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Research Article

Antimicrobial Potential and Phytochemical Analysis of *Dacryodes edulis* Against Selected Clinical Bacterial Isolates

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ABSTRACT

The antimicrobial potential and phytochemical analysis of *Dacryodes edulis* (African pear) was investigated using standard microbiological procedures. Phytochemical screening of the raw and boiled African pear seed and mesocarp showed the presence of glycosides, tanins, flavonoids, saponins, reducing compounds, alkaloids and polyphenol in their ethanol extracts while the absence of saponins, tanins and alkaloids was observed in aqueous extracts of both the raw and boiled African pear seed and mesocarp. The aqueous and ethanolic extracts of both the African pear seed and pulp at varying concentrations of 1g/10ml, 2g/10ml, 4g/10ml, 5g/10ml was tested against some selected human pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Staphylococcus aureus* and *Enterococcus faecalis.* The results of the ethanol extracts of the African pear seed showed marginally higher zones of inhibition against the clinical bacterial isolates tested. The organisms were resistant to the aqueous extracts of both the African pear pulp and seed. The African pear seed ethanol extracts showed a higher zone of inhibition of 21mm against *Klebsiella pneumoniae* and 18mm against *Proteus vulgaris* as compared to 18mm against *Klebsiella pneumoniae* and 17mm against *Proteus vulgaris* by Gentamycin used as standard antibiotic control. However, African pear (*Dacryodes edulis*) can be of immense benefit to pharmaceutical industries for the development of new antimicrobial drugs.

Keywords: Antimicrobial potency, phytochemical analysis, *Dacryodes edulis*, mesocarp and inhibition zone.

INTRODUCTION

Dacryodes edulis is commonly known as 'African pear and belongs to the Burseracaea family. They love shades and are dioecious plant species found in the humid tropical zone of non-flooded forest[1,20,33]. The fruit of *Dacryodes edulis* are ellipsoidal and their size varies approximately from 4 to 9cm long and from 2 to 5cm wide[2]. *Dacryodes edulis* fruits has seed that is enveloped or covered by a pulpy edible mesocarp, which is consumed cooked or raw and serves as a good source of oils, vitamins, minerals and protein[3]. Extensive knowledge has been acquired over the years by man concerning the usage of plants and herbs as food and traditional medicine[4-6]. These plants carry out a whole lot of biological and pharmacological activities such as antimicrobial, anti-inflammatory, anti-spasmodics, antihypertensive, laxative, and diuretics functions[7-9]. Phytochemicals have been linked to be one of the chemical constituents that bring about these aforementioned functions of plants[7,10,11]. *Dacryodes edulis* fruits possess medicinal properties as it is used as a continual treatment for aliments such as fever, oral problems and/or ear infection[12,33]. The resin of the plant in Nigeria is used for treating parasitic skin disease and Jiggers, while the pulped bark is used to cicatrize wounds[12]. The extract and secondary metabolites of the plant have been found to show biological activities such as antimicrobial, anti-oxidant and anti-sickle cell disease[13]. A wide range of chemical constitutes such as terpenes, flavonoids, tannins, alkaloids and saponins have been isolated from the plant[14]. Recently, it was reported that the leaves were made into plaster to treat snake bite in southwest Cameron[15], the stem exudates of the plant were reported to contain tannin, saponin and alkaloids[16]. 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. 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. 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) which have quite different action and structures when compared with antimicrobials conventionally used to control microbial growth and survival. 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 the public health threat arising from the activities of these drug resistant human pathogens.

MATERIALS AND METHODS

Sample collection

The African pear (*Dacryodes edulis)* samples were harvested aseptically from its tree located Calabar

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Municipality Local Government Area and placed in sterile food grade containers. These were transported in a cool box to the laboratory for analysis.

