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Antimicrobial Potential of *Dacryodes edulis* against Selected Clinical Bacterial Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors contributed in one way towards the research, writing of the article and its editing process. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

The antimicrobial potential of *Dacryodes edulis* was investigated to determine the antimicrobial properties of the collected raw *D. edulis* pulp and seed extracts on some medically important human pathogens. The research was investigated against the selected human pathogens using standard microbiological and biochemical procedures. The *D. edulis* samples were harvested aseptically between the periods of April to June 2016 from its tree located at IBB way, Calabar Municipality, Nigeria. The aqueous and ethanolic extracts of the seed and pulp at varying concentrations of 1 g/10 ml, 2 g/10 ml, 4 g/10 ml, 5 g/10 ml was tested against some selected human pathogens such as *Staphylococcus aureus, Klebsiella pneumonia, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris.* The antimicrobial susceptibility results of the ethanol extracts of *D. edulis* seed showed marginally higher zones of inhibition to the clinical bacterial isolates tested than the ethanol extract of the pulp tested against the same clinical isolates. The organisms were resistant to the aqueous extracts of both the pulp and seed. The seed ethanol

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extracts showed a higher zone of inhibition of 21 mm against *K. pneumoniae* and 18 mm against *P. vulgaris* as compared to 18 mm as against *K. pneumoniae* and 17 mm against *P. vulgaris* showed by Gentamycin used as standard antibiotic control. The result of these has shown that *D. edulis* could be of immense importance in our nation's young pharmaceutical industry for the development of new chemotherapeutic agent to address unmet therapeutic needs.

Keywords: Antimicrobial potential; Dacryodes edulis; bacterial isolates; antibiotic control and extracts.

1. INTRODUCTION

Dacryodes edulis is commonly known as 'African pear', it is known as eben among the Efik people, ube in Igbo, orumu in Benin, elemi in Yoruba, and safou in French [1,2]. It belongs to the family of Burseracaea. They are shade loving plant species, dioecious and found in the humid tropical zone of non-flooded forest [3,4]. The fruit of *D. edulis* are ellipsoidal and their size varies approximately from 4 to 9 cm long and from 2 to 5 cm wide [5]. *D. edulis* is a fruit that has seed and it is covered by a pulpy pericarp, it is an edible fruit which can be eaten cooked or raw and rich in minerals, vitamins, oils, and protein [6,7]. In spite, of its nutritional values, *D. edulis* is highly perishable and prone to microbial attack, as it is also rich source of nutrients to microorganisms [8,5]. Research have shown that microorganisms penetrate the intact cuticle of the fruits through natural openings or wound during harvest resulting to microbial deterioration of the fruit [5]. Microbial deterioration of *D. edulis* fruits leaves undesirable effect on the fruit quality which not only affects the texture but the organoleptic properties of the fruit [9].

Human over the years has acquired extensive knowledge regarding the use of plants around him as medicinal source and as food [10-13,14]. Plants that grows around mans' dwelling places have been proven to possess a wide range of pharmacological and biological activities. Some of which are laxative, antimicrobial, diuretics, anti-spasmodics, antihypertensive and anti-inflammatory [15,8,16,14]. These functions of plants are performed by plant chemicals which could be sugar, lipids, protein, vitamins, minerals and phytochemicals [16,17,14]. *D. edulis* fruits possesses medicinal properties besides the nutritional potentials, it is used as a perennial cure for a variety of aliment ranging from ear infection to fever and oral problems [3,18,19]. In Nigeria, the plant resin is used for treating parasitic skin disease and Jiggers, while the pulped bark is used to cicatrize wounds [18,19]. The extract and secondary metabolites of the plant have been found to show biological activities such as antimicrobial, antioxidant and antisickle cell anemia [20]. A wide range of chemical constitutes such as terpenes, flavonoids, tannins, alkaloids and saponins have been isolated from the plant [8]. Recently, it was reported that the leaves were made into plaster to treat snake bite in southwest Cameron [21], the stem exudates of the plant were reported to contain tannin, saponin and alkaloids [14].

Also recently, there is an alarming increase in the resistance of pathogens to antibiotics, as reports around the world have shown that several medical important human pathogens are proving resistance to even the most powerful antibiotics [7]. As a result of this, scientists are looking into nature in search of an alternative arsenal for an unending war against these emerging developments of drug resistant pathogens [11,12,22].

However, various researches have shown that medicinal plant could serve as such alternatives, as it has been proven that these plants have different bioactive constituents (identified as alkaloids, tannins, flavonoids, saponins amongst others) that can have quite different mode of action and structures when compared with antimicrobials conventionally used to control microbial growth and survival [10,12,6,23]. Nevertheless, with this in mind, there is need for search of plants with more effective and potent bioactive compounds, as they could be the main remedy in curtailing or curbing the public health threat arising from the activities of these drug resistances, human pathogens.

