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Phytochemical and Antimicrobial Screening of Root Extracts of *Dacryodes edulis*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TATA, JOI, MEK and PJN designed the study, wrote the protocol, approved the first draft of the manuscript. Authors PJN and Author JVA managed the analyses of the study. Authors PJN and JVA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To screen extracts of *Dacryodes edulis* phytochemically. To assay the said extracts for antimicrobial activity. To validate or otherwise claims by traditional medicine practitioners on *Dacryodes edulis*.
Place and Duration of Study: Department of chemistry, University of Agriculture, Makurdi, Nigeria, from October 2016 to November 2016.
Methodology: Roots of *Dacryodes edulis* extracted using n-hexane, ethyl acetate, and methanol, were phytochemically screened for presence of secondary metabolites. Anti-microbial assay of extracts was done using *Methicillin resist Staph. aureus, vancomycin resist enterococci, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Candida albicans, Candida krusei,* and *Candida tropicalis*.
Results: Results showed presence of steroid/triterpenes, phenols, reducing sugars, cardiac glycosides, alkaloids and flavonoids. Anti-microbial screening of extracts showed sensitivity against six microbes: *Staphylococcus aureus; Salmonella typhi; Pseudomonas aeruginosa, methicillin*

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resist, staphylococcus aureus, Candida albicans and *Candida krusei*. Ethyl acetate extract exhibited highest zones of inhibition against *Staphylococcus aureus* and *Candida albicans* (28 mm) and highest growth inhibitory effects (MIC) for the microbes at concentrations of 1.25 mg/mL and 2.5 mg/mL. All other extracts had Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC), values of 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively.
Conclusion: This study has justified the traditional use of the plant for treatment of various diseases that are caused by these organisms.

Keywords: Dacryodes edulis, phytochemical analysis, antimicrobial activity, root extracts, Minimum bactericidal/fungicidal concentration.

1. INTRODUCTION

Plants are the basis of traditional medicine system which have been used for thousands of years [1]. "Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat or to diagnose and prevent illnesses or maintain wellbeing" [2]. Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs [3]. In Nigeria and most developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine [4]. Substances derived from plants have recently being of great interest due to their versatility. Medicinal plants are the best bio-resource for drugs, for traditional and modern systems of medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [5-7].

Plant products derived from barks, leaves, flowers, roots, fruits and seeds have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information is valuable for synthesis of complex chemical substances [8–10].

In Nigeria, stems and roots of *Dacryodes edulis* are used as chewing sticks for oral hygiene and to treat cardiovascular diseases, while leaves are employed to cure skin conditions, such as rashes, scabies, ringworm and wounds [11,12]. Different parts of the plant, such as leaves stems (exudate), roots and fruits, reportedly, contain monoterpenes, sesquiterpenes, diterpenes and triterpenes [13–15].

Dacryodes edulis (G. Don) H.J Lam, (Burseraceae) African pear, is an evergreen fruit common to Central Africa and Gulf of Guinea region [16],(synonym: *Pachylobus edulis* G. Don). It has a relatively short trunk and deep dense crown; attains a height of 18-40 m in the forest but not exceeding 12 m in plantations. The plant prefers shady, humid, tropical forest areas. The natural range extends from Angola in the south, Nigeria in the North, Sierra Leone in the West and Uganda in the east [17]. It is also cultivated in Malaysia [18]. It bears fruits which are edible; the bark, leaves, stems, and roots are used as local medicine against some diseases [14,19,20].

Dacryodes edulis has a long history of use in folk medicine [21]. Traditional healers in Nigeria and in the Democratic Republic of Congo use the plant for treatment of parasitic skin diseases, jigger, mouthwash, tonsillitis and drepanocytosis [22,23]. This study investigates the phytochemicals present and the antimicrobial potentials of its root extracts from hexane, ethyl acetate and methanol.

2. MATERIALS AND METHODS

The roots of *D. edulis* were collected in Sime Tai, Tai Local Government Area of River State, Nigeria in Nov, 2016. The plant was identified and authenticated at the Department of Forestry, Rivers State University of Science and Technology, Port Harcourt by Dr. David Wisuator with general specie index of 2:255. Pulverized plant material (5 kg) was sequentially extracted with hexane (2500 mL); ethyl acetate (2500 mL) and methanol (2500 mL) via microwave assisted extraction, [24]. Microwave Assisted Extraction was done using a domestic microwave oven following methods described by Anyam et al. [24]. The powdered root (1 Kg) was distributed into 3 Winchester bottles (2.5 L each). Hexane, Ethyl Acetate and Methanol (2500 mL each) were introduced (833 mL/vessel) into the extraction vessels (Winchester bottles). The sample was then irradiated with microwaves (70

Watts/Defrost Function) using a modified domestic kitchen microwave (Mio-star, Model 7173.295, Germany) for 30 minutes, pausing every three minutes to effect cooling and vent pressure build up. Extracts were filtered and excess volume reduced in vacuum using rotary evaporator at 50°C, and then evaporated to dryness in a fumehood at room temperature prior to further analysis.

