Phytochemical Analysis and Anti-Tuberculosis Activity of Extracts of *Detarium senegalense* Bark and Root

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors KTO and AA designed the study. Author KTO wrote the protocol, performed the anti-tubercular analysis, data analysis and wrote the draft of the manuscript. Author AA performed the extraction process and phytochemical analysis. Authors YY, SBM and PO managed the literature search and made the necessary corrections in the write up. All authors read and approved the final manuscript.

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**ABSTRACT**

Tuberculosis [TB] remains one of the major global health threats leading to morbidity and mortality. *Detarium senegalense* J. F. Gmelin belongs to a group of medicinal plants that are used by traditional medicine healers for the treatment of venereal diseases, urogenital infections, hemorrhoids, rheumatism, stomach-ache, intestinal worms, diarrhea, bronchitis, tuberculosis, convulsion, malaria and leprosy. The present study was aimed to investigate the *in vitro* anti-tuberculosis effect of different solvent extracts of *Detarium senegalense* bark and root against attenuated strain of *Mycobacterium bovis* [BCG]. The crude extracts [n-hexane, ethyl-acetate, methanol] of the plant parts were successively extracted and tested *in vitro* by broth microdilution technique against *M. bovis*. The result shows that the crude extracts inhibited the growth of *M. bovis*. The result shows that the crude extracts inhibited the growth of *M. bovis*. *Corresponding author: E-mail: kto2k1@gmail.com;*
**INTRODUCTION**

*Mycobacterium tuberculosis* is a major culprit of tuberculosis [TB], which affects the lungs [pulmonary TB] and, less often other parts of the body [extrapulmonary TB] [1]. Tuberculosis [TB] remains a major global health problem which had caused ill-infectious agent, ranking above HIV/AIDS [2]. Tuberculosis prevalence is much greater among HIV seropositive patients due to weaken immune systems and people who smoke, drink alcohol, are diabetic and undernourished are also at higher risk. Multidrug-resistant TB [MDR-TB] remains a public health crisis and a health security threat. The world health organization [WHO] estimates that there were 600,000 new cases with resistance to rifampicin, of which 490,000 had MDR-TB [3]. Attenuated strains of *Mycobacterium bovis*, Bacillus Calmette Guerin [BCG], have been used as an alternative test organism for anti-tuberculosis activity screening [4].

In developing countries, the use of herbal medicine has increased, due to the lack of modern medicine to provide effective treatment for diseases and the emergence of multi-drug resistant organisms [5]. It is estimated that about 80% of the world population leaving in vast rural areas still depends mainly on medicinal plants [6]. *Detarium senegalense* J. F. Gmelin [Tallow tree] is common in tropical Africa, found close to the riverbank, and is well known for its rounded pods. In 1789, De Juisieu was the first to describe fruits produced by *Detarium senegalense* tree called Detar in Senegal. It occurs from Senegal and the Gambia east to Sudan, and south to northern Democratic Republic of Congo. In West Africa, the tree has common names, In English, it is called Sweet detar, Sweet dattock *Detarium microcarpum,* Tallow tree *Detarium senegalense.* In Arabic, it is called Abu leita and Abu Leila [7,8], in France it is called Grand detar [9]. *Detarium senegalense* is mostly enjoyed in West Africa still as traditional food plant [10]. Most of the plant parts are eaten fresh, but some are dried in the sun and sold in the market. The hard shell and dry pulp give them a distinctive shelf life and the sweet and sour flavor appeals to all palates. Despite of all the various uses of *Detarium senegalense* in food and as well as drug, their various phytochemicals have not been fully documented.

Studies have shown that *D. senegalense* possesses antidiabetic activity. The xylo glucan obtained from the seeds of *D. senegalense* reduces postprandial blood glucose and insulin concentrations in humans [16]. The seed extract of the plant also helps to reduce blood sugar levels and blood lipid levels [17]. This study is aimed to evaluate the anti-tuberculosis effect of different solvent extracts of *Detarium senegalense* bark and root to identify the potential efficacy of the extracts for TB chemotherapy development.

