBC) and minimum fungal concentration (MFC) test was carried out to by preparing mueller hinton agar and potato dextrose agar and sterilized by auto-cleaving. A sterilized swab stick was used to inoculate the content in the MIC dilution tubes unto the prepared media and inoculated (bacteria; 37^oC for 24 hours, fungi; 38^oC for 72 hours). After incubation, the cultured plates were observed for colony growth.

Results and Discussions

Proximate Content Analysis

The result of the proximate analysis are presented in Table 1.

Table 1: Result of the proximate analysis obtained from the seeds of *D. edulis*

Samples	Moisture (%)	Protein (%)	Ash (%)	Fibre (%)	Lipids (%)	CHO
D. edulis.	4.8	16.9	5.1	2.9	26.3	44.0

The seeds of some tropical fruits are edible because of their nutritional content and low toxicity [9]. The nutritional content of any substance is defined by its proximate values [10]. The table above indicated that the seed samples were very rich in carbohydrate (*Dacryodes edulis*, 44.0%), with the result higher than works by Onuegbu and Ihediohanma [11], (1.36% to 10.68%). The lipid was 26.3% which is typical of the plant though *D. edulis* oil has linoleic acid, an essential polyunsaturated fatty acid in human nourishment which can inhibit cardiovascular condition. The oil from *D. edulis* is naturally fortified with oleic acid which has oxidative stability, an important characteristics as frying oil [12].

The protein content was 16.9%, which was slightly different from report by Ogboloma *et al.,* [13] on the African pear for two locations as they reported 7.00±0.09 and 4.38±0.08 for Benue and Rivers State respectively. The ash content and crude fiber content of *Dacryodes edulis* was 5.1% and 2.9% respectively. Although the proximate content of the seed samples indicated the presence of nutritional components, the edibility of the seeds remains uncertain as toxicity and anti-nutritious components in them are yet to be determined [14].

Phytochemical Constituents Analysis

The result of the Qualitative and Quantitative analysis of the Phytochemical constituents of the seeds extract of *Dacryodes edulis* is presented in Table 2 and 3.

Table 2: Result of the qualitative analysis of the phytochemicals

Phytochemicals	*Dacryodes edulis*	
	Ethyl Acetate	n-Hexane
Flavonoids	+	+
Tannins	+	+
Phytate	+	+
Saponin	+	+
Alkaloids	+	+

Table 2 indicated that the ethylacetate and n-hexane extracts of both seeds showed the presences of the following phytochemicals; flavonoids, tannins, phytate, saponins, and alkaloids. It shows that the seed of the African pear is a natural source of the active components

Table 3: Result of the quantitative analysis of the phytochemicals

Phytochemicals	***Dacryodes edulis***	
	Ethyl Acetate	**n-Hexane**
Flavonoids (%)	1.24	0.83
Tannins (%)	0.477	0.174
Phytate (%)	2.31	3.00
Saponin (%)	4.13	4.79
Alkaloids (%)	0.84	1.14

The % flavonoids composition of the seed was 1.24 and 0.8 for ethylacetate and n-hexane extract. The data obtained are at variance with the results of Kindahunsi [15], both samples exhibited low values of flavonoids. Tannins result in percentages was 0.477% and = 0.174% for ethylacetate and n-hexane extracts. This is similar to reports by Osagie *et al.,* [16], on pigeon pea (0.1%) and plantain (0.51%). Phytate values were 2.31% and 3.00% for EAE and n-hexane. Though, phytate in considerable levels possess anti–oxidant properties and also inhibits cancers of the colon through the reduction of oxidative stress in the lumen of abdominal tracts [17]. Saponin value for *D. edulis* was EAE = 4.31 and n-hexane = 4.79, respectively. Alkaloid compositions of the investigated seeds were low. Most phytochemicals produced by plants are anti-nutritional in nature, and they mostly serve the function of protecting them from natural predators and infestations by pests (micro-organisms, insects etc.).

