Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1910-1915
Received: 01-05-2017
Accepted: 02-06-2017

Abubakar Sani Yelwa
Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Halilu Emmanuel Mshelia
Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Ogbiko Cyril
Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Sherifat Ibrahim Lawal
Department of Chemistry, College of Preliminary Studies, Yelwa-Yauri, Kebbi State Nigeria

Hafsat Buhari Bature
Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Abdulhamid Shamsiya
Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Emem Roland Christiana
Department of Biomedical Science Technology, Federal Polytechnic Mubi, Adamawa State Nigeria

Correspondence
Ogbiko Cyril
Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria

Phytochemical screening, acute toxicity study and evaluation of *in vitro* antimicrobial activities of the fractions of *Dacryodes edulis* against selected clinical bacterial isolates

Abubakar Sani Yelwa, Halilu Emmanuel Mshelia, Ogbiko Cyril, Sherifat Ibrahim Lawal, Hafsat Buhari Bature, Abdulhamid Shamsiya and Emem Roland Christiana

Abstract
Dacryodes edulis is a dioecious, small to medium-sized tree, reaching 20 to 25 m high. Different parts of the plant are used to treat many diseases including skin infections, digestive tract disorder and dysentery. The leaves were extracted exhaustively using methanol and then partitioned using n-hexane and pet ether. The fractions were evaluated for their phytochemical constituents and antibacterial potentials. Phytochemical analysis revealed the presence of carbohydrate, flavonoid, phenolics, anthraquinone, cardiac glycosides, steroids and proteins. The methanol and ethyl acetate fractions showed moderate to significant activities against all bacterial strains except that of *Pseudomonas aeruginosa*. The n-hexane fraction showed no activity in all the bacteria strains investigated. The highest and lowest antimicrobial activity was observed for the ethyl acetate and methanol fractions respectively when compared to the standard drug. The results revealed the leaf extracts of *Dacryodes edulis* showed notable antibacterial activity which can be used as medicine.

Keywords: *Dacryodes edulis*, leaves fractions, phytochemical, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration

Introduction
As a result of the emergence of multi-drug resistant strains partly due to the indiscriminate use of antimicrobial drugs coupled with the problem of side effect associated with orthodox medication, there is a huge pressure on antibiotherapy [1]. To circumvent this problem, there is a global attention to search for new antimicrobial substances from plant origin [2, 3, 4, 5]. Medicinal plants have extensively been used in both developing and developed countries where it holds promising potentials as presently used in various traditional, complementary and alternate systems of treatment of human diseases [6, 7]. The World Health Organization (WHO) reports that 80% of the world populations rely mainly on traditional therapies for their primary health care need which involve the use of plant extracts or their active substances [8]. This is so because plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found *in vitro* to have antimicrobial properties [6, 7]. Nigeria has a great variety of natural vegetation, which is used in trado-medicine to cure various ailments [9]. 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 [10, 11].
Dacryodes edulis belonging to the family Burseraceae is a small to medium-sized tree reaching 20-25 m high and has a long history in folk medicine [12]. The plant is a shade loving species, dioecious and found in the humid tropical zone of non-flooded forest [11, 13]. In addition to its fruit being edible either cooked or raw, it is a rich source of minerals, vitamins, oils, and protein [14, 15]. Different parts of the plant are used in different parts of Africa in the treatment of various ailments. A wide range of chemical constitutes such as terpenes, flavonoids, tannins, alkaloids and saponins have been isolated from the plant [16]. In Nigeria, the stem and root are also used as chewing sticks for oral hygiene, while the leaves are employed to cure skin diseases, such as rashes, scabies, ringworm and wound [17, 18]. The leave's preparation is employed to digestive tract disorders, toothache, skin problems such as ringworm, scabies and rashes as well as earache [12, 19].

The stem-barks are used to cure dysentery and anemia [19]. The resin from the bark heals scars and other skin diseases in Nigeria [20]. Recently, it was reported that the leaves were made into plaster to treat snake bite in southwest Cameron [21] and the stem exudates of the plant contains tannin, saponin and alkaloids [22]. To the best of our knowledge, there has been no documented report on the antibacterial activity of the methanol, ethyl acetate and hexane fractions of the leaves of the plant native to Nigeria. It is in this regard that we saw the necessity to determine the chemical constituents of the leaves of *D. edulis*, ascertain its oral acute toxicity profile and investigate its antibacterial activity on selected clinical isolates.

