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Antimicrobial activity of *Anchomanes difformis* (Blume) Engl. [family ARACEAE]

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ABSTRACT
Extracts of Anchomanes difformis (Blume) Engl. [family ARACEAE] was investigated for antimicrobial activity against Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Candida stellatoidea and Candida torulopsis using agar well diffusion methods and minimum inhibitory concentrations. All the bacteria tested were sensitive to the essential oils except for Pseudomonas aeruginosa that was only sensitive to the oils from the stem and root but not to that of leave. Virtually all the Candida species tested were resistant to the essential oils except for Candida torulopsis that was sensitive to the oil from stem. The minimum inhibitory concentrations ranged from 2.0mg/ml to 4.0mg/ml and were recorded for Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus in the stem, root and leaf extracts. Conclusively it was observed that inhibition of selected bacteria and fungi by essential oils of Anchomanes difformis makes it a promising antimicrobial agent.

Keywords: *Anchomanes difformis*], *rubefacients, purgative, abscesses , herbaceous*

INTRODUCTION

Anchomanes difformis (Blume) Engl. [family ARACEAE] is an herbaceous plant with prickly stem having huge divided leaf and spathe that arise from a horizontal tuber occurring in the forest of West Africa. It is sometimes called forest Anchomanes in English (Morton, 1961), while in Southwest Nigeria it is known as Ogirisako, Igo (Soladoye *et al*., 2005). The rhizome is eaten but only after special preparation that entails prolonged washing and cooking of early shooting stage (Morton, 1961). Aqeuos extract of the tubers has been used to cure dysentery by traditional healers (Oyatayo, 2007). Rubefacients and vesicants are made from the rhizome for external application against measles and smallpox (Dalziel, 1956). The root leaves and stems are purgatives. It has also been reported in the treatment of kidney-pains, oedemas and as diuretic for treating urethral discharge, jaundice and as poison antidote (Akah and Njike, 1990; Dalziel, 1937). Abscesses can be treated with application of the root pulped with potter's clay while lactogenic ability is found in the rhizome and eye-medicine can be obtained from the stem-sap (Akah and Njike, 1990; Kerharo and Bouquet, 1950). Alkaloids are found in Nigerian material (Adegoke *et al*., 1968). Rhizome aqueous extract is used in the treatment of pain, inflammation and fever (Akah and Njike, 1990; Almeida, 2003) The objectives of this study was to screen the essential oils of *Anchomanes difformis* against some selected bacteria and fungi implicated in nosocomial infections.

MATERIALS AND METHODS

Collection of Materials

Leaves, stem and root parts of *Anchomanes difformis* (Blume) Engl. [family ARACEAE] were collected from Ago-Iwoye. Nigeria in the month of June 2008 and was authenticated at *Elkalf Herbarium at Plant and Applied science department of Olabisi Onabanjo*

University, Ago-Iwoye. Nigeria. The materials were washed with clean water and air dried.

Microbial Strain

The microorganisms were supplied from the Department of medical Microbiology of the University and maintained on Nutrient agar (Merck, Darmasadt, Germany). The bacteria and fungi used were selected because they have been implicated with skin, oral and intestinal tract of man. Four species of bacteria *Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus* (ATCC 24213), and *Pseudomonas aeruginosa* (ATCC 9027) and three species of fungi *Candida albicans* (ATCC 10231), *C. stellatoidea*, and *C. torulopsis* were used in this study.

Essential oil extraction

Air-dried plant material (100 g) was introduced into round bottom flask with 100ml of water and plug with cotton wool. It was hydrodistilled for 3 h using a Clevenger type apparatus with a small quantity of n-Hexane (0.3ml) which was dried over anhydrous sodium sulfate and was kept in a sealed vial at 4^0C until analysis and tests.

Antimicrobial assays

The modified method of Muñoz-Mingarro *et al.* (2003) was used for the bioassay. Cultures of *Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus* (ATCC 24213) and *Pseudomonas aeruginosa* (ATCC 9027) were used for the *in-vitro* antibacterial assay. For the antifungal assay, *Candida albicans* (ATCC 10231), *C. stellatoidea*, and *C. torulopsis* were utilized for the studies. Bacterial strains were maintained on Mueller-Hinton broth and the yeast on Sabouraud's Dextrose agar. The diluted extract suspension was homogenized and the screening performed according to the liquid dilution method (Vanden Berghe and Vlietinck, 1991). Minimum inhibitory concentration (MIC) was determined by incorporating various amounts (2.0 – 4.0 mg/ml) of reconstituted extract solution into the medium. The MIC was interpreted as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the control.

