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The laboratory assessment of the efficacy of extracts from some plants on isolates from wounds

M. E. Abalaka*; A. Mann, S. O. Adeyemo and C. N. Abu

Department of Science Laboratory Technology, The Federal Polytechnic, Bida, Niger State, Nigeria.

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ABSTRACT: Extracts obtained from the plants *Piliostigma thoningii, Abrus precatorius* and *Cassia alata* were screened for antimicrobial activity using agar plughole method. The test organisms *Staphylococcus aureus, Klebsiella ozaenae, Proteus vulgaris* and *Pseudomonas aeruginosa* were subjected to the action of the extracts at various test concentrations. *Piliostigma thoningii* was highly active against all test organisms at the concentration of 10µg/ml, *Abrus precatorius* was active only on *Klebsiella ozaenae* at 100µg/ml while *Cassia alata* showed strong activity against all test organisms at 100µg/ml except *Pseudomonas aeruginosa*. The phytochemical screening revealed that the plants contain alkaloids, saponins, tannins and glycosides, which may be responsible for their antimicrobial activity and consequently their medicinal values.

Key Words:

Introduction

Herbal plants have been used to prevent or cure certain diseases that are caused mostly by micro-organisms. This is due to the occurrence of some natural chemicals in these plants. Plants parts have been reported to have various uses in folklore medical practices (Oboh and Abulu, 1997). A medicinal plant, according to Sofowora (1982) is one in which one or more of its organs contain substances that can be used for therapeutic purposes.

Habtemariam *et al* (1990) reported that *Premna schimper* (family verbenaceae) was useful in the treatment of inflammation and secondary infections associated with superficial wounds. Man *et al* (1997) reported the anti-microbial activity of the leave extracts of *Calotropis procera*. The anti-microbial activities of a number of cytotoxic C-benzylated flavonoids from Uvaia chamae have been determined. The minimum inhibitory concentration (MIC) values of these flavonoids and certain of their derivatives against *Staphylococcus aureus, Bacillus subtilis* and *Mycobacterium smegmatis* compare favourably with those of Streptomycin sulphate (Charles and William, 1983).

*To whom correspondence should be addressed.

Medicinal plants contain pharmacologically active agents, which over the years have been exploited in tradomedical practice for the treatment of various ailments (Adebanjo *et al*., 1983). Studies have shown such plants to contain alkaloids, phenolic compounds, saponins, tannins etc. (Odebiyi *et al*., 1978). Nature has endowed mankind with a rich storehouse of natural anti-microbial agents of plant origin.

Mann (1998) investigated folklore medical practices in Nupe land, Nigeria and reported the natives' claims on curative powers of some medicinal plants including *P. Thoningii, A. precatorius* and *C. alata*. The present research work was undertaken to ascertain these claims and to further authenticate his findings.

Materials and Methods

Collection of Plants:

The leaves of *Piliostigma thoningii*, *Abrus precatorius* and *Cassia alata* were collected from various locations in and around Bida, Niger State. The plants were immediately transported to federal Polytechnic, Bida and air-dried under fan.

Source of chemicals:

All chemicals used for the analysis were of the analytical grade (Analar) manufacture by British Drug House (BDH) Limited, England.

Extraction of active ingredients from the plants:

When well dried, the plants materials were ground to powder. 50g of the powdered leaves of each plant was extracted with 1.5 litres 95% ethanol for two weeks using the methods adopted by Mann *et al* (1997). The extracts were evaporated *in vacuo* to give extract of each plant labelled AX_1 (*Abrus precatorius*), PY_1 (*Piliostigma thoningii*) and CZ_1 (*Cassia alata*). The extract AX_1 was thoroughly mixed with chloroform water (75ml: 75ml) and allowed to stand for 10 min to yield three layers viz: water-soluble layer AX_2, chloroform soluble layer AX_3 and insoluble interface AX_4. The insoluble interface AX_4 was further extracted with a mixture of petroleum ether and 85% methanol (75ml: 75ml) to give two layers, which are petroleum ether soluble fraction AX_5 and methanol soluble fraction AX_6 (Fig. 1). This procedure was repeated with the other plants extracts PY_1 and CZ_1 and all the fractions were evaporated to give the required extracts using soxhlet apparatus (Quickfit Gallenkamp, England, AG 35 – 39).

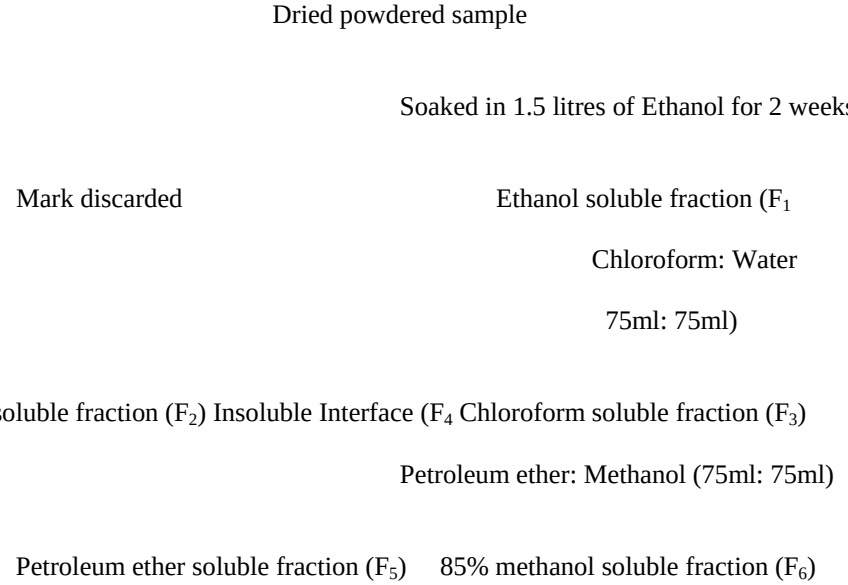

Figure 1: Flow chart for the extraction of active ingredients from plants materials (Mann *et al*., 1997).

