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STUDIES ON ANTIBACTERIAL EFFECT OF *Newbouldia laevis* AND *Aspilia africana*

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Abstract

Preliminary phytochemical analysis showed that the leaves and stem bark of *Newbouldia laevis* and the leaves and inflorescence of *Aspilia africana* possess phenolic compounds (including tannins) and saponins. Antimicrobial activity of aqueous, methanol and acetone extracts of the leaves and stem bark of *N.laevis* and the leaves and inflorescence of *A.africana* were studied by agar diffusion method. Our results confirm the basis of traditional use of these two plants in wound dressing. The methanol extracts of the stem bark of N. laevis and florescence of *A. africana* produced the strongest definite antimicrobial activities against *Streptococcus faecalis, Clostridium tetani, Clostridium perfringens, Nocardia asteriodes, Serratia marcescens* and *Proteus mirabilis*. The acetone extract of both plant parts produced less activity than the methanol extracts. The aqueous extracts did not exhibit any significant antibacterial activity. Warming to 60°C significantly increased the sensitivity of the acetone extract of *A. africana* to the test organisms. At pH 2 and pH 8 the sensitivity of the extracts to the test organisms was same to the nontreated extracts. The MIC (0.60-0.85mg/ml) and MBC (0.70-1.02 mg/ml) of the methanol extracts of both plant parts were higher than those of tetracycline and gentamycin (MIC- 0.20-0.35 and MBC 0.30-0.50).

Introduction

For centuries rural people in several communities have used traditional medicine to diagnose, prevent or treat diseases worldwide. In Abia State, Nigeria, herbalists use the stem, bark and the pulp juice of *Newbouldia laevis* (Family Bignoniaceae) as an external antiseptic for wound dressing. Some other people from the same area use the leaf and inflorescence of *Aspilia africana* (Pers) C.D.Adams (Family Compositae) for wound dressing and earaches caused by inflammation of the ear. They squeeze out the juice and then apply it to the affected part for several days.

Antimicrobial compounds are known to be present in the extract of some medicinal plants. Although there are a number of reports on isolation of antimicrobial substances from plants (Emeruwa, 1982; Akpata et.al., 1977; Rotimi et. al., 1987; Irobi et. al., 1993) nothing appears to be known about those of *Newbouldia laevis* and *Aspilia africana*. Because of the wide uses of these two traditional herbs in some parts of Nigeria, an attempt has been made to investigate the scientific basis of their traditional medicinal use.

Materials and Methods

2.1 *Source of Microorganisms.* Microorganisms used were isolates obtained from stock cultures in the Microbiology Laboratory of the Federal University of Technology, Minna. The aerobic bacteria among them include Salmonella *typhi, Streptococcus faecalis, Proteus mirabilis, Serratia marcescens* and *Nocardia asteriodes* while the anaerobic ones include *Clostridium tetani, Clostridium perfringens* and *Klebsiella* sp. The aerobes were maintained on MacConkey agar and the anaerobes were stored on blood agar at 4°C, prior to use.

2.2 *Plant Materials.* Leaves and stem bark of *N.laevis* were collected from the

outskirts of Minna town, Nigeria. The leaves and inflorescence of *A. africana* were collected from the School of Agriculture and Agricultural Technology Farm, Federal University of Technology, Minna. Both plants were identified and authenticated by Dr. M. I. S. Ezenwa, School of Agriculture and Agriciltural Technology, FUT, Minna (Keay and Onochie, 1960 and Ivens *et al.* 1978).

2.3 *Preparation of Extracts*. Leaves of *N.laevis* and inflorescence of *A.africana* were sundried to constant weight over five days and then kept dry for further use. The stem bark of *N.laevis* and *A.africana* were first chopped into small pieces and then dried similarly.

The dried plant materials were then reduced to fine powder using an electric blender (National MX 391N, Matsushita Electric). The powdered samples (50.0 gm) were separately extracted with 200-ml. water, methanol and acetone (BDH, Poole England) respectively over 24 hr. Extracts were then recovered by filtration using Whatman No.1 filter paper. A rotary evaporator was used, in vacuo at 40°C to concentrate the extracts by evaporating the solvents. The semi-solid material (yield 7-11%) obtained for each plant part was stored in the refrigerator for further use.

2.4 *Preliminary Phytochemical Studies*. A preliminary phytochemical analysis for saponins, sesquiterpenes, phenol compounds, tannins and anthraquinone was performed using the methods described by Odebiyi and Sofowora (1987) and Sofowora (1982).

2.5 *Antibacterial Sensitivity Tests*. Each of the semi-solid extracts was separately reconstituted in a minimum amount of the extracting solvent and then diluted with glycerol to get a final concentration of 2.0 mg/ml. The standard antibiotics, tetracycline and gentamycin, were diluted to required concentrations (2.0 mg/ml) with distilled water and used for comparison. The tests were carried out using the modified agar diffusion method of Garrod *et al.* (1981).

