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Full Length Research Paper

Antimicrobial activity of six selected plants against some strains of pathogenic organisms

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Crude ethanolic extracts selected six Nigerian plants based on ethnobotanical reports of their antifungal and antibacterial actions were subjected to phytochemical and antimicrobial screening. All the six plants namely *Acalypha fimbriata*, *Glaphae brevis, Vernonia amygdalina, Struchium sparganophora*, *Celosia argentea* and *Amaranthus spinosus* were screened against strains of *Candida albicans*, *Trichophyton metagophyte, Malassezia furfur*, *Aspergillus flavus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus*. The seventy percent (70%) aqueous ethanolic extract of *A. finbriata* was found to possess greatest activities against the pathogenic fungi; *C. albicans*, *T. metagophyte, M. furfur* and *A. flavus* at minimum inhibitory concentration (MIC) of 50 mg/ml. While *V. amygdalina* and *S. sparganophora* showed better antibacterial activity of MIC values of 25 to 50 mg/ml than *A. finbriata* which exhibited wider spectrum of activity against the three pathogenic bacteria of MIC 100 mg/ml. The phytochemical screening revealed the presence of alkaloids, saponins and combined anthraquinones in *A. finbriata* which justifies its antimicrobial activities as portrayed in this test. The results of this work justify the ethnobotanical uses of these plants in traditional medicine in Nigeria.

Key words: Nigerian plants, antimicrobial, phytochemical screening.

INTRODUCTION

The flora of tropical West Africa has for centuries provided a wealth of material for healing purposes, and its further investigation presents a challenge to scientists who seek to contribute to the search for a new means of alleviating and curing diseases. Tropical forest is a repository of many plants and about 30% of tropical plants have been so far screened for bioactive compounds (Wagner, 2005), and the plant kingdom of Nigeria still holds many species of plants of medicinal values which are yet to be discovered (Odebiji and Sofowora, 1979). The discovery of many plants in Nigeria has been facilitated by the unique rich folklore of the Yoruba tribe in West Nigeria than many other tribes.

Ethno-botany has been defined in many ways. It involves making use of the knowledge of traditional use of plants based on many years of experience of useful actions to assist in drug discovery. This experience may be written down in traditional texts or passed orally from generation to generation by folk healers, such as "abuelos sabedores" in Colombia (Cotton, 1996). In Nigeria there are so many licensed and unlicensed traditional healers. Some of the formulas of the traditional healers have been registered by relevant regulatory bodies having only assessed their safety for human consumption. However, the efficacy of many of such

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products, some of which are commercially sold has not been evaluated in the laboratory. It has been known that the practice of traditional medicine was borne out of the selection procedure, which has often been by trial and error, and at times has been dangerous to the extent of costing lives (Shellard, 1979). Unfortunately, this practice still persists in most African and third world countries, where majority of the population dwell in rural areas and traditional medicine is the mainstay of health care delivery (Shellard, 1979).

However, as with all forms of self-treatment, the use of herbal remedies presents a potential risk to human health. Globally, the use of natural medicines is estimated to increase by 13% each year, while in the US it has increased by 20% yearly since the1990s and the sale of herbal medicines is estimated at 4 billion dollars per year. In Germany, 40% of prescribed medicines are based on plant materials, including Echinacea, Chinese Ginseng and Aloe vera (Ferro et al., 2003).

The safety of these herbal remedies is of particular importance in that the majority of these products is self-prescribed and is used to treat minor and often chronic conditions. The extensive traditional use of plants as medicines has enabled those medicines with acute and obvious signs of toxicity to be well recognized and their use avoided. But, the premise that traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true or suffice not (Ferro et al., 2003).

The more subtle and chronic forms of toxicity, such as carcinogen city, mutagenicity and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicities that are of most concern when assessing the safety of herbal remedies (Mattocks, 1986).

