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Phytochemical Screening and Anti-Tb Activity of Root Extracts of *Guiera senegalensis (J. F. Gmel)*

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Abstract: The root of *Guiera senegalensis* is thought to possess medicinal properties according to Nigerian folklore. This study was undertaken to appraise the phytochemical constituents from polar and non-polar extracts (n-hexane, ethyl acetate and methanol) and anti-tuberculosis activities. Data obtained revealed that saponin and tannin appear as the only phyto-compounds in hexane extract, alkaloids, flavonoids, steroids, tannins and terpenoids were detected in the ethyl acetate fraction while alkaloids, cardenolides, flavonoids, phlobatanins, saponins, steroids, tannins and terpenoids were detected in the methanol extracts. Microplate Alamar Blue Assay (MABA) used for sensitivity study of *Mycobacterium tuberculosis* with 10µg/ml rifampicin revealed that the methanol extract from the root of *Guiera senegalensis* gave 22.71 ± 0.47 mm zone of inhibition whereas the ethyl acetate extract gave a 7.23 ± 1.35 mm zone of inhibition in comparison to 33.70 ± 0.64 mm obtained from the control. The minimal inhibitory content (MIC) of the methanol and ethyl acetate extracts were recorded at 2.8 ± 1.52 and 40.01 ± 1.20, while that of rifampicin was 0.38 ± 1.40. The n-hexane extracts did not show any inhibition. The results obtained suggested that the root of the studied plant possess anti-tuberculosis activities with the major activity tailored to the phyto-constituents from the methanol extracts.

Keywords: Phytochemical, *Guiera senegalensis*, Anti-Tb, Root Extract, Mycobacterium Tuberculosis

1. Introduction

Tuberculosis (TB) is disease caused by *Mycobacterium tuberculosis* which affects a third of the world's population with over 8 million new cases of infection diagnosed each year and is responsible for more than two million deaths per year [1], [2], [3], [4]. The greatest incidence of tuberculosis as of 2010 was reported in India (2.5 million affected), China (1.2 million affected), Indonesia (540,000 affected) and Pakistan (480,000 affected) with spatial distribution across Africa [5]. A study reported that Africa bore 29% of the global tuberculosis burden, accounting for 34% of the recorded TB-related deaths [6], despite the availability of anti-tuberculosis medication that has been commercially available for over three decades.

Researchers discovered that the emergence of drug resistant *Mycobacterium tuberculosis* strains is the primary factor for the persistent increase in TB infections globally [7]. In that report, resistance to antibiotics; Rifampicin and Isoniazid, commonly administered for the treatment of *Mycobacterium tuberculosis* infection brought about the realization of Multi Drug Resistant (MDR) strains of *Mycobacterium tuberculosis*. Drug-resistant strains of *M. tuberculosis* arise from spontaneous chromosomal mutations at a predictable low frequency. It has been suggested that of the over 400,000 cases of MDR variants of *Mycobacterium tuberculosis* infections a decade ago, about 40,000 of these occurred in Africa [8].

The plant kingdom holds new promise for the isolation and development of new drugs/compounds which can be used or manipulated towards the treatment of tuberculosis due to the unique chemical diversity of bioactive compounds that they synthesize [9], [10], [11]. Reports show that chloroform extracts of *Alpinia galanga* is effective against a virulent strain of *Mycobacterium tuberculosis* [12]. Other plants that have been investigated for anti-mycobacterial activity towards tuberculosis treatment include leaves of *Adhatoda vasica*, *Aegle marmelos*, *Tectona grandis* and the whole plant of *Solanum trilobatum* in other developing countries [13].

Furthermore, medicinal plants indigenous to countries facing the insurgence of TB have been found to possess anti-mycobacterial activity against virulent strains of the causative agent using Alamar Blue assay. These include *Allium sativum, Allium cepa, Syzygium aromaticum and Cinnamomum verum* [14]. A year later, other popular plants like *Acalypha indica, Adhatoda vasica* and *Aloe vera* were investigated and observed to be inhibitory towards the disease causing bacterium [15]. First line drugs like Rifampicin have been reportedly isolated from *Amycolatopsis mediterranei* [16].

