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In vitro Antimicrobial Screening on *Anchomanes difformis* (Blume) Engl. Leaves and Rhizomes Against Selected Pathogens of Public Health Importance

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Abstract: There is a need to establish scientific findings to many ethnobotanical uses of plants as phytotherapy which could find application in the pharmaceutical industry. Antimicrobial activity of *Anchomanes difformis* which is locally used in the treatment of diarrhea was investigated in this research. *A. difformis* leaf and rhizome were exhaustively extracted using ethanol, methanol and water as solvent in the ratio of 70:20:10. The antimicrobial activities were tested against pathogens of public health importance majorly the enterobacteriaceae and *Candida albican.* The zones of inhibitions ranged from 3-5 for rhizome extracts and 1-5 for leaf extracts at 100μl (0.1g/ml) MIC. The rhizome extracts contained phlebotannins, terpenoids and glycosides not found in leaf extracts while the leaf extracts contained steroids not found in rhizomes extracts among other phytochemicals present. *Pseudomonas aeruginosa* conferred resistance against the rhizome extract while *Shigella flexneri* conferred resistance against leaf extract. Differences in these observed susceptibility and resistance could be due to differences in their bioactive components. The rest of the organisms were found to be susceptible to both extracts showing that *A. difformis* had antimicrobial activity.

Key words: Antimicrobial · *A. difformis* · Rhizome · Leaf

INTRODUCTION

Plants have been selected and used empirically as drugs for centuries, initially as traditional preparations then as pure active principles, with this knowledge and accumulated practice passing from generation to generation [1]. The vast majority of people on this planet still rely on their traditional *materia medica* for their everyday health care needs. It is also a fact that one quota of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs and according to the WHO, 80% of the world's population (primarily those of developing countries) rely on plant-derived medicines for their healthcare [2, 3]. A large proportion of the population of developing countries uses traditional medicines, either as a result of the high cost of Western pharmaceuticals and health care, or because the traditional medicines are more acceptable from a cultural and spiritual perspective [4].

Plant derived medicine has made large contribution to human health and well being. Their role is two fold in the development of new drugs: They may become the base for the development of new drugs or a phytomedicine to be used in the treatment of diseases [5]. This study was carried out to investigate the antimicrobial activity of *A. difformis* leaves and rhizomes on bacteria of public health importance and on *Candida albican*.

MATERIALS AND METHODS

Collection and Identification of the Plant Materials: Fresh leaves and rhizomes of the plant were collected from Mesan, a village around Atan in Adodo Local Government of Ogun State, Nigeria. The plant was identified as *A. difformis* (Blume) Engl. (family= Araceae) at the Department of Biological Sciences, Covenant University, Canaanland, Ota, Ogun State, Nigeria.

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Fig. 1: Picture of leaves of *Anchomanes difformis*

Fig.2: Picture of the Rhizome of *Anchomanes difformis*

Description of the Plant (*A. difformis*): *Anchomanes difformis* (Blume) Engl. Is a plant in the family of Araceae. It is a large herbaceous plant with a perennial stout prickly green cylindrical stem to 2m high growing from a horizontal, irregularly whitish tuber up to 80cm long by 20cm across and a huge solitary leaf with a prickly petiole of 3m high. The foliage is very large. They are common in West African forest. They are also used as ornamental plant.

Common Names in West Africa

Nigeria: *EDO*- σlíkh.òr.òr, *EFIK*- eba enàη° = cow's udder, *FULA-FULFULDE (Nigeria)*- bugulli, gugulli the tuber, *GWARI*- chakara the tuber, hantsar gada = duiker's udder; the fruit. *HAUSA*- cakara, chakara the tuber (auctt.) hántsàr gàdaá = duiker's udder; the fruiting spadix with red berries, hántsàr giawaá = udder of the elephant. *IGBO (Owerri)*- olumahi, *IGBO (Umuahia)*- oje, *IJO-IZON (Kolokuma)*- bòù bèkèσdù = bush new-cocoyam, *IZON- (Oporoma)* bòù ódù = bush cocoyam. *KANURI*- gàs$_{\theta}$ nàngái. *YORUBA* ìgo lángbòdó, ògìrìòçákó the red berries and ripe spadix.

