

Full Length Research Paper

Preliminary phytochemical screening and antimicrobial evaluation of four medicinal plants traditionally used in Nigeria for skin infection

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Accepted 5 January, 2013

The antimicrobial effect of some selected Nigerian medicinal plants traditionally used by the local people for treating skin infections was evaluated on some microorganisms like *Candida albicans* MTCC 227, *Staphylococcus aureus* ATCC 2785, *Proteus mirabilis* ATCC 21784 and *Pseudomonas aeruginosa* ATCC 27856. The solvents used for extraction were water, ethanol, methanol and petroleum ether. The *in vitro* antibacterial activity was performed by agar disc diffusion method. Results showed that all the extracts were active but *Cassia alata* extracts were the most active. The activity of the extracts were concentration dependent and had zones of inhibition between 10 to 19 mm. It can be concluded that these medicinal plants extracts are as potent as standard antimicrobial drugs which justify their uses for the treatment of skin diseases by the local people.

Key words: Medicinal plants, dermatophytes, microbial activity, zones of inhibition.

INTRODUCTION

In Africa, the practice of using medicinal plants for ailments could be traced to early civilizations which used indigenous plants and materials from plants such as fixed and essential oil, resins, aqueous extracts e.t.c. for skin care (Sofowora, 1993). Within this period, there were no isolations of active constituents since in most cases the whole plant were used and all the chemical components work synergistically to achieve the purpose for which the plants were being used.

Also in Nigeria, a large percentage of the populace depends on herbal medication because the commercially available orthodox medicines are becoming increasingly expensive and out of reach (Lawal et al., 2012). Antibiotic resistance has become a global concern (Westh et al., 2004) and the clinical efficacy of many existing antibiotic is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003).

Natural products, either as pure compounds or as standardized plants extracts, provide unlimited opportunities for new drug leads because of the

unmatched availability of chemical diversity. In many developing countries, medicinal plants are still used on regular basis. There is also the renewed interest especially in developed countries in using plants to treat humans, livestock and pets.

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 (Rojas et al., 2003). Since the last quarter of the twentieth century, there has been a search for natural ways of treating humans, especially the skin being the largest organ, livestock and pets (Abels and Proksch, 2006).

Products from natural sources are gradually replacing synthetic drugs all over the world. Some of the reasons given are that the synthetic drugs, with time, tends to exhibit adverse effects on the users, unlike drugs produced using medicinal plants which are said to be less toxic and safer than synthetic drugs (Moulis, 1992). Also, medicinal plants as raw materials are easily accessible and abundantly available for production (Manisha and Vibsha, 2004).

The skin supports its own ecosystem of microorganisms, including yeasts and bacteria, which

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Table 1. Ethnobotanical information of the four medicinal plants selected for antimicrobial activity.

Plant specie	Family	Common name	Part used	Therapeutic use
<i>Cassia alata</i>	Caesalpinaceae	Asunwon oyinbo	Leaf	Laxative, skin diseases
<i>Cassia occidentalis</i>	Caesalpinaceae	Asunwon Egba	Leaf	Laxative and skin diseases
<i>Mitracarpus villosus</i>	Rubiaceae	Irawo ile	Leaf and stem	Skin diseases
<i>Acalypha wilkesiana</i>	Euphorbiaceae	Ewe lalalapa	Leaf	Skin diseases

cannot be removed by any amount of cleaning. Estimates place the number of individual bacteria on the surface of one square inch (6.5 cm²) of human skin at 50 million though this figure varies greatly over the average 20 square feet (1.9 m²) of human skin. Oily surfaces, such as the face, may contain over 500 million bacteria per square inch.

Microorganisms keep one another in check and are part of a healthy skin. When the balance is disturbed, there may be an overgrowth and infection, such as when antibiotics kill microbes, resulting in an overgrowth of yeast (Esipov and Sapiro, 1998). Proper skin hygiene is important because unclean skin favors the development of pathogenic organisms. Functions of the skin are disturbed when it is excessively dirty; it becomes more easily damaged, the release of antibacterial compounds decreases, and dirty skin is more prone to develop infections (Rook et al., 1979).

