PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND CYTOTOXICITY OF DIFFERENT EXTRACTS OF AFZELIA AFRICANA BARK

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Abstract

Afzelia africana bark was extracted by two methods; Macerated with distilled water and successive extraction method by soxhlet apparatus with different organic solvents in order of increasing polarity (n-hexane, ethyl acetate- butanol and methanol). The phytochemical screening of bark revealed the presence of the flavonoids, tannins, saponins, coumarins, reducing compound, anthocyanidines, alkaloids and steroids. The invitro anti-microbial activities of Afzelia africana bark extracts were examined against six standard microorganisms (four bacterial strains comprising of both Gram positive and Gram negative strains and two fungal strains) all extracts exhibited activities against bacterial strains tested. MICs of the extracts were also determined and extracts were favorably compared with standard antibiotics (amoxicillin and gentamicin). all extracts exhibited no antifungal activity against all fungal strains tested. On the other hand in vivo cytotoxic activity was studied for first time using Brine shrimp lethality bioassay; The maximum in vivo cytotoxicity was observed by n-butanolic extract on brine shrimp larvae with LD50 (0.95 μg/ml).

Keywords: Afzelia africana, phytochemical screening, antimicrobial, MIC, cytotoxicity, Brine shrimp.

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Available online: www.ijipsr.com January Issue 49
INTRODUCTION

The prevalence of multiple antibiotics resistance developed by microorganisms against the available synthetic antibiotics has increased astronomically in the last decade. This has prompted tremendous effort to explore for more potent antimicrobial agents, especially of natural origin to combat this development. One of the plants commonly used in Africa traditional medicine is *Afzelia africana*. Which is known as mahogany, and it belongs to the family Fabaceae. It is traditionally used for treatment of different ailments constipation, diarrhea, GIT infection, hernia in Nigeria [1], and as anti-malaria [2]. screening for antibacterial activity of *afzelia africana* was hold attention through different studies e.g [3] reported that the aqueous extract of chewing stick made from seed of *Afzelia africana* has inhibited some microbial isolates obtained from mouth washings. Another study confirms the antibacterial potentials of plant, but the extract showed no antifungal activity [4, 17]. This study aimed to evaluate the In vitro antimicrobial, phytochemical screening and in vivo cytotoxic activity of different extracts of *Afzelia africana* bark.

MATERIALS AND METHODS

Plant materials

*Afzelia africana* bark was collected from different areas in south Kordoufan by herb practioner during month of March 2012, then identified and authenticated by the Medicinal and Aromatic plants Research Institute, Khartoum, Sudan. The bark was cleaned, freed from dust and foreign material air-dried to constant weight, powdered and stored in an air-tight container for further use.

Preparation of extracts

The extraction of secondary metabolites from *Afzelia africana* bark was carried out in accordance to [18] with some modification by two methods; Maceration using distilled water and successive extraction method using soxhlet apparatus with different organic solvents in order of increasing polarity: (n-hexane, ethyl acetate, butanol and methanol).

Phytochemical screening

General chemical constituents of *Afzelia africana* bark were determined by Qualitative chemical analysis using successive extraction with solvents of different polarities with some modification as described by [5, 6].

Antimicrobial activity

**Preparation of standard micro-organisms:** The standard organisms were obtained from: ATCC: American Type Culture Collection, Rockville, Maryland, USA. And NCTC: National
Culture Type Collection, Colindale, England. The bacterial strains were first sub-cultured in nutrient broth and incubated at 37°C for 18 hours while the fungal strains were sub cultured on sabroude dextrose agar medium at 25°C for 72 hours.

**In vitro testing of extracts for antimicrobial activity**

Antibacterial activity was studied by agar–diffusion method. Each inoculate of the test organisms (1ml) were poured into sterile petri–dish. Media (about 45°C) was poured into petri dishes (20 ml). Then it was left to stand. Cups of 8 mm diameter were removed by cork porer as discs. The cups were marked then different plant extracts were pipetted into the cup using sterile micropipette. Plates were then incubated at 37°C for 24 hrs. The same manner was applied to fungal strain but here Plates were then incubated at 25°C for 4 days. The sensitivities of the test organisms to the plant extracts were indicated by clear zones of growth inhibition around the cups containing the plant extracts and diameter of the clear zone was taken as an index of the degree of sensitivity [7]. Then comparisons between Antimicrobial activities of Afzelia africana bark extracts at concentration of 10mg/ml and standard drugs (Antibiotics) (Amoxicillin and gentamicin) at different concentrations (0.04mg/ml-0.01mg/ml) were done against both Bacillus subtilis and Escherichia coli.

