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Studies on dacryodesedulis 1: Phytochemical and medicinal principles of raw seeds

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ABSTRACT

Dacroydesedulis is a plant with many trado-therapeutic claims.The seeds are used traditionally as a remedy for stomach problems like diarrhoea, dysentery etc. Raw seeds of D.edulis were exhaustively extracted using hexane, chloroform, ethyl acetate, and methanol. These extracts were phytochemically screened for presence of reducing sugars, anthroquinones glycosides, saponins glycosides, flavonoids, steroids,terpenoids, tannins and alkaloids. Resultsshowed presence of free anthraquinones, steroids/triterpenes, tannins, saponins, alkaloids and flavonoids. The anti-microbial screening of extractsshowed sensitivity against nine microbes:Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigelladysenteriae, Pseudomonas aeruginosa, Klebsiellapneumoniae and fungi: Candida albicans, Trichophytomrubrum and Microsporum sp. The chloroform extract, using diameter of zone of inhibition of the extracts as criteria for inhibitory strength, exhibited highest inhibition against the pathogens that were sensitive to the extract with Klebsiellapneumoniae being most inhibitive (23 mm). Chloroform 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 5 mg/mL. This lends credence the trado-medicinal claims on the seed ofDacroydesedulis as remedy for stomach problems.

Key words: *Dacryodesedulis*, Raw seeds extracts, Phytochemical analysis,Minimum inhibitory concentration, Minimum bactericidal concentration.

INTRODUCTION

Plants will remain important to man's struggle against disease in the foreseeable future. History is replete with accounts of medicinal plants and their place in man's battle against disease. The authors[1, 2],report that surviving scrolls, codices, manuscripts, parchments or papyri document phyto-medicinal practice among the ancient Aztecs and Maya, ancient Egyptians, Babylonians, ancient Chinese and Indian civilisations. Around the world, as pathogens are proving resistant to even the most powerful antibiotics, scientists are looking into nature in search of arsenal for an unending war against (parasitic) disease.

Dacroydesedulis, (G.D and H.J. Lam), is of the family:*Burseraceae*.This family is made up of resinous trees and shrubs with alternate leaves composed of many leaflets. They are of the order sapindales, subclass Rosidae and class Magnoliopsida. Burseraceae, also known as torchwood family is a family of moderate size trees and consist of 17-18 genera and about 540 species of flowering plants.

D.edulis, is commonly known by the following names:Ube (Igbo), Mzembe (Tiv). English names include:African pear, Bush butter tree, Bush fruit tree, Eben tree, Native pear and in French, Safoutier[3]. Various parts of the plant are used in the treatment of several diseases in different areas.In Gabon, bark of the plant is used for treating wounds[4];a decoction of the bark is taken orally for leprosy treatment and also used as gargle and mouth wash for treatment of tonsillitis in Democratic Republic of Congo, reports[5]; leaves are chewed with kolanut as an antiemetic; it has been reported by [5, 6] that the leaf sap is used as ear drop to treat ear trouble, vapour produced by leaf decoction is used to treat fever and headache. The bark resin is used in Nigeria to treat parasitic skin disease and jiggers [7, 8]. According to [9], when applied in creams and lotions, the resin smoothens the skin. The leaves are often crushed and juice is used to treat generalized skin diseases such as ringworm, scabies, rash etc while the stem and stem twigs are used as chewing sticks for oral hygiene [10, 11]. Seed is chewed by the Tiv people (Nigeria) as a remedy for stomach problems like diarrhoea, dysentery etc.*D. edulis's* pharmacological properties have been reviewed (Table 1.) [12].

Table 1.Previous Studies on *D. edulis*

Previous Study	Part of Plant Used	Study Findings
Phytochemical Analysis	seed	Flavonoids, Saponins, tanins, alkaloids etc. [13,14]
Antimicrobial Activity	seed	Antibacterial [13]
Antioxidant activity	Leaves and essential oil	Good antioxidant effects [14]
Cardiovascular Activity	Oil of plant	Good activity [17]
Anti-Sickle cell anaemia	Leaves	Normalized sickle cell blood erythrocytes [18]
Isolation	Leaves	Ethyl gallate, Quercitrin [15]
Isolation	Fruits Skin Zone and Pulp	Isoquercitrin, Isorhammetin, cyanidin, Petunidin, Rhamnoside [16]
Antimicrobial Activity	Essential oil	Good antimicrobial activity[14]

This study looks into the phytochemical constituents and antimicrobial activity of the seed of *D. edulis*and also to prove the ethno- medicinal claims on the seeds as a remedy for stomach problems.

