Phytochemical Screening and Antimicrobial Studies of Methanol, Ethyl Acetate and Hexane Extracts of *Vitex doniana*, Sweet. (Stem Bark and Leaf)

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ABSTRACT: The pulverized stem bark and leaf of *Vitex doniana* Sweet were extracted successively under soxhlet with hexane, ethylacetate and methanol. The extracts were qualitatively screened for the presence of some secondary metabolite, and then tested *in vitro* for activity against some common disease causing microbes. Both standard strains and clinical isolates were used in the antimicrobial screening. The zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined. The results of phytochemical screening revealed the presence of terpenes, sterols, alkaloids, flavonoids, tannins, saponins, glycosides, carbohydrates and balsams while resins were not detected. The *in vitro* antimicrobial screening using the well diffusion technique revealed the extract to have broad spectrum activity with zones of inhibition ranging from 19 to 24mm, MIC of 2.5 and 10mg/ml for all the sensitive organisms, and MBC and MFC of 2.5-5 and 10mg/ml respectively. The highest activity was an MIC of 1.25 mg/ml and MBC of 2.5mg/ml. The activity index (A.I) shows that the extracts were more active against some microbe especially the enteric bacteria like *Klebsiella pneumonia, Klebsiella ozaenae, Shigella dysenteriae, Basillus subtilis, Salmonella typhi* and *Staphylococcus Aureus*, while the Proportion index show that the methanol and water extract of the stem bark is a better broad spectrum antibiotic than the other extract. This study provides some scientific base for the use of the plant in traditional medicine. The activities observed could be due to the presence of some of the secondary metabolites like terpenes, alkaloids and flavonoids which have known antimicrobial activity. [Nature and Science 2010;8(8):177-185]. (ISSN: 1545-0740).

Key words: *Vitex doniana,* phytochemical, antimicrobial, MIC, MBC, MFC

INTRODUCTION

Plants and other substances of natural origin have being in used throughout the world for human and animal health care from time immemorial. This is especially in Africa where underdevelopment and poverty have made a large percentage of the people to rely almost entirely on traditional medical practices and folkloric use of plants (Ajoku et al 2001; Enzo, 2006;). More so, with the WHO Alma-Ata declaration of 1978 encouraging developing and poor countries to incorporate traditional medicines of proven efficacy into the orthodox practice in order to bridge the widening gap in accessing primary healthcare by the citizen and since more than 70% of the population in these countries already patronise traditional medicine [WHO 1978]. The efficacy of some of these traditional herbal remedies has been proven by various researchers (Kunle et al 2003; Lal *et al*. 1994 and Singh, 1994). One such plant renowned for it wide use in Africa native folklore is *V. Doniana* Sweet.

The plant is native to, Nigeria, Botswana, Ethiopia, Kenya, Lesotho, Namibia, Niger, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia. It is locally called vitex (English), dinya (Hausa), Ucha koro (Igbo) and oori-nla (Yoruba) (Burkill, 2000). *V.*

doniana, family Verbanaceae (Labiatae), is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown and a clear bole up to 5 m. Bark rough, pale brown or greyish-white, rather smooth with narrow vertical fissures. The bases of old trees have oblong scales. Leaves opposite, glabrous, 14-34 cm long, usually with 5 leaflets on stalks 6-14 cm long. Leaflets distinctly stalked, ovate, obovate-elliptic or oblong, entire, 8-22 cm long, 2-9 cm wide. Leaf tips rounded or emarginate, leaf bases cuneate, dark green above, pale greyish-green below, thickly leathery, with a few scattered stellate hairs on the upper surface, otherwise without hairs. Flower petals white except on largest lobe, which is purple, in dense opposite and axillary cymes. Flowers small, blue or violet, 3-12 cm in diameter, only a few being open at a time. Fruit oblong, about 3 cm long, green when young, turning purplish-black on ripening and with a starchy black pulp. Each fruit contains 1 hard, conical seed, 1.5-2 cm long, 1-1.2 cm wide (Burkill 2000).

