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IN-VITRO ANTIMICROBIAL PROPERTIES OF *ASPILLA AFRICANA* (COMPOSITAE).

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The in vitro anti-microbial activity of the petroleum ether, chloroform and methanol extracts of Aspilia africana (Compositae) was studied. The bacterial used for the antimicrobial analysis consisted of 3 clinical strains of Staphylococcus aureus, Bacillus subtilis, 2 clinical strains of Escherichia coli and Pseudomonas aeruginosa, Candida albicans and Aspergillus flavus. The petroleum ether extract was most active with a very good broad spectrum activity against all tested microorganisms. This was followed by the chloroform extract and then methanol. The cold extracts were generally more active than the soxhlet extracts. Only the cold petroleum ether extract showed a good activity against both C. albicans and A. flavus. The phytochemical screening for the whole plat of A. africana revealed the presence of alkaloids, saponin glycosides and tannins but absence of steroidal nucleus and anthraquinone.

Keywords: antimicrobial, Aspilia africana, Compositae,

Aspilia africana (Compositae) is a semi-woody herb from a perennial woody root stock up to 2m high, very polymorphic and occurring throughout the region on wasteland of the Savannah forested zones and widely distributed across tropical African (Dalziel, 1937). The plant is a weed grazed by cattle and sheep and is much used in the western state as food for rabbits and hares. It has a high crude protein content (Burkill, 1985). The plant is used in herbal medicine to treat various infections of bacterial origin such as gonorrhea, stomach trouble and corneal opacity. The plant is widely used as haemostatic agent (Dalziel, 1937). The fresh leaves are used on cuts, wounds and sores. A decoction has been recommended for use in treating pulmonary haemorrhages and haemostasis is thought to be due to vasoconstrienon (Irvine, 1961). In Tangayika, a root decoction is taken for tuberculosis and in Ghana, the leaves are made into cough medicine for children. In Uganda, a leaf decoction is taken for treatment of gonorrhea (Page *et al*, 1992).

There is little report on the chemical constituents of the plant. Page, *et al* (1992) reported the presence of fatty acid in the oil of seeds of *A. africana* and the presence of diterpenes, kaurenoic and grandiflorenic acids from the leave. *Aspilia mossambicensis* has been reported to possess antimalaria activity against *plasmodium falciparium*, galactogogue activity and used to alleviate menstrual cramps (ofulla-Avo *et al*, 1996; Page, et al, 1992).

There is no previous in-vtiro antimicrobial activities of Aspilia species in literature to the best of our knowledge. Hence in this paper, we report for the first time, the *in-vitro* antimicrobial activity of the whole plant of *A. africana*.

MATERIAL AND METHODS

Plant Material

The whole plant of *A. africana* was collected from the forestry hills of the Forestry Research Institute of Nigeria (FRIN), Ibadan during the dry season and authenticated by Mr. Gabriel, an herbarium officer at FRIN. (Herbarium Voucher Number FH 105352). The samples used in the following analysis were Imbally air dried and milled with a Hammer mill. Forty gram of the powdered plant was extracted three times by percolation for 3 days each (cold extraction) and another forty gram consecutively extracted with redistilled petroleum ether (60-80^{o}C), chloroform and methanol by soxhlet extraction. Each extract was filtered and concentrated *in-vacuo*. Extracts were stored in the refrigerator until needed for analysis.

Organisms

All the microorganisms used for the study consisted of 4 Gram-positive bacteria; *Staphylococcurs aurens* UCH 560, *S-aureus* 511, *S. aureus* 681 and *Bacillus subtilis*, 4 Gram negative bacteria: *Escherichia coli* UCH 270, *E. coli* UCH, 307 and 2 fungal strains (Table 1).

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Table1
List of Microorganisms used to access the antimicrobial activity of the crude plant extracts.

Microorganisms	Relevant Properties	Source
Staphylococcus aureu, UCH 560	Sensitive to all antibiotic	Clinical Isolate
S. aureus, UCH 511	Resistant to Te, amp	Clinical Isolate
S. aureus, UCH 681	Resistant to Te.	Clinical Isolate
Bacillus subtilis		Lab stock
Escherichia coli ,UCH 270	Resistant to Te, Am. and S&T	Clinical Isolate
E. coli ,UCH 307	Resistant to Te and S & T	Clinical Isolate
Pseudomonas aeruginosa UCH 649,	Resistant to Gn	Clinical Isolate
UCH 655	Resistant to Py and Gro.	Clinical Isolate
Candida albicans		Lab stock
Aspergillus flavus		Lab stock

Media
Nutrient broth No. 2pH 7.4,nutrient agar pH 7.4, tryptic soy broth pH 5.6 and Sabourand dextrose agar pH5.4, all products of oxoid laboratories, England were used in this study.

