Antibacterial activity of ethanolic extract of *Acalypha wilkesiana* leaves growing in Jos, Plateau State, Nigeria


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ABSTRACT

Antibacterial activity of ethanolic extract of *Acalypha wilkesiana* leaves growing in Vom, Jos, Plateau state, Nigeria was carried out to verify claims by the locals of its medicinal properties. We tested the extract for activity against *Staphylococcus aureus* (G+), *Yersinia enterocolitica*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes* (G-). The extract exhibited activity against the organisms in varying degrees. In the agar diffusion test, *Y. enterocolitica* showed the highest zone of inhibition (18 mm) at the highest concentration of extract tested (20 mg/mL) while *E. coli*, *S. aureus*, *S. typhi*, *P. aeruginosa* and *K. aerogenes* showed zones of inhibition of 17, 16, 15, 14 and 16 mm respectively. At extract concentration of 2.5 mg/mL, *Y. enterocolitica* and *P. aeruginosa* did not show any zones of inhibition while *E. coli*, *S. aureus*, *S. typhi* and *K. aerogenes* showed zones of inhibition of 7, 6, 6 and 7 mm respectively. Below extract concentration of 2.5 mg/mL, there was no zone of inhibition observed with any organism. The MIC of the extract against the organisms was 5 mg/mL except on *S. aureus* where the MIC was 2.5 mg/mL. The MBC of the extract was 10 mg/mL against *Y. enterocolitica*, *S. typhi*, *P. aeruginosa* and *K. aerogenes*, 5 mg/mL against *E. coli* and *S. aureus*. Preliminary phytochemical testing revealed the presence of tannins, steroids, flavonoids and cardiac glycosides while saponins, alkaloids and anthraquinones were not present. The result gives scientific backing to the use of the leaves by the local people in the treatment of conditions usually associated with the organisms tested.

Keywords: *Acalypha wilkesiana*, ethanolic extract, antibacterial activity, Jos Plateau State

INTRODUCTION

Since the discovery of the first antibiotic, penicillin, the need for antimicrobial agents is yet to be satisfied. This has been attributed to the emergence of antibiotic resistant strains of micro organisms (Davies, 1994). As a result, there is a continuous search for antimicrobials from plant sources. These plants are used either alone or in combination with known antibiotics for treating bacterial infections (Collin and Parecia, 1970).

During the last two decades, there has been a considerable increase in the study and use of medicinal plants all over the world especially in advanced countries. There was also increase in the international commerce and commercial exploitation of herbal medicines through over the counter labeled products. In some countries, herbal medicines are still a central part of the medical system e.g. China (Liu, 1987), Ethiopia (Desta, 1993), Argentina (Anesini and Perez, 1993) and Papua New Guinea (Nick et al., 1995). The renewed interest in the use of medicinal plants may be attributed to cheapness, availability, and accessibility by the local populace, high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts.

Medicinal plants also serve as the starting point for the discovery of semi synthetic chemical compounds. The chemical structures derived from plant substances can also be used as models for new synthetic compounds (Sofowora, 1989; Akerele, 1992). Medicinal plants have been used in Africa before the introduction of antibiotics and other modern drugs (Kabir et al., 2005).

About 80% of the populations in developing countries still use traditional medicine for their healthcare. Modern pharmacopeias contain at least 25% of drugs derived from plants and many others which are synthetic analogues build on prototype compounds isolated from plants (De Silva, 2005). Phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti infective agents from higher plants (Kluczek et al., 2005).

The genus "Acalypha" comprises about 570 species (Riley, 1963). *A. wilkesiana* belongs to the Euphobiaceae family. The plant is popularly used for the treatment of malaria, dermatological disorders, gastrointestinal disorders (Akinde and Odeyemi, 1987) and for its...

Antimicrobial screening has been carried out on the leaves of A. wilkesiana. Adesina and coworkers (2000) reported a seasonal variation in the distribution of the three natural antimicrobial phenols (geraniin, congalin and gallic acid) in the genus Acalypha. Previous antimicrobial screening of the leaves of A. wilkesiana were carried out using plants growing in south western part of Nigeria. This study was done using A. wilkesiana plants growing in Jos town in north central Nigeria which has different climatic conditions from south western Nigeria and some bacteria which had hitherto not been tested in those previous studies were also tested.