2.1 Preliminary Phytochemical Screening

Phytochemical tests were carried out on the extracts to identify secondary metabolites such as steroid/triterpenes, saponins, phenols, reducing sugars, cardiac glycosides, alkaloids and flavonoids using standard procedures [24,25].

2.2 Antimicrobial Studies

Antimicrobial screening of methanol, ethyl acetate, and n-hexane root extracts was determined using the following pathogens; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, methicillin resist. Staphylococcus aureus, vancomycin resist enterococci, Proteus mirabilis* and the fungi *Candida albicans, Candida krusei,* and *Candida tropicalis,* obtained from Department of Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria. All the clinical isolates were checked for purity and maintained in slant of nutrient agar. Well diffusion method was used to determine the antimicrobial activities of the extracts [26].

100 mg extract was dissolved in 10 mL of Dimethyl sulphoxide (DMSO) to obtain a concentration of 10 mg/mL. Mueller Hinton and Sabouraud dextrose agar were the media used for growth of the bacteria and fungi, respectively. All media were prepared according to the manufacturer's instruction, sterilized at 121°C for 15 minutes and poured into sterile petri dishes which were allowed to cool and solidify. The sterilized Mueller Hinton agar was seeded with 0.1 mL of standard inoculum of the test bacteria while Sabouraud dextrose was seeded with 0.1 mL of standard inoculum of the test fungi. The inocula were then evenly spread over the surface of the media using sterile swab. A sterile standard cork borer of 6 mm in diameter was used to cut a well at the centre of each inoculated medium. About 0.1 mL of solution of an extract of 10 mg/mL concentration was then introduced into the well on the medium. Incubation for bacteria was made at 37°C for 24 hrs and 30°C one week for fungi. Each plate was then observed for zone of inhibition of growth, which was measured with a transparent ruler and the result recorded in millimetres.

2.2.1 Minimum inhibition concentration (MIC)

Minimum inhibition concentration (MIC) of extract was carried out on an extract that had shown growth inhibitory on test organisms. It was done using broth dilution method [27] and modified by [28]. In this method, Mueller Hinton and Sabouraud dextrose broth were prepared according to the manufacturer's instruction. About 10 mL of broth was dispensed into test tubes, separated and sterilized at 121°C for 15 mins and allowed to cool. Mc-Farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared and used to make a turbid suspension of the microbes and 10 mL was dispensed into test tubes and test microbes were inoculated and incubated for 6 hrs at 37°C. Dilution of the micro-organism in the normal saline was continuously done until the turbidity (1.5×10^6 cfu/mL) matched that of Mc-Farland scale by visual comparison. Two fold serial dilution of an extract in sterile broth was done to obtain the following concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL and 0.313 mg/mL. Having obtained different concentrations of an extract in the broth, 0.1 mL of the standard inoculum of microbes was inoculated on to the different concentrations. Incubation for the bacteria, was made at 37°C for 24 hrs and at 30 °C for one week for fungi. The test tubes were then observed for turbidity. The lowest concentration of an extract in the broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC).

2.2.2 Minimum bactericidal/ fungicidal concentration (MBC/MFC)

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) were done to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabouraud dextrose agar were prepared according to manufacturer's instruction, sterilized at 121°C for 15 minutes and poured into sterile petri-dishes and the plates were allowed to cool and solidify. The contents of the MIC in serial dilution were then sub-cultured into the prepared media, were then incubated at 37°C for 24 hours for bacteria and at 30°C for one week for fungi, after which

the plates were observed for colony growth. The MBC/MFC were the plates with lowest concentration of extract without colony growth. The results were recorded after 24 hours. Methods were as described by [27] and modified by [29].

3. RESULTS AND DISCUSSION

3.1 Result for Phytochemical Screening

Phytochemical screening for secondary metabolites of root extracts showed presence of steroid/triterpenes, saponins, phenols, reducing sugars, cardiac glycosides, alkaloids and flavonoids (Table 1).