The isolation of active compounds from the bulk of unwanted plant parts has in no small measure reduce the toxicity of the plants and much also detailed pharmacological, toxicological and clinical studies have always been carried out on these isolated compounds/ medicines in orthodox practice all over the world before being marketed.

Though, temperate plants have provided most of the commercially valuable plant compounds today such as artemisinin, an antimalaria agent from annual Mugwort and taxol from yew trees. Taxol is used for the treatment of ovarian cancer and it is among the 20 best selling drugs. However there are many examples of herbs that are also widely studied by many laboratories whose active principles remain unknown even though therapeutics effects are proven. Some good examples are Valerian (*Valeriana officinalis*) and Echinacea (*Echinacea pallida, Echinacea augustifolia* and *Echinacea purpurea*) (Houghton, 1998).

The global trend of revival of interest in the traditional system of medicine especially in all developing countries of the world is also due to the realization of the world need to utilize locally available medicines and therefore to reduce the reliance on expensive imported drugs and its attendant economic benefit.

Traditional medicine may also provide a solution to the issue of drug resistance (WHO, 1984). Today a worldwide problem is the emergence of multidrug resistant pathogens which cause serious problems in hospitals, long-stay residential centre and in the community. There are also reports of changing bacterial sensitivities to antibiotics (WHO, 2002). In addition, it is thought that some antibiotics cause the release of endotoxins and other microbial products which can precipitate septic shock. There has also been growing awareness by governments and the scientific and medical communities of the importance of medicinal plants in health care systems in many developing countries. Medicinal plants are potential sources of new drugs and as starting products for the synthesis of drugs (Moody et al., 1998). So far, laboratory activities (which involved specimen screening, chemical analysis, pharmacological and toxicological processes, etc.) of some selected plant species, confirm that about 90% of the plants investigated, exhibit active curative properties as assumed by traditional medicinal users (Moody et al., 1998). Increasingly, the world is returning to nature's cure in the treatment and management of common prevalent diseases afflicting man. It is estimated that up to 80% of the world's population depend directly or indirectly on traditional medicine for their healthcare needs. Most of the herbal/traditional remedies on sale and in use particularly in non-industrial countries are not standardized and the efficacy as well as safety can not be readily ascertained (Moody et al., 1998). It has been known that 25% of all prescriptions dispensed in community pharmacies in the USA from 1059 to 1973 contained drugs extracted from higher plants and by 2008 they have spent up to $8 billion on plant based prescriptions (Sangobanwo, 2000). This led to the increased interest in research on plants, and even computerized data based programmes are used to predict bioactivity in plants. The major objective of this work was to verify the traditional uses of these six medicinal plants *Acalypha fimbriata, Glaphae brevis, Struchium spaganophora, Vernonia amygdalina, Celosia argentea* and *Amaranthus spinosus* as anti-microbial agents through phytochemical and antimicrobial screening. The plant that showed the greatest activity would be subjected to further studies with attempts to isolate and characterize its bio-active constituents.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of the six plants were collected from Sagamu local government of Ogun state, contaminants were hand picked then washed with clean water to remove sand and authenticated in Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu and the voucher specimen kept at Forest Research Institute of Nigeria, Ibadan. The botanical

Table 1. Botanical identity of the six selected Nigerian plants**.**

Botanical source	Family	Local/English name
Acalypha fimbriata	Euphorbiaceae	Jinwinin, acalypha
Glaphae brevis	-	Atori
Struchium spagarnophora	Astereaceae	Ewuro odo, water bitter leaf
Vernornia amygdalina	Astereaceae	Ewuro/bitter leaf
Celosia argentea	Amaranthaceae	Soko-yokoto, cockscomb
Amaranthus spinosus	Amaranthaceae	Tete-elegun-un, pricky amaranthus

identity of these selected Nigerian plants is as shown in Table 1.

Plant material

The plants were oven dried at 40°C and soaked in an absolute ethanol for 5 days and the extracts obtained were concentrated under reduced pressure (Rotavapor-R, Buchi). Dilutions of each dried extract were prepared in 50% ethanol to give final test concentrations of 100 mg/ml.