**Minimum Inhibitory Concentration**

The Minimum Inhibitory Concentrations of the plant extracts against the sensitive organisms were determined using the agar disc method. Serial dilutions of the plant extracts were prepared to obtain 5, 2.5, 1.25, 0.6, 0.3 and 0.1mg/ml. each of the inoculum (1ml) was poured into each petri-dish and the agar was latterly poured and allowed to set. Wells were bored using the sterile 3mm cork borer. Serial dilutions of the extracts were added into the marked wells. The plates were incubated at 37°CFor 24h. The growth was observed to determine the sensitivity of each organism using clear zones of no microbial growth. The least concentration of the plant extract that had inhibitory effect was taken As the Minimum Inhibitory Concentration MIC of that plant extract against such organisms [7].

**In vivo Cyto-toxic assay**

**Brine shrimp lethality bioassay**

Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay.
vials were prepared (positive control, negative control and four extracts); since the stock solution were prepared as 20mg of crude extract dissolved in 2ml of the respective solvent; then 500 μL, 50 μL and 5 μL of stock solutions was transferred to vials corresponding to 1000, 100 and 10 μg/mL, respectively. The solvent was evaporated overnight. After two days of hatching, 10 nauplii /larvae were placed into each vial and the volume was adjusted with sea water to 5ml per vial, and incubated at 27 °C for 24 hours under illumination. Then, the number of survivors were counted and recorded. all tests and analysis were done triplicate manner. Cyclophosphamide an anticancer drug was used as a positive control in the bioassay [8, 9, 10].

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. The data were processed using a Finney computer program [10] and LD_{50} values were obtained at 95% confidence intervals.

RESULTS

Phytochemical Screening tests

The results represented in table 1 Investigation on the phytochemical compounds of A. africana stem bark extract revealed the presence tannins, flavonoids, alkaloids, steroids and saponins [4], [11] agreed with Akinpelu but add other groups such as cardiac glycosides and carbohydrate. While were agree with Akinpelu, but disagree in the presence of steroids.

Table 1: Phytochemical Screening tests of Afzelia africana bark extracts

<table>
<thead>
<tr>
<th></th>
<th>Anthraquinone</th>
<th>Bornträger test</th>
<th>Coumarins</th>
<th>Ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liebermann-Burchard</td>
<td></td>
<td></td>
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<tr>
<td>Sterols</td>
<td></td>
<td>Mayer’s reagent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic Alkaloids</td>
<td>Basic Alkaloids</td>
<td>Wagner’s reagent</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Dragendorff’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Magnesium metal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavone</td>
<td></td>
<td>Aluminium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol extract</td>
<td>Fehling’s solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td>Sodium picrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol extract</td>
<td>Wagner’s reagent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids glycoside</td>
<td></td>
<td>Magnesium metal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqueous extract</td>
<td>Frothing test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
<td>alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyuronides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthocyanidines</td>
<td></td>
<td>HCL/ammonia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: (+): detected, (-): not detected.
Anti-microbial activity of *Afzelia africana* extracts against standard strains

The antimicrobial activities of *A. africana* stem bark extracts were investigated against some microbial strains at screening concentration of 10mg/ml. All extracts were exhibited activities against all bacterial strains tested comprising both Gram-negative and Gram positive organisms; except the n-hexane extract; which showed only low activity against *Bacillus subtilis*. On the other hand all plant extracts were exhibited no antifungal activity against all fungal strains tested as recorded in table 2.

The zones of inhibition exhibited by the crude extract ranged between (10-22mm for *E. coli* ATCC 25922), (10-14 mm for *S.aureus* ATCC 25923),(12-29 for *B. subtilis* NCTC 8236 )and (10-13 for P.aeroginosa ATCC 27853). When the activity of the plant extract was compared with that of the standard antibiotics, (Amoxicillin ,Gentamycin) results showed that the plant extracts compared favorably with those standard antibiotics table(2). The MICs were also determined and this ranged between (0.1and 1.25 mg/ml) Fig(1);the ethyl acetate extract of *Afzelia africana* bark showed the lowest MIC against both *Bacillus subtilis* and *Escherichia coli* which reflect its highest antibacterial activity against *Bacillus subtilis* with MIC (0.1mg/ml ) and *Escherichia coli* with MIC(0.3mg/ml ).

Methanolic, N-butanolic extracts exhibited the same antimicrobial activity against *Escherichia coli*, with MIC (0.6 mg/ml) and *Bacillus subtilis* with MIC (0.3mg/ml ) this result is supported by the study of [19]. While the minimal antimicrobial activity was exhibited by the aqueous extract as indicated in fig.1; since it showed the same MIC value against *Escherichia coli* and *Bacillus subtilis* with MIC 1.25mg/ml .this is compatible with [4].