Results and Discussion

The antimicrobial activity of *Anchomanes difformis* (Blume) Engl. [family ARACEAE] at different concentrations were determined by agar well diffusion method. A total of seven microorganisms that consisted of four bacteria and three fungi were tested. Standard antibiotics (Ampicillin-: Doyin Pharmaceuticals Nigeria Limited and Ketoconazole-: Oxoid Limited Hamspire England) were used as positive control while 75% methanol served as negative control. n-Hexane was used for the extraction. The percentage yield of essential oil obtained from hydrodistillation of the leaves, stem and root parts of *Anchomanes difformis* was 1.5%(v/w) As shown in table 1 the results obtained from the agar well diffusion method showed that *P.aeruginosa* (ATCC 9027) and *S.aureus* (ATCC 24213) were moderately sensitive to the essential oil from the stem part of the plant having the highest MIC values of 4.0mg/ml. High inhibitory activity of the oil was recorded against *B.subtilis* and *K.pneumoniae* (16.0±0.3mm and 17.0±0.3mm respectively) with a small MIC values of 2.0mg/ml.. Low values of 12.0±0.2mm and 13.0±0.3mm were actually recorded for *P.aeruginosa* (ATCC 9027) and *S. aureus* (ATCC 24213) for the inhibition zones.. Also, in table 2, *K. pneumoniae* and *S.aureus* (ATCC 24213) showed strong sensitivity (19.0±0.2 mm) against the oil obtained from the root part of the plant and equally recording the lowest MIC values of 2.0. Moderate inhibitory activity of the oil was recorded against, *B. subtilis* and *P.aeruginosa* (ATCC 9027). However, different values of MIC were recorded for the two microbes. *P.aeruginosa* (ATCC 9027) recorded the largest value of 4.0mg/ml.

Equally, in table 3, *S.aureus* (ATCC 24213) and *K.pneumoniae* were both found to be most sensitive with the lowest MIC values of 2.0mg/ml in the presence of essential oil obtained from leaves parts of *Anchomanes difformis.* Moderate inhibitory activity of the oil was observed against *B. subtilis* with MIC values of 4.0mg/ml from the leaves parts while *P.aeruginosa* was resistant to the oil. *C. torulopsis* was the only yeast sensitive to the oil from the stem part of *Anchomanes difformis* (table 4) with MIC values of 4.0mg/ml while *C. stellatoidea* and *C. albicans* were resistant to the same oil. Furthermore, all the *Candida* species tested against the essential oils from the roots and leaves of the plant were resistant to it (tables 5 and 6).

The demonstration of sensitivity by selected bacteria to the essential oil of *Anchomanes difformis* supports the claims of Akah and Njike, (1990) that the root pulp of this plant could be used for treatment of Abscesses. This observed inhibition of the bacteria by oil of *Anchomanes difformis* could be of significant importance in the pharmaceutical

industry, especially for treatment of diseases caused by some of the bacteria and fungi tested in this study. This activity of *Anchomanes difformis* made it of immense medicinal value and this is strongly supported by the findings of Almeida, (2003) who stated that Rhizome aqueous extract of this plant is used in the treatment of inflammation. Also the performance of the plant oil justify its ethno pharmacological claims as it compete favourably with ampicillin (especially oils from the Leave and root of the plant) against tested organisms especially *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (tables 1 and 2) which showed high sensitivity to the essential oil of the plant. This observation is perfectly in line with the assertion of Kerharo and Bouquet (1950), Akah and Njike (1990) who ascertained that *Anchomanes difformis* could be used for the treatment of conjunctivitis.The activity shown against *Candida* species further gives credit to the plant, as *Candida* species are known to be resistance to most antibiotic. *Candida* species are known to be involved in several diseases such as intertrigo, Diaper rashes and chronic mucocutaneous candidiasis all of which are skin disease.