In folkloric medicine, various parts of the plant are used as remedy for disease conditions such as infertility, anaemia, jaundice, leprosy, dysentery, colic, gonorrhea, backaches, headaches, febrifuge, conjunctivitis and other eye troubles, stiffness, measles, rash, fever, chickenpox , hemiplegia, as tonic galactagogue to aid milk production in lactating mothers, anodyne, ankylostomiasis (ancylostomiasis), rachitis, leprosy and liver disease, kidney troubles and lack of vitamin A and B. the twigs are used as chewing sticks for cleaning the teeth. The blackish extract obtained by boiling the leaves, bark, root and/or fruits is used as ink and dye for clothes (Burkill, 2000; Irvine 1961). The generic name, 'Vitex', is an old Latin name for the genus. To verify this folkloric use there is the need to screen this plant for known bioactive secondary metabolites and against common disease causing microbes which may be responsible for some of the disease conditions mentioned above. Apart from the economic relevance of this plant in timber and wood production, not much investigation on its chemical and antimicrobial activity has been done. It is in the light of the above that this study aims to link scientific facts with some of these traditional uses with the hope of attracting more research attention to plant in new lead/hit prospects in drug discovery.

MATERIALS AND METHODS

Solvents and reagent used in the study were of Analar grade and, unless otherwise stated, were sourced from Zayo-Sigma, Abuja, Nigeria.

Collection and Extraction of Plant Material

The plant collected in April 2010 from NIPRD medicinal plant garden, Idu Abuja, Nigeria and identified by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6415 was deposited at the herbarium of the department. The stem bark and leaves were collected from the plant in the garden and rinsed with clean water. The parts were separated and air-dried for three weeks, and then pulverized using a mortar and pestle. The pulverized plant parts were kept separately in an air-tight cellophane bag until used.

Phytochemical screening

The presence of some basic secondary metabolites in the pulverized plant material were determined using standard methods (Sofowora 2008 and Evans 2002). Proximate analysis was also carried out to determine the moisture content, total ash value, acid insoluble ash value, and alcohol and water extractive values.

Preparation of extracts

Exactly 400g each of the pulverized plant was macerated successively in Hexane, ethylacetate and 95% methanol for 48hrs each. The mixtures were then filtered under vacuum and the filtrates concentrated using a rotatory evaporator. The methanol concentrate was evapourated to dryness in a water bath. The extracts were stored in an airtight sample bottles and kept in a desiccator until used.

Preparation of Extract Stock Concentration for Antimicrobial screening

A test stock concentration of 10mg/ml for methanol and ethylacetate was prepared by dissolving 0.1g of each extract in 10mls of distilled water in separate test tubes. For the hexane extract a concentration of 20mg/ml was prepared by dispersing 0.2g in 10mls of distilled water. The positive control drugs were erythromycin (0.5mg/ml) and flouconazole (0.5mg/ml), all of sigma chemicals UK obtained from Zayo-Sigma Abuja Nigeria.

Antimicrobial Screening

Organism Source

The organisms used include standard strains, *Staphylococcus aureus NCTC 6571, Bacilluc subtilis NCTC 8236, Eschericia coli NCTC 10418, Pseudomonas aeruginosa NCTC 6750, Salmonella typhimurium ATCC 9184, Klebsiella pneumonia ATCC 10031* and *Staphylococcuc aureus ATCC 13704,* obtained from the department of Pharmaceutical Microbiology, Ahmadu Bello University (ABU) Zaria, Nigeria, and clinical isolates,

Staphylococcus aureus, Methicilin Resistant Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis, Corynebacterium ulcerans Listeria monocytogenes, Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Klebsialla ozaenae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas flourescense, Salmonella typhimurium, Shigella dysenteriae, Aspergillus fumigates, candida albicans, Microsporum gypseum and Trichophyton rubrum, obtained from the department of medical Microbiology Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria. All the organisms were checked for purity and maintained at 4^oC in slants of nutrient agar and sabouraud dextrose agar (SDA) for bacteria and fungi respectively. Well diffusion method described by Hugo and Russel (1992) was used to determine the antimicrobial activities (zone of inhibition) of the extracts against the organisms.

