

Phytochemical and Antimicrobial Properties of Four Herbs from Edo State, Nigeria.

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Abstract: Phytochemical and antimicrobial properties of four medicinal plants (*Bidens pilosa*, *Euphorbia heterophylla*, *Euphorbia hirta* and *Phyllanthus amarus*) used in the management of some diseases in Edo State, Nigeria were investigated in this study. The results revealed that tannins, saponins, flavonoids, cardiac glycosides, alkaloids and steroids were all present in *E. hirta*. While *P. amarus* contain all except cardiac glycosides. *E. heterophylla* contained saponins, flavonoids, cardiac glycosides and steroids whereas *B pilosa* contained saponins, alkaloids and steroids only. These substances are known components of medicinal plants and may explain the use of the preparations of the herbs under study for managing a number of common ailments including dysentery, diabetes, hypertension and some microbial infections among the indigenous communities in Edo State. When tested against *Escherichia coli*, *Streptococcus spp*, *Klebsiella spp*, *Pseudomonas spp* and *Staphylococcus spp*, aqueous and methanolic extracts of *E. hirta*, *P. amarus* and *B. pilosa*, showed varying degrees of inhibition to the growth of tested organisms. *E. heterophylla*, was effective as an antimicrobial agent only against *Streptococcus sp*. Extracts from all the plants tested had no effects on *Klebsiella sp* used in this study. Methanolic extracts of the plants were more effective than aqueous extracts in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to that of ofloxacin and ciprofloxacin used as positive controls. Presence of Phytochemical agents and antimicrobial properties in the tested plant species is confirmed. [Report and Opinion. 2009; 1(5):67-73]. (ISSN 1553-9873)

Key Words: Phytochemicals, antimicrobials, herbal medicine, Edo State.

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro *et al.*, 2000; Tanaka, 2002). Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu 2006). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno-medicine. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with good antibacterial activity will be used for the treatment of bacterial infections. This is because, according to Arora and Keur (1999), the success story of chemotherapy lies in the continuous search of new drugs to counter the challenges posed by resistant strains of micro organisms. Studies indicate that in

some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others which could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses (Arora and Keur, 1999; Okigbo and Omodamiro 2006).

The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.* 1996; Hammer *et al.*, 1999). Antibiotic resistance has become a global concern (Westh *et al.* 2004) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug-resistant pathogens (Bandow *et al.*, 2003). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of novel drugs because of the great diversity in their

chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rogas *et al.*, 2003). Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Benkeblia, 2004); and this has led to the screening of several medicinal plants for potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu *et al.* 1999).

In the present study, four herbal plants (*Bidens pilosa*, *Euphorbia heterophylla*, *Euphorbia hirta* and *Phyllanthus amarus*) which are used in the health care system of Edo State to manage some common ailments and infections were investigated. *Euphorbia* is one of the most diverse genera in the family Euphorbiaceae. Members of the family and genus are sometime referred to as spurs. The leaves of *Euphorbia heterophylla* are commonly used as a lactogenic agent by taking a decoction of it or massaging the breast with the poultice to induce milk flow (Dokosi 1998). They are also used in traditional medical practice as laxative and to treat gonorrhea, migraine and viral warts while the plant latex is used as fish poison, insecticide and ordeal poisons (Rodriguez *et al.*, 1976, Falodun *et al.*, 2003). Recently their anti-tumor/anticancer properties and their activities against the Human Immunodeficiency Virus (HIV) have also been reported in *E. heterophylla* leaf (Williams *et al.*, 1995).

Euphorbia hirta is also a member of the Euphorbiaceae family and its common names are Australian asthma plant, garden spurge and snake weed. A decoction of the leaves is taken to induce the flow of milk and the leaf can be chewed with palm kernel for the restoration of virility. A poultice of the leaves is externally applied to abscesses to bring it to a head and for faster pain relief. Similar uses of the plant have been reported in Ghana by Dokosi (1998)

The genus *Phyllanthus* also belongs to the family Euphorbiaceae. The most common species of this genus in Nigeria and most West African nations are *Phyllanthus amarus* and *Phyllanthus niruri*. The leaves of *Phyllanthus amarus* are made into a paste and licked to expel intestinal worms. The decoction of the plant is also used as a purgative. Topically, this plant has soothing effect on the skin and is used for genital and anal infections. Poultice is used for the treatment of certain types of ulcers or sores. The infusion of the root and leaf is a very good tonic and causes diuresis when taken in repeated doses (Oliver 1986). According to Sirajudeen *et al* (2006), administration of *P. amarus* extract is non-toxic with no signs of toxicity or mortality in experimental rats.