In case of aerobic bacteria, four holes were bored into each MacConkey plate previously seeded with 10^6 cells/ml of test bacteria. Using a sterile pipette, 0.5 ml of each extract was aseptically introduced into two holes in each plate, 0.5 ml of each of tetracycline and gentamycin were similarly introduced into the third and fourth holes respectively. Glycerol used as control was introduced into a second set of MacConkey agar plates instead of tetracycline and gentamycin in order to compare the activity between control and extracts. The plates were then incubated at 37°C for 24 hours.

To observe the activity against anaerobic bacteria, blood agar plates were seeded with 10^6 cells/ml of test bacteria before the extracts were applied. However the plates were kept at 37°C in an anaerobic jar for 24 hours.

2.6 *Effect of temperature and pH*. The effects of temperature and pH were studied using the method of Emeruwa (1982) For temperature, the suspensions were treated for 30 minutes at 30°C and 60°C respectively in a water bath. To see the effect of pH, the suspensions were adjusted to pH value between 2.0 and 8.0. Antibacterial activities of all the treated samples were determined by using the modified agar diffusion method described earlier.

2.7. *Determination of Minimum Inhibitory Concentration (MIC) of Extract*. The MIC of the methanol extracts of *N.laevis* and *A. africana* was estimated for *Clostridium tetani, Clostridium perfringens, Nocardia asteriodes, Streptococcus faecalis, Proteus mirabilis* and *Serratia marcescens*. To 0.5 ml of varying concentrations (0.2 – 2.0 mg/ml) of the reconstituted extract, 2.0 ml of MacConkey broth was added and then a loopful of the test organism previously

adjusted to a concentration of 10^6 cells/ml was introduced.

Aerobic bacteria (*Serratia marcescens, Proteus mirabilis, Nocardia asteriodes, Streptococcus faecalis*) were incubated at 37°C for 24 hours, while anaerobic bacteria (Clostridium tetani, Clostridium perfringens) were incubated in an anaerobic jar at the same temperature for 24 hours. For each of the selected bacterial species the procedure was repeated using gentamycin and tetracycline of varying concentrations in place of the extracts and incubated accordingly. A control containing broth only seeded with the same test organisms as described above was incubated similarly (Brock and Madigan, 1991).

2.8 *Determination of Minimum Bactericidal Concentration (MBC) of Extracts.* For each set of test tubes in the MIC determination a loopful of broth was collected from those tubes, which did not show any growth and inoculated on sterile agar plates (MacConkey for aerobes, Blood agar for anaerobes) by the streak method. Aerobes were incubated at 37°C for 24 hours while anaerobes were incubated similarly but in an anaerobic jar. The above procedure was also performed using the standard antibiotics for the purpose of comparison (Brock and Madigan, 1991).

Results

The preliminary phytochemical analysis showed that both plants contain phenolic compounds, tannins and saponins.The results are shown in Table 1. Antibacterial effects of different extracts of *N.laevis* and *A. africana* are shown in Table 2. The Table shows that the methanol and acetone extracts of parts of both plants possess significant antibacterial activities against *Clostridium tetani, Clostridium perfringens, Streptococcus faecalis, Nocardia asteriodes, Proteus mirabilis, Serratia marcescens, Klebsiella* sp, *Pseudomonas aeruginosa.* All the aqueous extracts demonstrated much less or no inhibitory action when compared with acetone and methanol extract or the standard antibiotics.

Effect of temperature on the antibacterial activity of acetone extracts of *N. laevis* and *A. africana* is shown in Table 3. Heating to 60°C increased the antibacterial activity of the acetone extract of the inflorescence of *A.africana* against *Streptococcus faecalis* and *Proteus mirabilis*. The increased temperature did not affect the activity of methanol extracts. When the extracts were kept at 30°C for 30 minutes, the antibacterial activity of the extracts was same as that of the nontreated extracts. There was no significant effect of pH on the antibacterial activity of the extracts.

The minimum inhibitory concentrations of both plant extracts were generally higher than those of tetracycline and gentamycin (Table 4). The MBC values of the extracts were slightly higher than their MIC values (Table 4).

Discussion

In this work a study has been made on the antimicrobial activities of the aqueous, methanol and acetone extracts of the plants *Newbouldia laevis* and *Aspilia africana.* According to Clark (1981) and Mather and Gonzalez (1982) tannin-like or phenolic compounds and saponins are capable of inhibiting bacterial growth and can protect certain plants against bacterial infections. Results of the preliminary phytochemical studies described here confirm the presence of phenol compounds or tannins from the two plants that possess antimicrobial activity. The solubility of tannins and phenols in methanol may explain the antibacterial activity shown by the methanol extracts (Wolinsky and Sote, 1984). Also acetone has the specific property of extracting tannins thus suggesting a basis for the antibacterial activity of the acetone extracts. The aqueous extracts did not show