**MATERIALS AND METHODS**

**2.1 Sample Collection and Identification**

The plant parts used were harvested from Bida basin, Niger state North Central Nigeria in the month of December, 2017. The Bida Basin (otherwise known as the Middle Niger or Nupe Basin) is a linear intracratonic sedimentary basin located in North Central Nigeria. The basin is about 350 km long and 75 km to 150 km wide...
2.3 Phytochemical Analysis

The plant extracts were screened to investigate presence of bioactive phytochemical constituents such as carbohydrates, saponins, tannins, terpenoids, phenols, anthraquinones, steroids, and alkaloids following standard procedures [18, 19, 20].

2.4 Test Organism

Mycobacterium bovis [BCG] [27290] was supplied by Microbiologics, [200 Cooper Avenue North, St Cloud, MN 56303] and cultured at the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development [NIPRD], Abuja, Nigeria.

2.5 Inoculum Preparation

Fifty micro-litre [50 μL] of the freshly thawed stock test organism [M. Bovis] was inoculated into 50 mL of sterile Middlebrook 7H9 (BD Difco) supplemented in albumin dextrose complex [ADC] media and incubated at 37°C with shaking for 5-7 days. The activity grown M. bovis culture had its optical density adjusted to between 0.2 - 0.3 at a wavelength of 650 nm on a UV-Visible spectrophotometer (Agilent Cary 60). The organisms were further diluted 1:1000 by diluting 50 μL of the organism in 50 mL 7H9/ADC broth.

2.6 Determination of Antimycobacterial Activity

Antimycobacterial activity susceptibility testing was conducted using the broth microdilution method in 96 well microtitre plates [21]. Crude extracts were first dissolved in DMSO and then diluted in Middlebrook 7H9 broth, to give a starting concentration of 1000 μg/mL. The dissolves extracts were diluted across the 96-well microtitre plate in a two-fold serial dilution to give final testing concentrations of 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 μg/mL. The same procedure was repeated for the control drug, Isoniazid [Sigma Aldrich Inc] at a concentration of 25 μg/mL used as positive control drug, and extracts/drug free medium with culture suspensions were used as negative control. Each extract concentration was assayed in duplicate. The plates were then incubated for 5-7 days at 37°C. After the 7th day, 25 μL of tetrazolium salt dye was added to all the wells, re-incubated over-night, and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest drug/extract concentration that prevented the color change of the tetrazolium dye to pink. Colourless well was interpreted as there is no mycobacterial growth and pink color was interpreted as growth occurrence.

3. RESULTS

Table 1 shows the result of the phytochemical screening indicating the presence of terpenoids...
and steroids in all the plant extracts screened, while phenols, saponins, tannins, and flavonoids were present in the ethyl-acetate and methanol extracts of the plant. Alkaloids were absent in all the plant extracts. Results from the antimycobacterial screening [Table 2] showed that ethyl-acetate extract of Detarium senegalense bark was the most effective extract in inhibiting the growth of M. bovis with a MIC of 7.8 µg/mL.

4. DISCUSSION

Plants have long been successfully used in the practice of traditional medicine for the treatment of infectious diseases. The widely distributed tropical plant, Detarium senegalense, used in the folkloric treatment of infectious diseases in Nigeria has not been fully investigated especially its antimycobacterial activity.

