Antimicrobial and Phytochemical Screening of the Leaf and Stem Bark Extracts of *Spondias Mombin*
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Abstract
Ethnobotany has brought about the rudimentary drugs derived from plants used in folk medicines and have been found to be beneficial in the treatment of many diseases, both physical and mental. This study was therefore undertaken to evaluate the medicinal potencies of the stem bark and leaf extract of *Spondias mombin* against some bacteria isolates of medical implications. These organisms include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*. The agar well diffusion assay was used while petroleum ether, ethanol and methanol were used as the solvents for the extraction of the bioactive compounds. At a concentration of 35mg/ml of the extracts, the zones of inhibition in petroleum ether and ethanolic bark extracts was recorded as 0.0 mm and 24.0 mm respectively while methanolic leaf extract of *S. mombin* against *K. pneumoniae* was 25.0mm. The zone of inhibition of the ethanolic bark extract of *S. mombin* against *P. aeruginosa* was 32.0 mm while *E. coli, S. aureus, B. cereus* were 20.0 mm, 20.0 mm and 30.0 mm respectively. The ethanolic extract also inhibited *E. coli, S. aureus, K. pneumoniae* and *P. aeruginosa* at 12.0 mm, 15.0 mm, 24.0 mm, 24.0 mm respectively. However, petroleum ether extract of both the stem bark and the leaf had no effect on the test organisms. The result of the phytochemical screening shows the presence of anthraquinone, steroids, phlobatannins in the ethanolic stem bark extract which is the more reason for its pronounced antimicrobial potencies against the selected pathogenic microorganisms. Also, the presence of tannin and terpenoid in the petroleum ether and methanolic leaf extract of the sample was equally noticed.

Keywords: Ethnomedicine Spondias mombin, Inhibition, bioactive, Phytochemicals, pathogenic microorganisms.

Introduction
Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing. Their role may include becoming a natural blue print for the development of new drugs as well as the development of phytomedicine to be used for the treatment of diseases [1]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world [2]. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin and lower costs [3]. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compound [4]. Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of Infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds. Medicinal plants are of great importance to the health of individuals and communities [5]. It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties [4]. Their actions include: antibacterial, antifungal, antiviral, anti-helminthic, antiallergic, anticarcinogenic, analgesic, larvicidal to mention a few. These medicinal values lie in some chemical substances they contain as inherent active ingredients. Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the

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synthesis of useful drugs [6]. According to the World Health Organisation, about three quarters of the world population relies upon herbs for the health care of its people. Medicinal plants not only cure different ailments, they provide food and shelter for the people. Many modern drugs have their origin in medicinal plants [7]. By the middle of the nineteenth century at least 80% of all medicines were derived from herbs. The synthetic drugs dominating the scene today have their prototypes from medicinal plants [8]. Aspirin, atropine, artimesin, colchicine, digoxin, ephedrine, morphine, physostigmine, quinine and quinidine are a few examples of what medicinal plants have given to us. Long before mankind observed the existence of microbes, the idea that certain plants had healing potentials, indeed, that they contained what we would currently characterize as antimicrobial principle, was well accepted [9]. Since antiquity, man has used plants to treat common infectious diseases. The aims and objectives of this project are to: Investigate the phytochemical activities of the leaf and bark extract of Spondias mombin, as well as determine the antimicrobial activities of the leaf and bark extracts of the Spondias mombin against some selected pathogens of medical importance.

Materials and Methods

Collection and identification of plant material

Samples were collected from the school farm at Ondo State University of Science and Technology, Okitipupa. The taxonomic identification of the plant material was confirmed by an expert in the Department of Biological Sciences (Botany option) of the same University.

Preparation of Extract

Leaves of the plant and barks were sundried for 10 days, dried samples were pulverized using an industrial blender (Excella). Thirty grams of each samples was soaked into 120 ml of each solvents (petroleum ether, ethanol and methanol) for 72hrs, the soaked samples were filtered using muslin cloth and filtrates were concentrated according to the method of Benedict and Brady.

Collection of bacterial isolates

Pure bacterial isolates: Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus and Pseudomonas aeruginosa were collected from Obafemi Awolowo Teaching Hospital, Ile-Ife, Osun-state and resuscitated in the laboratory on Nutrient agar slant.

Phytochemical Screening

Extracts of both plant's leaves and barks of Spondias mombin were obtained using the method of Sofowora and Odebiyi. The following phytochemicals were assayed for: Tannins, Alkaloids, Saponins, Phlobatannins, Anthraquinone, Steroid and Terpenoid while antimicrobial susceptibility assay of the barks and leaves of the plant was carried out using Agar well diffusion technique.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation. Statistical evaluation of the data was performed using one-way analysis of variance followed by Duncan’s multiple range test at 5% level of significance i.e. P ≤ 0.05 [10].