Preparation of the test micro-organisms

This process followed the previously established procedures for testing antimicrobial agents (Durodola, 1977; Cheruiyot et al., 2009). Standard cultures of bacteria were obtained from Olabisi Onabanjo University Teaching Hospital and their voucher numbers are as presented in Table 3. A Gram positive bacterium; *Staphylococcus aureus* OOUTH004SA, was used as a wound/skin pathogen and Gram negative bacteria; *Escherichia coli* OOUTH001EC, was used to represent pathogens that cause gastro enteritis while *Pseudomonas aeruginosa* OOUTH001SA, was used as an environmental pathogen. Standardized bacterial suspension was prepared by picking a colony of respective bacterium or fungus using sterile wire loop and suspending it in 5 ml Brain heart infusion liquid media or malt extract broth. The dilutions formed the bacterial or fungal stock solutions for use in the agar-well diffusion assays.

Preparation of culture media

Mueller Hinton agar (Becton Dickinson ® M.D USA) was used for direct sensitivity testing for the bacteria while malt extract broth was used for the fungus. The media was prepared and treated according to manufacturer's guidelines. 35 g medium was mixed with 1 L of distilled water, enclosed in a screw cap container and autoclaved at 121°C for 15 min. The medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 h at 37°C to confirm their sterility. When no growth occurred after 24 h, the plates were considered sterile.

Agar-well diffusion assay

A concentration of 1 g/ml of the plant extracts was designed from the stock solution for agar well diffusion assay. Cultures of *S. aureus*, *E. coli* and *P. aeruginosa* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. The sensitivity testing of the plant extracts was done using the agar well diffusion method (Reeves et al., 1979) whereby, wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. About 50 µl of 50% ethanol extract, of the concentration 1 mg/ml, was dispensed into respective wells and ciprofloxacin (Sigma, UK) (10 µg/ml) in 50% ethanol was used as a positive control for the anti-bacteria test since it is a broad spectrum antibiotic while fluconazole (Pfizer, U.K) (5 µg/ml) in 50% ethanol was used as a reference standard for the anti-fungal test. Physiological saline of 50% ethanol was used as negative control. All the tests were run in triplicates for quality results. The set up was incubated for 24 h at 37°C for anti-microbial test and incubated for 24 to 48 h at 27°C for the anti-fungal test. 24 or 48 h later, the zones of inhibition were measured using a ruler (AIM®) and a pair of divider, and then results reported in millimeters (mm). The inhibition was measured as a basis for activity (Abo and Ashidi, 1999).

Minimum inhibition concentration (MIC) evaluation

The MIC was evaluated on plant extracts that showed antibacterial activity in the agar well diffusion assay on any organism. This test was performed at five concentration of each extract (100, 50, 25, 12.5 and 6.25 mg/ml) employing doubling dilutions of plant extract in Brain heart infusion broth or malt extract broth up to the fifth dilution. 1 ml of the resultant broth was put in test tube and equal amounts of the extracts (1 ml) were added to the first test tube and serial dilution done with the last 1 ml being discarded. To complete the test, each organism was separately suspended in 5 ml of Brain heart infusion broth or malt extract broth and incubated overnight, after which 0.1 ml was added to all the test tubes and preparation incubated at 37°C for 18 or 36 h. After incubation, a loop full from each tube was sub cultured on nutrient agar to see if bacteria growth was inhibited. Growth of bacteria on solid media indicated that particular concentration of the extract was unable to inhibit the bacteria. The MIC was defined as the lowest concentration of an antimicrobial agent that inhibited the visible growth of a microorganism after overnight incubation (Cheruiyot et al., 2009).

Qualitative phytochemical analysis of the plant extracts

This was a qualitative analysis done at the Pharmacognosy Laboratory, Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University. A small portion of each extract was used for phytochemical screening test. Tests for alkaloids, saponins, tannins, akaloids, glycosides, cardiac glycosides and anthraquinones were carried out according (Ajibola, 1998).

