Preliminary Phytochemical and Antimicrobial Properties of Five Plants in Edo State Nigeria.

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Abstract
The current study was undertaken to evaluate the phytochemistry and antimicrobial properties of five species: Alchornea cordifolia (Schum and Thonn), Aspilia africana (Pers), Chromolaena odorata (L.), Jatropha curcas L. and Luffa cylindrica (Linn.) M.J. Roem in Edo State with a view to throw more light on their possible mechanism of action and justify their use as anti-microbial agents. Antimicrobial sensitivity assay was carried out by disc diffusion method where the alcohol extract of the some of the plants showed in-vitro potential of antimicrobial activities against the organisms tested. The extracts evaluated include cold water extract (CWE), hot water extract (HWE) and alcohol extract (AE) which was tested against four pathogenic organisms (Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli and Candida albicans) isolated from patients admitted in the Specialist Hospital, Irrua, Edo State, for various ailments. These plants contain tannins, saponins, alkaloids and flavonoids. When tested against Escherichia coli, Klebsiella pneumoniae, Candida albicans and Staphylococcus aureus, alcohol extracts of Chromolaena odorata and Aspilia africana showed varying appreciable degrees of inhibition to the growth of tested organisms (1-11mm). Alcohol extracts of the plants was found to be more effective than the water extracts in inhibiting the growth of the pathogenic bacteria under study.

Key words: Antimicrobial, Edo state, Extracts, Pathogenic organisms, Phytochemistry

Introduction
The scientific examination of the medicinal plants and plant based compounds for the healing of various infectious disorders was started in the late 19th century (Khan et al., 2007). Many new antibiotics have been introduced to combat with such bacterial infections but multiple resistance genes have appeared rapidly among bacteria which developed resistance to those antibiotics. Nature has been a foundation of therapeutic components for many decades. Larger number of recent drugs has been derived from medicinal plants including antimicrobial, with possibly novel mechanism of action and thus reducing antimicrobial resistance (Khan et al., 2012). Alchornea cordifolia in Nigeria is used for the treatment of gonorrhea, rheumatic pains, wound or cuts and cough (Gbile, 1986). Decotion of the leaf of Chromolaena odorata is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria in traditional medicine. Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Iwu et al., 1999). Most of Chromolaena odorata medicinal values lie in its phytochemical components such as alkaloids, tannins, flavonoids, and other phenolic compounds which produce a definite physiological action on the human body (Hill, 1952). Sap from the bark of Jatropha curcas is used to dress wounds and ulcers and can also be used to stop bleeding. When oil from the seed is mixed with benzyl benzoate, it becomes effective in combating with such bacterial resistance genes have appeared rapidly among bacteria which developed resistance to those antibiotics. Nature has been a foundation of therapeutic components for many decades. Larger number of recent drugs has been derived from medicinal plants including antimicrobial, with possibly novel mechanism of action and thus reducing antimicrobial resistance (Khan et al., 2012). Alchornea cordifolia in Nigeria is used for the treatment of gonorrhea, rheumatic pains, wound or cuts and cough (Gbile, 1986). Decotion of the leaf of Chromolaena odorata is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria in traditional medicine. Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Iwu et al., 1999). Most of Chromolaena odorata medicinal values lie in its phytochemical components such as alkaloids, tannins, flavonoids, and other phenolic compounds which produce a definite physiological action on the human body (Hill, 1952). Sap from the bark of Jatropha curcas is used to dress wounds and ulcers and can also be used to stop bleeding. When oil from the seed is mixed with benzyl benzoate, it becomes effective against scabies and dermatitis (Elewude, 1986). Aspilia africana is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores, in the treatment of rheumatic pains, bee and scorpion stings and for removal of opacities and foreign bodies from the eyes. It is commonly used to feed livestock particularly sheep and goats. It is believed to have nutritive and medicinal values (Agbor et al., 2012). Luffa cylindrica Syn. Luffa aegyptiaca commonly called sponge gourd or loofah has been reported to posses both medicinal and nutritional properties. Its seeds have been used in the treatment of asthma, sinusitis and fever (Indumathy et al., 2011).

The old antimicrobial technology was based either on poisons or heavy metals which may not have killed the micro-organisms completely allowing the microbe to survive the change and become resistant to the poisons or heavy metals. Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of related
disorders (Krishnan, 2006). However, with the development of antimicrobials, micro-organisms have adapted and become resistant to previous antimicrobial agents.

