Journal of Advances in Microbiology

21(1): 44-50, 2021; Article no.JAMB.65375
ISSN: 2456-7116

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

Kazeem Toyosi Olatunji[1*], Adamu Aliyu[2], Yakubu Ya'aba[1], Shehu Busu Mohammed[1] and Peters Oladosu[1]

[1]Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu, P. M. B. 21 Garki, Abuja, Nigeria.
[2]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, P. M. B. 21 Garki, Abuja, Nigeria.

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.

Article Information

DOI: 10.9734/JAMB/2021/v21i130318
Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Ali Abdel-hadi Mahoud Alsudani, University of Al-Qadisiyah, Iraq.
(2) Ayman Al-Mariri, Atomic Energy Commission, Syria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65375

Original Research Article

Received 10 December 2020
Accepted 16 February 2021
Published 13 March 2021

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.*

**Corresponding author: E-mail: kto2k1@gmail.com;*

bovis at concentrations ranging from 7.8 – 250 µg/mL. The ethyl-acetate extract of *Detarium Senegalense* bark was the most effective in inhibiting the growth of *M. bovis* with a minimum inhibitory concentration [MIC] of 7.8 µg/mL while the ethyl-acetate and methanol extract of the plant's root had MICs of 250 µg/mL. This study demonstrates the efficacy of extracts of *Detarium senegalense* bark and root as potential agents in the management of the tuberculosis disease.

Keywords: Mycobacterium bovis; anti-tuberculosis; Detarium senegalense; crude extracts; potential agents.

1. 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.

The seed contains a large amount of zyloglucan which is considered to be commercially potential in food, drugs, and pharmaceutical industries [11, 12]. Different parts of *Detarium senegalense* are extensively used in herbal medicine in Nigeria. The plant is used for lots of medicinal purposes and is also known to produce very nutritious fruits [12]. Pulp from the bark is used as a remedy for tuberculosis [13]. The stem bark of the plant is soaked into palm wine for treatment of bronchitis, pneumonia, and leprosy treatment [14]. The root and leaf decoctions are used for difficulties in delivery, paralysis, and meningitis while a decoction of the bark is taken for expelling the placenta during childbirth [15].

Studies have shown that *D. senegalense* possesses antidiabetic activity. The zyloglucan 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.

2. 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

trending Northwest-Southeast and extending from Kantagora (in the north) to just south of Lokoja (in the south) and is aligned approximately orthogonal to the Benue Trough. It is separated from the basal continental beds of the Sokoto Basin by a narrow outcrop of the crystalline basement rocks in the west and it is contiguous with the Anambra Basin in the east. Due to its large areal extent, the basin has been divided into two sectors which are the northern and southern Bida Basin. The Basin lies within the tropical climate marked by wet and dry seasons. The dry season sets in during November to March which is then followed by a wet or rainy season from April to October with maximal rainfall in June and September. The annual amount of rainfall within the area is between 1200 and 1500 mm. Relative humidity is about 90 percent in the morning and drops to about 80 percent in the dry season. The annual temperature range is between 16°C and 37°C. During the rainy season, the area is under the influence of moisture-laden southwest trade winds which give way to the dry and sometimes dusty northeast winds (harmattan) during the dry season.

The samples were identified and authenticated by the Taxonomist. Voucher specimens were deposited at the Herbarium Unit, National Institute for Pharmaceutical Research and Development [NIPRD], Abuja, Nigeria with the Herbarium numbers indicated. The plant parts were air dried at room temperature for about 14 days, pulverized, and stored in a well labeled container for further analysis.

2.2 Extraction of Plant Parts

The powdered samples were successively extracted with the aid of a soxhlet extractor, starting with n-hexane, ethyl-acetate, and then methanol. The n-hexane, ethyl-acetate, and methanol extracts were concentrated using the rotary evaporator. The concentrates were dried on a water bath. The dried extracts were stored in a well labeled sample bottle and kept in desiccators for further analysis.

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 37ºC 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 microlitre 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

Phytochemicals	N-hexane		Ethyl-acetate		Methanol	
Carbohydrate	-	-	-	-	+	+
Phenols	-	-	+	+	+	+
Tannins	-	-	+	+	+	+
Cardiac glycosides	-	-	+	+	-	-
Saponins	-	-	-	-	+	+
Flavonoids	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-

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*

	Minimum Inhibitory Concentration Values (µg/mL)						
Organism	N-hexane		Ethyl-acetate		Methanol		Isoniazid
	Bark	Root	Bark	Root	Bark	Root	25 µg/mL
M.bovis	31.3	125.0	7.8	250.0	15.6	250.0	0.04

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 anthocynadines) from the ethanolic stem bark extract of *D. senegalense* which successfully inhibited *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia*e. 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 pneumonia*e.