Data analysis

Data are expressed as mean ± SD. Students''t' test with 95%

Table 2. Result of phytochemical screening of the six plants.

Chemical constituents	*Acalypha fimbriata*	*Glyphae brevis*	*Stuchium sparganophora*	*Vernonia amygdalina*	*Celosia argentea*	*Amaranthus spinosus*
Tannin	+	--	--	--	+	--
Saponin	+++	--	+	+	--	--
Anthraquinone	--	++	--	++	--	+
Cardenolide	++	+	++	+++	--	+
Alkaloids	+++	--	++	+++	+	+

-- =Absent; + =Fairly present; ++ =Present; +++ Highly present.

Table 3. Names and sources of pathogenic organisms used in the anti-microbial screening of the six plants.

Name	Voucher number	Sources
Salmonela typhi	OOUTH001ST	Human blood
Escherichia coli	OOUTH001EC	Stool
Pseudomonas aeruginosa	OOUTH001SA	Human blood
Staphylococcus aureus	OOUTH004 SA	wound
Candida albican	OOUTH001CA	vagina
Trichophyton metagophyte	OOUTH001TM	Human dermatophyte
Malassezia furfur	OOUTH001MF	Human dermatophyte
Aspergillus flevus	OOUTH001AF	Human dermatophyte

confidence level was used for statistical analysis of the results.

RESULTS AND DISCUSSION

These six Nigerian plants have been selected for preliminary studies based on ethnobotanical reports of their medicinal uses (Burkill, 2000). The phytochemical screening of the six selected Nigerian plants revealed the presence of various secondary metabolites in different degrees in all the plants as shown in Table 2. *A. finbriata* tested fairly positive for the presence of combined anthraquinone and cyanogenetic glycosides, strong presence of saponin and a high concentration of alkaloids. *G. brevis* contained combined anthraquinones and cyanogenetic glycosides. Though, *A. fimbriata* and *G. brevis* are not being eaten, the remaining four plants are eaten as vegetable soup in West and East parts of Nigeria (Moody et al., 1996). *V. amygdalina* shows strong presence of alkaloids, anthraquinones, saponins and cardenolides. In accordance with the work of Oboh (2006), *S. sparganophora* indicated the presence of saponins, anthraquinones and alkaloids. *V. amygdalina* (Bitter leaf) and *S. spaganophora* (Ewuro odo) are eaten by people from West and East Nigeria (Odugbemi and Akinsulire, 2008). *S. sparganophora* and *V. amygdalina* are both reported to possess antibacterial activity (Burkill, 2000; Oboh et al., 2008). *V. amygdalina* is clearly distinguished by its intense bitter taste and the potency of its active constituents for many biological activities (Odugbemi and Akinsulire, 2008) *C. argentea* only showed the presence of tannins and alkaloids while *A. spinosus* indicated the presence of anthraquinones, alkaloids and mild presence of cardenolide. These chemical constituents of these herbs as revealed by this phytochemistry confirned their pharmacological properties (Carol et al., 1996) as portrayed in their ethnobotanical uses.

The antimicrobial screening of the 6 selected plants involved four (4) human pathogenic fungi isolates and four (4) human pathogenic bacteria isolates as shown in Table 3. All the six selected plants showed antibacterial activity of MIC ranging from 100 to 0 mg/ml for both Gram negative and Gram positive bacteria as shown in Table 4. *E. coli* was resistant to 50% aqueous ethanolic extracts of the two of the selected plants *V. amygdalina* and *G. brevis*, but sensitive to other four plants. *S. spaganophora* was the most effective with MIC 50 mg/ml which is also in agreement with the report of Oboh et al. (2008) while *A. fimbriata* had MIC 100 mg/ml. The 50% aqueous ethanolic extracts of all selected plants were effective against *Salmonella typhi* except *S. spaganophora* that showed no inhibitory activity within the concentration of the extracts used in the experiment. *V. amygdalina* showed the greatest activity of MIC 25 mg/ml against *S. typhi*. *A. finbriata* showed significant activity against three (3) pathogenic bacteria *E. coli* having (MIC 100 mg/ml, *S. aureus* MIC 100 mg/ml and *S. typhi* having, MIC 100 mg/ml. There was a marked antibacterial activity showed by *S. spaganophora* against

Table 4. Result of antibacteria and anti-fungal screening of 70% aqueous-ethanolic extracts of the six plants showing the minimum inhibitory concentration (MIC).