Materials and Method
Plant samples (leaves) of *Alchornea cordifolia* were collected from Emaudo village in Ekpoma, Esan West Local Government Area of Edo State; *Aspilia africana* and *Jatropha curcas* were collected from Irrua, Esan Central Local Government Area of Edo State while *Chromolaena odorata* and *Luffa cylindrica* were collected from Ewu, Esan Central Local Government of Edo State. The plants were identified in the Herbarium of the Department of Botany, Ambrose Alli University, Ekpoma, Edo State. The leaves were shed off and dried for 25 days. It was then milled into powder form and stored in air tight plastic containers. For each of the five specimens, one gram was analysed each for their tannin, flavonoids, alkaloid and saponin contents.

**Bacterial strains**
Assays were executed on different bacterial strains of gram negative *Escherichia coli*, gram positive *Staphylococcus aureus*, *Klebsiella pneumoniae*. The yeast *Candida albicans* was also used in this study with the three aforementioned bacterial strains. The strains were kept on agar slant.

**Antimicrobial Activity**

**Aqueous Extraction**
One gram of powdered sample was weighed and placed in a sterile container and properly labeled. 10ml of distilled water was added. Petri-dishes containing prepared medium were streaked with the different test organisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans*) and properly labeled. Using a pipette, 0.1ml of the extract was inoculated using a pipette.

**Alcohol Extraction**
One gram lot of powered sample was weighed and placed in a sterile container and properly labeled. Ten milliliters of alcohol was added. Petri-dishes containing prepared medium was streaked with the different test organisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans*) and properly labeled. Using a pipette, 0.1ml of the extract was inoculated using a pipette.

**Antimicrobial Determination**
Antibacterial sensitivity of the extract was tested using the disc diffusion method against the bacterial strains. In the agar diffusion method, wells were cut in seeded agar and the test sample was then introduced directly into these wells. After incubation, the diameter of the clear zone around the well were measured and compared against zones of inhibition produced by solutions of known concentrations of standard antibiotics.

Standard antibiotic discs and discs on which the solvent (used to dissolve the samples) was adsorbed and dried. These discs were then placed in Petri dishes (120mm in diameter) containing a suitable agar medium seeded with the test organism using sterile transfer loop for antibacterial screening. The test material diffuses from the discs to the surrounding medium. The plates were then kept in an incubator for 12 – 18 hours to allow the growth of the micro-organisms. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of millimetre. Zone diameter was recorded as the differences between extracts and any produced by the respective controls.

**Test for Alkaloids**
The method of Sofowora (1984) was used. About 2g of well ground plant materials was put in a test tube and treated with 10ml of 1% hydrochloric acid for 30minutes in a water reagent. 1ml of the filtrate was treated with a few drops of Mayer’s reagent and a second 1ml portion was treated with Dragendorff’s regent. Turbidity or precipitate with either of these reagents was taken as presence of alkaloids. Filtration was carried out using a Whatman filter paper.

**Test of Saponins**
The method of Sofowora (1984) was applied. About 0.5g of plant extract was shaken with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

**Test of Tannins**
The method of Sofowora (1984) was employed. About 5g of plant extract was sterilized with 10ml of distilled water, filtered with Whatman filter paper and ferric chloride reagent was added to the filtrate. Blackish-blue precipitate indicates the presence of hydrolysable tannin while blackish-green precipitate indicates the presence of condensed tannins.

Test of Flavonoids
The method of Cuiel (1982) was used. The alcoholic extracts of these plant was added to a few pieces of magnesium metal, concentrated hydrochloric acid was added. The formation of orange, red or crimson was taken as an evidence for the presence of flavonoids.

Results and Discussion
The plants under study with their esan and common names are presented (Table 1). The compounds that are responsible for medicinal property of most drugs are usually secondary metabolites. Tannins, saponins, flavonoid and alkaloids were observed in all the plant species (Table 2). Tannins which were present in all plants studied have been reported to irreversibly form complexes with proteins, resulting in the inhibition of cell protein synthesis. It also hinders the development of micro-organisms by their ability to precipitate and inactivate microbial adhesions enzymes and cell envelope proteins (Cowan, 1999). Saponins which are glycosides with soapy characteristic are often reported to possess bioactive agents (Sodipo et al., 1991). Some of the characteristics of saponin include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al., 2000; Okwu, 2004).