Mineral Element Concentration

The table shows the concentration (mg/kg) of mineral elements in the seeds.

Table 4: The concentration (mg/kg) of mineral elements in the seeds

Parameters	***Dacryodes edulis***
Zinc	11.20
Sodium	6.800
Potassium	50.10
Iron	120.0
Magnesium	30.65
Manganese	5.000

The table above showed that iron was highest with value at 120.0 mg/kg. The high level of iron shows that these seeds may be good for consumption because the presence of iron in the body helps in transport of oxygen to all parts of the body. Magnesium had significant value at 30.65 mg/kg, which indicates good sources of magnesium element. Weatherspoon [18], revealed that magnesium plays an active role the in over 300 enzymatic reactions within the body system. Potassium was 50.10% and it is needed to regulate fluid balance, muscle contraction, and nerves signals [19]. Zinc was11.20 mg/kg and researches have shown that micronutrients are usually higher in leaves of plants when compared with other parts above ground [20]. Manganese was 5.60 mg/kg and its presence plays big role in numerous activities within the body, which include metabolism of nutrients, activation of enzymes in metabolism, and other chemical processes within the body. Sodium was 6.80 mg/kg and it helps to maintain the balance of water within the body cells. These levels of the elements could make the seeds serve as good sources of elemental minerals needed in the body system.

Antimicrobial Assay

The result of the antimicrobial assay of ethylacetate and n-hexane extract are shown in Table 5.

Table 5: Antimicrobial of 1000 mg/L concentration of ethylacetate and n-Hexane extracts

Test Pathogen	Zone of Inhibition			
	D. edulis		Control	
	EAE	HE	TC	AmB
V. cholera	S(11.2)	S(10.8)	S(28.0)	R(0.00)
E. coli	S(13.9)	S(12.7	S(26.0)	R(0.00)
S. aureus	S(9.10)	S(6.20)	S(29.0)	R(0.00)
S. typhi	S(15.8)	S(14.1)	S(30.0)	R(0.00)
C. neoformans	S(17.3)	S(19.0)	R(0.00)	S(22.0)
R. mucilaginosa	S(14.8)	S(17.2)	R(0.00)	S(26.0)
C. glabrata	S(15.9)	S(13.8)	R(0.00)	S(28.0)
T. beigelii	S(16.1)	S(16.9)	R(0.00)	S(23.0)

Key: S = Susceptible, R = Resistant, Numeric value in brackets = Diameter of zone of inhibition in millimetres (mm), Negative control = Normal saline, EAE = Ethyl acetate extract, HE = n-Hexane extract, TC = Tetracycline, AmB = Amphotericin B.

At the concentration of 1000mgL^{-1} ethyl acetate and n-hexane had the highest activity on *Cryptococcus neoformans, T. beigelii, R. mucilaginosa,* while the least activity was for *S. aureus* and *V. cholera.* This shows that extract of African pear with active bioactive components have a little high activity of pathogens and bacteria. This could be attributed to the difference in their purity levels. The concept of purity in drug chemistry can never be over emphasized as purification process in drug production gets rid of interfering molecules that can lower drug activity/potency.

Table 6: Minimum Inhibitory Concentration (MIC) of n-Hexane and Ethyl acetate extract on Selected Microbial Pathogens

Pathogens	n-Hexane Extract (mg/L)							Ethylacetate extract (mg/L)						
	1000	100	10	1	0.1	0.01	0.001	1000	100	10	1	0.1	0.01	0.001
V. cholera	-	-	-	-	-	**	+++	-	-	-	-	**	++	+++
E. coli	-	-	-	-	**	+++	+++	-	-	-	-	**	+++	+++
S. aureus	-	-	-	**	+	+++	+++	-	-	**	+	+	++	+++
S. typhi	-	-	-	-	-	**	+++	-	-	-	-	-	**	+++
C. neofor.	-	-	**	+	+	++	++	-	-	-	**	+	++	+++
R. muci.	-	-	**	++	+++	+++	+++	-	-	**	++	+++	+++	+++
C. glabrat	-	-	-	-	**	+	++	-	-	-	**	+	++	+++
T. beigelii	-	**	+	+	++	+++	+++	-	-	**	++	++	+++	+++