Antimicrobial agents
The following chemotherapeutic agents were included in the test as positive controls: Gentamicin sulphate, 10µg/mL. (Nicholas Laboratories Limited, England), Ampicillin 25µg/ml. (Laboratory Oftalmiso, Spain) and Tioconazole 1%w/v (Pfizer Inc., New York).

Phytochemical screening
The qualitative chemical analysis of the powders as carried our for the presence of anthraquinone, tannins, saponins, steroids, cyanogenic glycosides, cardiac glycosides, and alkaloids using the method adopted in similar surveys (Harborne, 1973 and Sofowora, 1984).

Determination of antimicrobial activity
The antimicrobial activity of the extracts were determined using agar well diffusion technique (Adeniyi, et al, 1996). Nutrient agar plates were each seeded with 0.1 ml of an overnight culture of each bacterial (equivalent to 10^7 – 10^8CFU/mL), while the Sabourand dextrose agar plates were each similarly seeded with each fungal strain. The seeded plates were allowed to set and then dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar, into which was added 60uL. Solution of each extract resuspended in 50% methanoil at a concentration of 20 mg/ml. Nutrient agar plates with bacterial isolates were incubated at 37°C for 24 hours and Sabourand dextrose agar plates seeded with fungal strains were incubated at 25°C for 72 hours after which iameters of zones of inhibition were measured. Since each of the petroleum ether, chloroform, and methanol extracts was resuspended in 50% methanol before being tested, methanol was included in each plate as a solvent control besides the chemotherapeutic agents included as positive control. Gentamicin at 10ug/mL. And Ampicillin at 25ug/ml. Was used for bacterial and tioconazol at 2% was used for fungi as negative controls. All the assays were carried out in triplicate.

RESULTS

The percentage yield and macroscopic characteristics of the crude extracts by both cold and soxhlet extraction of the whole plant are presented in Table 2. The yield was generally more for the soxhlet extracts than cold extracts. Phytochemical screening of the whole plant (Table 3) showed that the constituents found in the plant are

Table 2
Extraction Yield and Macroscopic Characteristics of the Crude Extracts

Extracts (Whole plant)	% Yield	Macroscopical Characteristics
Cold Extraction		
Petroleum ether	1.1	A yellow-green shiny surface powder
Chloroform	2.15	A dark green shiny surfaced powder
Methanol	2.5	A brown crystalline powder
Soxhlet Extraction		
Petroleum ether	2.95	A yellowish-green powder
Chloroform	4.83	A dark-green shiny surfaced powder
Methanol	7.95	A brown crystalline powder

saponin glycoside, tannins and alkaloids while steroidal nucleus and anthraquinones glycosides were absent. The results of antimicrobial activity are presented in Table 4. All extracts were found to posses different degrees of antimicrobial activities. the zones of inhibition obtained in respect of the extracts of 20 mg/ml. Compared favorably with those obtained from ampicillin and gentamicin. The petroleum ether extracts showed a broad spectrum antimicrobial activities. the petroleum ether cold extract was the most potent of all the extracts. In most cases, the cold extracts showed better antibacterial activity than the soxhlet extracts. It is interesting to note that both *Candida albicans* and *Aspergillus flavus* were susceptible only to the petroleum ether cold extract and that the clinical strains of *S. aureus* and *P.aeruginosa* that were resistant to both ampicillin and tetracycline were found susceptible to all the cold extracts of *A. africana*.

Table 3
Phytochemical screening of *Aspilia africana* for secondary metabolites

Phytochemical groupings	**Whole Plant**
Alkaloids	+++
Tannin	++
Anthraquinone glycoside	-
Saponin glycosides	-
Steroidal glycosides	++
Cardiac glycosides	+
Cardenolides	+

Note - = Absent; += Low concentration
++ =Moderate +++ = High concentration.