In Nigeria, a variety of indigenous plants possesses ethno-medicinal properties and has been utilized in traditional medical practices for the treatment of numerous diseases. However, their application towards the treatment and efficacy against *Mycobacterium tuberculosis* has not been scientifically validated. One of such plants is *Guiera senegalensis* from the genus Guiera of the family Combretaceae and it is a native of tropical regions of Africa and known in Hausa language as kululu or saabaraa in the Northern parts of Nigeria (figure 1). A study reported that this plant produces the Tannin 3, 4, 5- tri-o-galloylguinic acid [17] and is used to treat a variety of microbial infections, consumed as vegetables or for timber. Traditional medical practice in Nigeria suggests that treatment of TB is possible when using this plant. In order to provide scientific evidence for this ethnomedical claim, this study was conducted to determine the efficacy and safety of the plants *Guiera senegalensis* via bioassay screening of its root extracts against virulent *Mycobacteria tuberculosis* H37RV (ATCC27294), using the Alamar Blue Assay.

Figure 1. *Sprouts of Guiera senegalensis.*

2. Materials and Methods

The roots of *Guiera senegalensis* were collected in fresh condition at Kufana and Basawa regions of Zaria locality in Kaduna state, Nigeria. Taxonomical identification was done at the herbarium unit of Biological Science department, faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The roots were then cut into pieces, washed and air dried, after which it was pulverized to powder form with mortar and pestle. The powdered roots were then kept in airtight container until required for further laboratory analysis. 1kg each of ground plant samples were exhaustively extracted using maceration extraction method [18]. The marc was extracted successfully using hexane, ethyl acetate and methanol using batch extraction methods. The extracts were concentrated at 40°C using a rotatory evaporator and later air-dried to give dried crude extracts. The extracts were weighed and their weights recorded. Stock solutions of 5.0mg/ml of rifampicin were prepared by dissolving 0.1g in 10ml of methanol.

2.1. Phytochemical Screening

Phytochemical screening was carried out on the hexane, ethyl acetate and methanol extracts for the qualitative determination of major constituents using methods previously described [18], [19], [20].

2.2. Antimicrobial Screening

All the Media were purchased from Sigma-Aldrich and were prepared in accordance with manufacturer instructions. The bacterial isolates were collected from Medical Microbiology Department of Specialist Teaching Hospital, University of Abuja, F. C. T., Nigeria on a slant Nutrient agar. The isolates were restored on Nutrient broth and confirmed using standard biochemical tests [21]. While the collected fungal isolates were identified using fungi chrome test kits in Specialist Teaching Hospital, University of Abuja, F. C. T., Nigeria. The isolates were collected on a Potato dextrose agar slant and restored in Potato dextrose broth. Agar diffusion method was adopted from [22] was employed.

2.3. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method [23]. Mueller Hinton broth was prepared, dispersed into test tubes and the broth was sterilized at 121°C for 15 minutes, the broth was allowed to cool. Normal saline was prepared, 10mls was dispersed into sterile test tube and the test microbes was inoculated and incubated at 39°C for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the McFarland's standard scale by visual comparison at this point, this test microbes has a concentration of about 1.5 x 10^8 CFU/ml. Two fold serial dilution of the extract in sterilized broth was made to obtain the concentration of 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, and 3.2µg/ml. The initial concentration was obtained by dissolving 6.0mg of the extract in 10mls of sterile broth. Having obtained the different concentration of the extracts in the sterile broth was observed for turbidity (growth), the lowest concentration of the extract in the broth which shows no turbidity was recorded to the MIC.

2.4. Determination of Minimum Bactericidal Concentrations

Minimum bactericidal concentration (MBC) was evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37°C for 24 hours (bacteria) after which the plates of the medium was observed for colony growth, the MBC was the plates with lowest concentration of the extract without colony growth.

2.5. Microplate Alamar Blue Assay (MABA)

Stock solutions of the individual plant extracts were prepared in 0.05% DMSO (Dimethyl sulfoxide) diluted to the final concentration of 20 and 40 µg/ml in sterile distilled water as part of experimental standardization. The sensitivity of *Mycobacterium tuberculosis* strain to the various extracts was demonstrated by agar diffusion method [24]. A sterile cork borer of 7mm diameter was used to bore holes into the inoculums seeded solidified nutrient agar. A 50µl volume of each 20 and 40µg/ml of the plant extracts was loaded into the labelled well in the prepared media plate using sterile pipette. The test was performed in triplicates and the plates were kept in a refrigerator for pre-diffusion of the sample and incubated at 37°C and 48 hours. Growth of *Mycobacterium tuberculosis* was observed after the incubation of 48 hours and the diameter of inhibition zone was measured subtracting the well size.