Preparation of the Inoculum

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test-tubes containing nutrient broth for bacteria and sabouraud dextrose liquid for fungi. The test-tubes were incubated for 24hrs at 37^oC for bacteria and for 48hrs at 30^oC for the fungi. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density of 10^8cfu/ml for the bacteria. For the fungi, fungal spores were harvested after 7 days old SDA slant culture was washed with 10ml normal saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spores suspension were standardized to 10^5cfu/ml.

Preparation of Media

The medium was prepared according to manufacturer's instruction (Oxoids Limited Basingstoke, Hampshire, England). 40g of Blood Agar (52g of SDA) were weighed into a conical flask 1000ml of distilled water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121^oC for 15mins. The media were allowed to cool to 45^oC and 20ml of the sterilized medium was poured into sterile petri-dishes and allowed to cool and solidify. The plates were labeled with the test microorganism (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the aid of a glass spreader. The plates were dried at 37^oC for 30mins and divided into two sets to be used for the well diffusion method and the disc diffusion method respectively.

Zone of Inhibition - Well Diffusion Method

A standard cork borer of 5mm in diameter was used to cut well at the center of each inoculated plate and the agar removed from the well. 0.1ml of the text solution (extract) was then introduced into the well created at the center for each plate. The bacteria plates were incubated at 37^oC for 24hrs while the fungal plates were incubated at 30^oC for 1-7days, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Sterilized distilled water was used as negative control.

Minimum Inhibitory Concentration - Broth Dilution Method

The MIC was determined using broth dilution method as described in Ibekwe *et al*, 2001. The nutrient broth and sabouraud dextrose liquid were prepared according to the manufacturer's instruction (10ml of each broth was dispensed into separate test-tube and was sterilized at 121^oC for 15mins and then allowed to cool. Two-fold serial dilution of the extract in the broth were made from the stock concentration of the extract to obtain 10, 5, 2.5, 1.25, 0.625mg/ml for methanol and ethylacetate, and 20, 10, 5, 2.5, and 1.25mg/ml for the hexane extracts. 0.1ml of the standardized inoculums of the microbes were then inoculated into the different concentrations of the extracts in the broth. The test tubes of the broth were then incubated at 37^oC for 24hrs and 30^oC for 1-7days for bacteria and fungi respectively and observed for turbidity of growth. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

Minimum Bactericidal/Fungicidal Concentration - Broth Dilution Method

This was carried out to check whether the test microbes were killed or only inhibited in growth. Blood and sabouraud media were prepared, sterilized at 121^oC for 15mins and was poured into sterile petri-dishes and left to cool and solidify. The contents of the MIC in the serial dilution were then sub-cultured onto the media and incubated at 37^oC for 24hrs and 30^oC for 1-7days for bacteria and fungi respectively, and observed for colony growth. The MBC/MFC was the plate with the lowest concentration of extract and without colony growth.

Determination of activity index
The activity index (Arya *et al* 2010) of the crude plant extract was calculated as

$$\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

Determination of proportion index
The proportion index (Arya *et al* 2010) was calculated as

$$\text{Proportion index (P.I.)} = \frac{\text{Number of positive results obtained for extract}}{\text{Total number of tests carried out for each extract}}$$

RESULTS

The results of phytochemical screening are shown in table 1 and 2, while that of microbial screening are as shown in table 3 to 6 below.