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 anti-mycobacterial activity against *M. tuberculosis.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerging Infectious Diseases. 1998;4(1): 59-70.
2. World Health Organization (WHO). Global tuberculosis report. Washington DC: WHO Global TB Programme; 2017.
3. World Health Organization (WHO). WHO meeting report of a technical expert consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF (WHO/HTM/TB/2017.04). Geneva: World Health Organization; 2017.
   Available:http://www.who.int/tb/publications/2017/XpertUltra/ en/,
   Accessed: 16 August 2017.
4. Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K, Oladosu P et al. Evaluation of in vitro antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases. African Journal of Biotechnology. 2008;7(11): 1630-1636.
5. World Health Organization (WHO). Antimicrobial resistance: Global report on surveillance. 2014.
6. World Health Organization (WHO). WHO traditional medicine strategy 2002-2005. World health organization, Geneva; 2002.
7. National Research Council (NRC). Sweet detar. Lost Crops of Africa: Vol.III, Fruits Washington. National Research Council, National Acadmies Press, Washington. 2008;330-337.
8. National Academy of Science (NAS). Fruits. Lost Crops of Africa: Vol.III, Washington. National Academy of Science, National Acadmies Press, USA; 2008.
9. Burkill HM. The useful plants of west tropical Africa, Detareium senegalense. Royal botanic gardens, Kew. 1995;3:102-105.
10. National Research Council (NRC). Tamarind. Lost Crops of Africa: Volume III: Fruits. National Research Council, Washington; 2008.
11. Akah PNC, Mbaoji F, Nwabunike I, Onyeto C. Genus Detarium: Ethnomedicinal, phytochemical and pharmacological profile. Phytopharmacology. 2012;3(2): 367-375.
12. Okwu DE, Uchegbu R. Isolation, characterization and antibacterial activity screening of methoxyamine tetrahydroxy-anthocyanidines from Detarium senegalense gmelin stem bark. African

Journal of Pure and Applied Chemistry. 2009;3(1):1-5.

13. Burkill HM. The useful plants of West tropical Africa. Royal Botanical gardens Kew London. 1995;3:101.
14. Kaey RWJ, Phil D, Biol TT. Trees of Nigeria. Oxford University Press London. 1998;204-207.
15. Onyechi UA, Judd PA, Ellis PR. African plant foods rich in non-starch polysaccharides reduce postprandial blood glucose and insulin concentrations in healthy humans' subject. British Journal of Nutrition. 1998;80:419-428.
16. Odoh UE, Osadebe PO, Ezeugwu CO, Ajali U. Effect of Detarium microcarpum seed extract on haematology and serum chemistry of rats. Journal Tropical Medicinal Plants. 2008;9(1):7-10.
17. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright-Science Technical, Bristol. 1975;57.
18. Harborne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis 1st Edition. Chapmen and Hall London. 1998;15.
19. Trease GE, Evans WC. Pharmacognosy, 13th Edition. Bailliere Tindall, London. 1989;176-180.
20. Clinical Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, 9th ed. 2012;32(2):18-19.
21. Uchegbu RI, Okwu D. An Evaluation of the Phytochemical and Nutrient Composition of the Seed and Stem Bark of *Detarium senegalense* Gmelin. Journal of Natural Sciences Research. 2012;2(5): 107-111.
22. Nweze EI, Okafor JI, Njoku O. Antimicrobial activities of Methanolic Extracts of Trema guineesis (Schumm and Thorn) and Morinda lucida Benth used in Nigerian Herbal Medicine Practice. Journal of Biological Research and Biotechnology. 2004;2(1):39-46.
23. Hassan MM, Oyewale AO, Amupitan JO, Abdullahi MS, Okonkwo EM. Preliminary phytochemical and antimicrobial investigation of crude extracts of root bark of *Detarium microcarpum*. Journal of Chemical Society of Nigeria. 2004; 29(1):26-29.
24. Sartoratto A, Machado AIM, Delarmelina C, Figupira GM, Duarte MCT, Rehder VG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology. 2004;35:275-280.
25. Nwaogu LA, Alisi CS, Ibegbulem CO, Igwe CU. Phytochemical and antimicrobial activity of etanolic extract of *Landophia oweriensis* Leaf. *African Journal of Biotechnology*. 2007;6(7):890-893.
26. Stephen SH. Analysis of Synthetic Industrial Essential oils in Northern Nigeria for some Toxic substances and antibacterial effects: A Ph.D. thesis submitted to the Department of chemistry, University of Maiduguri, Borno State, Nigeria; 2007.
27. Usman H, Osuji JC. Phytochemical and in-vitro antimicrobial Assay of the leaf extract of Newbouldia leaves. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4:474-480.
28. Kensil CR. Saponins as vaccine adjuvants. Critical Reviews in Therapeutic Drug Carrier Systems. 1996;13(1–2):1–55.
29. Rice-Evans C, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant acivities of plant-derived polyphenolic flavonoids. Free Radical Research. 1995;22(4):375-383.
30. Middleton E, Kandaswami C, Theohaorides TC. The effect of plant flavonoids on mammalian cells; Implications for inflammation, heart disease and cancer. Pharmacology Reviews. 2000;52(4):673-751
31. Sharma RK, Chatterji S, Rai DK, Mehta S, Rai PK, Singh RK, Watal G, Sharma B. Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. Journal of Medicinal Plants Research. 2009;3(11):944-948.
32. Scalbert A. Antimicrobial Properties of Tannins. Phytochemistry. 1991;30:3875-3883.
33. Cushine TPT, Lamp AJ. Antimicrobial activity of flavonoids. International Journal of antimicrobial agents. 2005;26(5):343-356.
34. Copp BR. Antimycobacterial Natural Products. Natural Product Reports. 2003; 20(6):535-557.
35. Sowemimo AA, Pendota C, Okoh B, Omotosho T, Idika N, Adekunle AA,

Afolayan AJ. Chemical Composition, Antimicrobial Activity, Proximate Analysis and Mineral Content of the Seed of *Detarium senegalense* JF Gmelin. African Journal of Biotechnology. 2011;10(48): 9875-9879.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/65375