Preliminary phytochemical screening and antibacterial activity of two Nigerian medicinal plants (*Ficus asperifolia* and *Terminalis catappa*)

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**ABSTRACT**

Ethanol and aqueous extracts of *Ficus asperifolia* and *Terminalis catappa* were studied for their *in-vitro* antibacterial activity against both Gram positive and Gram negative bacteria. The phytochemical screening of the extracts showed the presence of phenolic compounds such as tannins, saponins, flavonoids, alkaloids and glycosides. Both the ethanolic and hot water extracts had broad spectrum antibacterial effect against all the tested bacteria except *Pseudomonas aeruginosa* that was resistant to the extract of *F. asperifolia*. The observed activity of the investigated plants may be due to the presence of bioactive components.

**Key words:**  
*Ficus asperifolia*, *Terminalis catappa*, Phyto-components.

**Article Type:**  
*Full Length Research Article*

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**INTRODUCTION**

Plants with potent bioactive are regarded as components of phytomedicine. Plant based natural constituents can be derived from any part of the plant like leaves, bark, flowers, roots, fruits, seeds, etc (Parekh et al., 2006). The antimicrobial activities of plants are attributed to the variety of chemical substances synthesized by plants. These bioactive agents of plants include alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones, among others (Staffort et al., 2004). The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories (Nwankwo and Amaechi, 2013).

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

Given the alarming incidence of antibiotic resistance in bacteria of medicinal importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Bishmus et al., 2009).

*Ficus asperifolia* and *Terminalis catappa* whose common names are “sand paper tree” and “umbrella tree” respectively, were evaluated for the phytochemical analysis and antibacterial activity. The different extracts of these plants were screened for potential antibacterial activity against some bacterial strains of medical importance.

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MATERIALS AND METHODS

Plant extraction

Ethanol extraction

The collected plant materials were washed thoroughly, chopped into small pieces and soaked in 95% ethanol for 48 h. The crude extracts were filtered with Whatman No. 1 filter paper and evaporated to dryness in a steady air current. The extracts were stored in the refrigerator before being reconstituted and later used for the in-vitro study.

Aqueous extraction

50 g of the plant materials were boiled with 1000 ml of water after thorough washing. They were allowed to cool and then filtered with Whatman filter paper No. 1. The filtrates were evaporated to dryness in a steady air current. The crude extracts were kept in the fridge before being reconstituted and later used for the in-vitro study.

Collection of test organisms and preparation of stock culture

Test organisms were received from Federal Medical Center Owerri Microbiology laboratory and reconfirmed by Gram staining and sub-culturing in appropriate selective media. The organisms includes: Escherichia coli, Streptococcus pneumoniae, P. aeruginosa, Staphylococcus aureus and Proteus mirabilis.

Preparation of standard culture inoculum of test organisms

Three or four (3 or 4) isolated colonies were inoculated into 2 ml nutrient broth and incubated until the growth in the broth was equivalent with Mac-farland standard (0.5%) as recommended by NCCLS (1998).

Determination of zone of inhibition (ZOI)

The freshly prepared inoculum was swabbed all over the surface of the Mueller-Hintor agar plant using sterile cotton swab. Five (5) wells of 6 mm diameter were bored in the medium with the help of sterile cork-borer having 6 mm diameter and were labeled properly. 100 µl of the suspension of the different medicinal plant extract was poured into the wells with the help of micropipette plates and were left for some time until all the extracts diffused into the medium with the lid closed. These were incubated at 37°C for 24 h. Next, measurements were taken using scale and the mean were recorded after incubation. The plates were also observed for ZOI.

Determination of minimum bactericidal concentration (MBC)

Freshly prepared nutrient broth was used as diluents. Crude extract was diluted by two fold serial dilution method. 100 µl of the standard culture inoculum was added to each test tube. All tubes were incubated at 37°C for 24 h. The tube content was sub-culture in fresh nutrient agar separately; and MBC was determined as those that showed no growth.

Qualitative phytochemical analysis

F. asperifolia root and T. catappa fresh stem bark were tested for the presence of bioactive compounds by using the following standard methods.

Test for tannins

200 mg of crude plant extracts was mixed with 2 ml of 2% solution of FeCl₃. Blue-green or black colouration indicated the presence of tannins.

Test for flavonoids (alkaline reagent test)

200 mg of extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of dilated acid which indicated the presence of flavonoids.

Test for saponins

200 mg of extract was mixed with 5 ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides (Salkowski’s test)

200 mg of extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, that is, glycone portion of the glycoside.
Table 1. Results of phytochemical analysis of *F. asperifolia* (root) and *T. catappa* (fresh stem barks).

<table>
<thead>
<tr>
<th>Test</th>
<th><em>F. asperifolia</em></th>
<th></th>
<th><em>T. catappa</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>Hot water extract</td>
<td>Ethanol extract</td>
<td>Hot water extract</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = Present, - = absent.

Table 2. Zone of inhibition (mm) of extracts (100 µl/ml) against test bacterial isolates.