Some of the most common skin problems are acne, eczema, hives, impetigo, psoriasis, moles and rashes sometimes called basic eczema. There are three ways through which skin diseases can be managed. These include hygienic approach, pharmaceutical methods, and the traditional herbal approach. Good hygienic practices are usually advocated such as daily baths so as to prevent the proliferation of organisms causing skin diseases (Weller et al., 2008).

Traditional Medicines' herbal approach involves the use of medicinal plants in the fight against the microorganisms causing skin diseases (Iwu et al., 1999). Some plants have been identified and several have been used in the management of dermatosis by the local people for ages. These people have names for the various medicinal plants (Gbile, 1984), and use parts of the plants like the root, stem, leaves and flower to treat skin diseases (Table 1). Some of these plants include *Mitracarpus villosus*, *C. alata* (Figure 1), *Cassia occidentalis*, *Acalypha wilkesiana* (Figure 2), *Jatropha* species, *Celosia argentataeae*, *Ceae quailarass*, *Cnestis* spp, *Rauwolfia vomitora*, *Terminalia catapa*, *Vernonia amygdalina*, *Vitex doniana*, *Fromomium melegueta*. Several scientific researches have recommended the use of these plants as cheap and available antibiotic raw materials for topical and systemic application. For instance, the root and root bark of *Zanha Africana* Hiern methanolic extracts was found to possess strong antifungal activities against *Trichophyton rubrum* and

Trichophyton menthagraphytes using sabouraud's dextrose agar medium and mycobiotic agar medium (Sofowora, 1993).

Also, crude extracts of *Mitracarpus villosus* produced zones of inhibition (8 to 23 mm) against *Escherichia coli* and *Staphylococcus aureus*, the minimum inhibitory concentrations (MIC) of the effective extracts were in the range of 0.06 to 8.0 mg/ml while the minimum bactericidal concentrations (MIC) were in the range of 0.06 to 32.0 mg/ml (Olonitola and Aluko, 1995) also, Akinde et al. (2002) in their findings revealed that juices of fresh young and matured leaves of *Cassia alata* has effect on *Candida pseudotropicalis* which is concentration dependent and comparable to the activities of Acriflavine 6 mg/ml used as reference standard.

Alade and Irobi (1993) also showed that water and ethanol extracts of *A. wilkesiana* inhibited the growth of standard and local strains of *Staphylococcus aureus*, *T. rubrum* and *Candida albicans*, at the minimum inhibitory concentrations ranging between 1.25 and 32 mg/ml, while the minimum cidal concentraions were between 1.0 and 64 mg/ml.

The aim of this study is to evaluate the antimicrobial properties of four of these medicinal plants, examine their chemical composition for possible drug lead and possibly develop a safe and effective herbal dermatosis cream. This research is very significant in the sense that if the extracts are still bioactive in the body cream emulsions, then there would be a major breakthrough in the development of our indigenous herbal medicine in treating various skin diseases that plague our people mostly children, and at the same time reduce or remove if not completely the incidence of skin cancer caused by constant or prolonged use of synthetic chemicals in the treatment of the skin diseases (Abels and Proksch, 2006).

MATERIALS AND METHODS

Sample collection, identification and preparation

The plant samples were collected at the botanical garden of Nigeria Natural Medicine Development Agency, Lagos. It was identified by the Agency's botanist. Specimens were deposited in the Herbarium of the Forestry Research Institute of Nigeria, Ibadan. The plants were air dried in the shade to prevent decomposition of natural products contained in the plants. The dried materials were pulverized to fine-sized particles so as to increase the surface area



Figure 1. *Cassia alata*.



Figure 2. *Acalypha wilkesiana*.

of the sample during solvent extraction process and then stored in airtight bottles.

Preliminary phytochemical analysis

Qualitative phytochemical analyses of the crude powder of the four medicinal plants collected were determined as follows:

Tannins: 200 mg of plant material was dissolved in 10 ml distilled water and filtered. 2 ml filtrate was added to 2 ml FeCl_3 , blue-black precipitate was obtained which indicated the presence of Tannins.