Results

Table 1 shows the presence of phytochemicals such as anthraquinone and steroids in both the ethanolic and methanolic extract of the bark of Spondias mombin, while terpenoids was present in the ethanolic and methanolic extract of the leaf. Table 2 also shows the inhibition zones of methanolic and ethanolic extract of the stem bark against K. pneumonia as 25.0 mm and 20.0 mm respectively, while the methanolic and ethanolic extract of the leave against K. pneumonia were 25.0 mm and 20.0 mm respectively. The methanolic and ethanolic extract of the stem bark and the leave were pronouncedly displayed in their inhibition against some other test organisms used for the study as shown in table 2. However, there was a favourable comparism of the antimicrobial activities of these naturally obtained extracts with the standard antibiotics used for the study especially against S. aureus and B. cereus.

Table 1: Phytochemical screening of the leaf and stem bark extract of Spondias mombin

<table>
<thead>
<tr>
<th>Solvent for extraction</th>
<th>Sample</th>
<th>Anthraquinone</th>
<th>Steroids</th>
<th>Terpenoids</th>
<th>Tannin</th>
<th>Phlobatannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Leaf</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Stem bark</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>Leaf</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Stem bark</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Leaf</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Stem bark</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: - = Absence
+ = Presence
Table 2: Antimicrobial activity of the leaf, bark extracts and standard antibiotics against selected bacterial isolate.

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>CHL</th>
<th>GEN</th>
<th>MSB</th>
<th>ESB</th>
<th>PSB</th>
<th>LM</th>
<th>EL</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumonia</td>
<td>0.0±0.0</td>
<td>15.0±0.13b</td>
<td>25.0±0.18c</td>
<td>20.0±0.15c</td>
<td>0.0±0.0</td>
<td>25.0±0.18c</td>
<td>24.0±0.14c</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>15.0±0.10b</td>
<td>32.0±0.21d</td>
<td>0.0±0.0</td>
<td>18.0±0.12b</td>
<td>24.0±0.11c</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>15.0±0.11b</td>
<td>20.0±0.14c</td>
<td>0.0±0.0</td>
<td>19.0±0.16b</td>
<td>12.0±0.10a</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.0±0.14a</td>
<td>25.0±0.11c</td>
<td>12.0±0.13b</td>
<td>20.0±0.12c</td>
<td>0.0±0.0</td>
<td>23.0±0.19c</td>
<td>15.0±0.13b</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>B. cereus</td>
<td>10.0±0.12b</td>
<td>15.0±0.01b</td>
<td>13.0±0.10a</td>
<td>30.0±0.21d</td>
<td>0.0±0.0</td>
<td>18.0±0.12b</td>
<td>16.0±0.14b</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are means of triplicates ± SD; Samples carrying the same superscripts in the same row are not significantly different at (p<0.05)

Key
CHL - Chloramphenicol
GEN - Gentamycin
MSB - Methanolic extract of stem bark
ESB - Ethanolic extract of stem bark
PSB - Petroleum ether extract of stem bark
ML - Methanolic extract of leaf
EL - Ethanolic extract of Leaf
PL - Petroleum ether extract of Leaf

Discussion

Phytochemical analysis revealed the presence of anthraquinone, steroids, Phlobatannins in the ethanolic bark extract of S. mombin. Presence of tannin and terpenoid in the petroleum ether and methanolic leaf extract of S. mombin. Tannins (commonly refers to as tannic acid) are also known as antimicrobial agents. Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins as well as to form irreversible complexes with prolinerich protein [11] resulting in the inhibition of cell protein synthesis. [12] also reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. The growth of many fungi, yeasts, bacterial and viruses were inhibited in this compound. The tannin present in the petroleum ether leaf extract of S. mombin can be responsible for its antimicrobial efficacy against the growth of K. pneumonia [6]. The results of the antimicrobial activity of the extracts against the test organisms in this studies revealed that the ethanolic bark extract of S. mombin have the highest zone of inhibition against P. aeruginosa and B. cereus respectively which implies that these extracts can be used in the treatment of both urinary tract infection and gonorrhea. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [13]. The antimicrobial activity of the extracts on the test organisms may be due to the above phytochemical component identified in them.

Conclusion

All extracts assayed for in this research work have antimicrobial activities and this confirmed the historical use of both stem bark and leaf of S. mombin as antibacterial agents. The results of this study therefore form a good platform for selection of S. mombin for further phytochemical investigation and characterization and quantification of various active components present in the plant for their use in phytomedicine and as antimicrobial agent for the treatment of many infectious diseases.

Authors' Contributions

S conceived the study and participated in the coordination and helped to draft the manuscript.
KT participated in the design of the study and performed the statistical analysis and interpretation
ST involved in revising of the manuscript critically for important intellectual content.
VT participated in the carrying out of all assays as well as acquisition of data.

References