3. Results

3.1. Phytochemical Screening

Table 1 represents the phytochemical composition of n-hexane, ethyl-acetate and methanol extracts from the roots of *G. senegalensis*. The qualitative tests carried out on the extracts showed most activity in the methanol extract fractions whereby alkaloids, cardenolides, phlobatanins, steroids, tannins and terpenoids. The N-hexane extract was found to contain only saponins and tannins only. This minimal activity observed for the fraction maybe due to the solvent type. The ethyl acetate fraction showed the presence of trace amounts of alkaloids, terpenoids, tannins and flavonoids, while triterpenoids, glycosides, saponins, phenols and phlobatanins were absent.

It was observed that none of the fractions contain phenols, cardiac glycosides, glycosides, volatile oil and resins, hence may not have antioxidant potential that could enhance the body defense against pathology induced free radical generation [25]. It was observed that the solubility and reactions of the phytochemicals with the reagents are solvent dependent [26]. This observation indicates that cardenolides and phlobatanins are very polar compounds and hence only found in the in methanol layer (polar solvent).

Table 1. *Phytochemical screening of G. senegalensis roots.*

Phyto-constituents	N-hexane extract	Ethyl acetate extract	Methanol extract
Steroids	–	+	+
Triterpenoids	–	–	–
Glycosides	–	–	–
Saponins	+	–	+
Phenols	–	–	–
Alkaloids	–	+	+
Cardenolides	–	–	+
Terpenoids	–	+	+
Cardiac glycosides	–	–	–
Phlobatanins	–	–	+
Resins	–	–	–
Balsams	–	–	–
Volatile oils	–	–	–
Tannins	+	+	+
Flavonoids	–	+	–

(+) – Present (-) – Absent.

3.2. Antimicrobial and Anti-Mycobacterial Screening

The mean zones of inhibition of different extract against 10 bacterial species are summarized in Table 2. The methanol extract of *G. senegalensis root* were found more active against *Candida krusei* with 34.0 ± 0.55mm zone of inhibition followed by 33.0 ± 0.90mm against *Staphylococcus aureus* and 32.0 ± 0.35mm against *Shigella dysenteriae,* while the lowest value 20 ± 0.40 mm was recorded for *Methicillin Resistant Staphylococcus aureus*. The ethyl acetate extract showed the highest value of 35.0 ± 0.80 mm against *Staphylococcus aureus* followed by 34.0 ± 0.50 mm against *Candida albicans* while 24.0 ± 1.30 mm zone of inhibition was noted against *Candida tropicalis*. Using hexane, the root extracts were most resistant to *Candida albicans* and *Shigella dysenteriae* with a zone of inhibition of 35.0 ± 0.40 mm and 35.0 ± 0.00 mm. The least zone of inhibition of 22.0 ± 0.70 mm was observed against *Methicillin Resistant Staphylococcus aureus*. Ciproflaxin was used as standard for bacteria, ranging the value of zone of inhibition from 35 to 41 mm, while fluconazole was used as the control antifungal agent with a zone of inhibition ranging from 31 to 35 mm [27]. The data indicated that the root extract of *G. senegalensis was sensitive towards Salmonella typhi* as no zone of inhibition was observed using all three solvent extracts. Table 2 also reveals the anti-mycobacterial effectiveness of the respective plant extracts which was via agar diffusion method against virulent strain (H37RV) of *mycobacterium tuberculosis*. Zones of inhibition of *M. tuberculosis* isolates was observed for rifampicin (33.70mm), the methanol (31.24mm) and ethyl acetate (12.69mm) extracts of *G. senegalensis* whereas the n-hexane plant extracts did not show activity against this virulent strain.

Table 3 data shows the MIC and MBC of the different extracts of *G. senegalensis*. The methanol extract of *G. senegalensis* had the lowest MIC value against the virulent *Mycobacterium tuberculosis* (H37RV) strain. This was followed by the ethyl acetate extract of the same plant with MIC of 40.01mg/ml. The n-hexane extract did not generate

an MIC value against H37RV.