Phytochemical screening test of African Pear (Dacryodes edulis) pulp and seed

The phytochemical screening of the raw African pear seed and mesocarp was carried out using the method of[17,32] for alkaloids, glycosides and saponins, (Trease and Evans; 1989 method[17] for tannins, phlobatanin and anthraquinones, Onwuka, 2005 method[18] for reducing compounds, flavonoids and for polyphenol

Preparation and extraction of African pear (Dacryodes edulis) plant materials

The fruits were de-fleshed to separate the seed from the pulp. The fresh seed and pulp of African pear (*Dacryodes edulis*) plant were dried in a shaded place and moved to hot air oven set at 40ºC for 5-10 minutes[20]. The fresh seed and pulp of *D. edulis* were dried separately in the oven. The African pear seed and pulp were later grind to powder with an electronic grinder. Aqueous (water) and ethanol were the extraction solvents[20]. Ten (10) grams each of the powdered seed and pulp were then soaked into 100ml of water and ethanol in different beakers and the set up was allowed for 24 hours[20]. The suspensions were then shaken vigorously and filtered using Whatman No 1 filter paper[31]. For the preparations of crude extracts for antimicrobial screening, concentrations of 1g, 2g, 4g and 5g of the extracts were weighed and soaked in 10ml of ethanol and distilled water respectively. The concentrated extracts were stored in airtight bottles and labelled[20,21].

Sampling and confirmation of test bacterial isolates

Test bacterial pathogens 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[27,33].

Antimicrobial susceptibility test

The agar disk diffusion (Kirby-Bauer) method was used. The antibiotic discs that were used as control are erythromycin (15µg), ampicillin (30µg), ciprofloxacin (5µg), ofloxacin (5µg), and gentamicin (10µg). Morphologically identified and confirmed clinical bacterial isolates from 24-hour culture were suspended in 5ml physiological saline and incubated at 37ºC for 4 hours[20]. Mueller Hinton agar plate was evenly inoculated with the clinical cultures by surface inoculation and allowed to dry for 5 minutes[20,33]. The antibiotic discs were then applied to the surface of the seeded agar with a sterilized forceps. The seeded plates were then incubated at 37ºC for 24 hours, after which the diameter of growth inhibition around the discs was measured[22,28,29].

RESULTS

Table1 presents the results of the phytochemical screening of raw and boiled African pear seed. Alkaloids, Glycosides, tanins, saponins, flavonoids, reducing compounds and polyphenol were present in excess in the ethanol extracts while saponins, tannins and alkaloids were present in the aqueous extract of both the African pear raw and boiled seed. Other chemical constituents such as phlobatanins, anthraquinones were absent in both the ethanol and aqueous extracts of both the raw and boiled African pear seed.

Phytochemical screening of raw and boiled African pear mesocarp

Table 2 presents the results of the phytochemical screening of raw and boiled African pear mesocarp. It showed the presence of reducing compounds, alkaloids, saponins, flavonoids, glycosides polyphenol and tannins in the ethanol extracts of the raw and boiled pear mesocarp. However, the absence of saponins, alkaloids and tannins in the aqueous extract of the raw and boiled pearmesocarp was observed while other chemical constituents such as phlobatanins and anthraquinones were absent in both the raw and boiled pear mesocarp analysed. Table 3 presents the result of antimicrobial susceptibility testing of the African pear pulp extracts at different concentrations on the bacteria isolates collected. At 1g/10ml concentration, the African pear pulp ethanol extracts showed zone of inhibition with a diameter of 1.5mm on *Klebsiella pneumoniae,* 1.3mm on *Proteus vulgaris,* 2.3mm on *Escherichia coli,* while no zone of inhibition was observed on *Enterococcus faecalis Pseudomonas aeruginosa* and *Staphylococcus aureus*. The aqueous extract of the African pear pulp showed no zone of inhibition on all the tested bacteria isolates. At 2g/ml concentration, the African pear pulp ethanol extracts tested against the collected bacteria isolates showed a higher zone of inhibition (5.2mm) on *Klebsiella pneumonia*,4.7*mm* on *Proteus vulgaris* and *Escherichia coli* showed a zone of inhibition with diameter 6mm and no zone of inhibition showed with *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus 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 4g/10ml of the African pear pulp ethanol extracts, a higher zone of inhibition 10mm was observed with *Klebsiella pneumonia,* 9.5mm in *Proteus vulgaris* and 10.5mm in *Escherichia coli.* No zone of inhibition was observed when the African pear pulp ethanol extracts were tested against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus 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 5g/10ml tested against the bacteria isolates, zone of inhibition with a diameter of 12mm with *Escherichia coli,* 14mm with *Klebsiella pneumonia* and 15mm with *Proteus vulgaris* was recorded while no zone of inhibition was observed with *Pseudomonas aeruginosa, Enterococcus faecalis,* and *Staphylococcus aureus.* The aqueous extracts of the pear pulp at this concentration showed no zone of inhibition when tested against the bacteria isolates. Table 4 presents the results of antimicrobial susceptibility testing of the African pear seed extracts at different concentrations on the bacteria isolates collected. At 1g/10ml concentration, the African pear seed ethanol extracts gave a zone of inhibition with a diameter of 4.9mm with *Klebsiella pneumoniae,* 5.5mm with *Proteus vulgaris,* 3.5mm with