2. MATERIALS AND METHODS

2.1 Sample Collection

Dacryodes edulis samples were harvested aseptically between the period of April to June 2016 from its tree located at No 52 IBB way, Calabar Municipality, Nigeria and placed in sterile food grade containers. These were transported in a cool box to Microbiology Laboratory, University of Calabar, Nigeria for analysis.

2.2 Preparation and Extraction of *Dacryodes edulis* Plant Materials

The fruits were de-fleshed to separate the seed from the pulp. The fresh seed and pulp of *D. edulis* plant were dried in a shaded place and later transferred to the oven set at 40℃ for 5-10 minutes separately. The seed and pulp of the plant were then grind to fine powder with the aid of a mechanical grinder. Ethanol and water were the extracting solvents used in extracting the phytochemicals from *D. edulis* [3]. Ten grams each of the powdered seed and pulp were soaked in 100 ml of the solvents (ethanol and water) in separate beakers and the set up allowed to run for 18-24 hours [6]. After 24 hours of extraction, the suspensions were vigorously shaken and filtered with Whatmann No 1 filter paper. Preparation of crude extracts for antimicrobial screening was done by weighing 1.0 g, 2.0 g, 4.0 g and 5.0 g of the extracts and soaked in 10ml of ethanol and distilled water respectively. The concentrated extracts were stored in airtight bottles and labelled [3,22].

2.3 Sampling and Confirmation of Test Bacterial Isolates

Test bacterial pathogens (*Klebsiella pneumonia, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Pseudomonas aerugirosa, and Enterococcus faecalis*) were sourced for from the University of Calabar Teaching Hospital (UCTH) Calabar, Nigeria. The organisms were further identified and confirmed using standard protocols for cultural and morphological identification, as well as biochemical characterization of isolates [12]. The clinical isolates were immediately sub cultured onto fresh nutrient agar after which morphological and biochemical characterization (such as gram reaction, catalase, citrate, oxidase, sugar fermentation, urease, methyl red, Vogues Prokauer etc.) were employed to ascertain the identity of each clinical isolates.

2.4 Antimicrobial Susceptibility Test

The agar disk diffusion method was used (Kirby-Bauer method) [24]. The antibiotic discs that were used as control are erythromycin (15 µg), ampicillin (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg) and gentamicin (10 µg). Bacterial colonies from overnight culture were suspended in 5ml physiological saline and incubated for 4 hours at 37℃. Mueller Hinton agar plate was then evenly inoculated with the cultures and allowed to dry for 5 minutes [3,6]. The antibiotic discs were applied to the surface of the inoculated or seeded agar with the aid of a sterilized forceps. The diameter of growth inhibition around the discs was measured after 24 hours of incubation at 37℃ [12,25,26].

3. RESULTS AND DISCUSSION

Table 1 presents the result of antimicrobial susceptibility testing of the African pear pulp extracts at different concentrations on the bacteria isolates collected. At 1 g/10 ml concentration, the African pear pulp ethanol extracts showed zone of inhibition with a diameter of 1.5 mm on *Klebsiella pneumoniae,* 1.3 mm on *Proteus vulgaris,* 2.3 mm on *Escherichia coli,* while no zone of inhibition was observed on *Enterococcus faecalis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The aqueous extract of the African pear pulp showed no zone of inhibition on all the tested bacteria isolates.

At 2 g/10 ml concentration, the African pear pulp ethanol extracts tested against the collected bacteria isolates showed a higher zone of inhibition (5.2 mm) on *K. pneumonia,* 4.7 *mm* on *P. vulgaris* and *E. coli* showed a zone of inhibition with diameter 6 mm and no zone of inhibition showed with *S. aureus, P. aeruginosa* and *E. faecalis.* However, no zone of inhibition was observed when the aqueous extracts of the African pear pulp were tested against all the collected bacteria isolates.

At a concentration of 4 g/10ml of the African pear pulp ethanol extracts, a higher zone of inhibition 10 mm was observed with *K. pneumonia*, 9.5 mm in *P. vulgaris* and 10.5 mm in *E. coli.* No zone of inhibition was observed when the African pear pulp ethanol extracts were tested against *P. aeruginosa, S. aureus* and *E. faecalis*. The aqueous extracts of the pear pulp showed no zone of inhibition when tested against all the collected bacteria isolates.

At a concentration of 5 g/10 ml tested against the bacteria isolates, the extract showed a zone of inhibition with a 12 mm diameter on *E. coli,* 14 mm on *K. pneumonia* and 15 mm on *P. vulgaris* while no zone of inhibition was observed with *P. aeruginosa, E. faecalis,* and *S. aureus.* The aqueous extracts of the pear pulp at this concentration showed no zone of inhibition when tested against the bacteria isolates.