The data indicated that polar and nonpolar extracts of *G. senegalensis* were most active against *Staphylococcus aureus*, with MIC and MBC values of 3.12 and 12.5 mg/ml, respectively. *Candida albicans* and *Candida tropicalis* showed high resistance, with 12.5 and 25.0 mg/ml of MIC and MBC, respectively against the all three extracts.

Table 2. *Antimicrobial activity of G. senegalensis root extracts against test organisms Zone of inhibition (mm).*

Test microorganisms	N-hexane	Ethyl acetate	Methanol	Control[1]	Control[2]	Control[3]
Mycobacterium tuberculosis H37RV	NR	12.69 ± 0.55	31.24 ± 0.52	-	-	33.70 ± 0.64
Methicillin Resistant Staphylococcus aureus	22.0 ± 0.70	27.0 ± 1.20	20.0 ± 0.40	35.0 ± 0.30	-	-
Staphylococcus aureus	34.0 ± 1.10	35.0 ± 0.80	33.0 ± 0.90	37.0 ± 0.10	-	-
Shigella dysenteriae	35.0 ± 0.00	30.0 ± 0.40	32.0 ± 0.35	40.0 ± 0.11	-	-
Salmonella typhi	0	0	0	41.0 ± 0.55	-	-
Escherichia coli	27.0 ± 0.30	27.0 ± 0.25	25.0 ± 0.30	39.0 ± 0.20	-	-
Proteus mirabilis	27.0 ± 1.40	26.0 ± 0.45	25.0 ± 0.75	35.0 ± 1.15	-	-
Candida albicans	35.0 ± 0.40	34.0 ± 0.50	30.0 ± 0.65	-	35.0 ± 0.90	-
Candida krusei	30.0 ± 0.80	32.0 ± 0.30	34.0 ± 0.55	-	34.0 ± 0.35	-
Candida tropicalis	26.0 ± 0.10	24.0 ± 1.30	22.0 ± 0.60	-	31.0 ± 0.85	-

Control[1]= Ciproflaxin (30 µg/ml), Control[2]= Fluconazole (30 µg/ml), Control[3]= Rifampicin (10µg/ml).
NR = No reaction under experimental conditions.

Table 3. *Minimum Inhibitory Concentration (mg/ml ± SD) of G. senegalensis root extracts against test organisms.*

Test microorganisms	MIC/MBC	Concentration (mg/ml)		
		N-hexane	Ethyl acetate	Methanol
Mycobacterium tuberculosis H37RV	MIC	NR	40.01 ± 1.20	2.80 ± 1.52
Methicillin Resistant Staphylococcus aureus	MIC	3.12	3.12	3.12
	MBC	12.5	25.0	12.5
Staphylococcus aureus	MIC	3.12	3.12	3.12
	MBC	12.5	12.5	12.5
Shigella dysenteriae	MIC	3.12	3.12	12.5
	MBC	12.5	12.5	12.5
Salmonella typhi	MIC	12.5	12.5	12.5
	MBC	25.0	12.5	12.5
Escherichia coli	MIC	3.12	3.12	3.12
	MBC	12.5	25.0	12.5
Proteus mirabilis	MIC	3.12	3.12	3.12
	MBC	12.5	25.0	12.5
Candida albicans	MIC	12.5	12.5	12.5
	MBC	25.0	25.0	25.0
Candida krusei	MIC	3.12	3.12	3.12
	MBC	12.5	12.5	25.0
Candida tropicalis	MIC	12.5	12.5	12.5
	MBC	25.0	25.0	25.0

Each value represents mean (n = 3).