Bidens pilosa belongs to the family of *Asteraceae*. The plant is used for angina, diabetes,

dysentery, dysmenorrhea, edema, hepatitis, and laryngitis. In Peru, the leaf is used to treat toothache and headache (Dimo *et al.*, 1999; Rogas *et al.*, 2004). *B. pilosa* has been the subject of recent clinical studies which has explained many of its uses in herbal medicine (Dimo *et al.* 2001, Dimo *et al* 2002, Khan *et al* 2001). The selected microbes for this work were *Escherichia coli*, *Streptococcus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus* spp. which are among the most common bacterial isolates from human infections. (Brown and Anicelus, 2004 ; Donaldson and Gosbell, 2006).

Aims and Objectives: The objective of this study is to carry out an investigation on four medicinal plants (*E. hirta*, *E. heterophylla*, *B. pilosa* and *P. amarus*) to document their phytochemical and antimicrobial properties which would throw light on their possible mechanism of action and justify their use as antimicrobial agents.

Materials and Methods

The fresh leaves of *Bidens pilosa*, *Euphorbia heterophylla*, *Euphorbia hirta*, *Phyllanthus amarus* were collected randomly from secondary forests and open fields in Edo State of Nigeria. The leaves were washed under running tap water and dried in air for 24 hours. With the aid of grinder, the leaves of the plants were homogenized to fine powder and stored in airtight containers.

Phytochemical tests

One gram of powder was subjected to qualitative phytochemical tests for alkaloids (Myers Reagent), saponins (chloroform and H₂SO₄ tests), inulin (Molisch's Reagent) and tannins (Ferric salt tests) adopting the procedures described by Stephen (1970)²⁶ and Parekh and Chanda (2007)²⁷. Flavonoids were determined by the magnesium ribbon test while steroid was confirmed by the chloroform-acetic anhydride test.

Antimicrobial Activity

Aqueous Extraction

A 10g sample of air dried powder was added to 100ml of distilled water and boiled on slow heat for 2 hours. It was then filtered through 6 layers of muslin cloth and centrifuged at 5000g for 10 minutes. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121⁰ C and at 15 lbs pressure and stored at 4⁰ C.

Methanol Extraction

A 10g of air dried powder was taken in 100ml of 90% methanol in a conical flask plugged with cotton wool and then kept on a rotator shaker at 190-220 rpm for 24 hours. After 24hours the supernatant was cooled and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4°C in airtight bottles.

The methanolic and aqueous extracts were then tested against five bacteria species sourced from the Irrua Teaching Hospital, Edo State, Nigeria. The tested organisms are *Escherichia coli*, *Streptococcus spp*, *Klebsiella spp*, *Pseudomonas spp*. and *Staphylococcus aureus*. The antibiotics, ciprofloxacin and ofloxacin at 5µg (disc potency) were used as positive controls following standard methods.

Determination of Antimicrobial Activity of plant Extracts.

The test organisms and the standard strains were separately inoculated into nutrient broth and incubated at 37°C for 4-6 hours. Then 0.2ml (10⁵ cfu/ml) of the broth culture of the test bacteria were seeded on Mueller-Hinton agar plates and spread evenly.

Four wells of 6mm in diameter were cut on the seeded agar plates using a sterile cork borer. Two of the wells were each filled with 0.05ml of hot- water and ethanol extracts.

The remaining two wells contained hot water and ethanol each and these served as control wells. The plates were incubated at 37°C for 24hrs after which the zones of inhibition round the wells determined the antibacterial activity of the extracts. Zone diameter was recorded as the differences between extracts and any produced by the respective controls. This procedure was repeated for the plant extracts of the four species under study.