Alkaloids: 200 mg of plant materials was dissolved in 10 ml methanol and filtered. 2 ml filtrate was added to 1% aqueous HCl with 6 drops of Dragendroff reagent. Creamish precipitate obtained indicated the presence of alkaloids.

Table 2. Preliminary phytochemical analysis of screened medicinal plants.

Plant species	Tannins	Saponins	Flavonoids	Cardiac glycosides	Alkaloids
<i>Cassia alata</i>	+	+	-	-	+
<i>Cassia occidentalis</i>	+	+	-	-	+
<i>Mitracarpus villosus</i>	-	+	-	-	+
<i>Acalypha wilkisienna</i>	-	+	++	-	-

Saponins (frothing test): 0.5 ml of filtrate was added to 5 ml distilled water. Frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test): 2 ml filtrate was added to 1 ml glacial acetic acid, 1 ml FeCl₃ and 1 ml Conc. H₂SO₄ absence of green-blue colour indicated that cardiac glycosides were absent. Steroids (Liebermann-Burchard reaction): 200 mg of plant material was dissolved in 10 ml chloroform and filtered. 2 ml filtrate was added to 2 ml acetic anhydride and Conc. H₂SO₄, blue-green ring obtained indicated the presence of terpenoids.

Flavonoids: 200 mg plant materials was dissolved in 10 ml ethanol and filtered. 2 ml filtrate was added to Conc. HCl and magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids (Ogunyemi, 1979).

Extraction of plant material

Aqueous extraction

Twenty gram of air-dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 10 mins. The supernatant was collected. This procedure was repeated twice. After 6 h, the supernatant collected at an interval of every 2 h was pooled together and concentrated to make the final volume one-fourth of the original volume (Harbone, 1973). It was then autoclaved at 121 °C and 15 lbs pressure and stored at 4 °C.

Methanol/ethanol/petroleum ether extraction

Twenty gram of each pulverized plant samples were extracted with the appropriate solvents using soxhlet extraction method. Rotary evaporator was used to recover the solvent from the filtrate and the extract was subsequently obtained, dried and then stored in air tight containers.

Media preparation and antibacterial activity

The antibacterial assay was performed by agar disc diffusion method. A 28 g of the Nutrient agar was dissolved in 1 L of de-ionized water in a conical flask. The mixture was vigorously shaken and then sterilized in the autoclave for 15 min at 121 °C. To prepare each stock of 10,000 µg/ml, 0.2 g of accurately weighed extracts and 2.0 ml of mixture of Dimethyl sulphoxide (DMSO) and n-hexane were used. The stocks were then serially diluted to obtain concentrations of 1000, 100 and 10 µg/ml, respectively with each bottle containing 20 discs.

For the preparation of positive control solution, 0.3 ml of Gentamycin injection vials of concentration 80 mg / 2 ml was added to 1.7 ml of equal mixture of DMSO and n-hexane. For negative control solution, 10 ml of DMSO was mixed with 10 ml of n-hexane. After the sterilization, 10 ml of the sterilized agar solution were then poured into each Petri dish void of any contaminant, and allowed to cool after which they were inverted to prevent the condensing liquid

from returning so as not to destroy the agar film formed. The bottom of each of the Petri dishes was marked into 4 quadrants while they were still in inverted position. The loopfull of appropriate test organism was then streaked in a zigzag pattern on each plate using cotton bud.

C. albicans (MTCC 227), *S. aureus* (ATCC 2785), *Proteus mirabilis* (ATCC 21784), and *Pseudomonas aeruginosa* (ATCC 27856) were used. The different experimental discs were applied upon each inoculated dish of each organism in triplicates at the appropriate quadrant positions giving room for 10,000, 1000, 100 and 10 ppm. Positions for the positive and negative control discs were also created. Thereafter, the set up were then allowed to incubate for 24 h. After about 24 h of incubation, the plates were examined for growth. The result was obtained by measuring the diameter of zones of inhibition. The mean values of the triplicates were recorded.

