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Phytochemical Studies on Stem Bark Extracts of Dacryodes edulis and their Anti-Pathogenic Activity against some Common Clinical Isolates

Nna P.J.[1], Tor-Anyiin T.A.[2], Igoli J.O.[2]

[1]Department of Chemistry, Ignatius Ajuru University of Education, Port Harcourt, Nigeria.
[2]Department of Chemistry, University of Agriculture, Makurdi, Benue State, Nigeria.
Corresponding author: agaraprince@yahoo.com

Abstract *Dacryodes edulis* is a plant with many trado-therapeutic claims, the stem is used traditionally as a remedy for stomach problems like diarrhoea, dysentery and stomachache. The stem of *D. edulis* was exhaustively extracted using hexane, ethyl acetate, and methanol. These extracts were phytochemically screened for the presence of reducing sugars, saponins, cardiac glycosides, flavonoids, steroids/triterpenoids, phenols/tannins and alkaloids. Results showed the presence of steroids/triterpenes, tannins, saponins, alkaloids and flavonoids. The extracts showed sensitivity against seven microbes: *Staphylococcus aureus, Methicillin Resistant Staphylococcus, aureas, Vancomycin Resistant. Enterococci Pseudomonas aeruginosa, P.mirabilis and fungi: Candida albicans, Candida krusei.*The ethyl acetate extract, using diameter of zone of inhibition as criteria for inhibitory strength, exhibited highest inhibition against the pathogens that were sensitive to it with *Staphylococcus aureas* being most inhibitive (29mm). Ethyl acetate extract showed highest growth inhibitory effects (Minimum Inhibition Concentration) for the microbes at concentration of 1.25 mg/mL. All other extracts had MBC/MFC (Minimum Bactericidal/ Fungicidal Concentration) value of 10 mg/mL. The presence of some biologically active constituents as well as the sensitivity shown against the selected clinical pathogens lends credence to the trado-medicinal claims on the stem of *Dacryodes edulis* as remedy for stomach problems and other ailments.

Keywords *Dacryodes edulis*, Stem bark extracts, phytochemical analysis, Minimum inhibitory concentration, Minimum bactericidal concentration

Introduction

Plants are important in our everyday existence for their uses as medicine or food. The plant kingdom has proven to be the most useful in the treatment of diseases and thus provide an important source for the World's pharmaceuticals. The primary source of curing man's ailments in nature was traceable to the use of natural substances found around us [1]. Substances derived from plants and animals serve as basic ingredients for folk medicine and thus the use of plants for the treatment of diseases is dependent on the presence of some chemical substances known as secondary metabolites or phytochemicals.

The generic name Dacryodes is derived from the Greek word *Dakruon* meaning tear, referring to droplets on the bark surface of its members, while *edulis* means edible, emphasizing the importance of the nutritious fruit in the plant's cultivation [2].

Dacryodes edulis is of the plant family, Burseraceae, also known as the torchwood family, consisting of 17-18 genera and about 540 species of flowering plants. *Dacryodes edulis*, is commonly called; *ube* (Igbo) and *Mzembe* (Tiv), languages all in Nigeria. English name includes; African pear and French, Satoutier [3]. The plant stem is trado-medically used in the treatment of dysentery, diarrhea, cough, cramps and other muscle pains. This study was

therefore undertaken to screen phytochemically for secondary metabolites in the stem bark extracts of this plant as well as the antimicrobial activities of the extracts to ascertain the ethnomedical potentials of the plant.

Plant Collection and Extraction

The stem bark of *D. edulis* was collected from Bunu Tai, Tai Local Government Area, River State, Nigeria, in July 2016, identified and authenticated at the Department of Forestry, Rivers State University, Port Harcourt by Dr. David Wisuator with specie code 2:255. The ground stem (150g) was extracted sequentially with hexane, ethyl acetate and methanol (500mL) each by microwave assisted extraction as described by [4]. All extracts were allowed to evaporate to dryness in a fume cupboard.

