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Phytochemical and antimicrobial investigation of *Dissotis rotundifolia* (sm) Triana

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ABSTRACT

The crude ethanolic extract of *Dissotis rotundifolia* was partitioned between hexane, chloroform and ethylacetate. These extract including the remaining aqueous ethanolic extract were screened for secondary metabolites and tested for antimicrobial activities at concentration of 1000, 100 and 10 µg/ml on *Staphyloccocus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Shigella spp*, *Candida albicans* and *Aspergillus niger* using agar well diffusion method. The presence of saponin, tannin, flavonoid, anthraquinone, steroid, and cardiac glycosides were detected and the antimicrobial assay indicated a fairly concentration dependent activity of which higher concentrations of the crude and chloroform extracts showed strong activity on the fungi. The crude extract at concentration 1000 µg/ml was also found to strongly inhibit *S. aureus* while the hexane extract generally showed negligible activities.

INTODUCTION

Dissotis rotundifolia (sm) Triana belongs to the family Melastomataceae. It is wide spread in Nigeria and other West African countries. The genus has about 140 species of which most have been found to be medicinal[1]. *Dissotis rotundifolia* is a creeping herb with purple or sometimes pinkish flowers. Its medicinal uses include treatment of coughs, tooth ache, migraines, jaundices, blennorrhoea, bronchitis, fever, pneumonia, asthma, tubercular conditions, yellow fever, and diarrhoea[2].

Examination of *Dissotis rotundifolia* grown in Nigeria revealed absence of alkaloids[3]. Four C-Glycosylflavone: isoorientin, orientin, vitexin and isovitexin have been isolated from the methanolic and hydroalcoholic extracts of the plant using High Performance Liquid Chromatograhy (HPLC) coupled with UV diode-array (DAD) detection and thermospray (TSP-MS)[4]. The antitrypanosomal potential of the crude extract at different doses has been established in rats treated orally or intraperitoneally[5]. The plant also has been found to be rich in ascorbic acid (Vitamin C) when compared with some common garden fruits and vegetables[6].

Antimicrobial screening of plants continues to be relevant due to development of resistance by pathogenic organisms to orthodox synthetic drugs[7,8]. Also some medicinal plants exhibit stronger potency than the existing synthetic antimicrobial agents. *Plumeria acutifolia* for example showed better activities against some bacteria than conventional antibiotic such as gentamycin[9]. Drugs such as Plumbagin and Allicin are antimicrobial plant- drugs from *Plumbago indica* and *Allium sativum* for which no synthetic one is currently available; thus scientific study of traditional medicines and derivation of drugs through biopropecting are of great importance[10]. With the aforementioned, this study was set out to scientifically validate this claim or otherwise.

MATERIALS AND METHODS

Plant material

D.rotundifolia was collected at the premises of Uni-

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versity of Ibadan, Oyo state in the month of September, 2007. Identification and authentication were carried out at Forestry Research Institute of Nigeria (FRIN) Ibadan by Mr. Osiyemi and a voucher copy was deposited there. The herbarium number assigned to the plant was FHI 107817.

Extraction and partitioning

The air-dried and ground material (500g) was soaked in ethanol (95%) for a period of 72 h after which extract was filtered using Whatman No 1 filter paper and concentrated on rotator evaporator at 37^{0}C. The part of the resultant crude extract was reconstituted in aqueous ethanol and partitioned using separating funnel with hexane, chloroform and ethylacetate. Each of the extract including the residual aqueous ethanolic extract were concentrated and weighed.

Phytochemical analysis

The extracts were screened for secondary metabolites using standard techniques[11-13]. The presence of saponin, alkaloid, tannin, flavonoid, anthraquinone, steroid, reducing sugar, and cardiac glycosides were tested for.

Test organism

Two gram positive bacteria: *Staphyloccocus aureus* and *Bacillus subtilis*; three gram negative: *Salmonella typhi*, *Escherichia coli* and *Shigella spp* and two fungi: *Candida albicans* and *Aspergillus niger* were used. The bacteria were cultured on nutrient agar slants and 24 h old pure cultures were prepared for use while the fungi were cultured on potato dextrose agar for 72 h.

Preparation of test solutions

20 mg of each of the extract was dissolved in 2ml of Dimethylsulphoxide (DMSO) except for the crude and residual aqueous ethanolic extracts which were dissolved in 2 ml of distilled water. This gave a stock concentration of 10,000 µg/ml. The stock concentrations were serially diluted with distilled water to give concentrations of 1000, 100 and 10 µg/ml.

Test for antimicrobial activity

The antimicrobial activities of extracts were determined using agar well diffusion method. The microorganisms were inoculated uniformly and aseptically on prepared nutrient agar plates for bacteria and Potato dextrose agar for fungi using sterile cotton swabs. A sterile cork borer of 6 mm diameter was used to make wells on the agar. Graded concentrations of 10,100 and 1000 µg/ml of each extract were introduced into the wells. The plates were incubated at 37^{0}C for 24 h for bacteria while fungi were incubated at 25^{0}C for 72 h. Gentamycin at concentration of 5 µg/ml and a mixture of DMSO-water (1:1) were used as positive and negative controls respectively. The antimicrobial activity of the extracts was determined by measuring the diameter of the zone of inhibition.