Preparation of pure culture of test organisms:

The test organisms were isolated from wounds and obtained from department of Microbiology, University of Ilorin teaching Hospital and were transported to the Microbiology Laboratory of Federal Polytechnic, Bida and subsequently sub-cultured into nutrient broth. The organisms were *Staphylococcus aureus* B_{10}, *Pseudomonas aeruginosa* X_{13}, *Protens vulgaris* G_5 and *Klebsiella ozgenae* R_6. At each point of analysis 18-hour culture in both was used.

Investigation of the anti-microbialk activity of the plant extracts:

For the purpose of this analysis, plughole method was used. Using a sterile No. 4 cork borer, holes were made on prepared plates and were labelled corresponding to each dilution. Sterile molten agar was placed on each of the holes in order to seal the base to prevent seepage of the extract.

The agar surface was then flooded with the 18-hour pure culture of each of the test organisms. 0.1ml of each of the different fractions of the extracts was placed in each of the holes. The plates were incubated at 37°C for 24 hours in upright position. At the end of the incubation period, those plates that showed zones of inhibition (clearing) were measured.

Phytochemical screening of plant extracts:

Screening of plant extracts for active ingredients was carried out based on the methods adopted by Sofowora (1982).

Results

Table 1: Anti-microbial activity of extracts from *Abrus precatorius*, *Cassia alata* and *Piliostigma thoningii* on the test organisms showing the diameter (mm) of zones of inhibition.

Organism/Plant extract	***S. aureus***	***P. vulgaris***	***K. ozaenae***	***P. aeruginosa***
A. precatorius	Ni	Ni	14	Ni
C. alata	10	12	14	Ni
P. thoningii	16	15	14	18

Key = Ni = No inhibition.

Discussion

From the result obtained (Table 1), the extracts from *Piliostigma thoningii* and *Cassia alata* have strong activity of *Staphylococcus aureus* and *Proteus vulgaris* respectively while *Abrus precatorius* has weak activity on *Klebsiella ozaenae*. It has no activity on other test organisms at all test concentrations. *Cassia alata* and *Piliostigma thoningii* are both active against all test organisms except *Cassia alata* which has no activity on *Pseudomonas aeruginosa*. These results are in agreement with the claims by the local people over the curative efficacy of these plants on wounds (mann, 1998) since the test organisms are known to be associated with wounds and burns.

Susceptibility of microorganisms to any chemical or chemotherapeutic agent is largely dependent on the cell wall structures. If the cell structure of a microbe is less compact, it becomes more susceptible to a

chemical agent.(Rang *et al*., 1995). *Pseudomonas aeruginosa* is found to be very resistant to most drugs (Rang *et al*., 1995) and this could be due to the compactibility of the cell wall structure.

Chemically useful antibiotics are active against microorganisms at the level of at least 10ug/ml and for any plant extract to be a serious candidate for clinical use, it should be effective at the concentrations of 100mg/ml i.e. 1000ug/ml (Mitscher and Adesina, 1972). Such observation was noticed in this study with the three plants establishing the fact that extracts are effective herbs in the treatment of wound infections.

Phytochemical screening (Table 3) has revealed the plants as containing tanning, saponins, alkaloids and glycosides which confirms their basis for the treatment of wounds. *Abrus precatorius*, *Piliostigma thoningii* and *Cassia alata* with active ingredients could be a good source of remedy for wounds and burns infections.

Table 2: Diameter (mm) of zone of inhibition of standard antibiotics on test organisms.

Antibiotic	***S. aureus***	***K. ozaenae***	***P. vulgaris***	***P. aeruginosa***
Amp.	12.0	Ni	Ni	Ni
Gen.	16.0	13.0	10.0	12.0
Nal.	15.0	10.0	10.0	Ni
Nit.	15.0	2.0	Ni	Ni
Str.	20.0	13.0	Ni	NI
Tet.	13.0	10.0	Ni	Ni
Col.	16.0	Ni	6.0	6.0
Co-t	14.0	Ni	Ni	Ni

Key:
Ni - No inhibition
Amp - Ampicillin
Gen - Gentamycin
Nal - Nalidixic acid
Nit - Nitrofurantoin
Str - Streptomycin
Tet - Tetracycline
Col - Colustin sulphate (Colmycin^{R})
Co-t - Co-trimoxazole

Table 3: Phytrochemical screening of aqueous and ethanolic extracts of plant samples.

Phytochemical base	***A. precatorius***	***C. alata***	***P. thoningii***
Alkaloids	-	+	+
Saponins	-	+	+
Tannins	+	+	+
Glycosides	+	-	-

Key: + = Present
- = Absent.

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