4. Discussion

Plants are generally considered as an essential raw material which can be utilised for food, shelter, medicines, etc. The pharmacological worthiness of medicinal plants has been exploited for the treatment of many types of diseases in different parts of the developing world since treatment of such diseases by phyto-drugs are not prone to certain side effects commonly associated with the use of synthetic drugs. The phytochemical compounds within plants are being screened and studied to help elucidate which active compounds or synergy of compounds produce their antimicrobial activities thus making them effective drug candidates in addition to confirming the believe that local plants are the platform for traditional African medicine [28], [29]. A variety of illnesses including asthma, bronchitis, chest pain, diarrhoea, malaria, pneumonia and tuberculosis has been treated via traditional practice using indigenous plants [30]. In the northern part of Nigeria, *G. senegalensis* root extracts is used not only for fuel and for forage but also in medicine, particularly in the treatment of snakebites, dysentery, diarrhoea, stomach upsets and haemorrhoids [31]. Analysis of the data obtained from table 1 suggests that the root extract contained a higher degree of ethanol soluble compounds. The presence of Saponins, Cardenolides and Phlobatanins were the only significant difference between the methanol and ethyl acetate extracts. This result is similar to that obtained from studies performed by other researchers who investigated the phytochemical components of the same plant root extract [32], [33]. The success in treating diarrhoea and stomach upsets, attributed to *Staphylococcus aureus* and *Salmonella spp* infections could be due to the antibacterial effects of alkaloids, polyphenols, saponins and steroids [34]. Distorting the enzyme activity within bacteria is usually observed in the presence of phyto-tannins [31]. The anti-diarrheal effectiveness of alkaloids is

reportedly potent against intestinal infections associated with AIDS, thereby linking the screening of plants with a high presence of alkaloids to the treatment of HIV infections [35]. Other phyto-constituents observed included saponins which alongside alkaloids are known to be very effective against both gram positive and gram bacteria such as *Salmonella spp* and S*treptococcus spp* [36] used in this study. In this report, the respective root extracts of *G. senegalensis* from different solvents was tested against different microorganisms in an attempt to gauge their activity spectrum. The varied antimicrobial activity depicted (table 2) is indicative of the antibacterial and antifungal compounds present, thereby supporting the data obtained from the phytochemical studies (table 1). The result indicates that both polar and non-polar fractions of the root extract possess reasonable activity against gram positive and gram negative (*Proteus mirabilis*) in addition to their activity against yeast, thereby demonstrating the potential of the plant to be used in curing diseases caused by these organisms. The data revealed that the root extracts were general most effective against *Shigella dysenteriae* thus confirming claims that this plant can be used for the treatment of stomach upsets and infections. Furthermore, the antimicrobial properties of certain indigenous Nigerian plants has been attributed key phyto-metabolites such as alkaloids, tannins, saponins, etc [37], [38], [39].

The study showed that the polar and semi polar root extracts (methanol and ethyl acetate) of *Guiera Senegalensis* had anti-mycobacterial activity. The methanol extracts were most active in comparison with the control antibiotic, rifampicin (10µg/ml), against the virulent strain of *M. tuberculosis*. The methanol and ethyl acetate extracts of *G. senegalensis* were most active against the virulent H37RV strain of *Mycobacterium tuberculosis*. The potency of the methanol extract in comparison to the standard drug (rifampicin) was relatively close, thereby suggesting that certain phytochemical components either individually or in synergy with other soluble parts pose a unique advantage towards treatment or prevention of tuberculosis. The hexane extract however did not exhibit any anti-mycobacterial resistance compared to the other extracts. This could be credited to poor extractive value and absence or low quantity of phytochemicals (Table 1, 3). From the data obtained in this investigation it is conceivable that further research is needed to isolate and identify any active principle towards effective treatment of tuberculosis. The extracts of *G. senegalensis* is sensitive towards all tested microorganisms particularly *M. tuberculosis* at the MIC value.

The present study has revealed the importance of *G. senegalensis* to virulent strains of *M. tuberculosis* which are being a threat to human health and for the development of alternate, safe and effective medicines.

5. Conclusion

This study serves to validate traditional knowledge and adds to the growing literature on botanical sources identified as providing important novel anti-tuberculosis compounds. The information on the therapeutic effect of the root of this plant in the treatment of tuberculosis seems to be well known in some Northern Nigerian cultures. The findings in this study have hence provided scientific support for the ethnomedical anti–TB activity of extracts of the root of G. senegalensis, where the methanol fraction was found to have a significantly high MIC value when compared to the other extracts. More so, the standard drug (Rifampicin) as some closeness to the MIC value of the methanol fraction when compared. The phyto-chemistry of the plant shows that the methanol extract contains steroids, saponin, alkaloid, terpenoids and tannin. Hence the constituent with the anti-TB activity can be drawn down to be from the above phyto-constituents from methanol.

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