MATERIALS AND METHODS

Plant preparation and extraction

Pesticide free leaves of A. wilkesiana were collected from National Veterinary Research Institute Vom. The plant was identified at the Federal College of Forestry Jos. The leaves were washed with distilled water and dried in the oven at 40 °C. The dried leaves were pulverized using pestle and mortar in the laboratory. Extraction was done using the soxhlet apparatus. Briefly, 40 g of the powdered leaves was wrapped in a filter paper and placed in a soxhlet and extracted with absolute ethanol. The extraction was done until the solvent in the soxhlet turned lower layer. A reddish brown color at the interphase is indicative of the presence of steroidal ring.

Test for flavonoids

A 2 g powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5 mL of 20% sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids.

Preparation of various concentrations of extract

The extract was reconstituted in distilled water to obtain various concentrations of the extract thus: 2 g of extract was reconstituted in distilled water to obtain 100 mL of a 20 mg/mL solution. A portion of the 20 mg/mL solution was diluted with an equal volume of distilled water to obtain a 10 mg/mL solution. The double dilution procedure was continued to obtain lower concentrations of the extract.

Test organisms

The test organisms were clinical isolates from Department of Bacteriology, Federal College of Veterinary and Medical Laboratory Technology Vom, Plateau State. The following organisms were used: Y. enterocolitica, E. coli, S. aureus, S. typhi, P. aeruginosa and K. aerogenes.
Preparation of the 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) and incubated at 37 °C for 18 to 24 h. Turbidity produced was adjusted to match 0.5 McFarland standard about 10^8 cfu/mL. It was further adjusted to 10^5 cfu/mL.

Bacterial susceptibility testing

Agar Diffusion test
Sterile nutrient agar (NA) plates were prepared and 24 h old standardized cultures of bacteria were separately used to flood the nutrient agar surfaces of each plate and excess was drained off. A sterile cork borer of 5 mm diameter was used to make six ditches on each plate. 0.1 mL of reconstituted extract equivalent to 2 mg of the extract was dropped into each approximate labeled ditch and into the remaining two ditches gentamicin (40 mg/mL) and distilled water were used as positive and negative controls respectively. The inoculated plates were left on the table for 1 h to allow the extract to diffuse into the agar. The NA plates were incubated aerobically at 37 °C for 24 h and Y. enterocolitica at 30 °C for 24 h. Zones of inhibition produced after incubation were measured in millimeters (Abayomi, 1982; Okwori et al., 2007).

Determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration)
MIC was determined by the tube dilution method. (Baron and Fingold, 1990). The MIC was taken as the least concentration that inhibited the growth of the test organisms. MBC was determined by sub-culturing the test dilutions onto fresh solid medium and incubated further for 18 to 24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as the MBC.

RESULTS
The percentage yield of the extract was 10.16%. The phytochemical testing of the extract gave positive reactions for tannins, flavonoids, steroids and cardiac glycosides and negative reactions for saponins, alkaloids and anthraquinones as shown on Table 1.

The extract inhibited the growth of all the organisms tested in varying degrees at various concentrations as indicated by their zones of inhibition in Table 2. Y. enterocolitica showed zone of inhibition of (18 mm) at the highest concentration of extract tested (20 mg/mL) while E. coli, S. aureus, S. typhi, P. aeruginosa and K. aerogenes showed zones of inhibition of 17, 16, 15, 14 and 16 mm respectively. At extract concentration of 2.5 mg/mL, no zone of inhibition was observed with Y. enterocolitica and

Table 1: Qualitative Phytochemical analysis of Ethanolic extract of Acalypha wilkesiana leaves

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Not present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Not present</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Not present</td>
</tr>
</tbody>
</table>

Table 2: Antibacterial activity of Ethanolic extract of Acalypha wilkesiana leaves showing zones of inhibition at different concentrations of the extract

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Mean zone of inhibition diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td>18 11 10 0 0 20 0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>17 12 10 7 0 22 0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16 12 9 6 0 18 0</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>15 12 7 6 0 21 0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>14 11 6 0 0 17 0</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>16 10 9 7 0 21 0</td>
</tr>
<tr>
<td>Concentration of extract (mg/mL)</td>
<td>20 10 5 2.5 1.25 +C -C</td>
</tr>
</tbody>
</table>

+C = Gentamicin 40 mg/mL
-C = Sterile distilled water
P. aeruginosa while E. coli, S. aureus, S. typhi and K. aerogenes showed zones of inhibition of 7, 6, 6 and 7 mm respectively at that concentration (2.5 mg/mL). The positive control gentamicin had average zone of inhibition of 21 mm. The negative control did not show any zone of inhibition.