Materials and Methodology

Collection and Identification of Plant Material

Fresh *D. edulis* leaves were harvested between the periods of April to June 2017 from its tree located at No 5 trans-ekulu municipality, Enugu East local government area of Enugu State Nigeria. They were placed in sterile containers and transported to Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto Nigeria for analysis. Botanical identification and authentication of the sample was done by a plant Taxonomist of the Department of Botany, Faculty of Science, Usmanu Danfodiyo University Sokoto Nigeria.

Preparation and Extraction of *Dacryodes edulis* leaves

The fresh leaves were washed to remove earthy impurities and dried under shade for a period of 3 weeks for complete drying. The dried leaves were grounded to fine powder with the aid of a mechanical grinder. Methanol, n-hexane and ethyl acetate were the extracting solvents used in extracting the phytochemicals from *D. edulis* leaves. 300 grams each of the powdered leaves were extracted successively with 800 ml of the solvents in separate separating funnel for 24 hours to obtain the methanol extract (ME), n-hexane extract (HE) and the ethyl acetate extract (EA). After 24 hours of extraction, the suspensions were vigorously shaken and filtered with Whatmann No 1 filter paper. The filtrate was subsequently concentrated under reduced pressure at 45°C in rotator evaporator (Stuart RE 300) and dried at room temperature to constant weight.

Phytochemical analysis

Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites in the different extracts were done in accordance with standard methods [23, 24, 25].

Animal treatment

30 adult Swiss albino mice of both sexes were obtained from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto State Nigeria. The animals were housed in polypropylene cages with wood shavings and marked for easy identification. The animals were fed with standard laboratory animal food pellets.

Acute Toxicity Studies

Adult mice (15 – 23 g), nulliparous and non-pregnant of either sex were fasted overnight but with free access to water. The animals were randomly divided into five groups of 5 animals each and the crude methanol extract was administered orally at doses of 500, 1000, 2000 and 4000 mg/kg body weight to groups I, II, III and IV respectively while the control group V, received distilled water by same route. The test substance was administered in a single dose by gavage using a locally designed rat oral needle for acute toxicity study. General symptoms of toxicity and mortality in each group were observed within 24 hours. Animals that survived after 24 hours were observed for another 14 days (no extract administration) for any sign of delayed toxicity [26].

Collection of Bacterial strains

The antibacterial effect was tested on a panel of bacterial isolates including two Gram positive: *Staphylococcus aureus* and *Bacillus subtilis* and two Gram negative *Escherichia coli* and *pseudomonas aeroginosa* bacteria. All isolates were obtained from the Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto Nigeria.

Confirmation of test organisms

Biochemical analysis methods as described by MacFaddin [27], Fobres and Sham [28] and Leboffe and Pierce [29] were carried out on each of the test bacteria isolates for confirmation. The Bergey's Manual of Systematic Bacteriology [30] was used for species authentication.

Susceptibility Test

The susceptibility of the bacterial strains to the *D. edulis* leaves extract was investigated as described by Valgas *et al*., 2007 [31] with slight modification. Overnight cultures of the bacterial strains were spread on sterile Mueller-Hinton agar. With the aid of a sterile 6 mm cork borer, six equally spaced wells were bored in the agar plate with the fifth well at the center of the plate. Concentrations of 15 mg/ml, 20 mg/ml, 25 mg/ml and 30 mg/ml of the different fractions of *D. edulis* leaves were prepared by dissolving each extract in 10% tween-80. 50 µL of ME, HE and EA extracts each at the different concentrations were introduced into 4 of the 6 wells. The fifth well was filled with 50 µL of 10 % tween-40 while the central well (the sixth well) was filled with 20 µL of the standard antibiotic (ciprofloxacin 20 µg/mL) to serve as negative and positive controls respectively. The test was carried out in triplicate, incubated for 24 hours at 37 °C and examined for zones of inhibition. The diameter of the zone of inhibition was measured using a sliding caliper (mm) and the mean considered as the inhibition diameter.

