Antimicrobial Activity Of The Extract Of *Vernonia Ambigua* (Aerial Part)

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ABSTRACT: The *in vitro* antimicrobial activity of crude methanolic extract of the aerial part of *Vernonia ambigua* (Kotschy and Peyr) was investigated. The extract exhibited antimicrobial activities with zones of inhibition ranging from 19 to 24mm and the minimum inhibitory concentration (MIC) of 2.5 and 10mg/ml for all the sensitive organisms. The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were 2.5-5 and 10mg/ml respectively. There was appreciable activity against most of the bacterial investigated and was only active against *Candida albicans* among the fungi specie. Phytochemical screening confirmed the presence of saponin, tannin, alkaloids and flavonoids in the extracts. The ability of the crude methanolic extracts of *V. ambigua* to inhibit the growth of bacteria and fungi, though at a higher concentration of 2.5mg/ml, is an indication of its broad spectrum antimicrobial potential which may be exploited in the management of microbial infections and a source of new lead/hit for antimicrobials. [Researcher. 2010;2(6):74-80]. (ISSN: 1553-9865).

Key words: *Vernonia ambigua,* antimicrobial, MIC, MBC, MFC

INTRODUCTION

Enteric bacteria such as *Escherichia coli, shigella dysentariae* and *Salmonella typhimurium* are major causes of food-borne illnesses and gastrointestinal problems in the developing countries and human beings around the world. Some specie of *E. Coli* have been implicated in kidney failure and even death (Ajayi and Akintola, 2010). Some of these organisms of medical importance have developed resistance to currently available antibiotics, hence the constant need for new safe and more efficient therapeutic agents especially those of plant origin since some of these plants are being used traditionally in folklore (Igbinosa *et al*., 2009).

It is a well know fact that there is a growing change of focus from the use of the commonly known edible vegetables traditionally used as medical plants as a source of new leads/hit in drug discovery to traditional weeds considered hitherto non-edible and economically unimportant specie. *V. ambigua* locally called Orungo (Yoruba), Tab-taba/Tattaba (Hausa) and uses as remedies for cough and fever belong to the family Asteraceae/Compositae. It is a weed with no viable economic significance in Nigeria (Kunle and Egharevba, 2009). It is an annual herb, about 65cm in height, erect stem very hairy (long and dense); stem green in colour. The leaf is simple, sissile, very hairy and alternate, with new branch arising from leaf axil. The Leaf shape is oblanceolate with serrated margin, acute apex, slightly cordate or rounded base. The leaf nerves are prominent beneath and almost obscure above. Leaves are green in

colour are about 5cm in length and 1.5cm in width. The flowers are in capitulum, about 8mm to 10mm broad, more or less cluster at the end of spreading leafy branches. The florets are purple with calyx green and hairy. Fruits are tiny and ribbed with many light brown or dirty white pappus hair. Fruit with pappus is about 0.6cm (6mm) long. The preliminary antimicrobial screening of the whole plant at a concentration of 2mg/ml did not show any activity.

This work is aimed at screening the plant extract against common disease causing bacteria and fungi to determine their sensitivity, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) with a view towards further exploration as a source of new antimicrobials.

MATERIALS AND METHODS

All the solvents and chemicals used were of Analar grade and, unless otherwise stated, were obtained from Zayo-Sigma Nigeria.

Collection Plant Material

The Plant was collected in November 2009 from One-man village, Chaza, Suleja, Niger State, 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 was deposited at the herbarium of the department for reference. The flowers and roots was removed from the whole plant and the remaining aerial part was rinsed with clean water and air-dried for two weeks, and then pulverized using a mechanical grinder. The pulverized plant material was kept 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; Evans 2002). Proximate analysis was also carried out to determine the moisture content, total ash value, acid insoluble ash value, and alcohol and water soluble extractive values.

Preparation of extracts

Exactly 100g each of the pulverized plant was macerated with 70% methanol (Methnaol 70%, water 30%) for 48hrs. The mixture was then filtered and the filtrate was concentrated using a rotatory evaporator. The aqueous concentrate was freeze-dried and stored in an airtight sample bottle and kept in a desiccator until used. The extraction yield was 3.53% (w/w).