Table 1: Phytochemical screening of raw and boiled African pear seed

S. No.	Chemical constituents	Raw seed		Boiled seed	
		Ethanol extracts	Aqueous extracts	Ethanol extracts	Aqueous extracts
1	Alkaloids	++	+	++	-
2	Glycosides	+	++	++	+
3	Saponins	++	+	+	-
4	Tannins	++	+	+++	+
5	Flavonoids	+++	+++	+++	++
6	Reducing compounds	+++	+	+++	+
7	Polyphenol	+	++	+	+++
8	Phlobatanins	-	-	-	-
9	Athraquinones	-	-	-	-

Key: + = Present, ++ = Present in excess, +++ = Present in much excess, - = absent

Table 2: Phytochemical screening of raw and boiled pear mesocarp

S. No.	Chemical constituents	Raw mesocarp		Boiled mesocarp	
		Ethanol extracts	Aqueous extracts	Ethanol extracts	Aqueous extracts
1	Alkaloids	++	-	+	-
2	Glycosides	+	+	++	+
3	Saponins	+	-	+	-
4	Tannins	+++	-	+++	-
5	Flavonoids	++	++	+	+++
6	Reducing compounds	+	++	++	+++
7	Polyphenol	+	++	++	+++
8	Phlobatanins	-	-	-	-
9	Athraquinones	-	-	-	-

Key: += Present, ++ = Present in excess, +++ = Present in much excess, - = absent

Escherichia coli, while no zone of inhibition was observed with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus 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 2g/10ml, a zone of inhibition with diameter 9mm was observed when tested against *Klebsiella pneumoniae*, 10mm with *Proteus vulgaris* and 8mm with *Escherichia coli*, while a zone of inhibition with diameter (5mm) was observed with *Staphylococcus aureus.* No zone of inhibition was observed when the extracts were tested against *Pseudomonas aeruginosa* and *Enterococcus faecalis.* The bacteria isolates showed no zones of inhibition when tested against the aqueous extracts were tested. At 4g/10ml concentration, the African pear seed ethanol extracts gave a zone of inhibition with diameter of 16mm with *Klebsiella pneumoniae*, 15mm with *Proteus vulgaris*, 9mm with *Staphylococcus aureus*, 10mm with *E. coli* and 4.5mm with *Pseudomonas aeruginosa*, while no zone of inhibition was observed when tested against *Enterococcus faecalis.* The aqueous extracts of the African pear seed showed no zone of inhibition when tested against all the collected bacteria isolates except *Klebsiella pneumoniae* that gave an inhibition zone with a diameter of 2.1mm while *Proteus vulgaris* gave an inhibition zone with a diameter of 3.5mm. At a concentration of 5g/10ml, the bacteria isolates *showed* a zone of inhibition with a diameter of 21mm, when tested against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (9mm), 18mm with *Proteus vulgaris*, 11mm with *Staphylococcus aureus*, 15.5mm with *Escherichia coli* while no zone of inhibition was observed when the pear seed ethanol extracts was tested against *Enterococcus faecalis.* The aqueous extracts of the pear seed showed no zone of inhibition when tested against all the collected bacteria isolates except *Proteus vulgaris* and *Klebsiella pneumoniae* that showed a zone of inhibition with diameter of 4mm and 5mm respectively. A higher zone of inhibition of 21mm in *Klebsiella pneumonia* and 18mm in *Proteus vulgaris* was observed when tested against the African pear seed ethanolic extract compared to the zone of inhibition(18mm) for *Klebsiella pnuemoniae* and (17mm) for *Proteus vulgaris* observed when tested against gentamicin antibiotic.