The lowest MIC was observed with S. aureus at a concentration of 2.5 mg/mL. Other organisms tested showed MIC at 5.0 mg/mL concentration as shown on Table 3.

The MBC of the extract for all the organisms tested were slightly higher than the MIC except for E. coli where MIC and MBC were the same. For Y. enterocolitica, S. typhi, P. aeruginosa, and K. aerogenes the MBC was 10.0 mg/mL extract while for E. coli and S. aureus, the MBC was 5 mg/mL as shown on Table 4.

**DISCUSSION**

Phytochemical research based on ethnopharmacological informations is generally considered an effective approach to the discovery of antinfective agents from higher plants (Kloucek et al., 2005).

The presence of zones of inhibition on the seeded agar plates showed that the plant extract possesses antibacterial activity on the tested organisms which included both Gram positive and Gram negative organisms. Although the zones of inhibition were lower than that exhibited by the standard drug gentamicin, this could be due to the fact that the plant extract is crude and contains other constituents that do not possess antibacterial 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 drug were comparable.

Y. enterocolitica showed the highest zone of inhibition at the highest concentration of extract tested (20 mg/mL) but showed no zone of inhibition at lower concentrations where other organisms (E. coli, S. aureus, S. typhi and K. aerogenes) showed zones of inhibition. This suggests that higher concentration of the extract is needed to inhibit the growth of Y. enterocolitica but once that threshold is attained, it becomes very sensitive.

S. aureus had the lowest MIC (2.5 mg/mL). This shows that the organism is more sensitive to the extract than other organisms tested. This gives credence to its traditional use for the treatment of boils and wounds. All organisms tested except E. coli had higher MBC than MIC. This shows that higher concentrations of extract was needed to kill the bacteria than that required to inhibit their growth. For E. coli the concentration that inhibited growth of the organism was sufficient to kill the organism. This may make it a good bactericidal agent against E. coli.

Generally, the antibacterial activity of the extract against E. coli, S. aureus, and P. aeruginosa agrees with earlier works by Akinde and Odebiyi (1987), Adesina et al. (1980), Kabir et al. (2005) and Oladunmoye (2006). In addition the ethanolic extract of A. wilkesiana had antibacterial activity against Y. enterocolitica, S. typhi and K. aerogenes which had hitherto not been tested. Phytochemical testing of the extract showed that the extract contains cardiac glycosides, flavonoids, steroids and tannins while saponins, alkaloids, anthraquinones and terpenes were absent. This result differs slightly from Oladunmoye (2006) who reported the presence of saponins in the extract. This could be attributed to the different locations where the plants were collected: Jos in

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**Table 3:** Minimum inhibitory concentrations (mg/mL) of Ethanolic extract of *Acalypha wilkesiana* leaves

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Concentration of extract (mg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
</tr>
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<td>-</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>-</td>
</tr>
</tbody>
</table>

- = No growth
+ = Growth

**Table 4:** Minimum Bactericidal Concentration (mg/mL) of Ethanolic extract of *Acalypha wilkesiana* leaves

<table>
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</table>

- = No growth
+ = Growth
North Central Nigeria located at Lat. 9°56’ N and Long. 8°53’ E, 1.217 m above sea level and enjoys a more temperate climate than much of the rest of Nigeria and Akure located at Lat. 7°15’ N and Long. 5°11’ E 385 m above sea level with a more tropical climate. There is a relationship between chemical composition of plants and geographical location. Rao and Rout reported a variation in the composition of essential oils of *Jasminum sambac* (L.) collected from different parts of India (Rao and Rout, 2003). The composition of bee propolis has also been found to depend on geographical source (Evans, 2005). It contains flavonoids and phenolic esters in temperate regions but these compounds are absent in propolis obtained from tropical regions although both exert antibacterial property. Seasonal changes in the antimicrobial constituents of *A. wilkesiana* have also been reported. The report also attributed the antimicrobial property of the extract to its tannins: geraniin, corilagin and gallic acid (Adesina et al., 2000).

**CONCLUSIONS**

Ethanolic extract of *A. wilkesiana* leaves growing in Jos Plateau State Nigeria had antibacterial activity on some bacterial organisms tested to varying degrees. This supports the traditional use of the leaves for treatment of ailments associated with these bacteria. Research should be carried out on the toxicity of the plant in order to know the safety and toxicity of the plant and establish a safe dosage regimen since the infusion of the leaves is taken orally by local people for treating gastrointestinal disturbances and as an enema for children apart from its topical use for treating dermatological disorders.

**ACKNOWLEDGEMENT**

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**REFERENCES**