Preparation of Stock Test Concentration from the Plant Extracts

0.1g of freeze-dried extract was weighed and dissolved in 10mls of distilled water to obtain a concentration of 10mg/ml. The positive control drugs were sparfloxacin (0.2mg/ml), 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 antimicrobial screening was carried out on both standard strains and clinical isolates. The standard starins were obtained from the department of Pharmaceutical Microbiology, Ahmadu Bello University (ABU) Zaria, Nigeria, while the clinical isolates were obtained from the department of medical Microbiology Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria. 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, Staphylococcuc aureus ATCC 13704,* 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.* All the

organisms were checked for purity and maintained at 4°C in slants of nutrient agar and sabouraud dextrose agar (SDA) for bacteria and fungi respectively. Both well diffusion method described by Hugo and Russel (1992) and disc diffusion method described by Bauer-Kirby (1960) were used to determine the antimicrobial activities (zone of inhibition) of the extracts against the organisms [Hugo and Rusell, 1992; Bauer *et al* 1960).

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°C for bacteria and for 48hrs at 30°C 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°C for 15mins. The media were allowed to cool to 45°C 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°C 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°C for 24hrs while the fungal plates were incubated at 30°C for 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.

Zone of Inhibtion - Disc Diffusion Method

Filter paper disc of 8mm in diameter were cut and sterilized at 100°C for 30mins. The cut paper discs were then impregnated with the solution of the extracts, dried at 40°C and planted on the media seeded with the microorganisms. The plates were incubated at 37°C for 24hrs and 30°C for 1-7days for bacteria and fungi respectively, and observed for the zones of inhibition of growth. The zones were measured with a transparent ruler and the results recorded in millimeters. Discs dipped in sterilized distilled water served as negative control.

Minimum Inhibitory Concentration - Broth Dilution Method

MIC of the extracts were also carried out 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°C 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. 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°C for 24hrs and 30°C 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^{o}C 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^{o}C for 24hrs and 30^{o}C 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 microbial screening are shown in table 1 and 2, while that of phytochemical screening are as shown in table 3 & 4 below.

Table 1: Result of Zone of Inhibition by V. ambigua

S/N	TEST ORGANISM	STRAINS	ZONE OF INHIBITION (mm)			
			Va	Sp	Er	Fl
1	*Staphylococcus aureus*	NCTC 6571	20	29	22	0
2	*Bacillus subtilis*	NCTC 8236	0	20	22	0
3	*Escherichia coli*	NCTC 10418	21	22	24	0
4	*Pseudomonas aeruginosa*	NCTC 6750	0	24	0	0
5	*Salmonella typhimurium*	ATCC 9184	21	25	27	0
6	*Klebsiella pneumoniae*	ATCC 10031	19	25	29	0
7	*Staphylococcus aureus*	ATCC 13704	22	20	27	0
8	*Candida albicans*	ATCC 10231	20	0	0	22
9	*Staphylococcus aureus*	Clinical	22	20	21	0
10	*Methicilin Resistant Staph. aureua*	Clinical	0	0	27	0
11	*Streptococcus pyogenes*	Clinical	22	20	26	0
12	*Streptococcus*	Clinical	20	24	29	0

	faecalis					
13	*Corynebacterium ulcerans*	Clinical	24	25	30	0
14	*Listeria monocytogenes*	Clinical	20	25	24	0
15	*Bacillus subtilis*	Clinical	24	20	25	0
16	*Bacillus cereus*	Clinical	0	24	26	0
17	*Escherichia coli*	Clinical	23	27	20	0
18	*Klebsiella pneumoniae*	Clinical	20	26	19	0
19	*Klebsiella ozaenae*	Clinical	23	24	18	0
20	*Proteus mirabilis*	Clinical	0	22	20	0
21	*Proteus vulgaris*	Clinical	0	0	24	0
22	*Pseudomonas aeruginosa*	Clinical	0	19	22	0
23	*Pseudomonas flourescenses*	Clinical	15	0	24	0
24	*Salmonella typhimurium*	Clinical	19	20	22	0
25	*Shigella dysenteriae*	Clinical	20	20	20	0
26	*Aspergillus flavus*	Clinical	0	0	0	27
27	*Aspergillus fumigatus*	Clinical	0	0	0	23
28	*Candida albicans*	Clinical	17	0	0	24
29	*Microsporum gypseum*	Clinical	0	0	0	20
30	*Trichophyton rubrum*	Clinical	0	0	0	24