Key: **-** = No turbidity (no growth), ****** = MIC, **+** = Turbidity (light growth), **++** = Moderate turbidity, **+++** = High turbidity

The result for the minimum inhibitory concentration (mic) of the n-hexane extract shows that it is most potent against *vibrio cholera* and *salmonella typhi* (mic at 0.01mg/L) and least potent against *Trichosporon beigelii* (100mg/L). This interprets that the n-hexane extract can be used therapeutically to effectively control the infection and growth of vibrio cholera and salmonella typhii. For the ethyl acetate extract of *D. edulis,* MIC values indicated that it was most potent against *S. typhi* (0.01mg^{-1}) and least potent against *R. mucilaginosa* and *T. beigelii.*

Table 7: Minimum Bacterial/Fungal Concentration of n-Hexane extract on bacterial and fungal pathogens

Bacterial/	Concentration in mg/L						
Fungal	1000	100	10	1	0.1	0.01	0.001
V. cholera	-	-	-	-	-	**	+
E. coli	-	-	-	**	+	+	+
S. aureus	-	-	-	**	+	+	+
S. typhi	-	-	-	-	-	**	+
C. neofor.	-	**	+	+	+	+	+
R. mucilag.	-	**	+	+	+	+	+
C. glabrata	-	-	-	-	**	+	+
T. beigelii	**	+	+	+	+	+	+

**=MBC/MFC

The results from the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of this particular extract indicates that it is both bactericidal and fungicidal on the bacterial pathogens and fungal pathogens respectively. MBC of the extract on *V. cholera a*nd *S. typhi* was at 0.01 mgL^{-1} while that of *E. coli* and *S. aureus* was at 1.0 mgL^{-1}. On the other hand, MFC of the extract on *C. neoformans* and *R. mucilaginosa* was at 100 mgL^{-1} while that of *C. glabrata* was 0.1mgL^{-1} and *T. beigelii* was 1000 mgL^{-1}.

Table 8: Minimum Bacterial/Fungal Concentration of ethylacetate extract on bacterial and fungal pathogens

Bacterial/	**Concentration in mg/L**						
Fungal	**1000**	**100**	**10**	**1**	**0.1**	**0.01**	**0.001**
V. cholera	-	-	-	**	+	+	+
E. coli	-	-	-	**	+	+	+
S. aureus	-	-	**	+	+	+	+
S. typhi	-	-	-	-	-	**	+
C. neofor.	-	-	**	+	+	+	+
R. mucilag.	-	-	**	+	+	+	+
C. glabrata	-	-	-	**	+	+	+
T. beigelii	-	**	+	+	+	+	+

**=MBC/MFC

MBC and MFC also indicated that the extract is both bactericidal and fungicidal on the bacterial and fungal pathogens respectively. The MBC of the extract on *V. cholerae* and *E. coli* was at $1mg^{-1}$. On the other hand, MFC of the extract on *C. neoformans and R. mucilaginosa* was 10 mg/L while that of *C. glabarati* was 1 mg/L and *T. beigelii* was 100 mg/L.

Conclusion

The result obtained from this very research indicated that the seed samples are good reservoirs of nutritional components and bioactive phytochemicals. The seeds were very good sources of carbohydrates and lipids which can confer dietary benefits. MBC and MFC revealed that antimicrobial activities of the seeds on the pathogens were bactericidal and fungicidal, thereby bringing about cell death. The findings from the study justify the application of these seeds in traditional medicine in the control of infections and treatment of diseases.

