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Antimicrobial Studies of Some Local Plants Extracts on Isolates Obtained from Ear Infection

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Summary

Five local plants, *Musa sapiantum; Erythrina vogeli; Ocimum gratissimum; Aspilia africana* and *Juglan regia* were screened phytochemically for the presence of some important drug bases. The plants were found to contain alkaloids and saponins as well as tannins and cardiac glycosides. The aqueous and alcohol extracts of the medicinal plants were tested on *Pseudomonas aeruginosa* and *Streptococcus pyogenes* isolated from otitis media patients. Among the plants, *Musa sapiantum* and *Ocimum gratissimum* inhibited the pathogens. The importance of these phytochemical bases as potential sources of pharmaceutical drug preparation is discussed.

Key words: Antimicrobial property, plant extract, ear pathogens.

Introduction

In the developing countries, most communities depend on herbs for the treatment of different diseases.

There have been reports on the presence of active principles which are isolated from plants and used in modern medicine (1). These active principles include saponins, alkaloids, polyphenols, tannins, cardiac glycosides, phlabatannins, resins and mucilages.

Other studies have indicated that the plant kingdom represent a virtually untapped reservoir of new chemical compounds, many of which are biodynamic (2, 3, 4). For instance when *Acacia nilotica* was examined for its anti-diarrhoeal effects, it was found that this property is attributable to the presence of both condensed and hydrolysable tannins (4).

Other researches have identified some medicinal plants with antimicrobial properties which may be due to the presence of phytochemical bases (5, 6). Many of these plants are used in their natural state and in order to avoid complications which may arise, it is very necessary to identify the active ingredients and to investigate their antimicrobial properties thereby exploiting them as potential sources of drug bases.

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The aim of the present study was to identify the active ingredients in some medicinal plants and to investigate their antimicrobial activity on pathogens isolated from patients with otitis media infections.

Materials and Methods

Source of Plants

The plants used in this research were collected from Umofor Village Oquta Town in Imo State, Nigeria and identified by Dr. Madunagu of the Botany Unit, Department of Biological Sciences, University of Calabar. The plants were *Aspilia africana*, (Haemorrhage plant); *Ocium gratissimum* (Tea bush); *Juglan regia* (walnut); *Erythrina vogeli* (coral tree) and *Musa sapiantum* (plantain).

Media

Nutrient Agar; (Oxoid); Nutrient Broth (Oxoid); Mueller Hinton agar II (Lab M).

Isolation and characterization of microorganisms from swab samples

Swabs containing specimens taken from the middle ear of patients were inoculated onto Nutrient broth and incubated. Serial dilution of the sample and subsequent incubation gave rise to discrete colonies on Nutrient agar plates. The cultures were preserved on Nutrient agar and identified using standard method[7,8].

Phytochemical Screening and Extraction

The phytochemical components were analysed using standard methods (3, 9). For the extraction, 100g of dried, ground plant part was soaked overnight in 1 litre of water in reagent bottles and used as crude aqueous extract. The ethanol extracts were obtained by soxhlet extraction.

Standardization of inoculum

This was carried out by growing the culture in a shaker bath for 4h. The optical density (OD) at 480nm was read and the corresponding viable count taken. A plot of OD 480nm against viable count (c.f.u.) gave a linear graph. 1ml of the cell suspension gave 10^6 cells.

Antimicrobial Test

The aqueous and ethanolic extracts were tested on the organisms using the disc diffusion method (6). After incubation at 37°C for 24h, the zones of inhibition were measured.

Determination of minimum inhibitory concentration (mic)

This was carried out using the oily remnant of the ethanol extract. Cultures of *P. aeruginosa* and *S. pyogenes* each containing 10^6 cells/ml were inoculated on Mueller Hinton broth containing different concentration(50, 25, 12.5, 6.25 and 3.1 mg/ml) of extracts. These were prepared from doubling dilutions of the extract. Growth was observed after incubation for 24h at 37°C. Control tubes were not inoculated with any microorganism.

Results

Out of ten patients investigated, nine had predominantly Gram negative rods (90%) while one had Gram positive cocci (10%). Based on their cultural, biomedical and motility characteristics, the Gram negative rods were identified as *Pseudomonas aeruginosa* while the Gram positive cocci were *Streptococcus pygenes* as shown on Table 2.

The results of the phytochemical screening are shown on Table 3. All the plants examined contained alkaloids and saponins. *Aspilia africana* had five components, *Erythrina vogeli* had five components. Cardiac glycosides was absent in all plants except in *Musa sapiantum*. All the plants lacked reducing compounds.