The antimicrobial activity of flavonoids is may be due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall; thereby disrupting their membrane integrity (Vilioglu et al., 1998). However the extracts with higher phenolic content do not always have higher flavonoid content (Maisuthisakul et al., 2007). Alkaloids have been found to possess antimicrobial activity against organism such as Klebsiella pneumoniae, Mycobacterium smegmatis, and Candida albicans (Addae-Mensah, 1992). In this study, the antimicrobial activities of the extracts (hot and cold water, alcohol) were assayed to determine the zones of inhibition against four micro-organisms. Klebsiella pneumoniae was the most sensitive strain with the strongest inhibition zones (11mm) followed by Staphylococcus aureus (9mm). The result for the antimicrobial properties of the five plants (Table 3) showed that the cold water extract had no activity against the tested organisms while only the hot water extract of Alchornea showed inhibition to the growth of Klebsiella pneumoniae. Furthermore, the alcohol extract of Chromolaena odorata and Aspilia africana showed activity against Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae. However Jatropha curcas showed activity against Klebsiella pneumoniae only and Chromolaena odorata against Candidas albicans. It was noted that Luffa cylindrica had no activity whatsoever to the growth of any of the test organisms. With regards to the alcohol extract, Alchornea and Aspilia showed inhibition to Escherichia coli and Klebsiella pneumoniae. From the present study (Table 3), it shows that Aspilia africana has the highest inhibition of 11mm for Klebsiella pneumonia. Minimal values (6mm) were recorded for Chromolaena odorata against Escherichia coli and Alchornea cordifolia against Klebsiella pneumonia.

Table 1: Plants under study

<table>
<thead>
<tr>
<th>Family</th>
<th>Botanical name</th>
<th>Common name/Esan names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbiaceae</td>
<td>Alchornea cordifolia (Schum and Thonn)</td>
<td>Owhe</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Aspilia africana (Pers.)</td>
<td>Ebe-ugbo</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Chromolaena odorata (L.)</td>
<td>Awolowo</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>Jatropha curcas (L.)</td>
<td>Ebeborgbon</td>
</tr>
<tr>
<td>Curcurbitaceae</td>
<td>Luffa cylindrica (Mill)</td>
<td>Okhumerianlen</td>
</tr>
</tbody>
</table>

Table 2: Chemical constituents of the five plants investigated

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alchornea cordifolia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspilia africana</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chromolaena odorata</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Jatropha curcas</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Luffa cylindrica</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</table>

Present (+)  Absent (-)

Table 3: Zones of inhibition (mm) of extracts (cold water extract (CWE), hot water extract (HWE) and alcohol extract (AE) of selected plants on four micro-organisms
Although it has been observed that natural products are more extractable using alcohol solvents, the temperature variance of the hot and cold extracts needs to be investigated. Mensah et al., (2013) revealed that water is not a good solvent for the extraction of solute which has inhibitory activity. The extract of Chromolaena and Aspilia is therefore capable of inhibiting the growth of Staphylococcus, E. coli and Klebsiella pneumoniae. The leaves of Aspilia africana and Chromolaena odorata thus possess constituents capable of arresting wound bleeding, inhibiting the growth of microbial wound contaminants and also accelerating wound healing which suggest good potentials for use in wound care.

Umeh et al., (2005) reported that many of the phytoconstituents with antibacterial properties in plants are preferably concentrated in leaves. It is possible that some of the plants that were not active do not actually possess antibiotic properties. It could also be possible that the active constituents are not soluble in alcohol or water extracts. Thus further research should be carried out to show the effect of different solvent in extracting the efficacy of the plant extract against microbial agents.

The water extracts of the plants were tested because traditionally, plant extracts are prepared with water but the results were not surprising because other researchers have reported that antibacterial activities are low or most of the time not detectable (Matu and van Staden, 2003; Luseba et al., 2007). It has been observed that natural products are more extractable using alcoholic solvents. This effect could be due to the fact that the active ingredients are more soluble in alcohol solvent than others (Olaleye, 2007).

E. coli is the major causative agent in urinary tract infections (UTIs). It has been estimated that around 50-80% of women will be the victim of UTI at least once in their lifetime and 20-50% of women will faced repeated incidents (U-Syn and Young-Hyun, 2008). Staphylococcus aureus can be a major causative agent in a wide verity of infections ranging from minor skin infections to postoperative wound infections. Herbal medicines have been shown to have genuine utility and about 80% of most rural population depend on it. Countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin (Hassan et al., 2009).

However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

### Conclusion

The phytochemical screening of the plants studied shows that they are rich in alkaloid, flavonoids, saponins and tannins. The presence of phytochemical compounds in these plants are likely responsible for the observed biological activities of antimicrobial properties of these plants. The use of the active antibacterial plants under study could be useful in the management and treatment of ailments with bacterial causes in the locality. Subsequent evaluations of the antibacterial properties of various fractions of the secondary metabolites responsible for the different degrees of inhibition are encouraged. 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. Further studies using other extracts may be carried out to find out the active constituents.
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References