Table 1. Antimicrobial activity testing of African pear pulp extracts at different concentrations on the collected bacteria isolate

Bacteria isolate	Concentration of extracts (g/10 ml)	African pear pulp		Standard antibiotics used as control (zone of inhibitions in mm)				
		Ethanol extracts (zone of inhibitions mm)	Aqueous extracts (zone of inhibitions in mm)	*Gen* (10 µg)	*Amp* (10 µg)	*Cip* (5 µg)	*Ery* (15 µg)	*Oflox* (5 µg)
Klebsiella pneumonia	1	1.5	-	18	25	20	21	15
	2	5.2	-					
	4	10	-					
	5	14	-					
Proteus vulgaris	1	1.3	-	17	23	21	25	24
	2	4.7	-					
	4	9.5	-					
	5	15	-					
Escherichia coli	1	2.3	-	25	20	24	15	19
	2	6	-					
	4	10.5	-					
	5	12	-					
Staphylococcus aureus	1	-	-	25	21	29	30	26
	2	-	-					
	4	-	-					
	5	-	-					
Pseudomonas aerugirosa	1	-	-	21	10	32	12	18
	2	-	-					
	4	-	-					
	5	-	-					
Enterococcus faecalis	1	-	-	17	25	22	27	20
	2	-	-					
	4	-	-					
	5	-	-					

Key: mm= zone of inhibition, Gen= Gentamycin, Amp= Ampicillin Cip= Ciprofloxacin, E= Erythromycin, Oflox = Ofloxacin

Table 2 presents the results of antimicrobial susceptibility testing of the African pear seed extracts at different concentrations on the bacteria isolates collected. At 1 g/10 ml concentration, the African pear seed ethanol extracts gave an inhibition zone with a diameter of 4.9 mm on *K. pneumoniae,* 5.5 mm on *P. vulgaris,* 3.5 mm on *E. coli,* while no zone of inhibition was observed on *P. aeruginosa, S. aureus* and *S. pneumoniae.* The aqueous extracts of the African pear seeds showed no zone of inhibition when tested against all the collected bacteria isolates.

At a concentration of 2 g/10 ml, a zone of inhibition with diameter 9 mm was observed when tested against *K. pneumoniae,* 10 mm with *P. vulgaris* and 8 mm with *E. coli,* while a zone of inhibition with diameter (5 mm) was observed with *S. aureus.* No zone of inhibition was observed when the extracts were tested against *P. aeruginosa* and *E. faecalis.* The bacteria isolates showed no zones of inhibition when tested against the aqueous extracts were tested.

At 4 g/10 ml concentration, the African pear seed ethanol extracts gave an inhibition zone with diameter of 16 mm on *K. pneumoniae,* 15 mm on *P. vulgaris*, 9 mm on *S. aureus,* 10 mm on *E. coli* and 4.5 mm on *P. aeruginosa,* while no zone of inhibition was observed when tested against *E. faecalis.* The aqueous extracts of the African pear seed showed no zone of inhibition when tested against all the collected bacteria isolates except *K. pneumoniae* that gave an inhibition zone with a diameter of 2.1 mm while *P. vulgaris* gave a zone of inhibition with a diameter of 3.5 mm.

Table 2. Antimicrobial activity testing of African pear seed extracts at different concentrations on the collected bacteria isolate

Bacteria isolate	Concentration of extracts (g/10 ml)	African pear seed		Standard antibiotics used as control (zone of inhibitions in mm)				
		Ethanol extracts (zone of inhibitions mm)	Aqueous extracts (zone of inhibitions in mm)	*Gen* (10 µg)	*Amp* (10 µg)	*Cip* (5 µg)	*Ery* (15 µg)	*Oflox* (5 µg)
Klebsiella pneumonia	1	4.9	-	18	25	20	21	20
	2	9	-					
	4	16	2.1					
	5	21	5					
Proteus vulgaris	1	5.5	-	17	23	21	25	24
	2	10	-					
	4	15	3.5					
	5	18	4					
Escheriohia coli	1	3.5	-	25	20	24	15	19
	2	8	-					
	4	10	-					
	5	15.5	-					
Staphylococcus aureus	1	-	-	25	21	29	30	26
	2	5	-					
	4	9	-					
	5	11	-					
Pseudomonas aerugirosa	1	-	-	21	10	32	12	18
	2	-	-					
	4	4.5	-					
	5	9	-					
Enterococcus faecalis	1	-	-	17	25	22	27	20
	2	-	-					
	4	-	-					
	5	-	-					

