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# Antimicrobial Investigation of the Constituents of the Methanol Extract of the Rhizomes of *Anchomanes Difformis*, ENGL

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**The dried powdered rhizomes of *Anchomanes difformis*, Family-Araceae, was extracted exhaustively with methanol. The dried methanol extract was extracted with water and the aqueous solution was treated with chloroform to obtain aqueous and chloroform extracts. The residue from aqueous extraction was dissolved in petroleum ether to give petroleum ether extracts. The extracts (aqueous, chloroform and petroleum ether) were screened for antimicrobial activity. The chloroform extract was fractionated using preparative TLC, (coated with silica gel 245 HF) and developed with ethylacetate : isopropanol : ammonia (10:8:2), to obtain seven fractions ($S_1$ and $S_7$). The chemical class of each fraction was identified and screened for antimicrobial activity. The chemical class of the fractions were identified as steroidal glycosides ($S_6$-$S_7$), saponins ($S_1$ and $S_5$), alkaloid ($S_3$) and amines ($S_2$ and $S_4$). The steroidal glycosides $S_6$ and $S_7$ showed a antimicrobial activity.**

Herbal medicine has been practiced for many years by ancient man on trial and error basis[1]. The observed effects of various plant extracts on disease therapy have encouraged scientists to look into the extracts of roots, seeds, leaves, stems and stembarks of many plants with a view to harness their potential constituents for the treatment of man's maladies[2]. However, no matter how efficacious these extracts may be, it is still necessary to isolate and characterise the chemical constituents responsible for the activity.

*Anchomanes difformis* is said to have a wide application medicinally. The aqueous extracts of the rhizomes and leaves were used to mitigate the inflammation of the eye. It is used in some parts of Nigeria to achieve diuresis and purgation; and in the treatment of convulsions and fits of epilepsy. The methanol extract was used topically to treat some bacterial and fungal infections of the skin[3].

It has been reported that *Anchomanes difformis* contains amines, terpenoids, cyanogenetic compounds, alkaloids, saponins, tannins and phenolic acids. Important amines contained include canavorine, putrescine and skatole[4]. Two kinds of steroidal saponins have been recognised; the tetracyclic and pentacyclic triterpenoid types both of which have glycosidal linkage at C3 and have a common biogenetic origin[5]. The extraction, isolation[6], purification and characterisation[7] of some chemical constituents of rhizomes of *Anchomanes difformis* are described in this work. The antifungal and antibacterial effects of the isolates were also studied.

## EXPERIMENTAL

The following chemicals and reagents were obtained from commercial sources and were used as supplied : chloroform and ethanol from May & Baker; ammonia solution from BDH; ethylacetate, toluene, benzene from Vicker; isobutanol, isopropanol, silica gel 245 HF, methanol, dimethyl sulphoxide (DMSO) and acetone from Merck. The reference drugs used are nystatin$^R$ an antifungal from Squibb and chloromycetin$^R$ an antibacterial from Parke Davis.

The standard microorganisms used were *Bacillus subtilis* (NCTC 8326), *Staphylococcus aureus* (NCTC) 3761), *Escherichia coli* (NCTC 9001), *Pseudomonas*

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*aeruginosa* (NCTC 6750), *Aspergillus niger* (laboratory strain) and *Cadida albicans* (laboratory strain). These were standard cultures obtained from the Pharmaceutical Microbiology of the University.

The mature rhizomes of *Anchomanes difformis* were harvested in May 1997 from Nsukka and authenticated at the Department of Botany of the same University. The voucher specimens of the rhizomes and leaves are deposited at the herbarium of the Department of Pharmacognosy of the same University. The rhizomes were washed, sliced and dried in the oven at 60° and then ground into a coarse powder.

**Extraction with Solvents:**

The dried coarse powder (1.0 kg) was extracted with methanol (1500 ml) using a soxhlet extractor. The methanol extract (ME) was concentrated using a rotary evaporator. Twenty five grams of dried methanol extract was extracted with water (500 ml) and the residue dissolved in petroleum ether (500 ml) to give petroleum ether extract (PE). The aqueous extract was dried under reduced pressure and extracted with chloroform (500 ml). The residue from chloroform extract was re-dissolved in water (50 ml) to give aqueous extract (AE). The chloroform extract (CE) was evaporated to dryness in a rotary evaporator.

**Fractionation of the Chloroform extract:**

Many solvent systems were tried until ethylacetate:isopropanol:ammonia (10:8:2) was found to give the best separation. The chosen solvent system was thoroughly mixed and transferred into a developing tank and allowed to stand undisturbed for 2 h to attain equilibrium before use.

The PTLC plates (20 cm x 20 cm) were coated to a thickness of 0.25 cm with silica gel 254 HF using an standard method[6] with Kenso CJK 520 spreader. The coated plates were dried at room temperature and activated in the oven for 30 min at 110°. It was allowed to cool at room temperature before use.