Preliminary Phytochemical Screening

Phytochemical screening was carried out on the plant extracts to identify presence of pharmacologically active metabolites such as alkaloids, flavonoids, saponins, phenols/tannins, cardiac glycosides, reducing sugars and steroids/terpenoids using standard procedures as described by [4] and [5].

The Antimicrobial Screening

The antimicrobial activity of the plant extracts was determined using some clinical pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. The extract (0.1 g) was weighed and dissolved in 10 mL of DMSO to obtain a concentration of 10 mg/mL. This served as initial concentration for determination of the antimicrobial activity. Agar diffusion method was used for screening the extracts. Mueller Hinton Agar (MHA) medium was used for growth of microbes. The media were prepared according to manufacturer instruction, sterilized at 121 °C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify. The sterilized media were then seeded with 0.1 mL of the standard inocula test microbes. Inocula were spread evenly over the media surfaces by the use of sterile swabs. Using a standard cork borer (6 mm), a well was cut at the center of each inoculated medium. Solution of the extract (0.1ML) of concentrated 30mg/mL was then introduced into each well on the medium. The inoculated medium was then incubated at 37 °C for 24 hours for bacteria and at 30^0C, for 7 days for fungi, after which each plate was observed for inhibition zone of growth. Zone of inhibition was measured with a transparent ruler and the result recorded in millimeters as described by [6].

Minimum Inhibition Concentration (MIC)

The minimum inhibition concentrations of the extracts were determined using broth dilution method. Mueller Hinton broth was prepared; 10 mL was dispensed into test-tubes and sterilized at 121 °C for 15 minutes and allowed to cool. MC-Farland's turbidity standard scale number 0.5 was prepared to give turbidity solution. Normal Saline was prepared, 10 mL was dispensed into sterile test-tubes and the test microbe was inoculated and incubated at 37 ^{o}C for 6 hours. Dilution of the test microbe was done in the normal saline until the turbidity matched that of the MC-Farland's scale by visual comparison. At this point, the test microbe had a concentration of about 1.5×10^6 cfu/mL. Two-fold serial dilution of the extract was done and the sterile broth was made to obtain concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.33 mg/mL respectively. The initial concentration of the extract was obtained by dissolving 0.3 g of the extract in 10 mL of the sterile broth. Having obtained different concentrations of the extract in the sterile broth, 0.1 mL of the test microbe in normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 7 days for the fungi, after which the test tubes were observed for turbidity growth. The lowest concentration of an extract in the sterile broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC) as described by [6]

Minimum bactericidal concentration / Minimum fungicidal concentration

Minimum bactericidal concentration/Minimum fungicidal concentration was carried out to determine whether the test microbes were killed or not. Mueller Hinton and Sabour and dextrose agar were prepared and sterilized at 121 °C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify. Contents of the MIC in the serial

dilution were sub-cultured onto the prepared media, incubation were made at 37 °C for 24 hours for bacteria and 30 °C for 7 days for fungi, after which the plates of media were observed for colony growth. The MBC/MFC were the plates with lowest concentration of extracts without a colony growth.

Results

Table 1: Phytochemical screening of crude extracts obtained via microwave assisted extraction

Phytochemicals	**Results of Phytochemical Screening**		
	ME	**EA**	**HE**
Reducing sugars	++	+	-
Saponins	+	++	-
Steroids/triterpenoids	-	+	++
Cardiac glycosides	+	+	-
Phenols/tannins	+++	++	-
Alkaloids	+	+	-
Flavonoids	+	+	-

Key: ME = Methanol, EA= Ethyl acetate, HE= n- hexane, + = weakly positive, ++ = Positive, +++ = Strongly Positive, - = negative.