MATERIALS AND METHODS

Plant Collection and Extraction

Fruits of the plant were collected from Iyonov in Kwande local government, Benue state, Nigeria, in July 2013, identified and authenticated at Department of Wildlife and Range Management, University of Agriculture Makurdi.Fruits were dehulled to obtain seeds which were size reduced with mortar and pestle. Pulverised plant material was introduced into ten extraction bottles and immediately macerated with hexane.

Plant material (5 kg)was sequentially exhaustively extracted with hexane(10 Litres); chloroform(8 Litres), ethyl acetate (8 Litres) and methanol (10 Litres) via cold maceration, drying the marc before the next extraction. All extracts were concentrated using a rotary evaporator at 40^{o}C (50^{o}C for methanol). Yields of crude extracts were calculated as percentage crude extracts of plant material used.

Preliminary Phytochemical Screening

Phytochemical tests were carried out on the plant extracts to identify secondary metabolites such as alkaloids, reducing sugar, flavonoids, saponin glycoside, tannins, anthraquinone glycoside, cardiac glycosides and steroids/terpenes using standard procedures as described by [19, 20].

Antimicrobial studies

Antimicrobial screening of methanol, ethyl acetate, chloroform and hexane seed extracts of*Dacroydesedulis* was determined using the following pathogens; *Staphylococcus aureus; Escherichia coli; Salmonella typhi; Shigelladysenteriae; Pseudomonas aeruginosa; klebsiellapneumoniae* and the fungi *Candida albicans*, *Trichophytomrubrum*, *Microsporum sp., Aspergillusfumigatus*and *Aspergillusniger*. The microorganisms were obtained from Department of MicrobiologyA.B. U teaching hospital Zaria. All the 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.

Antibacterial screening was carried out using agar diffusion method as described by [21] with slight modifications. Mueller Hinton and Sabouraud dextrose agar were the medium used for growth of the bacteria and fungi. All media were prepared according to the manufacturer's instruction, sterilized at 121 ^{0}C for 15 mins and poured into sterile

petri dishes which were allowed to cool and solidify. The sterilized Mueller Hinton agar was seeded with 0.1mL of standard inoculums of the test bacteria and Sabouraud dextrose was seeded with 0.1mL of standard inoculums of the test fungi. The inoculums were then evenly spread over the surface of the media using sterile swab. A sterile standard cork borer of 6mm in diameters was used to cut a well at the centre of each inoculated medium. About 0.1mL of solution of an extract of 5 mg/mL of concentration was then introduced into the well on the medium. Incubation for bacteria was made at 37^0C for 24 hrs and 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.

Minimum inhibition concentration (MIC) of extracts was carried out on an extract that has shown growth inhibitory effect on a test organism. It was done using broth dilution method asdescribed by [22]and modified by [23]. 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 ^{o}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 6hrs at37^{o}C. Dilution of the micro-organism in the normal saline was continuously done until the turbidity ($1.5x10^6$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 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL and 0.3125 mg/mL. Having obtained different concentration of the extracts in the broth, 0.1mL of the standard inoculum of microbes was inoculated in to the different concentrations. Incubation for the bacteria, was made at 37^0Cfor 24hrs and at 30^0C for one week for fungi. The test tubes were then observed for turbidity. The lowest concentration of an extract in the broth which showedno turbidity was recorded as the minimum inhibition concentration (MIC).

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 ^{o}C 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 ^{o}C for 24 hrs for bacteria and at 30 ^{0}C for one week for fungi, after which the plates were observed for colony growth. The MBC/MFCwas plates with the lowest concentration of the extracts without colony growth. The results were recorded after 24 hrs as described by [22] and modified by [23].

RESULTS AND DISCUSSION

Preliminary phytochemical screening for secondary metabolites of *Dacroydesedulis*raw seed extracts showed presence of free anthraquinones, steroid/triterpenes, tannins, saponins, alkaloids and flavonoids. (Table 2)

Microbial sensitivity test for hexane, chloroform, ethyl acetate and methanol raw seed extracts of *Dacroydesedulis* showed sensitivity against nine microbes. These includes; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigelladysenteriae, Pseudomonas aeruginosa, klebsiellapneumoniae* and fungi; *Candida albicans, Trichophytomrubrum andMicrosporum sp*.The standard sparfloxacin was sensitive against 6 microbes,whereas,fluconazole andfulcin showed sensitivity against 1 and 4 microbes, respectively. The chloroform extract, using diameter of zone of inhibition of the extracts as criteria for inhibitory strength showed the highest inhibition against the pathogens that were sensitive to the extract and *Klebsiella pneumonia* was most inhibited (23 mm). The standard, sparfloxacin showed the highest inhibition. (Table 3).