DISCUSSION

Chemotherapeutic or antimicrobial properties of substances are useful and necessary weapons for microbiologists in the fight or eradication of (pathogenic) microorganisms most importantly in food industry (food spoilage) and in the treatment of infectious diseases, as their active components can inhibit the growth and interfere with the metabolism of microorganisms in a negative manner[23,30,33]. The antimicrobial of raw pulp and seed, ethanol and aqueous extracts of African pear (*Dacryodes edulis)* against selected medical important human pathogens were reported in a study carried out by Idu *et al.,* 2013, the result of the antimicrobial screening of the African pear pulp and seed extracts revealed that the test (clinical) isolates were more susceptible to ethanol extracts of the seed by showing a higher zone of inhibitions as compared to its counterpart which was the pulp extracts. The susceptibility of the tested (clinical) isolates increased with increasing concentrations of the extracts as observed

Table 3: Antimicrobial activity testing of African pear pulp extracts at different concentrations on the collected bacteria isolate

Bacteria isolate	Concentration of extracts (g/10ml)	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* (10µg)	*Amp* (10µg)	*Cip* (5µg)	*Ery* (15µg)	*Oflox* (5µg)
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	-					
Escheriohia 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: = No zone of inhibition, Gen= Gentamycin, Amp= Ampicillin Cip= Ciprofloxacin, E= Erythromycin, Oflox = Ofloxacin.

in the zones of inhibition obtained. This is an indication that the antimicrobial compounds in the investigated *Dacryodes edulis* plant could be polar as the inhibition zones of the tested organisms are function of relative antibacterial activity of the extracts[20,24]. However, this observation is in agreement with that of Idu *et al.,* 2013 and Ilusanya *et al.*, 2012[20,21], who reported a higher zone of inhibition when ethanol and chloroform leaf extracts of *Garcinia kola, Chrysophyllum albidum* and *Dacryodes edulis* were tested against some microbial pathogens. The phytochemical screening of the raw and boiled mesocarp and seed of African pear used in the research showed the presence of secondary metabolites such as Tannins, Alkaloids, Glyosides, Flavonoids, Reducing Compounds, Saponins, and Polyphenol in excess in the ethanol extracts of both the raw and boiled seed and mesocarp of the samples as compared to the aqueous extract of both the raw and boiled seed and mesocarp of the samples. This could have been the rationale behind the earlier observed susceptibility of the tested isolates to increasing concentrations of ethanol extracts of both the pulp and seed. Similar study by Idu *et al.,* 2013 reported that alkaloids are known to exhibit marked physiological activity when administered to animals, as these antioxidants are compounds that reduce the formation of free radicals or react to neutralize them. Thus, potentially protecting the cells from oxidative damage[20,27]. Saponins are responsible for most of the biological effects in herbal medicines, they are also key ingredient in traditional Chinese medicine[20,25,31]. Tannins and saponins have been reported for their astringency and foaming properties respectively[26]. However, this observation, suggest that the presence of these aforementioned bioactive phytochemicals in the extracts of the investigated *Dacryodes edulis* fruit might be responsible for its potency against some bacteria isolates.

CONCLUSION

The results of findings of the antimicrobial activity test of the pear (*Dacryodes edulis)* against selected human pathogens could of immense benefit to pharmaceutical industries for the development of new antimicrobial or chemotherapeutic drugs 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. The consumption of African pear will also serve

Table 4: Antimicrobial activity testing of African pear seed extracts at different concentrations on the collected bacteria isolate

Bacteria isolate	Concentration of extracts (g/10ml)	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 (10µg)	Amp (10 µg)	Cip (5µg)	Ery (15µg)	Oflox (5µg)
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	-	-					

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

as a defense in the body system due to the results from the antimicrobial activity detected in this study.

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