Flavonoids which are polyhydroxylated compounds are known as antioxidants and are also associated with the treatment and control of cardiovascular diseases [30]. Tannins are known for their antimicrobial, anti-helminthic and anti-diarrhoea properties [31]. Thus, the presence of flavonoids and tannins contribute to the various medicinal properties of the plant under study as they are known to have antiviral, antibacterial and antifungal properties. Cardiac glycosides serve as defences against cardiovascular diseases [32]. The presence of cardiac glycosides could explain its therapeutic effect against cardiovascular and digestive problems by herbalists. Steroids of plant origin have been shown moderate lipids profiles to acceptable levels [33], promote immune function and reduce inflammation [34]. Saponins are natural glycosides that act as hypoglycaemic, antifungal and serum cholesterol lowering agents in animals [35]. The presence of saponins in the sample could be responsible for their use in local oral hygiene (twigs of the plant are used as chewing sticks). Phenolics have been shown to possess antiseptic properties. Phenolics are also potent anti-oxidants and together with flavonoids are reported to have anti-mutagenic anticarcinogenic effects [36]. An *in vitro* and *in silico* study by Zafou et al. [37] have predicted possible use of *Dacryodes edulis* as an antimalarial.

Study by Omogbai and Eneh [16] on the aqueous and alchohol extracts of *Dacryodes edulis* seeds revealed the antimicrobial nature of the plant. Anyam et al. [24] have in a series of reports [24] examined and revealed medicinal principles in the boiled and raw seeds of *Dacyodes edulis*.

3.2 Result for Antimicrobial Sensitivity Test

Antimicrobial sensitivity test for ethyl acetate, methanol and hexane root extracts of *Dacryodes edulis* showed sensitivity against six microbes. *Staphylococcus aureus, Salmonella typhi. Pseudomonas aeruginosa*, *methicillin resist. Staphylococcus aureus* and fungi: *Candida albicans* and *Candida krusei.* Sparfloxacin ® was sensitive against 5 microbes, while Fluconazole showed sensitivity against 3 microbes. The Ethyl acetate extract exhibited considerable level of inhibition against the test organisms. The microbes *Staphylococcus aureus* and *Candida albicans* were most inhibited (28 mm); Sparfloxacin ® showed the highest inhibition of 34 mm and 35 mm, respectively (Table 2).

3.3 Result for Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for the ethyl acetate, hexane and methanol extracts showed inhibition against all the pathogens that were sensitive to the extracts with MIC at concentration of 1.25 mg/mL for *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Candida albicans*; however, *Methicillin resist aureus* and *Candida krusei* had MIC at 2.5 mg/mL in ethyl acetate extract. Hexane and methanol extracts had MIC values of 1.25 and 2.5 mg/mL, respectively (Table 3).

Table 1. Phytochemical screening of root extracts of *Dacryodes edulis*

Test	Methanol	Ethyl acetate	n-hexane
Cardiac glycosides	+	+	-
Flavonoids	+	-	-
Phenols	+++	+	-
Saponins	++	+	+
Steroids	+	+	+
Reducing sugars	++	+	-
Alkaloids	+	+	-

Key = + weakly positive; ++ positive; +++ strongly positive; - negative

Table 2. Sensitivity/Zone of Inhibition (mm) of extracts against test microorganisms

Test organism	Ethyl acetate	Methanol	n-hexane	Sparfloxacin ®	Fluconazole
Methicillin resist staph. aureus	S (25)	S (23)	S (20)	R	R
vancomycin resist enterococci	R	R	R	S (29)	R
Staphylococcus aureus	S (28)	S (25)	S (22)	S (34)	R
Escherichia coli	R	R	R	S (35)	R
Salmonella typhi	S (27)	S (24)	S (21)	S (30)	R
Proteus mirabilis	R	R	R	R	R
Pseudomonas aeruginosa	S (26)	S (22)	S (20)	S (31)	R
Candida albicans	S (28)	S (23)	S (21)	R	S (35)
Candida krusei	S (25)	S (22)	S (20)	R	S (34)
Candida tropicalis	R	R	R	R	S (32)

Key = S Sensitive; R= Resistant

Table 3. Minimum inhibitory concentration (mg/ml) of extracts against test microbes

Pathogen	Ethyl acetate						Methanol						n-hexane					
	10	5	2.5	1.25	0.625	0.313	10	5	2.5	1.25	0.625	0.313	10	5	2.5	1.25	0.625	0.313
Methicillin resist Staph. Aureus	-	-	0#	+	++	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Vancomycin resist enterococci	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	-	-	-	0#	+	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Escherichia coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Salmonella typhi	-	-	-	0#	+	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Proteus mirabilis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas aeruginosa	-	-	-	0#	+	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Candida albicans	-	-	-	0#	+	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Candida krusei	-	-	0#	+	++	++	-	-	0#	+	++	+++	-	-	0#	+	++	+++
Candida tropicalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: - = No turbidity (no growth), 0#= MIC, + = Turbidity (light growth), ++ = Moderate turbidity, +++ = heavy turbidity, R = pathogen is resistant to extract