The results of the effect of crude aqueous extract and the alcohol extract on the test organisms are illustrated in Table 4. The crude aqueous and alcohol extracts of *Aspilia africana* inhibited only P. aeruginosa but failed to inhibit *S. pyogenes*. in the case of *E. vogeli*, only the aqueous crude extract had inhibitory action on the test organisms. The alcoholic extract was not active against the organisms. The aqueous

Table 1: Plants studied and their local usage

Plant Name	Family	Part Used	Method and Usage	English Name
Aspilia africana	Asteraceae	Leaves	Used to treat ear diseases resulting in pus formation. The leaves are squeezed and the liquid introduced into the affected ear periodically.	Haemorrhage Plant
Ocimum gratissimum	Labiatae	Leaves	Used for treatment of infection of the middle ear. The leaf is flamed mildly and then squeezed to obtain a liquid which is used as ear drop.	Tea bush
Juglans regia	Olaceaceae	Leaves	Used as a therapeutic agent when pus discharge is observed in the ear. The leaf is flamed mildly and placed close to the affected ear. It is believed that healing is effected by emission of some chemical components of the leaf.	Walnut
Erythrina vogeli	Fabaceae	Leaves	Used when there is a discharge from the ear. The liquid from the leaf is used as ear drop.	Coral tree
Musa sapiantum	Musaceaceae	Skin	Used generally for the treatment of ear infection. The skin is flamed on fire for sometimes and the liquid part is squeezed out and used as a chemotherapeutic agent for otitis media.	Plantain

Table 2: Isolation and identification of Isolates from cases of Otitis media

					Fermentation					
Isolates	Pigment	Gram reaction	Haemolysis	Motility	Lac	Glu	Mal	Oxidase	Probable	Organism
1	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
2		+	Beta	—	+	—	+	+	*Streptococcus*	*pyogenes*
3	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
4	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
5	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
6	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
7	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
8	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
9	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*
10	+a	—		+	—	+	—	—	*Pseudomonas*	*aeruginosa*

+a:Pyocyanin; +: positive; -: negative; lac: lactose; Glu: Glucose; Mal: Maltose

Table 3: Phytochemical Composition of Plants

Phytochemical Components	*Aspilia africana*	*Erythrina vogeli*	*Ocimum gratissimum*	*Musa sapiantum*	*Juglan regia*
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Cardiac glycosides	–	–	–	+	–
Catechol tannin	+	+	–	–	–
Gallic tannin	–	–	–	–	+
Flavonoids	+	+	–	+	–
Polyphenols	+	+	–	+	–
Anthraquinones	–	–	–	+	–
Reducing Compounds	–	–	–	–	–

+ Present, – absent

and alcohol extracts of *O. gratissimum* and *M. sapiantum* inhibited all the test organisms. On the other hand, the crude aqueous extract of *Juglan regia* did not inhibit *Streptococcus pyogenes* but inhibited *P. aeruginosa*. However, the alcoholic extracts inhibited the two pathogens.

Results of the determination of minimum inhibitory concentration are illustrated in Table 5. The MIC

Table 4: Effect of Crude Aqueous and Alcoholic Extracts on the Test Organisms

Diameter Zones of Inhibition (mm)

Test Organism	Crude aqueous extract					Alcohol extract				
	PL_1	PL_2	PL_3	PL_4	PL_5	PL_1	PL_2	PL_3	PL_4	PL_5
Streptococcus pyogenes	—	20.70 ± 0.07	21.50 ± 0.03	22.00 ± 0.00	—	—	—	21.50 ± 0.31	25.50 ± 0.31	21.30 ± 0.41
Pseudomonas aeruginosa	20.70 ± 0.07	20.20 ± 0.01	22.50 ± 0.03	26.00 0.86	21.00 0.14	20.70 0.07	—	22.50 ± 0.03	27.50 ± 0.75	22.30 ± 0.03

PI_1: *Aspilia africana;* PI_2: *Erythrina vogeli;* PI_3: *Ocimum gratissimum;*

PI_4: *Musa sapiantum;* PI_5: *Juglans regia.*

Values are means of 3 measurements ± standard error; -: no inhibition.

Table 5: Minimum Inhibitory Concentration of Ethanolic extract of plants on *Ps. aeruginosa* and *S. pyogenes*

Test Organism	Concentration of Extract (mg/ml)									
	Musa sapiantum					*Ocimum gratissimum*				
	50	25	12.5	6.25	3.1	50	25	12.5	6.25	3.1
Streptococcus pyogenes	—	—	+	+	+	—	—	—	+	+
Pseudomonas aeruginosa	—	—	—	+	+	—	—	—	+	+

+ growth; — no growth.

of *M. sapiantum* extract against *S. pyogenes* was 25 mg/ml while that against *Ps. aeruginosa* was 12.5 mg/ml. The MIC of *Ocimum gratissimum* against *S. pygenes* and *Ps. aeruginosa* was 12.5 mg/ml, respectively.