Determination of the inhibitory parameters

Minimum inhibition concentration (MIC)

The MIC of the different extracts was carried out on an extract that has shown growth inhibitory effect on a test organism. It was done using broth dilution method as described by [32] and modified by [43]. 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 the extract in sterile broth was done to obtain the following concentrations of 15 mg/mL, 20 mg/mL, 25 mg/mL and 30 mg/mL. Having

obtained different concentration of the extracts in the broth, 0.1mL of the standard inoculums of microbes was inoculated into the different concentrations. Incubation for the bacteria was made at 37 ºC for 24 hrs. The lowest concentration of an extract in the broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC).

Minimum Bactericidal Concentration (MBC)

The MBC was 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 ºC for 15 mins and poured into sterile petri-dishes, and then plates were allowed to cool and solidify. The contents of the MIC in serial dilution were then sub-cultured on to the prepared plates and plates were then incubated at 37 ºC for 24 hrs after which the plates were observed for colony growth. The MBC plates with the lowest concentration of the extracts without colony growth were recorded [32, 33].

Results and Discussion

Preliminary phytochemical analysis

The preliminary phytochemical screening for the secondary metabolites of the different fractions of *D. edulis* leaves is presented in Table 1.

Table 1: Phytochemical screening result for the fractions of *D. edulis* leaf extract

Phytochemicals	Test		Observation (colour/ppt)			Inference		
			CH_3OH	EtOAc	n-hexane	CH_3OH	EtOAc	n-hexane
Saponin	a	Frothing	No frothing	No frothing	No frothing	-	-	-
	b	Fehling's	No colour change	No colour change	No colour change	-	-	-
	c	Lieberman Burchard's	No colour change	No colour change	No colour change	-	-	-
Carbohydrate	a	Molisch	violet ring formed	violet ring formed	violet ring formed	+	+	+
	b	Fehling's	brick red precipitate	brick red precipitate	brick red precipitate	+	+	+
	c	Tollen's	silver mirror	silver mirror	silver mirror	+	+	+
Alkaloid	a	Mayer's	Yellow solution	Yellow solution	Yellow solution	-	-	-
	b	Wagner's	Orange solution	Yellow solution	Yellow solution	-	-	-
Tannins	a	$FeCl_3$	Yellow solution	Yellow solution	Yellow solution	-	-	-
	b	Lead subacetate	Colourless	Colourless	Colourless	-	-	-
	c	Gelatin	No ppt	No ppt	No ppt	-	-	-
Flavonoid	a	$FeCl_3$ test	Deep green solution	Deep green solution	Deep green solution	+	+	+
	b	Alkaline reagent	yellow precipitate formed	yellow precipitate formed	yellow precipitate formed	+	+	+
	c	Lead acetate	milky precipitate formed	milky precipitate formed	milky precipitate formed	+	+	+
	d	Aluminium chloride	Yellow solution	Yellow solution	Yellow solution	+	+	+
Phenolics	a	Ferric chloride	intense coloration formed	No colour formed	No colour formed	+	-	-
	b	Folin Ciocalteu's	purple colour formed	No colour formed	No colour formed	+	-	-
Anthraquinone	a	Borntrager's	Pink to red colour formed	Pink to red colour formed	Pink to red colour formed	+	+	+
	b	Ferric chloride	Red colour formed	Pinkish red colour formed	Pinkish red colour formed	+	+	+
Protein	a	Xanthoproteic	yellow precipitate formed	yellow precipitate formed	yellow precipitate formed	+	+	+
	b	Ninhydrin	purple colour formed	purple colour formed	purple colour formed	+	+	+
Cardiac glycosides	a	Keller-killiani	violet ring formed	violet ring formed	violet ring formed	+	+	+
Steroids/triterpenoid	a	Lieberman Burchard's	Brown ring formed	Brown colour formed	Brown ring formed	+	+	+

Key: CH_3OH = methanol; EtOAc = ethyl acetate extract. - = absent, + = present.

The results indicated the presence of several phytochemicals which are responsible for the diverse tradomedical application of the plant (Table 1). The disparity in reports on the phytocemical composition of the leaves of *D. edulis* could be attributed to the variation in the harvesting period, differences in soil composition, nature of processing and probably due to differences in the genetic variability between the plants used [34].

Antibacterial activity

The *in vitro* susceptibility pattern and inhibition parameters at different concentrations of the methanol, ethyl acetate and n-hexane fractions of *D. edulis* challenged with the tested organisms are indicated below (Table 2 and 3).