<table>
<thead>
<tr>
<th>Test isolate</th>
<th>Ethanol extract</th>
<th>Hot water extract</th>
<th>Control antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>26.69±0.03</td>
<td>14.76±0.04</td>
<td>31.35±0.01</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>30.17±0.01</td>
<td>6.40±0.06</td>
<td>29.17±0.08</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>7.76±0.01</td>
<td>18.34±0.03</td>
<td>14.75±0.21</td>
</tr>
<tr>
<td><em>S. auereus</em></td>
<td>12.45±0.01</td>
<td>8.79±0.01</td>
<td>26.50±0.06</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>10.58±0.06</td>
<td>0.00±0.00</td>
<td></td>
</tr>
</tbody>
</table>

Key: 1 = *F. asperifolia*, 2 = *T. catappa*.

Test for alkaloids

200 mg of extract was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1 ml of the filtrate was added 6 drops of Wagner's reagent. Brownish-red precipitate indicated the presence of alkaloids.

Statistical analyses

Paired sample T-test was conducted to analyze the diameter of the zone of inhibition. Values are reported as means of duplicate determination ± standard deviation.

RESULTS

Phytochemical analysis of the ethanolic and hot water extracts of *F. asperifolia* and *T. catappa* are shown in Table 1. The presence of the following phyto-components were observed; tannins, flavonoids, saponin, glycosides and alkaloids. The result obtained in Table 2 shows that the ethanolic extracts of both plants showed a higher antibacterial activity than the hot water extracts. Ethanolic extract of *F. asperifolia* showed strong inhibition of *S. pneumoniae* (30.17±0.01). Both the ethanol and hot water extracts of *T. catappa* showed moderate inhibition potential against *P. aeruginosa* (ZOI=18.34±0.03 and 16.18±0.02, respectively). The activities of all the extracts against *E. coli* range from weak to strong inhibition.

The results of the minimum inhibitory concentration (MIC) and MBC of the extracts as shown in Table 3 reveals that while some extracts showed bactericidal effects, others were bacteriostatic.

DISCUSSION

The antibacterial potentials of the *F. asperifolia* and *T. catappa* plant samples were investigated in this study. Two solvents (ethanol and hot water) were employed for the extraction of the plant samples and the phytochemical analyses revealed the presence of alkaloid, saponins, Tanins, and flavonoids which has been reported to confer antimicrobial effect on the plant extracts.

The ethanolic and hot water extracts of the plants used inhibited the growth of majority of the test isolates. This indicates that the extracts possess substances such as phenolic compounds which have been linked with the healing properties of plants. Some of the ethanolic and hot water extracts contained similar phytochemical constituents. However, the presence of these secondary metabolites may not always indicate higher antibacterial activity. For example, the hot water extract of *T. catappa* contained virtually all the secondary metabolites that are said to be responsible for antibacterial activity, yet this
extract showed low antibacterial activity. Lack of antibacterial activity in this extract, in spite of the presence of secondary metabolites, indicates that active principles are heat labile, that is, they have been destroyed by heating. This finding contradicts the observations made by Geyid et al. (2005) who reported that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Also as stated by Cowan (1999), plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids and polyphenols are generally superior in their antimicrobial activities. Some researchers had also reported that there was a relationship between the chemical structures of the most abundant compounds in the tested extracts and their antimicrobial activity (Farag et al., 1989; Deans and Svoboda, 1989).

*S. pneumoniae* and *S. aureus* which are both Gram positive were more susceptible to the extracts than the Gram negative bacteria - *P. mirabilis* and *P. aeruginosa* as shown in Table 2. Antimicrobial studies have shown that Gram negative bacteria show a higher resistance to plant extracts than Gram positive bacteria (Palambo and Sample, 2001; Kuchi et al., 1999). This may be as a result of the variation in the cell wall structure of Gram positive and Gram negative (Palambo and Sample 2001). More especially, Gram negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Palambo and Sample, 2001).

Ten thousand milligram (10,000 mg) of *F. asperifolia* fresh stem back produce antibacterial activity equivalent to the activity produced by ten microgram of Gentamycin as seen in Table 2. More so, the zone of inhibition obtained especially against the *S. pneumoniae* of the ethanol extract of *F. asperifolia* root (30.17±0.07 mm) was found to be comparable to that of the primary standard antibiotic - Gentamycin (29.17±0.08 mm) used in this study. These findings denote that the effectiveness of the extracts is comparable with that of the primary standard antibiotics.

The 0.50 index value of the MIC/MBC ratio obtained for the tested *P. aeruginosa* pointed to a moderate antimicrobial activity of the extract of *T. catappa* against *P. aeruginosa* which is known to have posed a very big problem in the treatment of infections caused by it as a result of its resistance to most antibiotics.

### Conclusion

The present work has shown that the studied plants are potentially a good source of antimicrobial agent and demonstrate the importance of these plants in medicine and in assisting primary health care in this part of the world.

### REFERENCES