significant antibacterial activities, probably due to the low concentrations of the active ingredients in the aqueous extracts. Furthermore preparing plant materials in water lead to the release of a number of phenolases and hydrolases (Emeruwa, 1982). These enzymes may interact with the active principles in the aqueous extracts and so prevent them from expressing their antimicrobial activity. All the extracts demonstrated a lesser antimicrobial action than the standard antibiotics (tetracycline and gentamycin) probably because the extracts were in crude form and may contain substances, which could modulate the activity of the extracts. The bacteria that showed susceptibility to the plant extracts are those commonly isolated from wounds and burns. (Croshaw, 1983).It was shown that heating to 60 °C increased the antibacterial activity of the extract of the inflorescence of *A. africana* against *Streptococcus faecalis* and *Proteus mirabilis*. This contrasts with the work of Emeruwa (1982). One may attribute this to a reduced effect by other components of the crude extract. Since pH had no significant effect on the extract, the active principle in the extract may not be sensitive to pH. Both the MIC and MBC of the plant extracts were generally higher than those of tetracycline and gentamycin. Furthermore the MBC values of the extracts were slightly higher than the MIC values. These results suggest that the plants *Newbouldia laevis* and *Aspilia africana* possess antimicrobial activities comparable to those of gentamycin and tetracycline and thus confirm the basis of traditional use of these two plants in wound dressing.

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Table 1: Preliminary phytochemical analysis of *N. laevis* and *A. africana*.

Phytochemical Components	*N. laevis* leaf	*N. laevis* bark	*A.africana* Inflorescence	*A.africana* leaf
Sesquiterpenes	-	-	-	-
Anthraquinones	-	-	-	-
Phenolic compounds	+	++	++	++
Tannins	+	+	++	+
Saponins	++	++	+	+

++ indicates active component present in high amount
+ ,, ,, ,, ,, in moderate amount
- ,, ,, ,, absent

Table 2: Antibacterial effects of different extracts of *N.laevis* and *A. africana*.

Plant extracts /antibiotic	Zone of inhibition diameter (mm)							
	A	B	C	D	E	F	G	H
Methanol Extract								
Leaf: *N.laevis*	2.0	5.0	6.5	6.0	6.0	7.0	1.7	2.5
Bark: *N.laevis*	8.2	11.2	2.0	8.5	9.0	9.0	1.4	2.0
Leaf: *A.africana*	1.5	2.8	2.1	2.8	2.0	4.0	2.5	2.0
Inflo: *A.africana*	5.4	5.6	8.0	5.6	2.0	4.3	3.2	1.2
Acetone Extract								
Leaf: *N.laevis*	1.2	2.9	1.4	1.5	4.0	5.5	1.5	1.5
Bark: *N.laevis*	7.0	8.0	1.5	4.3	5.6	6.6	1.4	1.8
Leaf: *A.africana*	1.2	2.0	1.6	1.0	1.0	1.2	1.2	1.0
Inflo: *A.africana*	4.0	4.8	2.1	1.4	1.3	1.0	2.8	1.0
Glycerol Control	0	0	0	0	0	0	0	0
Gentamycin	9.0	11.7	10.0	11.9	8.6	12.6	9.6	5.0
Tetracycline	9.4	12.7	8.6	12.6	9.9	7.7	8.2	3.5

A- *Proteus mirabilis* B- *Strep.faecalis* C- *Serratia marcescens* D- *Nocardia asteriodes* E- *Clostridium tetani* F-*Clostridium perfringens* G-*Klebsiella* sp.H- *Salmonella typhi*

Table 3: Effect of temperature on antibacterial activity of the acetone extracts of *N. laevis* and *A. africana*.

Bacterial species	Zone of inhibition (mm)			
	Non-treated		Treated (60°C)	
	IF	SB	IF	SB
Proteus mirabilis	4.0	8.0	5.5	2.2
Streptococcus faecalis	4.7	8.0	10.0	8.5
Nocardia asteriodes	6.0	8.0	8.2	2.2
Clostridium perfringens	4.0	9.0	4.0	4.4

Key: IF = Inflorescence of *A. africana*; SB = Bark of *N. laevis*;

Table 4: Determination of MIC and MBC of methanol extracts of *N. laevis* and *A. africana*.

Bacterial species	MIC (mg/ml)				MBC (mg/ml)			
	IF	SB	TE	GE	IF	SB	TE	GE
S. faecalis	0.60	0.60	0.20	0.30	0.80	0.75	0.35	0.40
C.perfringens	0.60	0.65	0.20	0.35	0.90	0.90	0.35	0.35
N. asteriodes	0.55	0.70	0.30	0.35	0.85	1.02	0.30	0.30
P. mirabilis	0.80	0.65	0.25	0.20	0.75	0.85	0.45	0.35
S. marcescens	0.72	0.70	0.35	0.30	0.90	0.70	0.40	0.40
C. tetani	0.65	0.85	0.20	0.25	0.80	0.90	0.35	0.50

IF = Inflorescence of *A. africana*, SB = Bark of *N. laevis*,
TE = Tetracycline, GE = Gentamycin.