Table 2. Phytochemical Screening Result for raw seed extracts of *Dacryodesedulis*

CONSTITUENTS	TEST		OBSERVATION				INFERENCE			
			CH_3OH	EtOAc	Chloroform	Hexane	CH_3OH	EtOAc	Chloroform	Hexane
Carbohydrate	a.	Molisch test								
	b.	Fehling test	Green solution	Blue solution	Blue solution	Blue solution	_	_	_	_
Anthraquinones	a.	Free anthraquinone	Red solution	Pink soln	Creamy	Colourless	+	+	_	_
	b.	Anthraquinone glycosides	Colourless	Colourless	Colourless	Colourless	_	_	_	_
Saponin	a.	Frothing test	No frothing	Frothing	No frothing	Nofrothing	_	+	_	_
Steroid/ and triterpenes	a.	Lieberman Burchard's test	Brown ring	Reddish brown. Reddish brown	Violet ring	Brown ring	+	+	+	+
	b.	Salkowski test	Reddish brown		Reddish brown	Reddish brown	+	+	+	+
Tannins	a.	Lead subacetate	Coloured ppt	Colourless	Colourless	Colourless	+	_	_	_
	b.	$FeCl_3$ test	Greenish black ppt	Yellow solution	Yellow solution	Yellow solution	+	_	_	_
Alkaloids	a.	Mayer's test	Yellow soln	Yellow soln	Yellow sol	Yellow sol	_	_	_	_
	b.	wagner's test	Orange solution	Yellow solution	Brown ppt	Yellow solution	_	_	+	_
	c.	Dragendoff's test	Orange soln	Orange soln	Orange sol	Orange sol	_	_	_	_
Cardiac glycosides	a.	Kella-killiani test	Yellow ppt	Creamy solution	Pale green solution	Yellow solution	_	_	_	_
Flavonoid	a.	Sodium hydroxide	Brownish red	Pale yellow	Cloudy	Cloudy	_	_	_	_
	b.	$FeCl_3$ test	Deep green	Brown soln	Yellow sol	Yellow sol	+	_	_	_
	c.	Shinoda's Test	Pale orange solution	Cloudy solution	Colourless	Colourless	_	_	_	_

Key: EtOAc = ethyl acetate extract, - = absent, + = present, CH3OH = methanol, Soln = solution

Table 3.Microbial Sensitivity Test and Diameter Zone of Inhibition (mm) of extracts and standards.

Test Organism	Hexane	Chloroform	Ethyl acetate	Methanol	Sparfloxacin	Fluconazole	Fulcin
Staphylococcus aureus	S/20	S/21	S/19	S/20	S/37	R	R
Escherichia coli	S/21	S/20	S/19	S/19	S/35	R	R
Klebsiella pneumonia	S/22	S/23	S/18	S/19	S/40	R	R
Shigelladysenteriae	S/20	S/21	S/19	S/18	S/42	R	R
Salmonella typhi	S/19	S/20	S/20	S/20	S/37	R	R
Pseudomonas aeruginosa	S/18	S/19	S/18	S/17	S/34	R	R
Candida albicans	S/19	S/20	S/17	S/20	R	S/35	R
Aspergillusfumigatus	R	R	R	R	R	R	S/32
Aspergillusnigre	R	R	R	R	R	R	S/30
Microsporumsp	S/19	S/21	S/17	S/19	R	R	S/37
Trichophytonrubrum	S/18	S/21	S/17	S/18	R	R	S/34

Key: S = sensitivity R = Resistance

Table 4. Minimum Inhibitory concentration (MIC) for hexane, chloroform, ethyl acetate and methanol extracts of *Dacroydesedulis*