Table 1: Result of the phytochemical screening

Phytochemical constituents	Stem bark			Leaf		
	M	E	H	M	E	H
Flavonoids	-	-	+	-	-	+
Tannins	+	-	-	+	-	+
Saponins	+	+	+	-	-	-
Anthraquinones	+	+	-	+	-	-
Balsam	+	+	+	+	+	+
Carbohydrate	-	+	+	-	+	+
Resin	-	-	+	+	-	+

Key: + = Present; - = Absent; W=water; M=methanol; E=ethylacetate; H=heaxane

Table 2: Result of the proximate analysis

Parameter	Values (%)	
	Stem bark	Leaf
Moisture content	9.29	11.73
Water-soluble extractive value	18.04	26.14
Alcohol-soluble extractive value	6.78	7.72
Total ash value	5.40	3.44
Acid-insoluble ash value	0.53	0.41

Table 3: Result of Zone of Inhibition by *V. doniana*

S/N	TEST ORGANISM	STRAINS	ZONE OF INHIBITION (mm)										
			Leaf			Stem bark					Control		
			wl	ml	el	hl	wsb	msb	esb	hsb	Sp	Er	Fl
1	*Staphylococcus aureus*	NCTC 6571	27	22	27	0	30	32	19	19	29	22	0
2	*Bacillus subtilis*	NCTC 8236	29	27	28	0	32	32	27	0	20	22	0
3	*Escherichia coli*	NCTC 10418	0	0	0	18	0	30	0	20	22	24	0
4	*Pseudomonas aeruginosa*	NCTC 6750	31	0	27	20	29	27	24	19	24	0	0
5	*Salmonella typhimurium*	ATCC 9184	29	27	24	0	30	29	0	18	25	27	0
6	*Klebsiella pneumoniae*	ATCC 10031	29	0	24	20	32	27	22	20	25	29	0

7	*Staphylococcus aureus*	ATCC 13704	24	22	26	0	27	0	25	0	20	27	0
8	*Candida albicans*	ATCC 10231	0	0	0	0	0	0	0	0	0	0	22
9	*Staphylococcus aureus*	Isolate	22	27	24	14	29	27	29	0	20	21	0
10	*Methicilin Resistant Staph. aureua*	Isolate	24	26	0	15	27	29	0	17	0	27	0
11	*Streptococcus pyogenes*	Isolate	25	0	27	13	22	24	0	0	20	26	0
12	*Streptococcus faecalis*	Isolate	22	0	27	14	20	26	30	0	24	29	0
13	*Corynebacterium ulcerans*	Isolate	0	27	29	0	29	27	29	14	25	30	0
14	*Listeria monocytogenes*	Isolate	0	0	24	0	18	32	28	0	25	24	0
15	*Bacillus subtilis*	Isolate	27	30	0	15	29	30	0	19	20	25	0
16	*Bacillus cereus*	Isolate	25	27	0	15	32	29	30	14	24	26	0
17	*Escherichia coli*	Isolate	0	0	27	14	0	27	0	0	27	20	0
18	*Klebsiella pneumoniae*	Isolate	24	27	22	16	22	27	30	17	26	19	0
19	*Klebsiella ozaenae*	Isolate	25	29	0	0	27	25	29	16	24	18	0
20	*Proteus mirabilis*	Isolate	0	0	29	0	0	29	30	0	22	20	0
21	*Proteus vulgaris*	Isolate	0	30	29	0	0	29	30	0	0	24	0
22	*Pseudomonas aeruginosa*	Isolate	27	29	30	17	22	30	29	0	19	22	0
23	*Pseudomonas flourescenses*	Isolate	0	29	0	14	14	0	0	0	0	24	0
24	*Salmonella typhimurium*	Isolate	22	24	27	0	20	28	27	17	20	22	0
25	*Shigella dysenteriae*	Isolate	22	27	27	14	24	30	29	16	20	20	0
26	*Aspergillus flavus*	Isolate	0	0	0	0	0	0	0	0	0	0	27
27	*Aspergillus fumigatus*	Isolate	0	0	0	0	0	0	0	0	0	0	23
28	*Candida albicans*	Isolate	0	17	0	0	0	0	0	0	0	0	24
29	*Microsporum gypseum*	Isolate	0	0	0	0	0	0	0	0	0	0	20
30	*Trichophyton rubrum*	Isolate	0	0	0	0	0	0	0	0	0	0	24

wl = *V. doniana leaf water extract; ml=V. doniana leaf methanol extract; el= V. doniana leaf ethylacetate extract; hl= V. doniana leaf hexane extract; wsb= V. doniana stem bark water extract; msb= V. doniana stem bark methanol extract; esb= V. doniana stem bark ethylacetate extract;* hsb = *V. doniana stem bark hexane extract;* Sp= Sparfloxacin; Er = Erythromycin; Fl = Flouconazole