The 20 cm by 20 cm coated plates were spotted (band) with chloroform solution of the chloroform extract at a distance of 1cm from the origin. The spotted plates were left to dry at room temperature and were developed in the solvent system (ethylacetate, isopropanol, ammonia (10:8:2). The developed plates were viewed under UV light and some were sprayed with standard reagents[7] to locate the bands. The located bands were scrapped out and each washed with enough quantity of chloroform. The chloroform filtrates were evaporated to dryness using a rotary evaporator. The Rf values and the weights of the isolated compounds were determined.

**TABLE 1 : SOME PHYSICAL AND PHYTOCHEMICAL PROPERTIES OF THE CHLOROFORM FRACTIONS**

| Fraction | Rf value | Nature of Residue | % yield[a] (w/w) Chemical nature of Residue |
|---|---|---|---|
| $S_1$0.04 | Whitish powder | 8.3 | Saponin |
| $S_2$0.11 | Greenish brown powder | 7.4 | Amine |
| $S_3$0.22 | Brown powder | 20.5 | Alkaloid |
| $S_4$0.30 | Dark brown powder | 10.0 | Amine |
| $S_5$0.41 | Light brown powder | 20.0 | Saponin |
| $S_6$0.56 | Golden brown | 10.0 | Steroidal glycoside |
| $S_7$0.89 | Dark brown | 23.0 | Steroidal glycoside |

a = % yield calculated on the basis of dried quantity of chloroform extract.

TABLE 2 : ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY (MIC, UG/ML) OF *ANCHOMANES dIFFORMIS* DIFFERENT EXTRACTS AND CHLOROFORM FRACTIONS

| Sample | *S. aereus* | *B. subtilis* | *P. aeruginosa* | *E. coli* | *A. niger* | *C. albicans* |
|---|---|---|---|---|---|---|
| $S_1$ | - | - | - | - | - | - |
| $S_2$ | - | - | - | - | - | - |
| $S_3$ | - | - | - | - | - | - |
| $S_4$ | - | - | - | - | - | - |
| $S_5$ | - | - | - | - | - | - |
| $S_6$ | - | 13.0 | 13.0 | 15.0 | 48.0 | 13.0 |
| $S_7$ | - | 12.0 | 12.0 | 25.0 | 43.0 | 12.0 |
| ME | - | 750.0 | 750.0 | 800.0 | 2000.0 | 750.0 |
| CE | - | 300.0 | 300.0 | 350.0 | 980.0 | 300.0 |
| PE | - | - | - | - | - | - |
| AE | - | - | - | - | - | - |
| Chloromycetin | 6.5 | 6.5 | 6.5 | 10.0 | - | - |
| Nystatin | - | - | - | - | 20.0 | 14.0 |

$S_1$ to $S_7$ = Chloroform fractions, ME = Methanol extract, PE = Petroleum ether extract, CE = Chloroform extract AE = Aqueous extract

**The Sensitivity Screening of the Chloroform Fractions:**

The antibacterial and antifungal activity of the fractions were studied using agar diffusion assay method[8]. Dimethylsulphoxide (DMSO) was used to dissolve the extracts. The minimal inhibitory concentration (MIC) was determined by the agar dilution method[9], Nystatin and chloromycetin were used as standard drugs. The test was carried out in triplicate using the bacterial cultures and solutions of the extracts in DMSO. Two-fold serial dilution of the fractions and standard drugs in DMSO and water were made, respectively. A 0.04 ml of these different dilutions are introduced into wells bored in nutrient agar plates already seeded with a standard (about $10^5$ cfu/ml) inoculum of the test microorganisms. After incubation at 37° for 48 h, the zones of inhibition (1ZD) are measured and the MIC obtained form the intercepts of log concentration axis of a graph of 1 $ZD^2$ against log of concentration.

## RESULTS AND DISCUSSION

The ME gave 12% w/w yield of the dried plant material while PE, AE and CE gave 35, 45, 20% w/w respectively of the methanolic extract.

The results of antimicrobial Screening have been presented in Table 2. It was found that the methanolic extract showed antimicrobial activity and on further solvent fractionation only CE has antimicrobial activity. From the extraction method, it means that the antimicrobial component of this plant are soluble in methanol and chloroform, and are polar. On further fractionation of chloroform extract, seven bands were observed in the developed plates. The result of the Rf values, physical appearances, chemical classes, percentage compositions of these bands were reported in Table 1.

The result of the antibacterial and antifungal sensitivity to the micro-organisms is shown in Table 2. It was found that only fractions $S_6$ and $S_7$ have antifungal and antibacterial activities. The sensitivity bio-guided extraction indicated that the chloroform extract to be the only fraction that has antifungal and antibacterial activities. Further fractionation of this extract confirmed the active component to be fractions $S_6$ and $S_7$ which are steroidial glycosides. These fractions can be assumed to have the same bio-synthetic origin hence the same basic structural unit responsible for antibacterial and antifungal activities in this plant.

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