Table 2: Microbial sensitivity test and diameter zone of inhibition (mm) of the methanol, ethyl acetate and n-hexane extracts of *D. edulis* and the ciprofloxacin standard.

Bacteria Isolates	Concentration (mg/mL)	Zone of Inhibition in mm of the fractions of *D. edulis*			10% Tween-40 (50 µL)	Ciprofloxacin standard (20 µg/mL)
		methanol	ethyl acetate	n-hexane		
Bacillus subtilis	15	S/1.0	S/3.0	R	R	S/11.6
	20	S/1.3	S/3.5	R		
	25	S/1.5	S/4.0	R		
	30	S/3.0	S/4.5	R		
Pseudomonas aeruginosa	15	R	R	R	R	S/14.5
	20	R	R	R		
	25	R	R	R		
	30	R	R	R		
Staphylococcus aureus	15	R	S/1.0	R	R	S/8.7
	20	S/1.0	S/2.5	R		
	25	S/2.0	S/3.0	R		
	30	S/3.0	S/4.0	R		
Escherichia coli	15	R	S/1.0	R	R	S/8.0
	20	R	S/2.5	R		
	25	R	S/3.0	R		
	30	R	S/4.0	R		

Key: S = sensitivity R = resistance

Table 3: Inhibition parameters of the methanol, ethyl acetate and n-hexane extracts of *D. edulis* and the ciprofloxacin standard.

Bacteria Isolates	Inhibitory parameters (mg/mL)	*D. edulis* fractions			10% Tween-40 (50 µL)	Ciprofloxacin standard (20 µg/mL)
		methanol	ethyl acetate	n-hexane		
B.subtilis	MIC	18	16	ND	ND	0.42
	MBC	14	15	ND	ND	0.42
	MBC/MIC	0.78	0.94	ND	ND	1.0
P. aeruginosa	MIC	ND	ND	ND	ND	0.42
	MBC	ND	ND	ND	ND	0.42
	MBC/MIC	ND	ND	ND	ND	1.0
S. aureus	MIC	29	16	ND	ND	0.42
	MBC	21	13	ND	ND	0.42
	MBC/MIC	0.72	0.81	ND	ND	1.0
E. coli	MIC	ND	19	ND	ND	0.42
	MBC	ND	17	ND	ND	0.42
	MBC/MIC	ND	0.89	ND	ND	1.0

B. subtilis = Bacillus subtilis; P. aeruginosa = Pseudomonas aeruginosa; S. aureus = Staphylococcus aureus; E. coli= Escherichia coli; Cipro = Ciprofloxacin; MBC= Minimum Bactericidal Concentration; MIC= Minimum Inhibitory Concentration; ND = Not Determined.

While the methanol and ethyl acetate fractions showed varying degree of activity against some of the bacteria isolates, the n – hexane fractions showed no activity against all the bacteria isolates employed. Similarly, all the fractions of *D. edulis* (methanol, ethyl acetate and n-hexane extracts) showed no activity when challenged with *P. aeruginosa* at the tested doses. The susceptibility of the tested isolates increased with increasing concentrations of the extracts as observed in the zones of inhibition obtained. Using diameter of zone of inhibition of the extracts as criteria for inhibitory strength, the best and least activities were exhibited by the ethyl acetate and n-hexane fractions respectively (Tables 2 and 3). The antimicrobial activities demonstrated by the methanol and ethyl acetate fractions of the plant may be attributed to the presence of anthraquinone, steroid/triterpenes and flavonoids identified in all the fractions. Studies have shown that while terpenoids exhibit properties like anti-inflammatory [35], antibacterial [36], antifungal [37], antiviral [38], and antitumor [39], natural anthraquinones possess astringent, purgative, anti-inflammatory, antiviral, moderate anti-tumour and bacteriocidal effects [40]. The ratio of MBC/MIC revealed that the methanol and ethyl acetate fractions of *D. edulis* leaves was not bactericidal on all the clinical isolates investigated (MBC/MIC < 1) whereas the standard drug ciprofloxacin was bactericidal (MBC/MIC=1) on all the clinical isolates investigated. The standard drug ciprofloxacin exhibited a 100% or near 100% activity. The disparity between the activities of the extracts and the standard antimicrobial drug may be due to the mixtures of bioactive compounds present in the extracts compared to the pure compound contained in the standard antibiotics [41]. The researchers therefore agree with the statement of Agbo and Mboto [42] who stated that African medicinal plants not only supply nutrients to the body but also serve medicinal purposes. This then opens the frontiers for prospective industrialists to explore the use of this plant in their constant search for new antimicrobial agents.