Ghana: *ADANGME-KROBO*- kwai-aŋma-tɸ. *AKAN*- σpє. *AKAN-ASANTE*- σpє = dry season, alluding to the plant's renewal of growth at this time. *DAGBANI*- lukpogu. *FANTE*- atõe, nyame kyin = God's umbrella. *GA*- bata foia kani = bush-pig's cocoyam. *GBE-VHE*- deviofє-tsini = children's umbrella, dσli. *TWI*- ópє = dry season, alluding to the plant's renewal of growth at this time.

Ivory Coast: *ABURE*- kohodié. *AKAN-ASANTE*- eupé, niamatimi. *AKYE*- alomé. *ANYI*- tupain. *BAULE*- niamé kwanba, séréusso kwama, topi topi. *BRONG*- pê. *DAN*- dina tali, linna batari. *GUERE* -don, vianakwäï. *GUERE (Chiehn)* dobli, dobré-dobré, yaa plè, yaprè. *GUERE-(Wobe)* méapolodè. *KRU-BETE*- bédro-bédro. *KULANGO*- dé. *KWENI* -diri dobli dobli. *KYAMA*- niamitma. *MANDING-DYULA*- dé. *'KRU'* blima.

Senegal: *DIOLA*- éken = sucker.

Sierra Leone: *KISSI*- n-dσndσ. *MENDE*- kipσnσ. *MENDE-KPA*- kalilugbo. *SUSU-DYALONKE*- alatala-kunde-na. *TEMNE*- a-thoŋbothigba.

Togo: *TEM*- nau. *TEM- (Tshaudjo)* nau (Gaisser)

Upper Volta: *MANDING-DYULA*- dé

Preparation of the Plant Extract: The leaves and rhizomes were collected and dried to a constant weight at 18°C in an enclosed air conditioned research laboratory. The leaves and rhizomes were spliced to pieces to ensure proper drying. The dried rhizomes and leaves were grounded to powder forms to increase the surface area for extraction. Rhizomes and leaves were extracted exhaustively by cold method of extraction, i.e by soaking in a solvent for four days. The solvents used were a mixture of ethanol, methanol and water in the ratio of 70:20:10 to get the ethanolic, methanolic and aqueous extracts. About 100g of the grounded rhizomes powder yielded 20g of the extract. Powdered leaves (75g) yielded 9g of the extract. The extracts were dried and then reconstituted using dimethylsulfoxide (DMSO). Varying weight (0.3g, 0.2g and 0.1g) of the extracts was dissolved individually in 1ml of DMSO to give concentrations of 0.3, 0.2 and 0.1g/ml respectively. These concentrations apply to both extracts.

Phytochemical Analysis: Phytochemical test were carried out on the leaves and rhizomes extract as described by Sofowora [6], Trease and Evans [7] and Harbone [8]. All chemicals used were freshly prepared.

Antimicrobial Assay: Pure isolate of test organisms were inoculated in a nutrient broth and incubated for 6 hours to ensure that the organism were at their exponential phase of growth. The organisms were serially diluted using double sterilized distilled water and the 10^{-7} dilution corresponding to 0.5 MacFarland standard were used as the inoculum. Mueller Hinton Agar (Oxoid) for bacteria and Saubouraud Dextrose Agar (Oxoid) for yeast were used. Both media were measured and dissolved in appropriate volume of distilled water, following the manufacturer's guideline; and was sterilized by autoclaving. 1ml of the standardized inoculum was mixed with the media in a sterile container to ensure that the test organisms were evenly distributed and poured into sterile Petri dishes and allowed to set. Each plate contains equal volume of the media. The antimicrobial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by Irobi *et al*. [9]. The plates were incubated at 37°C for bacteria and 28°C for yeast. The plates were observed for zones of inhibition after 24 h for bacteria. It was observed that the yeast produced discrete colonies within 24 hours, thus all the plates were read after 24hours. Two controls were used in this research: Organism viability control to check for the viability of the organisms. This implies that any clear zone of inhibition observed is due to the activity of the extract. All the organisms showed viability with colonies covering 100% surface of the plate. The second control is to test the activity of the solvent (DMSO) used to dissolve the extract to ensure that the activity is not due to action of the solvent on the organisms. The solvent showed zero activity on all the organisms. Plates were read by measuring observed clear zones (area without growth) of inhibition around the wells containing the extract. Measuring ruler in millimeter was used to take the measurement from the edge of the well to the end of the clear zone of inhibition. No measurement was taken if no clear zone of inhibition was observed. The estimation of MIC of the crude extracts was carried out using the method of Kinpelu and Kolawole [10].

