Phytochemical Screening and Antimicrobial Activity of *Terminalia catappa* (L.) seed oil obtained from Anyigba, Kogi State, Nigeria, on Some Selected Clinical Isolates

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*All Res. J. Biol.*, **2020**, 11, 1-5
Phytochemical Screening and Antimicrobial Activity of *Terminalia catappa* (L.) seed oil obtained from Anyigba, Kogi State, Nigeria, on Some Selected Clinical Isolates

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Grafical Abstract

Collection of *Terminalia catappa* seeds from tree

Oven drying of seeds

Mechanical removal of nuts from seeds

Pulverization of seeds

Extraction of oil using soxhlet apparatus

Use of oil for bioassay

Abstract:

This research was carried out in a bid to seek for alternative ways of dealing with strains of microorganisms that have become resistant to conventional antibiotics. The study was carried out to evaluate the antimicrobial efficacy of *Terminalia catappa* seed oil obtained from Anyigba, Kogi State Nigeria, on some selected clinical isolates of selected microorganisms (*Aspergillus niger*, *Staphylococcus aureus*, *Escherichia coli*). The oil was extracted from the seeds using the soxhlet extraction method with n-hexane as the solvent. The Mueller Hinton (well diffusion method) was used to test the susceptibility of the strains of the microorganisms to the oil, using Ciproflaxin as standard positive controls, alongside Tween and DMSO as negative control. Experiments were carried out in duplicates. The results obtained revealed that *Terminalia catappa* oil was unable to create any inhibition zones on the clinical isolates. From this research, it can be concluded that *Terminalia catappa* oil had no Antibacterial activity.

Keywords: Antimicrobial, *Terminalia catappa*, Essential oils, seed

Introduction

Although many different antibacterial agents are available in the field of medicine, many of these agents are increasingly being incapacitated by the microorganisms through the evolution of different mechanisms that amount to resistance to these drugs.¹ Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also are often with adulterations and side effects.² There is therefore a continuous and urgent need to discover new antimicrobial components with diverse chemical structures and novel mechanisms of actions because of the increase in the incidence of new and re-emerging infectious diseases³ to replace those that have lost their
Belonging to the family Combretaceae, *Terminalia catappa* Linn. is naturally occurring and widespread in the subtropical and tropical zones of the Indian and Pacific Oceans, and is planted extensively in many countries as an ornamental tree. The type of oil that is obtained from *Terminalia catappa* seeds is safe for consumption, in addition Oliveria *et al.*, (2000) indicated that the oil content of *Terminalia catappa* (583.0 g/kg dry matter) is comparable to that of other oil seeds such as peanut, rapeseed, and sunflower. *Terminalia catappa* is used primarily as an ornamental shade, and salt-tolerant street tree, but the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with similar oils. On the Malay Peninsula and through the Canary islands this tree is known as the tropical almond. *Terminalia catappa* has been claimed to have therapeutic effects for liver related diseases. It is attributed with cholagogue action. In India, it is used as cardiac stimulant. Its leaves are widely used as a folk medicine in Southeast Asia for the treatment of dermatosis and hepatitis. More and more pharmacological studies have reported that the extract of *Terminalia catappa* leaves and fruits have anticancer by (Fan et al., 2004), antioxidant by (Zhai et al., 2001), anti-HIV reverse transcriptase, anti-inflammatory, and anti-diabetic effects as stated by (Xu et al., 2000) and hepatoprotective activities as stated by (Nagappa et al., 2003) but the effective components and related mechanisms remain unknown. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. The efforts of scientists in establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with high antimicrobial property have been elucidated. This work was carried out to determine the phytochemical components as well as evaluate the antimicrobial activity of *Terminalia catappa* seed oil.

**Methodology**

The essential oil used in this study was extracted from nuts of Indian almond. Ripe fresh seeds were collected directly from the tree plant and dried in an oven for 5 days to reduce moisture content and enable easy breaking to get reasonable amount of the nuts. The seeds were cracked open with a nut cracker and the nuts are pounded using mortar and pestle into a smooth powder to increase surface area so that the solvent can permeate properly into it to extract the oil.