RESULT AND DISCUSSION

Five bacteria and two fungi were used as trest organisms for hexane, chloroform, ethyl acetate and water extracts of *Dissotis rotundifolia* at different concentrations. The extracts were in addition screened for secondary metabolites. The extracts showed inhibitory activities against some of the test organisms though in varying degrees with the chloroform extract showing the highest inhibitory activity. At 1000µg/ml and 100µg/ml, the chloroform extract showed activities against all the test organisms while at 10µg/ml it was active against five of the test organisms. All three concentrations of ethyl acetate extract were active against all the test bacteria and one fungus (*A. niger*). *Candida albicans* was totally resistant to the ethyl acetate extract. The hexane extract had no activity on the test fungi except at concentration of 1000µg/ml on *C. albicans*.

TABLE 1 : Extraction yield.

Weight (g)		% (w/w)
Plant material	Crude	
430	12	2.8

The inhibitory activities exhibited by the extracts fall in line with an earlier report by Elmahmood *et al*[14]. They reported that the antimicrobial properties of plants can be linked to the presence of bioactive secondary metabolites like alkaloids, tannins, saponins, flavonoids, phenols, glycosides and diterpenes. These metabolites present in the extract enable the partitioning of the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable and susceptible to lyses. Extensive leakage from bacterial cells or the exit of critical molecules and

ions will lead to death[15]. Numerous extracts have been tested for *in vivo* and *in vitro* antimycotic activity and some demonstrated to be potential antifungal agents. Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration[16].

TABLE 2 : Phytochemical screening of extracts.

Secondary metabolite	CDE	HXE	CFE	EAE	AEE
Saponin	-	-	+	+	-
Alkaloid	-	-	-	-	-
Tanin	+	+	-	+	+
Flavonoid	+	-	+	+	+
Anthraquinone	-	-	+	-	-
Steriod	+	+	+	+	-
Cardiac glycoside	+	+	+	+	-

CDE= Crude extract; HXE= Hexane extract; CFE= Chloroform extract; EAE= Ethylacetate extract; AAE= Aqueous ethanol extract

TABLE 3 : Antimicrobial screening of extracts.

Extract	Concentration (µg/ml)	Diameter of zone of inhibition (mm)						
		S.t	S.s	E.c	S.a	B.s	C.a	A.n
CDE	1000	NA	14	17	25	18	16	22
	100	NA	4	16	16	13	16	22
	10	NA	4	14	16	10	4	NA
HXE	1000	NA	2	NA	4.5	4	2	NA
	100	NA	2	NA	3	NA	NA	NA
	10	NA	NA	NA	NA	NA	NA	NA
CFE	1000	7.5	19	7	6	8	25	20
	100	6	15	7	2	8	8	20
	10	NA	9	6	2	5	2	NA
EAE	1000	17.5	22	11	16	16	NA	15
	100	15	18	9.2	16	15	NA	15
	10	14	11	8	13	10	NA	NA
AEE	1000	NA	20	8	15	NA	6	20
	100	NA	13	3	11	NA	4	12
	10	NA	13	3	11	NA	3	NA

S.t=Salmonella typhi, S.s= Shigella spp, E.c= Echerichia coli, C.a= Candida albicans, A.n= Aspergillus niger, S.a= Staphyloccocus aureus, B.s= Bacillus subtilis, NA=No activity

Jigna *et al.* explained that presence of oil, wax, resin or fatty acids which makes the bacterial cell wall impervious to active secondary metabolites maybe the reasons why some of the extracts were not active against the bacteria[17]. The antimicrobial activity varied between solvents. Chloroform had the highest activity followed by ethyl acetate, water, crude extract and hexane. It has been reported that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity[18].

TABLE 4 : Antimicrobial screening of controls.

CONTROL	Diameter of zone of inhibition (mm)						
	S.t	S.s	E.c	S.a	B.s	C.a	A.n
Gentamycin (µg/ml)	25	22	26	22	21	16	19
DMSO & H_2O (1:1)	NA	NA	NA	NA	NA	NA	NA

The crude extract, at concentration of 1000µg/ml strongly inhibited *S. aureus*. This is interesting in view of the fact that prevalence of *S. aureus* resistance to conventional antibiotics is on the increase. Therefore, this plant could be a source of an alternative remedy to treatment of *S. aureus* infections[19,20].

The Gram negative bacterium *Salmonella typhi* was the most resistant of the bacteria tested. Doris *et al* hypothesized that plants produce compounds can be effective antimicrobials if they find their way into the cell of the pathogen especially across the double membrane barrier of Gram negative bacteria[21]. Gram-positive cell walls consist of many layers of peptidoglycan and do not possess a lipid outer membrane. Gram-negative cell walls on the other hand have only one or a few layers of peptidoglycan but posses an outer membrane consisting of various lipid complexes which aids in resistance.

The standard antibiotic used, Gentamycin had a range of inhibition zone of between 16mm and 26mm. It displayed greater potency when compared to the extracts. This may be due to the fact that these conventional antibiotics are refined and purified products while extracts of plants are a mixture of various constituents some of which can interfere with antimicrobial activities and are subject to degradation and decomposition on storage[22].

The various activities recorded in the tested extracts could be attributed to the presence of secondary metabolites in the plant[23]. It was observed that none of the extracts showed the presence of alkaloid and reducing sugar. This is in agreement with Burkhill that *D. rotundifolia* found in Nigeria has no alkaloids[24]. However, all the extracts except the aqueous ethanol extract showed the presence of cardiac glycosides and steroids while anthraquinones was detected only in the

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chloroform extract. Tannin was present in all the extracts except the chloroform extract while saponin was detected in chloroform and ethyl acetate extracts. Flavonoids was found to be absent only in the hexane extract. The presence of these secondary metabolites elicits some biological activities in man. Polyphenolic substances such as flavonoids and tannins are antioxidant in nature; they help scavenge free radical in biological systems.

This study justifies the acclaimed therapeutic properties and folkoric usage of *D. rotundifolia.*

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