References

[1]. Okwu, D. E. (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure Applied Science*, 7, 455–459.

[2]. Ajibesin, K. K. (2011). *Dacryodes edulis* (G. Don) H.J. Lam: A review on its medicinal, phytochemical and economical properties. *Research Journal of Medicinal Plant*, 5 (1), 32-41.

[3]. Duru, M., Amadi, C., Ugbogu, A., Eze, A. & Amadi, B. (2012). Phytochemical, vitamin and proximate composition of *Dacryodes edulis* fruit at different stages of maturation. *Asian Journal of Plant Science and Research*, 2(4), 437-441.

[4]. Khare, C. P. (2010). Indian herbal remedies: Rational Western Therapy, Ayurvedic, and Other Traditional Usage, Botany. Pp. 25-28.

[5]. Sofowora, A.B. (2010). The state medical plants in Nigeria, University of Ibadan, Nigeria. Pp. 22-25.

[6]. Association of Analytical Chemist (AOAC). (2000). Saponification number of fats and oil, Official method 17th Edition.

[7]. Association of Official Analytical Chemists. (A.O.A.C.) (1990). Official Methods of Analysis of the AOAC, 15th Edn., Washington, D.C.

[8]. Nna, P. J., Tor-Anyiin, T. A. & Igoli, J. O. (2019). Fagaramide and Pellitorine from the stem bark of zanthoxylum zanthoxyloides and their antimicrobial activities. *South Asian Research Journal of natural Products*, 1-8.

[9]. Gibsons, E. L., Wardel, J. & Watts, C. J. (1998). Fruits and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite*, 31, 205-228.

[10]. Mathai, K. (2000). Nutrition in the Adults years. In Krause's food, nutrition, and diet therapy, 10th ed., ed. L. K. Mahan and S. Escott-Styump, 271, 274-275.

[11]. Onuegbu, N. C. and Ihediohanma, N. C. (2008). Some Proximate Analysis of African Pear (*Dacryodes Edulis*). *Journal of Applied Science and Environmental Management.* 12(1) 83-85.

[12]. Ikhuoria, E. U. & Maliki, M. (2007). Characterization of Avocado pear (Persea American) and African pear (*Dacryodes edulis*) extracts. *African Journal of Biotechnology* 6 (7): 950-952.

[13]. Ogoloma, J. U., Npkaa, W. K., Akaninwor, O. J. & Uwakwe, A.A. (2013). Proximate, Phytochemical and Mineral Elements Compositions of Some Edible Fruits Grown in Oil Producing Community of Rivers State, Nigeria. *Journal of Environmental Science, Toxicolgy and Food Technology.* 5(2), 38-46.

[14]. Harborne, J. B. (1999). An overview of the antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animals feeding, Caygill, J. C. and Mueller-Harvey, I, eds, Nottingham Univ. Press, UK, 7-16.

[15]. kindahunsi, A. A. (2005). Phytochemical screening, nutrient and anti-nutrient composition of selected tropical green leafy vegetables, *African Journal of Biotechnology.* 4:54-60.

[16]. Osagie, U. A., Muzquiz, M., Burbano, O. C., Cuadado, C., Ayet, G. & Castano, A. (1995). Some antinutritional constituents in ten staple food items grown in Nigeria. *Tropical Scienc*e. 36, 109-115.

[17]. Reddy, R. N. & Shridhar, K. (2001). Food phytates. Boca Raton. CRC.

[18]. Weatherspoon, D. (2017). Why do we need magnesium? Medical News Today. Retrieved from www.medicalnewstoday.com/articles/286839.php on the 29th December 2019.

[19]. Ryan, M. (2017). What does potassium do for your body? Healthline publication retrieved from www.healthline.com/nutrition/what-does-potassium-do on the 29th December 2019.

[20]. Basgel, S. & Erdenmoglu, S. B. (2005). Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Science and Total Environment,* 359, 82-89.