**Table 2: Invitro anti-microbial activity of Afzelia africana bark extracts against standard Microorganisms**

<table>
<thead>
<tr>
<th></th>
<th>Methanolic</th>
<th>n-Butanol</th>
<th>Ethyl acetate</th>
<th>Water</th>
<th>n-Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDIZ (mm)</td>
<td>22</td>
<td>14</td>
<td>22</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (<em>Sa</em>)</td>
<td>12</td>
<td>27</td>
<td>13</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (<em>Bs</em>)</td>
<td>27</td>
<td>29</td>
<td>20</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (<em>Ec</em>)</td>
<td>20</td>
<td>28</td>
<td>13</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (<em>Pa</em>)</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida albicans</em> (<em>Ca</em>)</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Aspergillus niger.</em> (<em>An</em>)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: MIZD (mm) =Mean inhibition Zone Diameter, *Sa* =*Staphylococcus aureus* ,*Bs* = *Bacillus subtilis* . *Ec* = *Escherichia coli* ,*Pa* = *Pseudomonas aeruginosa* *Ca* = *Candida albicans* ,*An* = *Aspergillus niger.*
Fig. 1: Antibacterial activity of *Afzelia africana* bark extracts against bacterial strains and their MICS.

MIC: Minimum inhibitory concentration mg/ml. *Bs* = *Bacillus subtilis*. *Ec* = *Escherichia coli*

Fig. 2: The comparison between the *Afzelia africana* extracts bark (n-butanol, Ethyl acetate, methanol) (10mg/ml) and standard drug (Amoxicillin and gentamycin) at concentration of (0.4 mg/ml) Against *B.S*, *E.c.*

MIZD (mm) = Mean inhibition Zone Diameter. *Bs* = *Bacillus subtilis*. *Ec* = *Escherichia coli*.

**In vivo Cyto-toxic assay**

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plants. The maximum activity was observed by n-butanol extract on brine shrimp.
larvae with LD₅₀ (0.95 μg/ml) as compared to cyclophosphamide LD₅₀ (16.3 μg/ml). Followed by methanolic extract with LD₅₀ (1.2μg/ml) and ethyl acetate extract with LD₅₀ (1.68μg/ml). The minimum activity was shown by n-hexane extract with LD₅₀ (200μg/ml) as compared to cyclophosphamide LD₅₀ (16.3 μg/ml). All most all *Afzelia africana* bark extracts showed higher activity than cyclophosphamide drug which was used as positive control; except n-hexane extract that showed no toxicity. The brine shrimp lethality bioassay was found to be concentration dependent. As shown in fig.3

![LD₅₀ Bioassay](image)

**Fig.3: The brine shrimp lethality bioassay of *Afzelia africana* bark extracts**

**DISCUSSION**

Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs. Thus, there is need to search for new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens [4]. Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. The studies of medicinal plants used in folkloric remedies have attracted the attention of many scientists in finding solutions to the problems of multiple resistances to the existing synthetic antibiotics. [4]. Phytochemical Screening tests of *Afzelia africana* bark extracts revealed the presence of the flavonoids, tannins, saponins, coumarins, reducing compound, anthocyanidines, alkaloids and steroids. These compounds were reflected a high antibacterial activity compared to gentamicin at different concentration. The presence of tannins in *A. africana* supports the traditional medicinal use of this plant in the treatment of different ailments [12].

**Available online:** www.ijipsr.com  
**January Issue**
revealed the importance of tannins for the treatment of inflamed or ulcerated tissues. While Scalbert[13] was reported that tannins is the most secondary metabolites as antimicrobial compound which act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells. Herbs that have tannins are used for treating intestinal disorders such as diarrhea and dysentery [5, 4]. Flavonoids which are also one of the constituents of A. africana stem bark extract exhibit a wide range of biological activities and antimicrobial, anti-inflammatory, activities considered as one of the major activities [14]. Saponins which were tested positive in A. africana stem bark extract are responsible for numerous pharmacological properties in addition to antimicrobial activity, it inhibit mould, and protect plants from insect attack [15]. Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms [15].Coumarin itself has a very low antibacterial activity, but compounds having long chain hydrocarbon substitutions show activity against a wide spectrum of Gram +ve bacteria and Gram –ve. The presence of coumarins in the plant could confirm the antibacterial potentials of the Afzelia africana bark [16].

CONCLUSION

it is concluded that Afzelia africana bark extracts have good antimicrobial activity beside invivo cytotoxicity that supported by being rich with phytochemical groups which suggest Afzelia africana bark as good source for antimicrobial agent in the future.

REFERENCES


Available online: www.ijipsr.com January Issue 56


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