Table 4: Activity Index

S/N	TEST ORGANISM	STRAINS	Activity Index (A.I)							
			Leaf				Stem bark			
			wl	ml	el	hl	wsb	msb	esb	hsb
	Proportion Index		**0.57**	**0.57**	**0.60**	**0.47**	**0.67**	**0.73**	**0.57**	**0.43**
1	*Staphylococcus aureus*	NCTC 6571	1.23	1.00	1.23	0.00	1.36	1.45	0.86	0.86
2	*Bacillus subtilis*	NCTC 8236	1.32	1.23	1.27	0.00	1.45	1.45	1.23	0.00
3	*Escherichia coli*	NCTC 10418	0.00	0.00	0.00	0.75	0.00	1.25	0.00	0.83
4	*Pseudomonas aeruginosa*	NCTC 6750	1.29	0.00	1.13	0.83	1.21	1.13	1.00	0.79
5	*Salmonella typhimurium*	ATCC 9184	1.07	1.00	0.89	0.00	1.11	1.07	0.00	0.67
6	*Klebsiella pneumoniae*	ATCC	1.00	0.00	0.83	0.69	1.10	0.93	0.76	0.69

7	*Staphylococcus aureus*	10031 ATCC 13704	0.89	0.81	0.96	0.00	1.00	0.00	0.93	0.00
8	*Candida albicans*	ATCC 10231	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	*Staphylococcus aureus*	Isolate	1.05	1.29	1.14	0.67	1.38	1.29	1.38	0.00
10	*Methicilin Resistant Staph. aureua*	Isolate	0.89	0.96	0.00	0.56	1.00	1.07	0.00	0.63
11	*Streptococcus pyogenes*	Isolate	0.96	0.00	1.04	0.50	0.85	0.92	0.00	0.00
12	*Streptococcus faecalis*	Isolate	0.76	0.00	0.93	0.48	0.69	0.90	1.03	0.00
13	*Corynebacterium ulcerans*	Isolate	0.00	0.90	0.97	0.00	0.97	0.90	0.97	0.47
14	*Listeria monocytogenes*	Isolate	0.00	0.00	1.00	0.00	0.75	1.33	1.17	0.00
15	*Bacillus subtilis*	Isolate	1.08	1.20	0.00	0.60	1.16	1.20	0.00	0.76
16	*Bacillus cereus*	Isolate	0.96	1.04	0.00	0.58	1.23	1.12	1.15	0.54
17	*Escherichia coli*	Isolate	0.00	0.00	1.35	0.70	0.00	1.35	0.00	0.00
18	*Klebsiella pneumoniae*	Isolate	1.26	1.42	1.16	0.84	1.16	1.42	1.58	0.89
19	*Klebsiella ozaenae*	Isolate	1.39	1.61	0.00	0.00	1.50	1.39	1.61	0.89
20	*Proteus mirabilis*	Isolate	0.00	0.00	1.45	0.00	0.00	1.45	1.50	0.00
21	*Proteus vulgaris*	Isolate	0.00	1.25	1.21	0.00	0.00	1.21	1.25	0.00
22	*Pseudomonas aeruginosa*	Isolate	1.23	1.32	1.36	0.77	1.00	1.36	1.32	0.00
23	*Pseudomonas flourescenses*	Isolate	0.00	1.21	0.00	0.58	0.58	0.00	0.00	0.00
24	*Salmonella typhimurium*	Isolate	1.00	1.09	1.23	0.00	0.91	1.27	1.23	0.77
25	*Shigella dysenteriae*	Isolate	1.10	1.35	1.35	0.70	1.20	1.50	1.45	0.80
26	*Aspergillus flavus*	Isolate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	*Aspergillus fumigatus*	Isolate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	*Candida albicans*	Isolate	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00
29	*Microsporum gypseum*	Isolate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	*Trichophyton rubrum*	Isolate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A.I. used Erythromycine as standard wherever active, and Sparfloxacin or Fluconazole where not active; the P.I result were wl= 0.57; ml=0.57; el=0.60; hl=0.47; wsb=0.67; msb=0.73; esb=0.57; hsb=0.43.

