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Research Article

STUDIES ON *DACRYODESEDULIS II*: PHYTOCHEMICAL AND MEDICINAL PRINCIPLES OF BOILED SEEDS

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Abstract

This study was carried out to investigate the claims on seed of *Dacroydesedulis*as a remedy for stomach ailments. Boiled seed extracts of *D.edulis* were subjected to phytochemical and antimicrobial analysis. Results of phytochemical screening showed presence of reducing sugar, free anthraquinone, saponins, tannins, steroid/ triterpenes, flavonoids and alkaloids. The anti-microbial screening result of extracts (hexane, chloroform, ethyl acetate and methanol)showed sensitivity against the following disease causing human pathogens: *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella dysenterea, Pseudomonas aeruginosa, klebsiella pneumoniae*, *Candida albicans*, *Trichophytom rubrum and Microsperum sp.* Chloroform extract exhibited highest inhibition against the pathogens that were sensitive to the extracts, the pathogen*Klebsiellapneumoniae* was most inhibited (32 mm).For Minimum inhibitory concentration **(**MIC), these extracts showed inhibition against all the pathogens. Chloroform, hexane and methanol extracts showed inhibitory effects at concentration of 1.25 mg/mL for most of the pathogens with MIC value of 0.625 mg/mL for *Escherichia coli, Klebsiellapneumoniae and Shigelladysenteriae.*The minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) of extracts showed inhibition against all the pathogens at values of 5, 2.5 and 1.25 mg/mL. This justifies the trado-medicinal claims on *Dacroydesedulis* as remedy for stomach ailments.

Keywords: *Dacroydesedulis,* boiled seed extracts, Phytochemical analysis,*Minimum inhibitory concentration, Minimum bactericidal concentration.*

Introduction

Dacroydesedulis is of the plant family, *Burseraceae*, also known as the torchwood family consisting of 17-18 genera and about 540 species of flowering plants. *Dacroydesedulis,* is commonly called; ube (Igbo) and Mzembe (Tiv) languages, Nigeria. English names includes; African pear,bush butter tree, bush fruit tree, Eben tree, native pear and French, Safoutier (Burkill, 1985).

Several studies have been carried out on different parts of *D. edulis*such as phytochemical analysis, antimicrobial activity, antioxidant activity, antisickle cell anaemia etc. Positive results have been reported. Ajibesin, etal., 2011, identified two compounds, ethyl gallate and quercitrin from the leaves of *D. edulis;* these were found to be responsible for the antibacterial effect of the plant. Also, flavonols such as quercitrin, isoquercitrin, isorhamnetin and rhamnoside, as well as anthocyanins such as petunidin and cyanidin were reported to be present in the fruit skin zone and pulp of *D. edulis* during ripening (Missangetal., 2003).

Materials and Methods

Plant Collection and Extraction

Fruits of *D. edulis* were collected from Iyonov in Kwande local government, Benue state, Nigeria, in July 2013, identified and authenticated at the Department of Forestry and Wildlife Management, University of Agriculture Makurdi.

Ground boiled seed(300 g) was extracted sequentially with hexane (600 mL), chloroform (400 mL), ethyl acetate (400 mL) and methanol (500 mL) by maceration. All extracts were concentrated using a rotary evaporator at 40^{o}C (50^{o}C for Methanol).

Preliminary Phytochemical Screening

Phytochemical screening analysis was carried out on the plant extracts to identify presence of pharmacologically active metabolites such as alkaloids, flavonoids, saponins, tannins, anthraquinones, cardiac glycosides and steroids/terpenes according to procedures described byTrease and Evans, (2002) and Edeoga et al., (2005).

