Inhibitory effects of methanolic extract of acalypha wilkesiana on candida albicans and Staphylococcus Aureus

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ABSTRACT

The effect of Acalypha wilkesiana on Candida albicans and Staphylococcus aureus was investigated. Methanol extraction method was used for the leaf specimen. C. albicans and S. aureus used as test organisms were standardized, and cultured in the leaf extract agar, after which growth of the organisms and zones of inhibition were observed. An increased antimicrobial activity of C. albicans and S. aureus was observed with a corresponding increase in the concentration of the leaf extract. At 100% concentration, extract of A. wilkesiana gave the highest inhibition zone in C. albicans while the least was recorded at 6.25%. Also, at 100% concentration highest inhibition zone in S. aureus was observed while the least was at 12.5%. Extracts of the leaf at 100% also compared favourably with Amplicox in the in vitro inhibition of the growth of C. albicans, while Rifampicin, Norfloxacin and Chloramphenicol also compared favourably well with the extract in S. aureus.

Keywords: Acalypha wilkesiana, Candidiasis, Mycoses, Antimicrobial activity.

INTRODUCTION

Fungi are eukaryotic saprophytes that are ubiquitous in nature. They are sometimes referred to as the true fungi or Eumycota (1). Fungi are important to humans in both beneficial and harmful ways. They degrade complex organic materials in the environment to simple organic compounds and inorganic molecules. In this way carbon, nitrogen, phosphorous and other critical constituents of dead organisms are released and made available for living organisms (2).

Fungi especially the yeast, are essential to many industrial processes involving fermentation. Examples include the making of bread, wine, and beer. Fungi also play a major role in the preparation of some cheeses, Soy sauce, and fufu; in the commercial production of many organic acids (citric, garlic) and certain drugs (ergometrine, cortisone); and in the manufacture of many antibiotics (Penicillin, griseofulvin) (2).

On the other hand, fungi are a major cause of disease. Plants are particularly vulnerable to fungal disease because fungi can invade leaves through their stomata. Fungi also cause many diseases of animals and humans. About 20 new human fungal pathogens are documented each year (2). The diseases caused by fungi in humans are known as mycoses. Candidiasis is the mycoses caused by the dimorphic fungus, Candida albicans or Candida glabrata. In contrast to the other pathogenic fungi C. albicans and C. glabrata are members of the normal microbiota within the gastrointestinal tract, respiratory tract, vaginal area, and mouth. In healthy individuals they do not produce disease because growth is suppressed by other microbiota and other host resistance mechanisms. However, if anything
upsets the normal microbiota and immuno-competency, candida may multiply rapidly and produce candidiasis. Because Candida can be transmitted sexually, it is also listed by the Center for Disease Control (CDC) as a sexual transmitted disease (3). Most infections involve the skin or mucous membranes. This occurs because candida is a strict aerobe and finds such surfaces very suitable for growth. Cutaneous involvement usually occurs when the skin becomes overtly moist or damaged. (3).

Medicinal plants also serve as the starting point for the discovery of Semi-synthetic chemical compounds. The chemical structures derived from plant substances can be used as models for new synthetic compounds (4). Medicinal plants have been used in Africa before the introduction of antibiotics and other modern drugs (5). About 80 percent of the populations in developing countries still use traditional medicine for their health care. Modern Pharmacopoeias contain at least 25 percent of drugs derived from plants and many others which are synthetic analogues build on prototype compounds isolated from plants (6). Phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (7).

The genus “Acalypha” comprises about 570 species. The plant is popularly used for the treatment of malaria, dermatological disorders, gastrointestinal disorders (8), and for its antimicrobial property (9, 5, 10, 11). It is widely used in southern Nigeria as a remedy for the treatment of undefined skin infections in children (12). The use of plant, plant extract or plant-derived chemicals to treat diseases; topical, subcutaneous and systemic, has stood the test of time (10). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (13). Relatively, few studies have demonstrated the phytochemical constituents of A. wilkesiana. Akinde, (1986) reported the presence of sesquiterpenes, monoterpenes, interpenoids and polyphenols. While Adesina et al, (2000) reported the presence of gallic acid, corallagin, geranlin, quercetin 3-0-rutinoside and kaempferol 3-0 rutinoside in the leaves of A. wilkesiana. In another study, Oladunmoye, (2006) reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of A. wilkesiana. A. wilkesiana has antibacterial and antifungal properties Alade and Irobi (1993); Oladunmoye, (2006). The expressed juice or boiled decoction is used for the treatment of malaria, gastrointestinal disorders and fungal skin infections as pitynasis versicolor, Impetigo conlaqosa, candida intetrigio, Tinea versicolor, Tinea corporis and Tinea pedis (14). In traditional medicine the leaves of this diuretic plant are eaten as vegetable in the management of hypertension.