Table 5: Minimum Inhibitory Concentration (MIC)

S/N	TEST ORGANISM	STRAINS	Minimum Inhibitory Concentration (MIC) mg/ml							
			Leaf				Stem bark			
			wl	ml	el	hl	wsb	msb	esb	hsb
1	*Staphylococcus aureus*	NCTC 6571	2.5	2.5	2.5	-	1.25	1.25	5	10
2	*Bacillus subtilis*	NCTC 8236	2.5	2.5	2.5	-	1.25	1.25	2.5	-
3	*Escherichia coli*	NCTC 10418	-	-	-	10	-	1.25	-	5
4	*Pseudomonas aeruginosa*	NCTC 6750	2.5	-	2.5	5	2.5	2.5	2.5	10
5	*Salmonella typhimurium*	ATCC 9184	2.5	2.5	2.5	-	1.25	2.5	-	10
6	*Klebsiella pneumoniae*	ATCC 10031	2.5	-	2.5	5	1.25	2.5	2.5	5
7	*Staphylococcus aureus*	ATCC 13704	2.5	2.5	2.5	-	1.25	-	2.5	-
8	*Candida albicans*	ATCC	-	-	-	-	-	-	-	-

		10231								
9	*Staphylococcus aureus*	Isolate	2.5	2.5	2.5	10	2.5	2.5	2.5	-
10	*Methicilin Resistant Staph. aureua*	Isolate	2.5	2.5	-	10	2.5	2.5	-	10
11	*Streptococcus pyogenes*	Isolate	2.5	-	2.5	10	2.5	2.5	-	-
12	*Streptococcus faecalis*	Isolate	2.5	-	2.5	10	2.5	2.5	1.25	-
13	*Corynebacterium ulcerans*	Isolate	-	2.5	2.5	-	2.5	2.5	2.5	10
14	*Listeria monocytogenes*	Isolate	-	-	2.5	-	5	1.25	2.5	-
15	*Bacillus subtilis*	Isolate	2.5	1.25	-	10	2.5	1.25	-	10
16	*Bacillus cereus*	Isolate	2.5	2.5		10	1.25	2.5	1.25	10
17	*Escherichia coli*	Isolate	-	-	2.5	-	-	1.25	-	-
18	*Klebsiella pneumoniae*	Isolate	2.5	2.5	2.5	10	2.5	2.5	1.25	10
19	*Klebsiella ozaenae*	Isolate	2.5	2.5	-	-	2.5	2.5	2.5	10
20	*Proteus mirabilis*	Isolate	-	-	2.5	-	-	2.5	1.25	-
21	*Proteus vulgaris*	Isolate	-	1.25	2.5	-	-	2.5	1.25	-
22	*Pseudomonas aeruginosa*	Isolate	2.5	2.5	1.25	10	2.5	1.25	2.5	-
23	*Pseudomonas flourescenses*	Isolate	-	2.5	-	10	5	-	-	-
24	*Salmonella typhimurium*	Isolate	2.5	2.5	2.5	-	2.5	2.5	2.5	10
25	*Shigella dysenteriae*	Isolate	2.5	2.5	2.5	10	2.5	1.25	2.5	10
26	*Aspergillus flavus*	Isolate	-	-	-	-	-	-	-	-
27	*Aspergillus fumigatus*	Isolate	-	-	-	-	-	-	-	-
28	*Candida albicans*	Isolate	-	5	-	-	-	-	-	-
29	*Microsporum gypseum*	Isolate	-	-	-	-	-	-	-	-
30	*Trichophyton rubrum*	Isolate	-	-	-	-	-	-	-	-

Table 6: Minimum Bactericidal (Fungicidal) Concentration (MBC/ MFC))