Table1: Phytochemical analysis of screened medicinal plant species

Plant Species	Tannins	Saponins	Flavonoids	Cardiac glycoside	Alkaloids	Steroids
<i>Euphorbia hirta</i>	+	+	+	+	+	+
<i>Euphorbia heterophylla</i>	-	+	+	+	-	+
<i>Bidens pilosa</i>	-	+	-	-	+	+
<i>Phyllanthus amarus</i>	+	+	+	-	+	+

+ =Present

- =Absent

Table 2: Traditional uses of four selected herbs from Edo State of Nigeria

Plants	Traditional uses of selected plants
<i>Euphorbia hirta</i>	Dried leaf as vermifuge. Leaf infusion to increase breast milk flow, and to treat diarrhea/dysentery, splenomegally, asthma and whooping cough.
<i>Euphorbia heterophylla</i>	Extract used to treat ear pain. Poultice induces milk flow. Poultice taken in pap to increase sperm quality.
<i>Phyllanthus amarus</i>	Malaria, Chronic stomach pains, oral or vaginal thrush, alcoholic liver disease, hyperglycaemia, urinary tract infection and venereal disease. Taken in honey as aphrodisiac.
<i>Bidens pilosa</i>	Poultice topically applied to sores, for ear aches and intestinal infections. Infusion taken for coughs and colics. Healing of peptic ulcers. Hot infusion of leaves for conjunctivitis.

TABLE 3: Zones of inhibition (mm) of crude aqueous and alcoholic extracts (100mg/ml) of selected herbs, ciprofloxacin and ofloxacin on five bacteria species.

Plant species	Extract	Zones of inhibition (mm) of extract on selected microbials				
		<i>Streptococcus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>
<i>Euphorbia heterophylla</i>	Aqueous	0	0	0	0	0
	Alcohol	5	0	0	0	0
<i>Phyllanthus amarus</i>	Aqueous	5	10	6	0	7
	Alcohol	6	6	10	0	11
<i>Euphorbia hirta</i>	Aqueous	0	6	8	0	12
	Alcohol	0	12	6	0	14
<i>Bidens pilosa</i>	Aqueous	0	0	5	0	0
	Alcohol	0	0	5	0	0
Controls	Ofloxacin	0	0	17	30	21
	Ciprofloxacin	8	14	19	0	0
	Sterile Distilled water	0	0	0	0	0
	Sterile Distilled water	0	0	0	0	0

0= No inhibition of growth.

Results and Discussion

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs (Yakubu *et al.*, 2005). Phytochemical screening of the leaves of the selected plants revealed the presence of saponins, alkaloids, steroids and tannins as the major phytochemical components (Table 1).

In the present study, *Biden pilosa*, *Euphorbia hirta*, *Euphorbia heterophylla*, and *Phyllanthus amarus* recorded different phytochemicals of medicinal importance although some of their therapeutic properties are exhibited by more than one plant, hence these plants are used for different ailments in the communities under study (Table 2). The results revealed that tannin was present in *E. hirta* and *P. amarus* but absent in *E. heterophylla* and *B. pilosa*. While saponin was present in all the plants, flavonoids were present in *E. hirta*, *E. heterophylla* and *P. amarus*. Cardiac glycosides were present in *E. hirta* and *E. heterophylla*, but absent in the other two plants studied (Table 1). Alkaloids, Steroids, and saponins were present in all plants examined while alkaloids were present in all except *E. heterophylla*. The use of *Euphorbia hirta* can be explained by its high content of various phytochemical agents such as

tannins, saponins, alkaloid, cardiac glycosides and steroids as it is used by the indigenes to manage a number of ailments. The dried plant is used as vermifuge. It is also used to increase the production of breast milk, an effect likened to that of both oxytocin and prolactin. Its use in the treatment of diarrhoea/dysentery and application to wounds may be due to its antimicrobial actions (Table 2). Ogueke *et al* (2007) reported that the plant is used in the treatment of sores, boils, wounds and control of dysentery and diarrhoea among the Igbo ethnic group in Nigeria. In the current investigations the aqueous as well as the methanolic extracts of this plant exhibited antimicrobial properties, thus confirming previous records that the plant has antibacterial properties on certain bacterial species (Gill, 1992)

Similarly, *Euphorbia heterophylla* contains saponins, cardiac glycosides flavonoids, tanins and steroids. Traditionally the leaf of the plant is used to treat ear pain (otitis media or externa) while the poultice induces milk flow from breast. *Phyllanthus amarus* is also an important herb used in Edo State for the management of malaria, chronic stomach pains, and thrush or candidiasis. The three species belonging to the Euphobiaceae family (*E. hirta*, *E. heterophylla* and *P. amarus*) are used to manage cardio-vascular related health problems, properties that can be attributed to the high content of cardiac

glycosides which is used in treatment of heart failure. Cardiac glycosides were originally prepared from crude digitalis, a preparation from the dried leaf of the foxglove plant *Digitalis purpurea*. Their blood sugar lowering properties may also not be unrelated.