Antimicrobial studies

The following disease causing human pathogensobtained from the Department of MicrobiologyA.B. U Teaching Hospital Zaria,were used for the antimicrobial screening of boiled seed extracts of *D. edulis*:*Staphylococcus aureus; Escherichia coli; Salmonella typhi; Shigelladysenterea; Pseudomonas aeruginosa; klebsiellapneumoniae* and fungi *Candida albicans*, *Trichophytomrubrum, Microsperum sp., Aspergillusfumigatus*and *Aspergillusniger.*

Agar diffusion method was used for the antimicrobial activity described by (Lino and Deogracios, 2006).Mueller Hinton and Sabourand dextrose agar were used as the growth medium for the bacteria and fungi. Test was performed in sterile petri dishes. Sterilized Mueller Hinton agar was seeded with 0.1 mL of the standard inoculums for bacteria strains and Sabourand dextrose was seeded with 0.1 mL of the 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 diameter was used to cut a well at the centre of each inoculated medium. About 0.1 mL of solution of extract of 5 mg/mL of concentration was then introduced into the well on the medium. Incubation for bacteria was made at 37 ^{0}C for 24 hrs and one week for fungi. Each plate was observed for zone of inhibition of growth.

Minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of extracts were carried out using broth dilution method asdescribed by (Vollekova, etal., 2001) as modified by (Usman, etal., 2007). 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 Sabourand dextrose broth were prepared according to the manufacturer's instruction. Broth (10 mL) was dispensed into test tubes 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. 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 comparism. Two fold serial dilution of an 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 concentrations of anextract in the broth, 0.1mL of the standard inoculum of microbes was inoculated into the different concentrations. Incubation forbacteria was made at 37 ^{0}C for 24 hrs and at 30 ^{0}C for one week for fungi. Test tubes were then observed for turbidity and colony growth. The lowest concentration of an extract in the broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC). The MBC/MFCwas plates with the lowest concentration of the extracts without colony growth.

Results and Discussion

Result of phytochemical screening of seedextracts of *Dacroydesedulis* showed presence of reducing sugars and tannins in methanol extract, free anthraquinone in methanol and ethyl acetate extracts, saponins in methanol extract,flavonoids in methanol and hexane extracts, alkaloids in methanol and chloroform extracts and steroid/ triterpenes in all extracts.(Table 2). Presence of these metabolites is most likely to be responsible for the antimicrobial properties of the seed of *D. edulis*as they have been linked to various bioactivities. For example, Ayo et al., 2014, have reported that alkaloids exhibit antimicrobial, antidiarrhoeal and antihelmintic properties by intercalating into cell wall and DNA of parasites and inhibiting release of autocoids and prostaglandins.

Several plants which are rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms (Banso and Adeyemo, 2007). Tannins have also been reported as being responsible for preventing and treating urinary tract infections and other bacterial infections (Shimbe and Tor-Anyiin, 2014). Doss et al., 2009, reported that the ability of tannin compounds to cause the bacterial colonies to disintegrate may result from their interference with the bacterial cell wall;thus inhibiting the microbial growth.

Flavonoids have been reported to exhibit anti-diarrhoea, anti-dysentery and antifungal activities as well as a broad biological and pharmacological activities (Shimbe and Tor-Anyiin, 2014).

Microbial Sensitivity Test and Diameter Zone of Inhibition

Boiled seed extracts of *Dacroydesedulis*showed sensitivity against the following nine microbes; *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigelladysenterea, Pseudomonas aeruginosa, klebsiellapneumoniae* and fungi; *Candida albicans*, *Trichophytomrubrum and Microsperum sp.*The chloroform extract showed the highest inhibition against the pathogens that were sensitive to the extracts and *Klebsiellapneumoniae* was most inhibited (32 mm). Thus, the chloroform extract was most active. The control, sparfloxacin was sensitive against 6 microbes; fluconazole andfulcin showed sensitivity against 1 and 4 microbes respectively. The standard antibiotic, sparfloxacin showed the highest inhibition (Table 3).

Minimum Inhibition Concentration (MIC)

Boiled seed extracts showed inhibition against all the pathogens that were sensitive to the extracts. (Table 4). Chloroform, hexane and methanol extracts showed inhibitory effects at concentration of 1.25 mg/mL for most of the pathogens and MIC value of 0.625 mg/mL for *Escherichia coli, Klebsiellapneumoniae and Shigelladysenteriae.* Ethyl acetate extract had MIC value of 1.25 mg/mL for all the pathogens, except *Klebsiellapneumoniae*(0.625 mg/mL).