S/N	TEST ORGANISM	STRAINS	MBC/MFC (mg/ml)							
			Leaf				Stem bark			
			wl	ml	el	hl	wsb	msb	esb	hsb
1	*Staphylococcus aureus*	NCTC 6571	5	5	5	-	2.5	2.5	10	20
2	*Bacillus subtilis*	NCTC 8236	5	5	5	-	2.5	2.5	5	-
3	*Escherichia coli*	NCTC 10418	-	-	-	20	-	2.5	-	20
4	*Pseudomonas aeruginosa*	NCTC 6750	5	-	5	20	5	5	10	20
5	*Salmonella typhimurium*	ATCC 9184	5	5	10	-	2.5	5	-	20
6	*Klebsiella pneumoniae*	ATCC 10031	5	-	10	20	2.5	5	10	20
7	*Staphylococcus aureus*	ATCC 13704	10	10	5	-	5	-	5	-
8	*Candida albicans*	ATCC 10231	-	-	-	-	-	-	-	-
9	*Staphylococcus aureus*	Isolate	10	5	10	20	5	5	5	-
10	*Methicilin Resistant Staph. aureua*	Isolate	5	5	-	20	5	5	-	20
11	*Streptococcus pyogenes*	Isolate	5	-	5	20	10	5	-	-

12	*Streptococcus faecalis*	Isolate	10	-	5	20	10	5	2.5	-
13	*Corynebacterium ulcerans*	Isolate	-	5	5	-	5	5	5	20
14	*Listeria monocytogenes*	Isolate	-	-	10	-	10	2.5	5	-
15	*Bacillus subtilis*	Isolate	5	2.5	-	20	5	2.5	-	20
16	*Bacillus cereus*	Isolate	5	2.5	-	20	2.5	2.5	2.5	20
17	*Escherichia coli*	Isolate	-	-	5	-	-	2.5	-	-
18	*Klebsiella pneumoniae*	Isolate	10	5	10	20	10	5	2.5	20
19	*Klebsiella ozaenae*	Isolate	5	5	-	-	10	5	5	20
20	*Proteus mirabilis*	Isolate	-	-	5	-	-	5	2.5	-
21	*Proteus vulgaris*	Isolate	-	2.5	5	-	-	5	2.5	-
22	*Pseudomonas aeruginosa*	Isolate	5	5	5	20	10	2.5	5	-
23	*Pseudomonas flourescenses*	Isolate	-	5	-	20	10	-	-	-
24	*Salmonella typhimurium*	Isolate	10	5	5	-	10	5	5	20
25	*Shigella dysenteriae*	Isolate	10	5	5	20	5	2.5	5	20
26	*Aspergillus flavus*	Isolate	-	-	-	-	-	-	-	-
27	*Aspergillus fumigatus*	Isolate	-	-	-	-	-	-	-	-
28	*Candida albicans*	Isolate	-	10	-	-	-	-	-	-
29	*Microsporum gypseum*	Isolate	-	-	-	-	-	-	-	-
30	*Trichophyton rubrum*	Isolate	-	-	-	-	-	-	-	-

DISCUSSION

V. doniana stem bark extract appears to be active than leaf extract on the overall assessment. The water and methanol extract of the stem bark were the most active against wide range of microorganisms than the others. Only the methanol extract of the leaf was active against cadida. It is also worthy of note that at the active concentration, some of the extract exhibited larger zone of inhibition compare to the control drugs, suggesting the possibility of a stronger activity. The extract did not show strong activity against E. coli.

The secondary metabolites like flavonoids, tannins, saponins, resin, anthraquinone and balsam revealed in the phytochemical screening of the plant may be responsible for some of the antimicrobial activities (Kunle and Egharevba, 2009). Work is still ongoing in the authors' laboratory to isolate some of compounds responsible for the antimicrobial activities.

CONCLUSION

V. doniana is highly exploited traditionally for ethnomedicinal purposes and this study actually justify its wide traditional application. The study also shows that the plant may be a good as an antibacterial preparation but may not be very useful as antifungi.

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