Bidens pilosa is an important herb which also serves as vegetable in times of need (Dosoki 1998). It contains alkaloids, saponins, and steroids as its major chemical components. Poultice from the leaves is externally applied to sores, and is also used for ear aches and intestinal infections. Leaf infusion is taken for coughs and abdominal colics. *Bidens pilosa* is widely used as an antibiotic (Table 2). The exhibited antibacterial properties of *B. pilosa* can be attributed to the presence of saponins, steroids and alkaloids in the leaves. This agrees with Tomas-Barberan *et al.*, (1990), who reported that these might have complimented or potentiated the saponins in the antibacterial activities in the plants where both alkaloids and saponins were present. According to Dimoi *et al* (1999)³³ *Bidens pilosa* is used to cure angina, diabetes, dysentery, dysmenorhea, oedema, hepatitis, jaundice, laryngitis and worms in Peru. A decoction of the root is used for treating hepatitis and intestinal worms. In the present study, the crude alcoholic extract of the plant exhibited moderate inhibitory effect on the growth of *E. coli*, thus justifying its use by local communities for managing some microbial infections in wounds, and some intestinal bacterial infections.

This antimicrobial study showed that the Gram-negative bacterial strains used (*E. coli* and *Pseudomonas* spp.) were generally more susceptible to the extracts than gram-positive bacteria strains (*S. aureus* and *Streptococcus* spp). *S. aureus* and *Streptococcus* spp exhibited smaller zones of inhibition at the concentration used compared to *E. coli*, and *Pseudomonas* which are gram negative species (Table 3). That the leaf extract of three of the herb used in the present study is capable of inhibiting the growth of *S. aureus* and *Streptococcus* spp at all is good news since multi-drug resistant strains of the organism are on the increase in both hospital and community environments in Nigeria against orthodox antibiotics (Lamikanra and Ndep, 1993; Chamber, 2001; Brown and Anicelus, 2004). *S. aureus* is reported to be highly resistant to ampicillin, cephalixin, methicillin and vancomycin; and is also resistant to gentamycin, rifampicin and chloramphenicol (Onanuga *et al.*, 2005; Donaldson and Gosbell, 2006). Tannins have been isolated from some medicinal plants (Mitcher *et al.*, 1988; Hasfermaria *et al.* 1993). Egwim *et al.* (2000) have earlier demonstrated the presence of tannins in *Euphorbia hirta* and opined that it may account for its antimicrobial activity against *Salmonella typhi* *in vitro*. Tannins may decrease protein quality by

decreasing digestibility and palatability. It may equally interfere with absorption of iron and this may explain why herbs with high tannin concentrations are not administered to anaemic individuals and so should be used with caution in protein deficient/kwashiorkor patients.

Tannin can be toxic to bacteria, filamentous fungi and yeast (Harborne, 1973). *Phyllanthus amarus* root and leaves provides a very good tonic and cause diuresis when taken cold in repeated doses. A poultice of the leaves with salt cures scabies infection and without salt is applied on bruise and wounds (Oliver, 1986). In the present study, the crude extract was observed to inhibit the growth of *E. coli*, *Streptococcus* and *S. aureus*. However its effects are low when compared with standard antimicrobial agents such as ofloxacin and ciprofloxacin which was used in this study as positive controls (Table 3). However, the present results revealed that the alcoholic extracts were more effective than the aqueous extract in inhibiting the growth of the test microbes. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused changes to occur in some of the chemical constituents found in these plants. Thus future research will centre on the effects of different solvents and drying methods on the efficacy of the plant extracts as microbial agents.

Conclusion

The four plants studied in this report contain various amounts of phytochemicals such as tannins, saponins, steroids, flavonoids, and alkaloids which are known for their therapeutic effects. This study has confirmed and justified the use of the herbal preparations amongst the people especially those in the rural communities where the practice has become prevalent owing to easy accessibility to the plant and the relatively low cost of the herbal preparations. It is believed that the plants mentioned and used in this research work could be potential sources of drugs if the active ingredients are identified and adequately characterized.

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