This study therefore, investigates the effect of methanolic extracts of A. wilkesiana on some microbial activities in humans in vitro.

MATERIALS AND METHODS

Equipments, agars, and chemicals used were of standard stock. Soxhlet extractor was used in the extraction of Acalypha wilkesiana leaves with methanol as the extracting solvent.

Collection of Plant Materials

Fresh leaves of A. wilkesiana were obtained from the surroundings of Cross River University of Technology (CRUTECH), Calabar, Nigeria. The identity of the plant was authenticated by a Botanist in the Department of Biological Sciences, CRUTECH. The methanolic extract of the plant was carried out in the analytical chemistry laboratory, while the rest of the work was done in the chemistry laboratory of the Department of Chemical Sciences, CRUTECH.

Plant preparation and Extraction

The leaves were thoroughly washed in running tap water to remove debris, air dried for 3 weeks, and subjected to a temperature of 60°C for 30 minutes in an oven to obtain a uniform powder of the leaves. The dried leaves were pulverized into uniform powder using piston and mortar in the laboratory. Extraction was done using the soxhlet apparatus. Exactly, 40g of the powdered leaves was wrapped in a filter paper and placed in a soxhlet and extracted with absolute methanol (250ml). The extraction was done until the solvent in the soxhlet turned colorless. The extract was concentrated by recovering the solvent using the soxhlet apparatus until the extract became just pourable. It was poured into a pre-weighed beaker and placed in the oven at 40°C to dry.

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Preparation of various concentrations of the extract
The extract was reconstituted in distilled water to obtain various concentrations of the extract thus: 1ml of extract was reconstituted in 1ml of distilled water to obtain a 100 percent concentration of the extract. A portion of the 100 percent concentration was diluted with an equal volume of distilled water to obtain a 50 percent concentration. The serial dilution procedure was continued to obtain lower concentrations of the extract as follows; 100, 50, 25, 12.5, 6.25, 3.13, and 1.57 per cent.

Test organisms
The test organisms were clinical isolates from the University of Calabar Teaching Hospital, Calabar, Nigeria. The following organisms were used Candida albicans and Staphylococcus aureus

Preparation of test organisms
The isolates were sub-cultured onto selective and differential solid media and re-identified using biochemical tests.

Standardization of inoculum
Five colonies of each organism were inoculated into nutrient broth (NB) to revive the organisms. This was done by adding glucose into the NB for the organisms to obtain energy to grow. The nutrient broth was incubated at 37°C for 18 to 24 hours. Turbidity produced was diluted to match a turbidity standard.

Preparation of culture medium
Exactly 10gms of Mueller–Hinton agar was dissolved into 270ml of distilled water in an Erlenmeyer flask and sterilized in an autoclave at 121°C for 1 hour and later poured into sterile Petri dishes, after being allowed to cool down for 45 minutes.

Susceptibility testing
Disk diffusion test using Kirby-Bauer method (15)
After the double dilution procedure was completed, 16 disc of a 5mm diameter of whatman No.1 filter paper was poured into each of the 7 diluted tubes containing the extract and allowed to stand for 1 hour in order to allow the extract to be absorbed into the disc. It was then dried in an oven at 60°C for 5 minutes. Exactly 1.0ml each of the 24-hour old standardized cultures of the test organisms were separately poured into each of the 7 dishes. The prepared Mueller-Hinton agar was used to flood the surface of the inoculated organisms and shaken gently for the agar to spread evenly. After the agar surface has dried for about 5 minutes, the already impregnated disc containing the extract was dropped into each approximate labeled ditch using sterilized forceps. Series of antibiotic impregnated discs were used to inoculate the centre of the ditches which served as controls respectively. The inoculated plates were left on the laboratory bench for 1 hour to allow the extract and antibiotic to diffuse into the agar. The plates were incubated aerobically at 37°C for 24 hours. (16).

Measurement of zones of inhibition
Zones of inhibition produced after incubation was measured in millimeters (17).

Determination of MIC (minimal inhibitory concentration)
MIC was determined by the plate method (16). The MIC was taken as the least concentration that inhibited the growth of the test organisms.

Determination of MBC (minimal bacteriocidal concentration)
MBC was determined by sub-culturing the test plates onto fresh solid medium and incubated further for 18 to 24 hours at 37°C. The lowest plate that yielded no single colony on a solid medium was taken as the MBC.