	Plants minimum inhibitory concentration (MIC) in mg/ml							
Pathogenic bacteria / fungi	***G. brevis***	***A. finbrata***	***C. argentea***	***V. amygdalinana***	***A. spinosus***	***S. sparganophora***	**Ciprofloxacin**	**Fluconazole**
Salmonela typhi	100	100	50	25	100	0	$1x\ 10^{-5}$	NA
Escherichia coli	0	100	100	0	100	50	$1x\ 10^{-5}$	NA
Salmonella aeruginosa	0	0	0	0	0	0	$1x\ 10^{-5}$	NA
Staphylococcus aureus	100	100	100	100	0	50	$1x\ 10^{-5}$	NA
Candida albican	0	50	0	0	0	0	NA	5
Trichophyton metagophyte	0	50	0	0	0	100	NA	5
Aspergillus flevus	0	50	0	100	0	0	NA	5
Malassezia furfur	0	50	0	0	o	0	NA	5
Control	0	0	0	0	0	0	0	0

NA = Not applicable; 0 = Nil.

E. coli with MIC 50 mg/ml and *S. aureus* with MIC 50 mg/ml. *V. amygdalina* also proved appreciably effective against *S. aureus* with the MIC 100 mg/ml and *S. typhi* having MIC 25 mg/ml. *A. fimbriata* showed wider spectrum of antibacterial activity while *S. sparganophora* and *V. amygdalina* exhibited greater degree of antibacterial activity than *A. finbriata*; although, all the antibacterial activities of these plants were not significant ($P>0.05$) in comparison to the standard drug ciprofloxacin with MIC 10 µg/ml. The tested plant extracts were most active against Gram positive *S. aureus* than most of the Gram negative microorganisms. This is in an agreement with the previous reports of several workers (Buwa and Staden, 2006).

Moreover it has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally, ion leakage from the cells of microorganisms (Parekh et al., 2005).

Moreover, human pathogenic fungi used to evaluate the antifungal activities of the six 6 selected plants (Table 3); *A. finbriata* showed significant activity ($P<0.05$) when compared with the remaining five plants against the four chosen pathogenic fungi. *S. sparganophora* showed a slight activity against *Trichophytum metagophyte* and *V. amygdalina* also showed a slight activity against *Aspergillus flavus* with MIC 100 mg/ml. The antifungal activity showed by *A. finbriata* was similar to the activity of other species in the family and this highlights its chemical relationship with other species of the Acalypha family which have been shown to possess antifungal activities (Moody et al., 1998.) The ethanolic extract was used being the conventional solvent used by local herbalists for their preparations for use, and it has been known that high activity is associated with ethanolic extracts of local herbalists. (Allero and Afolayan, 2006).

A fimbriata have the most promising antifungal properties indicating the potential for discovery antifungal principles from it. Further phyto-chemical studies are required to determine the types of compounds responsible for the antifungal effects of this plant. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful sources for new antimicrobial agents.

Conclusion

The six plants used in this study have shown promising pharmacological potentials, some of which have been evaluated. The evaluation of the toxicity profile on organ systems should be carried out to ascertain their safety to humans, though, certain physicochemical factors like pH and polarity of the medium that affect the transfer of biologically active agents across membranes may be altered in the process of extraction and purification. Further work is to isolate chemical constituents responsible for pharmacological activities observed in these plants.

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