RESULTS

Zones of inhibition were generally wider with rhizome extract than with leaves extract. The zones of inhibition of rhizome extract at 0.1, 0.2 and 0.3g/ml ranged from 3-5, 3-6 and 4-7mm, respectively while leaf extract ranged from 1-5, 2-6 and 2-7mm at 0.1, 0.2 and 0.3g/ml, respectively (Fig.3).

Table 1: Phytochemical composition of A. difformis rhizome and leaf extract

Phytochemical Components	Rhizome	Leaf
Tannins	+ + +	+ + +
Phlebotannins	+	_
Steroids	_	+
Terpenoids	+ +	_
Cardiac glycosides	+ + +	+ + +
Glycosides	+	_
Saponins	++ +	+ + +
Alkaloid	+ +	+
Flavonoid	+ +	+ +

+ = Positve - = negative

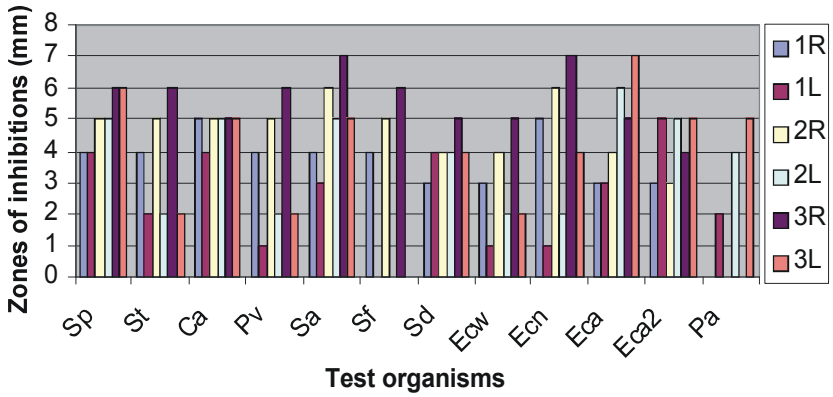

Fig. 3: Zones of inhibition of rhizome and leave extract on test organism at various concentration

Sp= *Samonella paratyphi*, St = *Salmonella typhi*, Ca = *Candida albican*, Pv = *Proteus vulgaris*, Sa = *Staphylococcus aureus*, Sf = *Shigella Flexneri*, Sd = *Shigella dysenterae*, Ecw = *Escherichia coli* (wild strain), Ecn = *Escherichia coli* (NCTC 12900), Eca = *Escherichia coli* (ATCC 35218), Eca2 = *Escherichia coli* (ATCC 25922), Pa = *Pseudomonas aeruginosa*, 1R, 2R, 3R = 0.1, 0.2, 0.3g/ml of rhizome extract respectively, 1L, 2L, 3L = 0.1, 0.2, 0.3g/ml of leaf extract respectively.

Zones of inhibition that ranged from 1-5 and 3-5 correspond to MIC values of leaf and rhizome extract respectively. This showed that rhizome extract had wider inhibition zones at the MIC value used (Fig.3). However, *Pseudomonas aeruginosa* was resistant to the rhizome extract and *Shigella flexneri* resistant to the leaf extract (Fig.3). These resistances were conferred at all concentrations tested. Statistical analysis using paired t-Test showed that there is no significant differences in the zones of inhibitions on the entire organism, however, differences exist when values were compared individually.