Minimum bactericidal concentration/minimum fungicidal concentration

The minimum bactericidal concentration/minimum fungicidal concentration of boiled seed extracts of *D. edulis* showed bactericidal/ fungicidal effects against the pathogens with MFC/MBC values varying of 5, 2.5 and 1.25 mg/mL.(Table 5). Results of antimicrobial activity test have proven that the boiled seed of *D. edulis* has great antimicrobial property which is related to the presence of secondary metabolites present in the seed.

Table 1: Uses of*Dacryodes edulis*

Area	Part of plant used	Medicinal uses
Gabon	Bark	To cicatrize (treat) wounds [A]
Democratic Republic of Congo	Decoction of bark	Leprosy, tonsillitis [B]
Nigeria	Bark resin	Parasitic skin disease, jiggers. [C]
		Smoothens and protects the skin. [D]
	Leaves	Antiemetic. [B]
	Leaf sap	Ear trouble. [B]
	Decoction of leaves	Fever and headache. [B]
	Juice from leaves	General skin disease. [E]
	Stem/ stem twigs	Chewing stick. [E]

KEY A-Walker and Silans, 1961 B- Ajibesin, 2011 C- Dalziel, 1937; Hutchinson etal., 1963 D- Ekpa, 1993 E- Igolietal., 2005; Ajibesinetal., 2008

Table 2. Phytochemical Screening Result for Seed Extracts of *Dacroyde sedulis*

CONSTITUENTS	TEST	OBSERVATION				INFERENCE			
		CH_3OH	EtOAc	Chloroform	n-Hexane	CH_3OH	EtOAc	Chloroform	n-Hexane
Carbohydrate	a. Fehling test	Brick red ppt	No ppt	Blue solution	Blue ppt	+	–	–	–
Anthraquinones	a. Free anthraquinone b. Anthraquinone glycosides	Red solution Colourless	Pink solutn Colourless ppt	Creamy Creamy solution	Colourless Colourless ppt	+ –	+ –	– –	– –
Saponin	a. Frothing test	Frothing	No Frothing	No frothing	No frothing	+	–	–	–

Steroid and triterpenes	a. Lieberman Burchard's test	Brownish ring	Reddish brown ring	Brownish ring	Reddish brown ring		+	+	+	+
	b. Salkowski test	Reddish brown ring	Reddish brown ring	Reddish brown ring	Reddish brown ring		+	+	+	+
Tannins	a. Lead subacetate	Coloured ppt	Colourless	Colourless	Colourless		+	–	–	–
	b. $FeCl_3$ test	Greenish black ppt	Yellow solution	Nogreenish/ black ppt	Yellow solution		+	–	–	–
Alkaloids	a. Mayer's test	Yellow soln	Yellow soln	Yellow sol	Yellow sol		–	–	–	–
	b. wagner's test	Yellow solution	Yellow solution	Brown ppt	Yellow solution		–	–	+	–
	c. Dragendoff's test	Deep Orange	Red solution	Deep Orange	Orange sol		+	–	–	–
Cardiac glycosides	a. Kella-killiani test	Yellow ppt	Creamy solution	Yellowish ppt	Yellow ppt		–	–	–	–
Flavonoid	a. Sodium hydroxide	Faint yellow	Brown soln	Cloudy Yellow sol	Colourless		+	–	–	+
	b. $FeCl_3$ test	Greenish black	Yellow solution		Yellow sol		+	–	–	–
	c. Shinoda's Test	Faint Pink solution	Colourless solution	No pink/red sol	Colourless		+	–	–	–

Key: EtOAc = ethyl acetate extract, - = absent, + = present, CH_3OH = methanol, soln/ sol = Solution, ppt = Precipitate