Discussion

From the ten cases of otitis media examined, *Pseudomonas aeruginosa* was the most prevalent. This suggests that there was a higher probability of *Pseudomonas* causing this disease in every ten patients sampled.

The plants studied have been shown to contain important phytochemical components especially alkaloids and saponins. Preparative thin layer chromatography analysis of extracts of *Canthium subcordatum* (10) revealed the presence of glycosides and saponins in all organs of the plant. It has been reported that these components were responsible for its antimicrobial property. Thus the antimicrobial property of the plants studied is strongly related to the presence of alkaloids and saponins.

Four medicinal plants containing cardiac glycosides and alkaloids were examined for antimicrobial properties (5). It was concluded that in some plants, one or both components were responsible for inhibitory activity, whereas in others, the inhibitory action was attributable to components other than cardiac glycosides and alkaloids. The findings in this study were in conformity with published data. Furthermore, the medicinal action of a plant may be due to the combined effect of two or more components

(11). For instance, sage tea, effective as a gargle in cases of sore throat is due to combined effect of both essential oils and tannin.

Aqueous and alcoholic extract of *Aspilia africana* did not inhibit *S. pyogenes* but showed inhibition only on *Ps. aeruginosa*. It is claimed by herbalists that *Erythrina vogeli* is effective in the treatment of otitis media but the alcohol extract failed to inhibit the pathogens showing that the phytochemical components responsible for the antimicrobial property were not soluble or were inactivated by alcohol since the crude aqueous extract inhibited all the organisms.

Among the plants examined, *Musa sapiantum* and *Ocimum gratissimum* had relatively better inhibition on the bacteria studied. Thus if the safety of the extracts is established, the extracts of these plants could be incorporated into drugs used for the treatment of otitis media.

Conclusion

The medicinal plants studied contained alkaloids, saponins and cardiac glycosides which are pharmacologically important. Drugs like quinine and morphine contain alkaloids and cardiac glycosides which are useful for the treatment of dropsy and other diseases related to malfunctioning of the heart. In conclusion therefore, the present work has established that the plants examined especially *Musa sapiantum* possessed medicinal properties and can be exploited in drug formulations for treatment of otitis media.

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References

1. Cuilei, I. (1982). Methodology for Analysis of Vegetable Drugs, p. 67. Practical manuals of the Industrial utilisation of Medical and Aromatic plants. Bucharest, romania. Bucharest Office of Joint UNIDO.
2. Schultes, E.R. (91972). The Future of plants as sources of new biodynamic compounds. In: *plants in the Development of modern medicine Swain*, T. (Ed.) harvard University Press, Cambridge, pp. 103-105.
3. Sofowora, A. (1984). *Medicinal Plant and Traditional Medicine in Africa*. pp. 256-257, New York John Wiley.
4. Benjamin, J.V. (1979). Phytochemical and biological analysis of some local antieczimatic plants. In the 4th O.A.U./STRC Inter-African Symposium on Traditional Pharmacopeia and African Medicinal Plants, Abidjan - Ivory Coast pp. 496-498. OAU/STRC Secretariat, Lagos-Nigeria.
5. Ebana, R.U., Madunagu, B.E.; Ekpe E.R. and Otung, I.N. (1991). Microbiological exploitation of Cardiac glycosides and alkaloids from *Garcinia Kola, Borrelia ocymoides, Kola nitida and Citrus aurantifolia. J. Appl. Bacteriol.* **71**: 398-401.
6. Ekpe, E.D. Ebana R, U.B. and Madunagu, B.E. (1990). Antimicrobial activity of four medicinal plants on pathogenic bacteria and phytopathogenic fungi. *West Afr. J. Biol. & Appl. Chem.* **35**: 1-4.
7. Cowan, S.T. and Steel, K.J. (1974). *Manual for the Identification of Medicinal Bacteria*. Cambridge University Press, London pp. 33-40.
8. Cruickshank, R., Duguid, J.P; Marinion, B.P. and Surian, R.A. (1977). *Medical Microbiology* 12th edn. pp. 180-188 London: Churchill-Livingstone.
9. Harbone, J.B. (1973). Pytochemical method: *A guide to modern Techniques of plant Analysis*. pp. 4-25, London: Chapman & Hall.
10. Ampofo, S.A. and Badu, D. (1979). Preliminary Pharmacological and Phytochemical study of *Canthium subcordatum*. In the 4th O.A.U./STRC Inter-African Symposium on Traditional Pharmacopeia and African Medicinal Plants, Abidjan, Ivory Coast, pp. 685-692. OAU/STRC Secretariat, Lagos, Nigeria.
11. Schauenberg, P. and Ferdinand, P. (1977). *A guide to Medicinal Plants*, Lutter Work Press Guid Ford and London, pp. 115-120.