Semi-quantitative screening for phytochemicals showed that tannins, cardiac glycosides and saponins are major constituent of *A. differmis* rhizome and leaf (Table1). Phlebotannins, terpenoids and glycosides were present only in the rhizome extract and steroid was found only in the leaf extract (Table1). The presence and absence of each of the phytochemical components in the extracts were designated as positive (+) and negative (-) respectively. More than one positive signs corresponding to some phytochemical components indicated the extent to which the components were present compared to others based on the rate and intensity of colour formation.

DISCUSSION

A. differmis rhizome and leaf extract showed antimicrobial activity against the test organism. Wider zones of inhibition were generally observed with rhizome extract than leaf extract. The Minimum Inhibitory Concentration (MIC) of the extracts was 0.1g/ml, being the minimum concentration that inhibited growth. Nevertheless, statistical analysis using paired t-Test showed that there is no significant differences ($P>0.05$) in the zones of inhibitions between the rhizome and leaf extract because the activities of the extracts were analyzed collectively on all organisms such that their differences cancel out. Differences were observed when the activities of the extracts were compared individually. The antimicrobial activities of these extracts were due to the presence of bioactive compounds reported in the phytochemical studies. Steroidal compounds had been found to exhibit antibacterial activities on some bacterial isolates [11]. Nweze *et al*. [12] also documented that presence of steroids, phlebotannins, terpenes, alkaloids, cardiac glycosides, flavonoids and some other phytochemical components have been associated with antimicrobial activities and thus have a curative properties against pathogens. Flavonoids which are hydroxylated phenolic substances might be responsible for their therapeutic effectiveness against wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [13]. Antimicrobial activity of flavonoids was also described by Hodek *et al*. [14]. Antimicrobial activity of *A. differmis* could be traceable to these phytochemicals. Other secondary metabolites observed in *A. differmis* rhizome and leaf extracts was alkaloid. One of the most common biological properties of alkaloid is their toxicity against cells of foreign organisms. Some alkaloids and saponins have been found to possess antimicrobial activity [15]; hence, the activities being exhibited by extracts of *A. differmis* could be also due to the presence of alkaloids and saponins in the extracts. Most of the organisms that were susceptible to either rhizome or leaf extracts of *A. differmis* were responsible for intestinal disorders (enterobacteriaceae), this account for the effectiveness of *A. differmis* in the treatment of intestinal disorder. Dharmananda [16] reported that herbs that have tannins as their main components are astringent in nature and are used for treating disorder such as diarrhea and dysentery. The presence of tannins widely distributed in *A. differmis* added to their activity against these enteric bacteria. Our result complied with other documented evidences that *A. differmis* rhizomes soaked in water is used for treating cases of dysentery, fever and respiratory diseases [17, 18]. It has also been documented that *A. differmis* have antimicrobial effect against methicillin resistant *S. aureus* (MRSA) with zone of inhibition of about 13mm at $4mgml^{-1}$ concentration [17]. The observed resistance to rhizome extracts and susceptibility to leaf extract by *P. aeruginosa* might be due to certain phytochemicals not found to be present in the rhizome extract such as steroids which had been documented to have antimicrobial activity [11]. Likewise, the resistance and susceptibility conferred to leaf and rhizome extract respectively by *S. flexneri* might be due to phytochemical present in the rhizome extract not found to be present in the leaf extract such as phlobotannins, terpenoids and glycosides which could exhibit antimicrobial activity. Further investigation should be geared towards the effect of steroids on *P. aeruginosa* and the effect of phleobotanins, terpenoids and glycosides on *S. flexneri.*

In conclusion, A. differmis rhizome and leaf extract had shown to exhibit antimicrobial activity against a wide range of bacteria of public health importance which are mostly enterobacteriacea, showing that they could be used to treat enteric infection or disease condition. It had also shown to be effective

against *Candida albican* and could be used in the treatment of candidiasis caused by *Candida albican*. This paper had also confirmed the ethnobotanical use of *A. differmis* in the treatment of diarrhea which is mainly due to enteric infection. Variation in the susceptibility and resistance of *A. differmis* leaf and rhizome extract to *P. aeruginosa* and *S. flexneri* are index toward further investigation to discover particular bioactive compound/compounds that is/are responsible in inhibiting these organism.

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