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

Test Organism	Hexane	Chloroform	Ethyl acetate	Methanol	Sparfloxacin	Fluconazole	Fulcin
Staphylococcus aureus	S(25)	S(26)	S(24)	S(24)	S(37)	R	R
Escherichia coli	S(27)	S(29)	S(26)	S(27)	S(35)	R	R
Klebsiellapneumoniae	S(30)	S(32)	S(28)	S(26)	S(40)	R	R
Shigelladysenteriae	S(29)	S(27)	S(25)	S(28)	S(42)	R	R
Salmonella typhi	S(24)	S(24)	S(22)	S(24)	S(37)	R	R
Pseudomonas aeruginosa	S(22)	S(24)	S(21)	S(20)	S(34)	R	R
Candida albicans	S(24)	S(26)	S(22)	S(23)	R	S(35)	R
Aspergillus fumigates	R	R	R	R	R	R	S(32)
Aspergillusnigre	R	R	R	R	R	R	S(37)
Microsperumsp	S(22)	S(24)	S(20)	S(23)	R	R	S(34)
Trichophytonrubrum	S(20)	S(22)	S(20)	S(21)	R	R	S(34)

KEY: S = Sensitivity R = Resistance EOAc=Ethyl acetate fraction. Figures in brackets are zone of inhibition in mm.

Table 4. Minimum Inhibitory concentration (MIC) hexane, chloroform, ethyl acetate and methanol seed extracts of *Dacroydes edulis*

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	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++
Escherichia coli	_	_	_	0*	+	_	_	_	0*	+	_	_	0*	+	++	_	_	_	0*	+
Klebsiellapnuemoniae	_	_	_	0*	+	_	_	_	0*	+	_	_	_	0*	+	_	_	_	0*	+
Shigelladysenteriae	_	_	_	0*	+	_	_	_	0*	+	_	_	0*	+	++	_	_	_	0*	+
Salmonella typhi	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++
Pseudomonas aeruginos	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++
Candida albicans	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++
Aspergillus fumigatus																				
Aspergillus nigre																				
Microsporumsp	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++
Trichophyton rubrum	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++.

Key: – =No turbidity (No growth), 0* = MIC, + =Turbidity (light growth), ++ = moderate turbidity, +++ = high turbidity.

Table 5. MBC and MFC hexane, chloroform, ethyl acetate and methanol seed 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	_	0*	+	++	+++	_	0*	+	++	+++	_	0*	+	++	+++	_	0*	+	++	+++
Escherichia coli	_	0*	+	++	+++	_	_	0*	+	++	_	0*	+	++	+++	_	_	0*	+	++
Klebsiellapnuemoniae	_	_	0*	+	++	_	_	0*	+	++	_	_	0*	+	++	_	0*	+	++	+++
Shigelladysenteriae	_	_	0*	+	++	_	0*	+	++	+++	_	0*	+	++	+++	_	_	0*	+	++
Salmonella typhi	_	0*	+	++	+++	_	0*	+	++	+++	0*	+	++	+++	+++	_	0*	+	++	+++
Pseudomonas aeruginos	0*	+	++	+++	++++	_	0*	+	++	+++	0*	+	++	+++	+++	0*	+	++	+++	++++
Candidasalbicans	_	0*	+	++	+++	_	0*	+	++	+++	0*	+	++	+++	+++	_	0*	+	++	+++
Aspergillusfumigatus																				
Aspergillusnigre																				
Microsporumsp	0*	+	++	+++	++++	_	0*	+	++	+++	0*	+	++	+++	+++	_	0*	+	++	+++
Trichophytonrubrum	0*	+	++	+++	++++	0*	+	++	+++	++++	0*	+	++	+++	+++	0*	+	++	+++	++++

Key: – =No Colony growth, 0* = MBC/MFC, += Scanty Colonies growth, ++ = moderate colonies growth+++ = Heavy colonies growth

Conclusion

This study showed that boiled seed of *D. edulis* have antibacterial and antifungal properties, which is dependent on the secondary metabolites present in the seed. These metabolites have been linked with various bioactivities. This justifies the traditional usage of this plant as remedy for stomach ailments. Similar study on the raw seed showed that the boiled seed of *D. edulis* exhibited higher antimicrobial activity than the raw seed (Anyam, etal., 2015). Thus, boiled seed has higher potential for medicinal value.The information provided in this study on the medicinal potency of boiled seed of *D. edulis* would serve as a useful tool for proper evaluation of the seed for therapeutic applications. Further research is on going to isolate and characterize the active principle(s)in the seed of *Dacroydes edulis.*

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