**Extraction of essential oil by soxhlet method**

The extraction of essential oil was done using n-hexane in electro-thermal soxhlet extractor (Gallenkamp, England). 30g of powdered seeds was weighed and put into the thimbles of the soxhlet extractor, the apparatus was mounted and allowed to run for 6 hours, after which the mixture of essential oil and n-hexane was collected in a beaker and evaporated over a hot water bath to collect the oil as residue. The method was repeated thrice to get sufficient amount of oil.

**Determination of relative density of oil**

Two density bottles were washed and dried in an oven and allowed to cool in a desiccator. Each of the bottles was then weighed empty and the weights recorded. One was filled with water and the other with *Terminalia catappa* seed oil. Both was weighed again and the weight of the water and oil were determined by the difference in weight.

\[
\text{Relative density} = \frac{\text{Weight of oil}}{\text{Weight of an equal volume of water}}
\]

Where:

- Weight of empty bottle = W1
- Weight of empty bottle + oil = W2
- Weight of empty bottle + water = W3

**Determination of the phytochemical constituents of oil**

Chemical tests is carried out on the seeds oil of *Terminalia catappa* using standard procedures to identify the constituents as described by (sofowora, 1982), (Trease and Evans, 1989) and (Harborne,1973).

**Alkaloids**: About 0.5ml of the extracts is warmed with 2% of the H\textsubscript{2}SO\textsubscript{4} for two minutes. It was filtered and a few drops of Dragondoff reagent is added. Orange red precipitate indicates the presence of alkaloids.

**Tannins**: Small quantity of extracts is mixed using water and heated on water bath and filtered. Few drops of ferric chloride is added to filtrate. Dark green solutions indicates the presence of tannins.

**Glycosides**: Extracts is hydrolyzed using HCL and neutralized with NaOH solution. Few drops of Fehling’s solution is added. Red precipitate indicates the presence of glycosides.

**Saponins**: About 0.5ml of the extract is shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

**Flavonoids**: Extract of about 0.5ml is dissolved in diluted NaOH and HCL added. A yellow solution that turns colourless indicates the presence of flavonoids.

**Total phenol**: Extract of about 0.5ml is dissolved in Dennis solution. A red colour precipitate indicates the presence of phenol.

**Culture of Clinical Isolates**

Clinical isolates (*Aspergillus niger, Staphylococcus aureus, Escherichia coli*) were obtained from the Department of Microbiology, Faculty of Natural Sciences, Kogi State, All Res. J. Biol, 2020, 11, 1-5.
University, Anyigba. Overnight cultures were obtained using 10ml Nutrient agar and used for the study.

**Well Diffusion Assays**

Antimicrobial activity testing was carried out by using agar diffusion method. Three dilutions (30, 60 and 90%v/v) of *Terminalia catappa* seed oil were made using 0.005%v/v Tween 20 with 0.005%v/v Dimethyl sulfoxide (DMSO) in the ratio 1:1 as a co-diluent. Mueller-Hinton sterile agar plates were flooded with 2ml indicator microbial stains (*Aspergillus niger*, *Staphylococcus aureus*, *Escherichia coli*), and the excess drained. A cork borer was flamed and used to bore five wells which contained 0.005% tween and 0.005% DMSO (1:1) (negative control); 30%, 60%, and 90% v/v of oil (treatments), and 0.5mg/L Ciprofloxacin (positive control) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured using venier caliper and a meter rule after 18 to 24 hours of the incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The experiments were done in duplicates.

**Statistical Analysis**

One way Analysis of Variance (ANOVA) was used to compare the mean inhibition zones of the different treatment groups and Duncan Multiple Range Test (DMRT) was used to separate means where significant. P < 0.05 was considered significant.

**Results**

Results revealed that essential oil of *Terminalia catappa* seed contained tannins, flavonoids and glycosides (Table 1)

### Table 1: Phytochemical Components of Oil Extract

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test/Reagent</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>No colour change</td>
<td>(-)</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>Dark green colour</td>
<td>(+)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>No frothing</td>
<td>(-)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>NaOH-HCL test</td>
<td>Slight yellow colour</td>
<td>(+)</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Fehlings A and B test</td>
<td>Slightly red colour</td>
<td>(+)</td>
</tr>
<tr>
<td>Phenols</td>
<td>Dennis test</td>
<td>No colour change</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Key: + Present    - Absent