Key: mm = zone of inhibition, Gen = Gentamycin, Amp = Ampicillin, Cip = Ciprofloxacin, Ery = Erythromycin, Oflox = Ofloxacin

At a concentration of 5 g/10 ml, the bacteria isolates gave an inhibition zone with a diameter of 21 mm, when tested against *K. pneumonia* and *P. aeruginosa* (9 mm), 18 mm with *P. vulgaris*, 11 mm with *S. aureus*, 15.5 mm with *E. coli* while no zone of inhibition was observed when the pear seed ethanol extracts was tested against *E. faecalis*. The aqueous extracts of the pear seed showed no zone of inhibition when tested against all the collected bacteria isolates except *P. vulgaris* and *K. pneumoniae* that gave an inhibition zone with diameter of 4 mm and 5 mm respectively.

A higher inhibition zone of 21 mm in *K. pneumonia* and 18mm in *P. vulgaris* was observed when tested against the African pear seed ethanolic extract compared to the zone of inhibition (18 mm) for *K. pnuemoniae* and (17 mm) for *P. vulgaris* observed when tested against gentamicin antibiotic.

Chemotherapeutic properties of substances are useful weapons in the hands of microbiologist in the fight against microbes most importantly in the treatment of infectious pathogenic diseases and in food spoilage, as their active components usually interfere with growth and metabolism of microorganisms in a negative manner [13,3]. The antimicrobial properties of raw pulp and seed, ethanol and aqueous extracts of *Dacryodes edulis* against selected medically important human pathogens were analyzed in this study. The antimicrobial screening result of *D. edulis* pulp and seed extracts revealed that the clinical or test isolates were more susceptible to ethanol extracts of the seed by showing a higher zone of inhibitions as compared to the

ethanol extracts of the pulp extracts [6]. The susceptibility of the tested isolates increased with increasing concentrations of the extracts as observed in the zones of inhibition obtained. This is an indication that the phytochemical compounds in the *D. edulis* plant might be polar as the inhibition zones of the tested organisms are function of relative antibacterial activity of the extracts [3,2]. However, this observation is similar to that of Idu et al. [3], who had a higher zone of inhibition when chloroform and ethanol leaf extracts of *Chrysophyllum albidum, D. edulis* and *Garcinia kola* were tested against selected microbial pathogens.

From the Table 1 above it can be seen that at 5 g/10 ml concentration, the ethanol extract of the plant gave 14 mm zone of inhibition to *K. pneumonia* which is quite close that of Ofloxacin (15 mm) and Gentamycin (18 mm). In the same vein, *P. vulgaris* was inhibited with a zone of 15 mm by 5 g/10 ml concentration ethanol extract of the plant which is equally close to that of Gentamycin (17 mm). Also, 5g/10 ml concentration ethanol extract of the plant inhibited *E. coli* by 12 mm which is close to that of Erythromycin (15 mm). The above findings are similar to the works of Agbo and Mboto [12].

From the Table 2 above it can be seen that at 5 g/10 ml concentration, the ethanol extract of the plant gave 21 mm zone of inhibition to *K. pneumonia* which is almost the same with that of Ciprofloxacin (20 mm), Ofloxacin (20 mm), Erythromycin (21 mm) and Ampicillin (25 mm). *Proteus vulgaris* was inhibited with a zone of 18 mm by the 5g/10ml concentration ethanol extract of the plant, the result is equally quite close to that of Gentamycin (17 mm) and Ciprofloxacin (21 mm). Also, 5g/10ml concentration ethanol extract of the plant inhibited *E. coli* by 15.5 mm which is close to that of Erythromycin (15 mm) and Ofloxacin (19 mm). The findings in this research work agrees with that of other researchers [11,12,13,15,3,22]

According to Table 1 above, it can be seen that African foods [12] and fruits do not only supply nutrients to the body but also serve medicinal purposes. Prospective industrialists can tap from this, in their constant search for new antimicrobial agents.

4. CONCLUSION

The results of these has shown that consumption of African pear will not only provide nourishment to the body but will also help in combating enterics as can be seen in the results from the antimicrobial activity or the medicinal potential detected in this research. The findings of the antimicrobial activity or medicinal potential of the pear (*Dacryodes edulis)* against selected human pathogens could be of immense importance to our Nigerian young pharmaceutical industries for the development of new chemotherapeutic agent to address unmet therapeutic needs as such screening of various natural organic compounds and identification of active agents is the need of the hour for saving life and providing good health to humanity. Our government should make plans to ensure that while clearing lands for construction of governmental establishments, recreational centers and amusement parks, botanists, ecologist environmental scientists and pharmacognosists should all be consulted. So as to help save some plants that are of pharmacological importance from been destroyed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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