Acute Toxicity Studies

Table 4 shows that the LD_{50} was above 4000 mg/kg in the treated mice. The administered graded doses of the methanol extract of *D. edulis* did not result in lethality over the 24 hour period. No death and latent toxicity was observed in the animals after keeping them for extra 14 days. Hence, the acute toxicological results showed that the plant is relatively safe but further toxicity evaluations using mammalian tissues and organs will be necessary in the future.

Table 4: Acute toxicity profile of *D. edulis* methanol leaf extract.

Dose (mg/kg)	Mortality Ratio	% Mortality
500	0/5	0
1000	0/5	0
2000	0/5	0
4000	0/5	0

Conclusion

The results of this research shows that the consumption of *D. edulis* not only provide nourishment to the body but also help in combating infections as seen in the results from the antimicrobial activity and that the oral administered extract of *D. edulis* is relatively safe at the highest tested dose of 4 g/kg in mice. This finding is of immense importance for the development of new chemotherapeutic agent to address unresolved therapeutic needs of the populace. This validates its use in traditional medicine for treating infections.

References

1. Ahmad I, Mahmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial propreties. J. Ethnopharmacol. 1998; 62:183-193.
2. Clark A. Natural products as source of new drugs. Pharmaceutical research. 1996; 13:1133-1141
3. Finch RG. Antibiotic resistance. J. Antimicrobial Chemotherapy. 1998; 42:125-128
4. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J. (Ed) Perspectives on New Crops and New Uses. ASHS press, Alexandria, 1999, 457-462.
5. Kunin CM. Resistance to antimicrobial drugs a worldwide calamity. Annals of Int. Med. 1993; 118:557-561
6. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal Plants and Natural Products. Indian J Pharmacol. 2000; 32:581-589.
7. Cowan MM. Plant products as anti-microbial agents. Clinical microbiology reviews. 1999; 12:564-582.
8. Owolabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. Afr. J. Biotechnol. 2007; 6(14):882-85.
9. Egwaikhide PA, Gimba CE. Analysis of the phytochemical content and antimicrobial activity of *Plectranthus glandulosis* whole plant. Middle-East J. Scientific Res. 2007; 2:135-138.
10. Ahmed I, Arina Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogen. J. Ethnol-Pharm. 2001; 74:114-123.
11. Aidu M, Erhabor JO, Towuru GE. Antimicrobial effects of the chloroform and ethanolic leaf extracts of *Dacryodes edulis* (G. Don) H. J. Lam, *Garcinia Kola Heckel* and *Chrysophyllum Albidum* G. Don on some test isolates. Med. Sci. 2013; 1(3):63-66.
12. Ajibesin KK, Etienne EE, Saburi AA. Antibacterial constituents of the leaves of *Dacryodes edulis* Afr. J. Pharm and Pharmacol. 2011; 5(15):1782-1786.
13. Leakey RRB, Tchoundjeu Z, Schreckenberg K, Schacleton SE, Shackleton CM. Agroforestry tree products (AFTPS): Targeting poverty and enhanced livelihoods. Int J. Agric. Sustainability. 2005; 3(1):1-23.
14. Bidu M, Oshomoh EO, Ovuakporie-Uvo PO. Phytochemical and antimicrobial properties of *Chlorophora excels*, *Cedrelan odorata* and *Tectona grandis*. Top-class J. Herbal Med. 2013; 2(11):248-253.
15. Sofowora L. Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria, Spectrum Books Ltd, 2008, 289-291.
16. Ajibesin K. Dacryodes edulis: A Review of its medicinal, phytochemical and economic properties. J. Biotech. 2011; 57:32-34.
17. Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Traditional medical practices among the Igede people of Nigeria. Part II. Afr. J. Trad. CAM. 2005; 2(2):134-152.
18. Ajibesin KK, Ekpo BJ, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. J. Ethnopharmacol. 2008; 115(3):387-408.
19. Ayuk ET, Duguma B, Franzel S, Kangué J, Mollet M, Tiki-Manga T *et al*. Uses, management and economic potential of *Dacryodes edulis* (Burseraceae) in the humid lowlands of Cameroon. Econ. Bot. 1999; 53(3):292-301.
20. Burkill HM. The Useful Plants of West Tropical Africa (Edition 2). Families A-D. Royal Botanic Gardens, Kew. 1985; 1:21-26.
21. Onocha PA, Ekundayo O, Oyelola O, Laakso I. Essential oils of *Dacryodes edulis* (G. Don) *HJ Lam* (African pear). Flavour Fragrance. 1999; 14:135-139.
22. Okwu DE, Nnamdi FU. Evaluation of the chemical composition of *Dacryodes edulis* and *Raphia hookenimann* and wendl exudates used in herbal medicines in South Eastern Nigeria. Afri. J. Trad. CAM. 2008; 5(1):194-200.
23. Stalh E. Drug analysis by chromatography and Microscopy. A Practical Supplement to Pharmacopoeias. 1st ed., Ann Arbor, Michigan, 1973, 219-224.
24. Sofowora A. Screening Plants for Bioactive Agents. In Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd. Ibadan, 1982, 128-161.
25. Trease EA, Evans WC. Pharmacognosy, 11th Edition. Churchill Livingstone Harcourt Health service, London, 1978, 3.
26. Lorke D. A new approach to practical acute toxicity testing. Archive of Toxicol. 1983; 54:275-287.
27. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd Edition. Williams and Wilkins, Lippincott, New York, 2000, 23-32.
28. Fobres BD, Sham A. Weissfield. Diagnostic Microbiology. 11th Edition, Andrew Allen Press, Canada, 2002, 12-29.
29. Leboffe MJ, Pierce BE. Microbiology Laboratory Theory and Application. 1st Edition, Morton Publishing, USA, 2002, 45-53.
30. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's Manual of Systematic Bacteriology. Vol., Williams and Wilkins, Philadephia, 1986; 2:22-29.
31. Valgas C, De Souza S, Smânia E, Smânia A. Screening methods to determine antibacterial activity of natural products. Brazilian J. Microbiol. 2007; 38:69-380.
32. Vollekova AD, Kostalova S, Sochorova R. Folia Microbiology. 2001; 46:107-111.
33. Usman H, Abdulrahman FI, Ladan AH. Phytochemical and antimicrobial activity of the stem-bark of *Gardenia aqualla* Stapf & Hutch (Rubeacea). Res. J. Biol. Sci. 2007; 2:244-247.
34. Capecka E, Mareczek A, Leja M. Antioxidant activity of fresh and dry herbs of some *Lamiaceae species*. Food Chemistry. 2005; 93:223-226.
35. Fu L, Zhang S, Li N, Wang J, Zhao M, Sakai J *et al*. Three new triterpenes from *Nerium oleander* and