Results of this study showed that the extracts of Detarium senegalense contain a varied number of phytochemical constituents that have been reported to possess antimicrobial properties against different microorganisms. Terpenoids and steroids were present in all the plant extracts screened for phytochemical analysis. Uchegbu and Okwu [12,21], revealed the presence of alkaloids, flavonoids, tannins, phenols, and saponins in the seed and stem bark of Detarium senegalense used as soup thickener and in herbal medicine in South Eastern Nigeria which is consistent with the result of the phytochemical analysis in this study except for alkaloids which is completely absent. Terpenoids and flavonoids have been known for their antibacterial and curative properties against several pathogenic bacteria species [23,24,25,26,27,28]. Saponins are bioactive compounds produced mainly by plants, they generally occur chemically as glycosides of steroids or polycyclic triterpenes [29]. Saponins inhibit the growth of cancerous cells, stimulate the immune system, and are very good in reducing the blood cholesterol level [20]. They are also known to ease coughing by making the bronchial secretion more liquid and by reducing the congestion of the bronchi [9]. This also may be the reason the plant fruit pulp is used in herbal medicine for the treatment of cough [9] and the pulp from the bark is used as a remedy in the treatment of tuberculosis [13]. Flavonoids act as ‘biological response modifiers’ such as anti-allergic, anti-inflammatory, antioxidant, anti-microbial, hepatoprotective, anti-thrombic, anti-viral, and even the in vitro studies show it has anti-cancer activities [30-32]. Tannins have been found to form irreversible complexes with proline proteins resulting in the inhibition of cellular protein synthesis [33]. They are also potent antioxidants and are also used for the treatment of diarrhea and dysentery [34]. A review of antimycobacterial natural products by Copp [35], showed that compounds such as terpenes, monoterpenoids, diterpenes, sesquiterpenes and triterpenes, steroids and alkaloids were found to possess antimycobacterial activity.

Table 1. Phytochemical analysis of n-hexane, ethyl-acetate, and methanol extracts of Detarium senegalense bark and root

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>N-hexane</th>
<th>Ethyl-acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: (+) present, (-) absent

Table 2. Minimum inhibitory concentrations (MICs) values of n-hexane, ethyl-acetate and methanol extracts of Detarium senegalense bark and root against M.bovis

<table>
<thead>
<tr>
<th>Organism</th>
<th>N-hexane</th>
<th>Ethyl-acetate</th>
<th>Methanol</th>
<th>Isoniazid 25 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bark</td>
<td>Root</td>
<td>Bark</td>
<td>Root</td>
</tr>
<tr>
<td>M.bovis</td>
<td>31.3</td>
<td>125.0</td>
<td>7.8</td>
<td>250.0</td>
</tr>
<tr>
<td></td>
<td>15.6</td>
<td>250.0</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>
Previous studies on this plant have shown to have good antimicrobial activities. Okwu and Uchegbu [21], isolated an anthocyanidine alkaloid (2-methoxyamine 3,4,5,7 – tetrahydroxy anthocynamines) from the ethanolic stem bark extract of D. senegalense which successfully inhibited Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae. Sowemimo et al. [35] reported the antibacterial activity of the oil from the seed of D. senegalense, against Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis and Klebsiella pneumoniae.

Findings from this study also showed that the different extracts screened exhibits some degree of antimycobacterial activity. The ethyl-acetate of Detarium Senegalense bark was the most effective extract against M. bovis with a MIC of 7.8 µg/mL whereas the root extract had an MIC of 250.0 µg/mL. The methanolic extract of the plant’s bark and root had MICs of 15.6 µg/mL and 250.0 µg/mL respectively while the n-hexane extract of the plant’s bark and root had MICs of 31.3 µg/mL and 250.0 µg/mL respectively. It can be deduced from the result that the bark extract of the plant is more effective than the root extract against M. bovis.

5. CONCLUSION

The study has shown that the extracts of D. senegalense possess some level of anti-tuberculosis properties thus justifying its traditional usage as a health remedy in the treatment of tuberculosis. All these studies pull together, point to the potential antimicrobial and anti-tubercular usefulness of Detarium senegalense. Further studies should also be carried out on other parts of the plant such as the leaves and seeds to determine the antimycobacterial activity against M. tuberculosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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35. Sowemimo AA, Pendota C, Okoh B, Omotosho T, Idika N, Adekunle AA,

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