Table 2: Antibacterial activity of the essential oil of the root part of *Anchomanes difformis* (Blume)

Microorganism	Essential oil (10ul/ml)		Ampicillin (10 ug/ml)
	IZ (mm)	MIC (mg/ml)	IZ (mm)
B.subtilis	17.0±0.2	2.0±0.0	19.0±0.2
P.aeruginosa	15.0±0.1	4.0±0.0	21.0±0.3
K.pneumoniae	19.0±0.2	2.0±0.0	23.0±0.1
S. aureus	19.0±0.2	2.0±0.0	25.0±0.2

IZ, inhibition zone including diameter of Well (6 mm); MIC, minimum inhibitory concentration; na, not active;

Table 3: Antibacterial activity of the essential oil of the leave part of *Anchomanes difformis (Blume)*

Microorganism	Essential oil (10ul/ml)		Ampicillin (10 ug/ml)
	IZ (mm)	MIC(mg/ml)	IZ (mm)
B.subtilis	16.0±0.2	4.0±0.0	20.0±0.2
P.aeruginosa	na	na	22.0±0.1
K.pneumoniae	18.0±0.2	2.0±0.0	21.0±0.3
S. aureus	18.0±0.3	2.0±0.0	23.0±0.2

IZ, inhibition zone including diameter of Well (6 mm); MIC, minimum inhibitory concentration; na, not active;

Table 1: Antibacterial activity of the essential oil of the stem part of *Anchomanes difformis (Blume)*

Microorganism	Essential oil (10ul/ml)		Ampicillin (10 ug/ml)
	IZ (mm)	MIC (mg/ml)	IZ (mm)
B.subtilis	16.0±0.3	2.0±0.0	17.0±0.2
P.aeruginosa	12.0±0.2	4.0 ±0.0	22.0±0.1
K.pneumoniae	17.0±0.3	2.0±0.0	18.0±0.2
S. aureus	13.0±0.3	4.0 ±0.0	20.0±0.1

IZ= inhibition zone including diameter of well (6 mm); MIC = minimum inhibitory concentration.

Table 4: Antifungal activity of the essential oil of the stem part of *Anchomanes difformis* (Blume)

Microorganism	Essential oil (10ul/ml)		Ketoconazole (10 ug/ml)
	IZ (mm)	MIC (mg/ml)	IZ (mm)
C. albicans	na	na	12.0±0.2
C. stellatoidea	na	na	10.0±0.3
C. torulopsis	7±0.3	4.0±0.0	11.0±0.2

IZ, inhibition zone including diameter of Well (6 mm); MIC, minimum inhibitory concentration; na, not active;

Table 5: Antifungal activity of the essential oil of the root part of *Anchomanes difformis*

Microorganism	Essential oil (10ul/ml)		Ketoconazole (10 ug/ml)
	IZ (mm)	MIC (mg/ml)	IZ (mm)
C. albicans	na	na	10.0±0.1
C. stellatoidea	na	na	8.0±0.2
C. torulopsis	na	na	12.0±0.3

IZ, inhibition zone including diameter of Well (6 mm); MIC, minimum inhibitory concentration; na, not active;

Table 6: Antifungi activity of the essential oil of the leaves part of *Anchomanes difformis*

Microorganism	Essential oil (10ul/ml)		Ketoconazole (10 ug/ml)
	IZ (mm)	MIC (mg/ml)	IZ (mm)
C. albicans	na	na	12.0±0.2
C. stellatoidea	na	na	10.0±0.1
C. torulopsis	na	na	11.0±0.3

IZ, inhibition zone including diameter of well (6 mm); MIC, minimum inhibitory concentration; na, not active;

CONCLUSION

Anchomanes difformis (Blume) Engl. [family ARACEAE] oil extract has both antifungal and antibacterial properties, although it is more noticeable against bacteria. The minimum inhibitory concentration of the oil extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 4.0mg/ml, while *Bacillus subtilis and Klebsiella pneumoniae* minimum inhibitory concentration was 2.0mg/ml, while *Candida stellatoidea* and *Candida albicans* were resistant. The antifungal activity can be found in the stem, but its antibacterial activity is concentrated in the root than in any other part of the plant. *Anchomanes difformis* is a medicinal plant with high antimicrobial activity and this justifies its use for treatment of such ailments as Abscesses, inflammation, intertrigo, Diaper rashes and conjunctivitis.Pharmacological and toxicological studies of the plant extract can be done in future to identify its pure compound and elucidate the components responsible for these antimicrobial activities.

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