biological activity of the isolated compounds. J. Nat Prod. 2005; 68:198-206.
36. Katerere DR, Gray AI, Nash RJ, Waigh RD. Phytochem. 2003; 63:81-88.
37. Yuan WH, Yi YH, Xue M, Zhang HW, La MP. *In vitro* evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* leaves. Chin J.Nat Med. 2008; 6:105-108.
38. Zhu YM, Shen JK, Wang HK, Cosentino LM, Lee KH. Bioorganic and Medicinal Chemical Letters. 2001; 11:3115-3118.
39. Saxena BB, Zhu L, Hao M, Kisilis E, Katdare MO, Bomshteyn OA *et al*. Bioorganic and Medicinal Chemistry Letters. 2006; 14:6349-6358.
40. Manojlovic NT, Solujic S, Sukdolak S, Krstic LJ. *In vitro* antimicrobial activity of the methanol extract and compounds from the wood of *Ficus elastica* Roxb. ex Hornem. aerial roots. J. Serb Chem Soc. 2000; 65(8):555-560.
41. Gatsing D, Nkeugoauapi CFN, Nkah BFN, Kuiate JR, Tchouanguep FM. Antioxidant and antimicrobial activities of solvent fractions of *Vernonia cinerea* (L.) Less leaf extract. Int. J. Pharmacol. 2010; 6:173-182.
42. Agbo BE, Mboto CI. Phytochemical and antibacterial evaluation of selected locally produced herbal medicines sold in Calabar, Nigeria, Arch. Applied Sci. Res. 2012; 4(5):1974-1990.