Micro organisms	Concentration (mg/ml)																			
	Hexane extract					Chloroform extract					ethyl acetate extract					Methanol extract				
	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125
Staphylococcus aureus	_	_	**µ**	+	++	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++
Escherichia coli	_	_	**µ**	+	++	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +
Klebsiellapnuemoniae	_	_	**µ**	+	++	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +
Shigelladysenteriae	_	_	**µ**	+	++	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +
Salmonella typhi	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++	_	_	**µ**	+	++	_	_	**µ**	+	++
Pseudomonas aeruginos	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +
Candidasalbicans	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++
Aspergillusfumigatus																				
Aspergillusnigre																				
Microsporumsp	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +
Trichophytonrubrum	_	**µ**	+	+ +	++ +	_	_	**µ**	+	++	_	**µ**	+	+ +	++ +	_	**µ**	+	+ +	++ +

Key: *– =No turbidity (No growth),* ***µ*** *= MIC, + =Turbidity (light growth), ++ = moderate turbidity, +++ = high turbidity.*

Ethyl acetate, chloroform, hexane and methanol extract showed inhibition against all the pathogens. Chloroform extract showed highest inhibitory effects with MIC at concentration of 1.25 mg/mL for all microbes, except for *Pseudomonas aeruginosa* (which was 2.5 mg/mL). Ethyl acetate extract, had MIC value of 2.5 mg/mL for all microbes but 1.25 mg/mL for *Salmonella typhi.* Hexane and methanol extracts had MIC values of 1.25 and 2.5 mg/mL, respectively(Table4.).

The minimum bactericidal concentration/minimum fungicidal concentration, generally for all the extracts was5 mg/mL. (Table 5).

The antimicrobial activities demonstrated by the seed extracts are dependent on the presence of the following secondary metabolites; free anthraquinone, steroid/triterpenes, saponins, tannins, alkaloids and flavonoids identified in the seed of *D. edulis*. Presence of these secondary metabolites also accounts for the usage of the seed in traditional medicine, as a remedy for stomach ailments.Antibacterial and antifungal properties of these metabolites have been reported in previous studies; tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidalcompounds and anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders[24]. Also, that tannin not only heals burns and stop bleeding, but they also stop infection while continue to heal the wound internally. Tannins have also been reported to have anti-viral [25], antibacterial[26] and antiparasitic effects [27].

Saponins from plant origin have been reported to have interesting properties like spermicidal [28], molluscicidal[29], antibacterial and anti-inflammatory [30].saponins exhibit haemolytic, antifungal, molluscicidal activity[31];saponinsalso contribute as important constituents in various herbal drugs and folk medicines that exhibit pharmacological properties.

Studies have shown that terpenoids exhibit properties like anti-inflammatory[32], antibacterial [33], antifungal [34], antiviral [35], and antitumor [36]. Natural anthraquinones possess astringent, purgative, anti-inflammatory, antiviral, moderate anti-tumour and bactericide effects[37].

Table 5. MBC and MFC for hexane, chloroform, ethyl acetate and methanol extracts of *Dacroydesedulis*

Micro organisms	Concentration (mg/mL)																			
	Hexane extract					Chloroform extract					ethyl acetate extract					Methanol extract				
	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125
Staphylococcus aureus	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Escherichia coli	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Klebsiellapnuemoniae	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Shigelladysenteriae	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Salmonella typhi	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Pseudomonas aeruginos	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Candidasalbicans	**µ**	+	++	+++	++++	µ	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Aspergillusfumigatus																				
Aspergillusnigre																				
Microsporumsp	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++
Trichophytonrubrum	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++	**µ**	+	++	+++	++++

Key: *– =No Colony growth, **µ** = MBC/MFC, += Scanty Colonies growth, ++ = moderate colonies growth+++ = Heavy colonies growth*

CONCLUSION

Phytochemical screening for secondary metabolites of *Dacroydesedulis*raw seed extracts showed presence of free anthraquinone, steroid/triterpenes, tannin, and saponin. The anti-microbial screening of methanol, ethyl acetate, chloroform and hexane extracts of *D.edulis* showed sensitivity against nine microbes; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigelladysenteriae, Pseudomonas aeruginosa, klebsiellapneumoniae* and fungi; *Candida albican, Trichophytomrubrum and Microsporum sp.*The chloroform extract showed the highest inhibition against the pathogens that were sensitive to the extract;*Klebsiella pneumonia* was most inhibited (23 mm). The moderate antibacterial and antifungal activities of these extracts could not be unrelated to the presence of secondary metabolites detected in the plant. This justifies the traditional usage of the seed as remedy for stomach problems. Further research is on-going to isolate and characterize the medicinal principles of *Dacroydesedulis.*

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