Va = *V. ambigua*, Sp = Sparfloxacin, Er = Erythromycin, Fl = Flouconazole

Table 2: MIC and MBC/MFC of V. ambigua

S/N	TEST ORGANISM	STRAINS	*V. ambigua*		
			MIC	MBC/MFC	A.I
1	*Staphylococcus aureus*	NCTC 6571	2.5	10	0.91
2	*Bacillus subtilis*	NCTC 8236	-	-	0.00
3	*Escherichia coli*	NCTC 10418	2.5	10	0.88
4	*Pseudomonas aeruginosa*	NCTC 6750	-	-	0.00
5	*Salmonella typhimurium*	ATCC 9184	2.5	10	0.78
6	*Klebsiella pneumoniae*	ATCC 10031	5	10	0.65
7	*Staphylococcus aureus*	ATCC 13704	2.5	10	0.82
8	*Candida albicans*	ATCC 10231	5	10	0.91
9	*Staphylococcus aureus*	Clinical	2.5	10	1.05
10	*Methicilin Resistant Staph. aureua*	Clinical	-	-	0.00
11	*Streptococcus pyogenes*	Clinical	2.5	10	0.85
12	*Streptococcus faecalis*	Clinical	2.5	10	0.69
13	*Corynebacterium ulcerans*	Clinical	2.5	10	0.80
14	*Listeria monocytogenes*	Clinical	2.5	10	0.83
15	*Bacillus subtilis*	Clinical	2.5	10	0.96
16	*Bacillus cereus*	Clinical	-	-	0.00
17	*Escherichia coli*	Clinical	2.5	10	1.15
18	*Klebsiella pneumoniae*	Clinical	2.5	10	1.05

19	*Klebsiella ozaenae*	Clinical	2.5	10	1.28
20	*Proteus mirabilis*	Clinical	-	-	0.00
21	*Proteus vulgaris*	Clinical	-	-	0.00
22	*Pseudomonas aeruginosa*	Clinical	-	-	0.00
23	*Pseudomonas flourescenses*	Clinical	5	10	0.63
24	*Salmonella typhimurium*	Clinical	5	10	0.86
25	*Shigella dysenteriae*	Clinical	2.5	10	1.00
26	*Aspergillus flavus*	Clinical	-	-	0.00
27	*Aspergillus fumigatus*	Clinical	-	-	0.00
28	*Candida albicans*	Clinical	5	10	0.71
29	*Microsporum gypseum*	Clinical	-	-	0.00
30	*Trichophyton rubrum*	Clinical	-	-	0.00

A.I. used Erythromycine as standard wherever active; the P.I result was 0.63.

Table 3: Result of the phytochemical screening

Phytochemical constituents	Results
Terpenes	-
Alkaloid	+
Flavonoids	+
Tannins	+
Steroids	-
Saponins	+
Anthraquinones	-
Phlobatannin	-

Key: + = Present; - = Absent

Table 4: Result of the proximate analysis

Parameter	Values
Moisture content	8.80%
Water-soluble extractive value	20.10%
Alcohol-soluble extractive value	3.65%
Total ash value	13.8%
Acid-insoluble ash value	3.15%

DISCUSSION

Vernonia ambigua was most effective against *S. aureus, S. typhi, E. coli Klebsiella pneumoniae* and *Klebsiella ozaenae.* From their activity index, the extract exhibited a comparable activity with the standard antibiotic erythromycin at a low concentration of 2.5mg/ml which is higher that the concentration previously tested (Kunle and Egharevba, 2009). The plant's activity against the fungi *C. albicans* was also above average as with some of the other enteric bacteria isolates like *Streptococcus pyogenes, Streptococcus faecalis, Corynebacterium ulcerans, Bacillus subtilis* and *Salmonella typhimurium.* Hence *V. ambigua* could be a potential source of a very potent antimicrobial comparable to existing one. The proportion index of 0.63 shows that the extract exhibited a broad spectrum activity against more than half of the 30 microbes screened and therefore could be used as a broad spectrum antibiotic.

The phytochemical screening and proximate analysis of the plant (Table 3 & 4), shows no significant seasonal variation in its composition when compared to previous studies (Kunle and Egharevba, 2009). The presence of alkaloids, saponins, tannins and flavonoids were detected in the plant. Some of these metabolites may be responsible for the antimicrobial activity exhibited (Reuben *et al*., 2008). More work is

currently ongoing in the authors' laboratory to isolate some of bioactive principles responsible for the antimicrobial activity.

CONCLUSION

V. ambigua is a plant yet to be thoroughly explored and could be a plant of great promises on antimicrobials. The activities exhibited against the bacteria and fungi strains is of great interest and this may be a pointer to a potent antimicrobial and other pharmacologically active substances which may lead to new drug discovery.

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