Table 2: Sensitivity/ Zone of Inhibition

Test pathogen	**E.A**	**ME**	**HE**	**SF**	**FZ**
MRSA	S (28)	S (23)	S (21)	R(0)	R(0)
VRE	S (26)	S (22)	S(20)	S(29)	R(0)
S. aureas	S (29)	S (24)	S (21)	S(34)	R(0)
E. coli	R (0)	R (0)	R (0)	S(35)	R(0)
S. typhi	R (0)	R (0)	R (0)	S(30)	R(0)
P.mirabilis	S (26)	S (24)	S (22)	R(0)	R(0)
P. aeruginosa	S (27)	S (23)	S (21)	S(31)	R(0)
C.albicans	S (25)	S (22)	S (21)	R(0)	S(35)
C. krusei	S (26)	S (23)	S (22)	R(0)	S(34)
C. tropicalis	R (0)	R (0)	R (0)	R(0)	S(32)

KEY: S = Sensitive, R = Resistant, Numeric value in brackets = Diameter of zone of inhibition in millimetres (mm)\ Negative control = Normal saline, EA= Ethyl acetate, HE=Hexane, ME=Methanol, FZ= Fluconazole, VRE= *Vancomycin Resistant. Enterococci,* SF= Sparfloxan, Negative Control = Normal Saline
MRSA=*Methicillin Resistant Staphylococcus aureas.*

Table: 3 Minimum Inhibition Concentrations (MIC) (mg/mL) of Extracts against Test Microorganism (mm)

Test pathogen	**EA**						**ME**						**HE**					
	10	**5**	**2.5**	**1.25**	**0.625**	**0.312**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.312**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.312**
MRSA	-	-	-	ox	+	++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
VRE	-	-	-	ox	+	++	-	-	ox	+	++	+++	-	-	Ox	R	R	R
S. aureas	-	-	-	ox	+	++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. typhi	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
P.mirabilis	-	-	ox	+	++	+++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
P. aeruginosa	-	-	-	ox	+	++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
C.albicans	-	-	ox	+	++	+++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
C. krusei	-	-	ox	+	++	+++	-	-	ox	+	++	+++	-	-	Ox	+	++	+++
C. tropicalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: - = No turbidity (no growth), ox = MBC/MFC, + = Turbidity (light growth), ++ = Moderate turbidity, +++ = heavy turbidity, R = pathogen is resistant to extract, VRE= *Vancomycin Resistant. Enterococci,* MRSA =*Methicillin Resistant Staphylococcus aureus*

Table 4: Minimum Bactericidal Concentrations (MBC) / Minimum Fungicidal Concentrations (MFC) (mg/ml) of Extract against Test Microorganism (mm)

Test pathogen	EA						ME						HE					
	10	**5**	**2.5**	**1.25**	**0.625**	**0.312**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.312**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.312**
MRSA	-	-	ox	+	++	+++	-	ox	+	++	+++	++++	ox	+	++	+++	+++	++++
VRE	-	ox	+	++	+++	++++	ox	+	++	++	+++	++++	ox	+	++	+++	+++	++++
S. aureas	-	-	ox	+	++	+++	-	ox	+	++	+++	++++	ox	+	++	+++	+++	++++
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. typhi	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
P.mirabilis	-	ox	+	++	+++	++++	-	ox	+	++	+++	++++	ox	+	++	+++	+++	++++
P. aeruginosa	-	-	ox	+	++	++++	-	ox	+	++	+++	++++	ox	++	+++	+++	+++	++++
C.albicans	-	ox	+	++	+++	++++	ox	+	++	+++	+++	++++	ox	+	++	+++	+++	++++
C. krusei	-	ox	+	++	+++	++++	-	ox	+	++	+++	++++	ox	+	++	+++	+++	++++
C. tropicalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: - = No turbidity (no growth), ox = MBC/MFC, + = Turbidity (light growth), ++ = Moderate turbidity, +++ = high turbidity, ++++ = Heavy turbidity, R = pathogen is resistant to extract, VRE= *Vancomycin Resist. Enterococci*, MRSA =*Methicillin Resistant Staphylococcus aureas*