RESULTS

Phytochemical analysis

Results from alkaloidal test proved positive for *C. alata*, *C. occidentalis* and *M. villosus*, however, it was negative for *Acalypha wilkisienna*. Saponins were present in all the medicinal plants. Traces of tannin were present in the *Cassia* species but absent in the other two medicinal plants. Cardiac glycosides were absent in all the samples. Presence of flavonoid was confirmed in *A. wilkisienna* but absent in the other 3 medicinal plants (Table 2).

Antimicrobial activity

The results of the antimicrobial assay were shown from Tables 3 to 6. Zones of inhibition were mean of triplicate experiment performed. Maximum antibacterial activity against all the test microorganisms was exhibited by *C. alata* with its activity ranging from 12 mm against *S. aureus* to 19 mm against *P. aeruginosa*.

DISCUSSION

The preliminary phytochemical investigation carried out on the leaves of the medicinal plants revealed the presence of alkaloids in *C. alata*, *C. occidentalis* and *M. villosus*, however, it is absent in *A. wilkisienna*. The other

Table 3. Zones of inhibition of samples against test micro organisms at 10, 000 µg/ml in mm.

Plant extracts	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>
<i>Cassia alata</i>				
Aq.	18	12	17	19
Me	14	16	18	15
Et.	15	12	19	12
Pet.Ether	11	20	11	12
<i>Cassia occidentalis</i>				
Aq.	12	15	15	13
Me	12	11	12	12
Et.	13	12	16	10
Pet.Ether	12	15	12	15
<i>Mitracarpus villosus</i>				
Aq.	13	14	10	16
Me	15	12	12	13
Et.	11	12	10	12
Pet.Ether	10	13	12	12
<i>Acalypha wilkisiensis</i>				
Aq.	14	15	12	13
Me	10	13	13	09
Et.	13	13	12	15
Pet.Ether	11	10	12	11

Inhibition Zones measured in mm: - = implies no zone of inhibition, Aq= Aqueous extract, Me=Methanol extract, Et=Ethanol extract, Pet. Ether= Petroleum Ether.

secondary metabolites such as saponins were present in all the plants. Only *A. wilkisiensis* contains flavonoids, tannins were present in trace amount while cardiac glycoside were completely absent in all the plant samples. These metabolites have been shown to be responsible for therapeutic activity of plants (Trease and Evans, 1985).

Also plants containing these metabolites usually demonstrate stronger antimicrobial properties than others (Manisha and Vibsha, 2004). Tannins have been reported to inhibit growth of microorganisms by precipitating microbial pattern and making nutritional proteins unavailable for them (Ekpendu, 1995). Saponins are special class of glycosides that have been shown to be an antifungal agent (Ekpendu, 1995). The potential for developing antimicrobials from plants appears rewarding as it will lead to the development of phytomedicines to act against microbes. Plant-based antimicrobials have been shown to achieve result with lesser side effects often associated with synthetic antimicrobials (Iwu et al., 1999).

Maximum antimicrobial activity was shown by *C. alata* aqueous extracts which were active against all the test microorganisms. However, its activity was concentration dependent and it had zones of inhibition between 10 to 19 mm against all the test microorganisms (Table 3). *C. occidentalis* extracts were active against the test microorganisms but not as active as *C. alata* extracts

(Tables 3 and 4). At lower concentrations, activity of *M. villosus* and *A. wilkisiensis* greatly reduces and became more selective (Tables 5 and 6).

It is however, observed that these medicinal plants at higher concentrations have antimicrobial and antifungal activity, which is as potent as standard antimicrobial drugs against certain microorganisms. This probably explain why the local people use them for the treatment of skin diseases. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

ACKNOWLEDGEMENTS

Dr. Oladosu O. O., Department of Chemistry, University of Ibadan, Nigeria, Mr Oche E. O., Department of Botany, Nigeria Natural Medicine Development Agency. Mr Kwaptoe E and Mr Fashina F. O., Laboratory Department, Nigeria Natural Medicine Development Agency.

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