Table 4
Antimicrobial Activity of Crude extracts of *Aspilia africana*

Test organisms			Extracts/Diameter of Zones of Inhibition (mm)*							
	Pc	Ps	Cc	Cs	Mc	Ms	Am	Gn	Ti	50% M
s aureus UCH 560	18±0.3	14±0.3	10±0.3	R	R	18±0.3	R	14±0.3	NT	R
S. aureus UCH 511	14±0.3	R	R	R	R	R	R	12±0.4	NT	R
S. aureus UCH 681	1±0.5	10±0.4	12±0.4	16	12±0.3	R	R	10±0.2	NT	R
B.subtilis	15±0.7	11±0.5	12±0.3	10	NT	R	R	16±0.2	NT	R
E. coli UCH 270	14±0.5	12±0.2	15±0.2	R	20±0.9	R	R	12±0.5	Nt	R
E. coli UCH 307	17±0.9	17±0.5	14±0.2	12	17±0.2	16±0.3	12	12±0.5	NT	R
P.aeruginosa UCH 649	16±0.3	R	12	R	12±0.3	R	R	10±0.4	NT	R
P. aeruginosa UCH 655	13±0.3	12±0.4	14±0.2	14±0.3	R	R	R	R	NT	R
C. albicans	20±0.4	R	R	R	R	R	NT	NT	16	R
A. flavus	17±0.2	10±0.2	R	R	R	R	NT	NT	14	R

DISCUSSION

The antibacterial activity of the cold extract appears to be broad spectrum especially since both Gram positive and gram-negative bacterial were sensitive to the extracts. The petroleum ether ether extracts especially its cold extract were active than chloroform and methanol extracts probably because they contain some active compounds absent in the other extracts or they contain a higher concentration of the active compounds. More interesting, the cold extracts perhaps because of one or more of the active constituents are thermolabile. Fractionation oif the extracts and further bioassay will reveal the nature of these active compounds. Some of the bacteria that are susceptible

have been implicated in diseases such as diarrhoea, oral and dental infections, wound sepsis and dysentery (Hugo and Russel, 1983; Sleigh and Timbury, 1981). The susceptibility of both *C.alcicans* and *A. flavus* to the cold petroleum ether extracts in an indication that this plant possess a great potential as a source of antifungal agent but which may be thermolabile. The antimicrobial activities demonstrated by crude extracts of this plant may, therefore justify some of the ethnopharmacological claims about this plant for the treatment of disease like dysentery, wound sepis, cough and even mycobacterium infections. The antimicrobial properties exhibited by these plants could be traced to its possession of alkaloids and tannis noted for their use as astringent in wound healing based on the ability of tannin to bind to proteins of exposed tissues thus, precipitating the protein. This then form a mild antiseptic protective coat under which regeneration of new tissues take place in the process which leads to healing of wounds. Odebiyi (1985) reported the antimicrobial properties of the extracts of *Jatropha podogrica* stem bark which contains both alkaloids and tannins. Hans (1952) also attributed the antimicrobial and haemostatic activities of plant extracts on fresh wounds to tannins.

Since there is no reported adverse or toxic effects of this plant used in different African communities including Nigeria for various ailments, phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity.

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REFERENCE

Adeniyi, B.A., Odelola, H.A. and Oso, B.A (1996): Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). Afr. J. Med. Med. Sci. 25:221 – 224.

Burkill, H.M. (1985): The useful plants of West Tropical Africa, Vol. 1, 2nd Edition, Families A-D, P. 446-447, Royal Botanic Gardens, KEW.

Dalziel J.M. (1937): The useful plants of West Africa. Crown Agents. London (1935).

Han, N. (1952): Tannin as a factor in the resistance of chestnut *Castanea spp.* To the chest nut blight fungus *Endothia parasitica* (Murr) A and A. Phytopath.; 43:32.

Harborne, J.B. (1973): "Phytochemical Methods". Chapman and Hall, London, pp 41-48.

Hugo W.B. and Russel, A.D (1983): Pharmaceutical microbiology, rd Edition. Blackwell Scientific publication, 47.

Irvine, F.R. (1961): Woody plants of Ghana. Oxford University Press, London.

Odebiyi, O.O. (1985): Antimicrobial and antifungal properties of the extractives of J. podogrica stem. Fitoterapia 56, 297 – 299.

Offulla, A.V., Rukunga, G.M., Chege, G.M.M., Kiarie, F., Muthaura, C.N., Githure, J. I., and Kofi-Tsekpo, W.M. (1996) Antimalaria activity of fractions isolated from *Albizia gummifera* and *Aspilia mossambicensis* crude extract. African Journal of Health Sciences, 3 (2), 44 – 46.

Page, J.E., Balza, F., Nishida, T., Towers, G.H.M. (1992): Biologically active diterpenes from Aspilia mossambicensis-a chimpanzee medicinal plant. Phytochemistry, 31:10, 3437 – 3439.

Sofowora, A (1984): Medical plants and traditional medicine in Africa. Chichester. John Wiley and Sons, 142 – 146.

Sleigh J.D. and Timbury, M.C. (1981): Notes on medical bacteriology. Churchill Livingstone Edinburgh, London, Melbourne and New York, 130.

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