Table 4. Minimum bactericidal/fungicidal concentration (mg/ml) of extracts against test microbes

Test microbes	Ethyl acetate						Methanol						n-hexane					
	10	5	2.5	1.25	0.625	0.313	10	5	2.5	1.25	0.625	0.313	10	5	2.5	1.25	0.625	0.313
Methicillin resist Staph. aureus	-	***µ***	+	++	+++	+++	-	***µ***	+	++	+++	++++	***µ***	+	++	+++	+++	++++
Vancomycin resist enterococci	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	-	-	***µ***	+	++	+++	-	***µ***	+	++	+++	++++	***µ***	+	++	+++	+++	++++
Escherichia coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Salmonella typhi	-	-	***µ***	+	++	+++	-	***µ***	+	++	+++	++++	***µ***	+	++	+++	+++	++++
Proteus mirabilis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas aeruginosa	-	***µ***	+	++	+++	+++	***µ***	+	++	++	+++	++++	***µ***	+	++	+++	+++	++++
Candida albicans	-	-	***µ***	+	++	+++	-	***µ***	+	++	+++	++++	***µ***	+	++	+++	+++	++++
Candida krusei	-	***µ***	+	++	+++	+++	***µ***	+	++	+++	+++	++++	***µ***	+	++	+++	+++	++++
Candida tropicalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

*Key: - = No turbidity (no growth), **µ** = MBC/MFC, + = Turbidity (light growth), ++ = Moderate turbidity, +++ = high turbidity, ++++ = Heavy turbidity, R = pathogen is resistant to extract*

3.4 Result for Minimum Bactericidal Concentration/ Minimum Fungicidal Concentration (MBC/MFC)

For minimum bactericidal concentration/minimum fungicidal concentration, ethyl acetate extract showed MBC and MFC of 2.5 mg/mL against *Staphylococcus aureus, Salmonella typhi,* and *Candida albicans* while *Methicillin resist Staph. aureus, Pseudomonas aeruginosa,* and 5 mg/mL for *Candida krusei*. Methanol extract showed MBC and MFC of 10 mg/mL for *Pseudomonas aeruginosa* and *Candida krusei* while *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans*, methicillin resist. *Staph aureus* were killed at 5 mg/mL. n-Hexane extracts killed all the pathogens at 10 mg/mL (Table 4).

The antimicrobial activities demonstrated by the root extracts are dependent on the presence of secondary metabolites (steroid/triterpenes, saponins, tannins, alkaloids, Cardiac glycosides, Reducing sugars, flavonoids and Phenols)identified in the root of *D. edulis*. A previous study on the stem bark of the plant of *D. edulis* by Zafou et al. [37] has indicated great potential for the plant in the battle against malaria. Anyam et al. [24] investigated activity of extracts of *D. edulis* against some clinical isolates, showing the plant to have great potential in treatment of disease.

4. CONCLUSION

This study was motivated by the strategic role played by *D. edulis* in West-Central African ethnobotanical traditions. The phytochemical study of the plant shows that many secondary metabolite classes (steroid/triterpenes, tannins, alkaloids, cardiac glycosides, flavonoids and phenols) known to exhibit antimicrobial behaviour and impact physiological effects are present in its root back. Extracts of the plants were sensitive against six test microbes (*Salmonella typhi, Staphylococcus aureus, methicillin resist. Staphylococcus aureus Pseudomonas aeruginosa,* and fungi: *Candida krusei and Candida albicans*) responsible for some common human ailments. *C. albicans* and *C. krusei* are implicated in many diseases of the old, very young and immune-compromised. Extracts were also delightfully sensitive against both *Staphylococcus aureus* and *methicillin resist. Staphylococcus aureus*; the two which bothersome in bedridden patients causing noisome ulcers and bedsores. Typhoid has become an emerging threat due to growing anti-microbial resistance to standard drugs; that the extracts were showed activity against *Salmonella typhi* is consoling when one thinks of potential drugs to be uncovered by a later study. As has been a recurring pattern in many antimicrobial studies involving plant extracts, the ethyl acetate extract showed the most promising activity against test pathogens. A future isolation could unravel the principle behind this activity. Extracts of this plant hold great promise in this unending war against disease. Subject to toxicity studies use of *D. edulis* roots in topical herbal applications is hereby encouraged. Also from these results the plant could be exploited for use in the formation of cheap alternative antimicrobial drugs that could cure and control infectious diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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