**Relative Density of the Oil**

The relative density of *Terminalia catappa* oil obtained was 0.875 (Table 2)

### Table 2: Relative Density of *Terminalia catappa* Oil

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of Empty Density Bottle</th>
<th>Weight of Empty Bottle × Sample</th>
<th>Mass of Substance</th>
<th>Density g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>9.55</td>
<td>18.35</td>
<td>8.80</td>
<td>0.875</td>
</tr>
<tr>
<td>Water</td>
<td>9.55</td>
<td>19.61</td>
<td>10.06</td>
<td></td>
</tr>
</tbody>
</table>

**Effect of the Extracts on Isolates**

Results showed that the extract (*Terminalia catappa* seed oil), at 30% (A), 60% (B), and 90% (C) v/v had no significant effect on the test organisms, the three organisms being *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* respectively. The negative control (NC), like the extract, had no effect as well on all the test organisms. The positive control (PC) however had effects on *E.coli*, *S. aureus*, *A. niger* with inhibition zones of 12mm, 17mm and 22.5mm respectively (Table 3; Fig 1 -3).

### Table 3: Antimicrobial activity of *Terminalia catappa* seed oil extract on a few selected pathogenic organisms

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>NC</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.0 ± 2.0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.0 ± 3.0</td>
</tr>
<tr>
<td>A. niger</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22.5 ± 2.5</td>
</tr>
</tbody>
</table>

**Pictorial Presentation of the Agar Plates**

*Figure 1*: Effect of *Terminalia catappa* seed oil on *Escherichia coli* : NC = Negative Control (0.5% DMSO and 0.5% Tween 20) ; A = 30% oil extract ; B = 60% oil extract ; C = 90% oilextract; PC = Positive Control (0.5mg/L ciprofloxacin)
Discussions

The seed oil extract of *Terminalia catappa* was found to have tannins, flavonoids and glycosides; but had no inhibitory or antimicrobial activity against the micro-organism isolates (*Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger*) this was confirmed by its failure to exert any form of inhibition zone around the wells containing the oil. This could be due to the fact that the concentration of the phytochemicals present in the oil was not enough to exert antimicrobial activity against the organisms and the low concentration of the phytochemicals could be due to the soxhlet method used in extracting the oil, which has been reported to be less effective than methods like steam distillation. Mikusanti *et al* (2019) reported that 0.5% *Terminalia catappa* seed oil showed no antibacterial effect on *Streptococcus mutans* (with inhibition zone of 0 mm²), but on ethanolation of the oil the resultant product had antimicrobial effect on the same isolate (with inhibition zone of 183 mm²). The lack of antimicrobial activity of the oil may also be as a result of its inability to diffuse through the medium properly due to its poor solubility.

Other parts of *Terminalia catappa* have however shown antimicrobial activities. Studies by Babayi *et al.* (2004) using leaf extracts of *Terminalia catappa* containing saponins, tannins and steroids had antimicrobial activity against *Escherichia coli* and *staphylococcus aureus* which are well known to be involve in cutaneous diseases, wounds, burns and other different ailments. The antimicrobial activity of the leaf extracts of *Terminalia catappa* may be due to the presence of saponins and steroids present which are absent in the seed oil. Studies by Payaw and Pal (2002) on the chloroform extracts of *Terminalia catappa* root also show good antimicrobial activity against Gram-positive and Gram-negative microorganisms.

Conclusion

The phytochemical analysis carried out on the oil extract revealed the presence of tannins, flavonoids and glycosides and the absence of saponins, alkaloids and phenols. The antimicrobial investigations carried out on *Terminalia catappa* seed oil base on this study prove had no antimicrobial activity against *Escherichia coli*, *Aspergillus niger*, and *Staphylococcus aureus*.

Recommendations

Though *Terminalia catappa* oil had no significant effect on the organisms tested in this research, it may be further tested on other microorganisms at different concentrations. Adoption of more suitable and effective method of extraction and isolation of active compounds such as stem distillation and column chromatography is suggested. Other uses of the oil should be investigated; such as the possibility of its use as a cooking oil and in the manufacture of cosmetics.

References


seeds from Pachira aquatica Aubl, Sterculia striata St Hil et Naud and Terminalia catappa Linn. Food Chemistry, 70:185-91.