Discussion

Phytochemical screening of stem bark extracts of *Dacryodes edulis* showed the presence of reducing sugars, cardiac glycosides, phenols/tannins, alkaloids, saponins and flavonoids in ethyl acetate and methanol extracts. Steroids and triterpenoids were found to be present in ethyl acetate and n- hexane extracts (Table 1). Investigations into the mode of action of secondary metabolites have indicated that tannins have anti-viral, antibacterial and antiparasitic effects [7]. Alkaloids are used in medicine as anaesthetic and antiinfective agents and are also noticed for their microbial effects on small intestine and antihypertensive potencies [8]. Saponins have been reported to have antihypercholesterol, hypertensive and also responsible for antimicrobial, antifungal, anti-inflammatory and antidote activities [9]. This enhances its features as an antifeedant and help to protect plant against microbes and fungi. Flavonoids have shown anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [10]. The presence of steroids account for the usage of the plant in the treatment of theraupeutic applications as arrow poisons or cardiac drugs as laxatives, thus, it serves as an antioxidant agent. Triterpenoids have been reported for their anti-cancer, anti-inflammatory and anti-bacterial potentials [11]. Cardiac glycoside was present in the sample and it has been reported as stimulants in treatment of cardiac failure and diseases [12].

The antimicrobial activity of the stem bark extracts of *D. edulis* against 10 pathogenic microorganisms by the broth dilution method and diffusion method is reported in (Table 2 and 3). The results obtained revealed anti-pathogenic activity of stem bark extract of *Dacryodes edulis* against *Vancomycin Resistant Enterococci, Methicillin Resistant Staphylococcus aureas, Pseudomonas aeruginosa, Candida albicans*, *Staphylococcus aureus, Candida krusei* and *Proteus mirabilis* were also sensitive to the extracts (Table 3). In this regard, ethyl acetate extract showed highest sensitivity/ zone of inhibition against *Staphylococcus aureus and Methicillin Resistant Staphylococcus aureas* at 29 mm and 28 mm, respectively (Table 2). Ethyl acetate extract had the lowest minimum inhibition concentration (MIC) of 1.25 mg/mL against *Methicillin Resistant Staphylococcus aureas*, *Vancomycin Resistant Enterococc,* and *Pseudomonas aeruginosa* pathogen (Table 3). For hexane and methanol extracts, MIC of 2.5 mg/mL was observed against test pathogens except *Escherichia coli*, *Salmonella typhi* and *Candida tropicalis* (Table 3). The minimum bactericidal concentrations of the extracts against test pathogens were found to be 1.25 mg/mL for ethyl acetate extract against *Vancomycin Resistant Enterococci*, *Pseudomonas aeruginos,* for hexane extract it was 10 mg/mL against all sensitive tested pathogens (Table 3). Methanol extracts showed (MBC) activities at (10 mg/mL) against *Vancomycin Resistant Enterococci and Candida albicans* (Table 3). Escherichia coli, *Salmonella typhi* and *Candida albicans* were resistant to all extracts. The antibacterial and antifungal activities of these extracts could be related to the presence of secondary metabolites detected in the plant. This justifies the traditional usage of the stem as remedy for stomach problems.Minimum bactericidal/ fungicidal concentration of the stem extracts of *Dacryodes edulis*

presented in Table 4, showed that, the test organisms could be inhibited at concentration of 0.132mg/mL except *E. coli, S. typhi* and *C. albicans* in all three solvent extracts used in the study and extract could not be unrelated to the presence of the plant secondary metabolites detected.

Conclusion

The presence of the metabolites in the stem bark of *Dacryodes edulis* justifies its traditional claims in trado-medicine. However, the high level of pathogenic sensitivity and activity against the selected clinical pathogens with comparable activity to the reference drugs under study also account for the antimicrobial potentials of the plant. Thus, providing some scientific evidences for the folkloric claims as documented by relevant literatures. The isolation and possible spectroscopic characterization of the bioactive constituents from the extracts of this plant species as possible antimicrobial agent is recommended.

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