RESULTS
The extract inhibited the growth of the organisms tested in varying degrees at various concentrations as indicated by their zones of inhibition in tables 1 and 2.
Table 1: Zones of Inhibition for *C. albicans* at different Concentrations of extract

<table>
<thead>
<tr>
<th>Antibiotic/Concentration</th>
<th>Antibiotic zone of inhibition (mm)</th>
<th>Means zone of inhibition diameter of extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of extract (%)</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Gentamycin (10mcg)</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>Levofloxacin (20mcg)</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>Chloramphenicol (30mcg)</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Ciprofloxacin (10mcg)</td>
<td>43</td>
<td>18</td>
</tr>
<tr>
<td>Ampiclox (20mcg)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Gentamycin (10mcg)</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>Amoxil (20mcg)</td>
<td>45</td>
<td>21</td>
</tr>
<tr>
<td>Ciprofloxacin (10mcg)</td>
<td>44</td>
<td>18</td>
</tr>
</tbody>
</table>

*C. albicans* showed zones of inhibition of 18, 19, 20, 18, 20, 18, 21, and 18mm respectively on all the plates at the highest concentration of extract tested (100 per cent). While *S. aureus* showed zones of inhibition of 24, 10, 15, 35, 18, 25, and 34mm on all the plates.

At extract concentration of 50 per cent, *C albicans* showed zones of inhibition on all the plates; 13 15, 18, 13, 15, 13, and 15mm. While *S. aureus* showed zones of inhibition of 13, 11, 25, 16, 14, and 15mm.

*C. albicans* had zones of inhibition of 11, 15, 11, 13, 10 and 11mm. while *S. aureus* had zones of inhibition of 14, 18, 14, and 10mm at extract concentration of 25 per cent.

At extract concentration of 12.5 per cent, *C. albicans* showed zones of inhibition of 14, 10 and 8mm. while *S. aureus* showed no zone of inhibition.

At extract concentrations of 6.25, 3.13 and 1.57 per cent, no zone of inhibition was observed with *C. albicans* and *S. aureus*.

The antibiotics used as controls were Gentamycin (GN), Levofloxacin (LEV), Chloramphenicol (CH), Ciprofloxacin (CPX), Ampiclox (APX), Rifampicin (RD), Erythromycin (E), Amoxil (AML) and Norfloxacin (NB). AML, CPX and CN showed the highest zones of inhibition of 45, 44, and 40mm respectively, while APX had the lowest zone of inhibition of 20mm on the isolate *C. albicans*; NB, AML and E showed the highest zones of inhibition of 43, 40 and 38mm respectively, while no zone of inhibition was observed with APX on the isolate *S. aureus*.

The lowest MIC was observed with *S. aureus* at a concentration of 1.57 per cent of extract. *C. albicans* showed MIC at 3.13 per cent of extract.

The MBCs for *S. aureus* and *C. albicans* was observed at 1.57 per cent concentration of extract. (see tables 3 and 4)
The results of the in vitro inhibitory activity of the methanol extract are quite appreciable when compared to the mean inhibition zones produced by the control antibiotics and the fact that the standard antibiotics are in the purified and concentrated form whereas the extracts are crude and harbor both pharmacologically and non-pharmacologically active compounds. Although the zones of inhibition were lower than that exhibited by the standard antibiotics, this could be due to the fact that the plant extract is crude and contains other constituents that do not possess antimicrobial property. Also, the ability of the extract to diffuse through the gel may be hindered because of large molecules (stearic hindrance). At higher concentrations of the extract, the zones of inhibition with the standard antibiotics were comparable.

*S. aureus* showed the highest zones of inhibition at the highest concentration of extract tested (100 per cent) but showed no zone of inhibition at lower concentrations. This suggests that highest concentration of the extract is needed to inhibit the growth of *S. aureus* but once that threshold is attained, it becomes sensitive. *C. albicans* showed the highest zones of inhibition at the highest concentration of extract tested (100 per cent) but showed varied zones of inhibition at extract concentration of (12.5 per cent) which differed from *S. aureus* while at lower concentration of extract, it showed no zone of inhibition.

Interestingly, the standard antibiotic ampiclox, showed no zones of inhibition with the test organism (*S. aureus*). Whereas, *S. aureus* showed zones of inhibition when tested with the extract.

This shows that the extract could be used as an alternative in treating staphylococcal infections pending when the active ingredients of this plant would be isolated, chemically identified and purified for commercial use.

*S. aureus* had the lowest MIC (1.57 per cent). This shows that the organism is more sensitive to the extract than (*C. albicans*). This gives credence to its traditional use for the treatment of boils and wounds. While *C albicans* showed MIC at extract concentration of (3.13 per cent) which suggests that higher concentrations of extract may inhibit their growth. MBCs for *S. aureus* and *C. albicans* was observed at extract concentration of (1.57 per cent).

Generally, the antibacterial and antifungal activity of the extract against the organisms tested agrees with earlier works by Akinde and Odeyemi (1987), Adesina et al. (2000); Kabir et al, (2005) and Oladunmoye, (2006).

**REFERENCES**


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