

## TRITERPENE FROM THE STEM BARK OF PSOROSPERMUM SENEGALENSE (SPACH) INHIBITS TUBERCLOSIS

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### ABSTRACT

Psorospermum senegalense which is traditionally used in the treatment of tuberculosis (TB) in Northern Nigeria was investigated. Preliminary antimicobacteria studies on the ethyl acetate (EA), hexane (HE), dichloromethane (DCM) and methanol (ME) extracts showed activity at a minimum inhibitory concentration (MIC) of 2.5 mg/mL for EA extract, while other extracts did not show activity. Chromatographic purification of the EA extract led to the isolation of a triterpene (P2) whose structures was established to be  $\alpha$ -amyrin, by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and by comparison with spectral data from literature. Biological evaluation of P2 showed that it inhibited the growth of *Mycobacterium bovis* (BCG) at MIC of 125  $\mu\text{g}/\text{mL}$  and 62.5  $\mu\text{g}/\text{mL}$  against *Shigella dysenteriae*. These results clearly shows that *P. Senegalensis* has potential that can be explore in the search for anti-TB drug from nature.

**KEYWORDS:** Psorospermum senegalense, antituberculosis activity, Chromatography,  $\alpha$ -amyrin, *Mycobacterium bovis*, BCG.

### INTRODUCTION

Tuberculosis, a major chronic infectious disease, remains one of the most serious medical and social challenges of our days. It is responsible for about 3 million deaths per year and around 8 million cases of first-recorded disease. The advances in the Chemotherapy of tuberculosis in the mid-20th century have recently givenway to anxiety over the evolution of drug resistance based on the genetically fixed mutations of *M. tuberculosis*. And the long regime of treatment has contributed greatly in the spread of resistance to effective drugs and mortality, due to inability to complete treatment. Similarly, nearly all drugs used for the treatment of tuberculosis and possessing different mechanisms of activity are associated with adverse side effects on the human organism. These have placed a demand for the search of new classes of drugs with less side effect, low toxicity and shorter regime of treatment. The search has led others to look at nature for a possible solution; <sup>[1]</sup> reported the anti-TB activity of tricyclic diphenol ether engelhardion. Pyrrolnitrin and banegasine isolated from the zoobacterium aristabacterneator act synergetically against TB. <sup>[2]</sup> Triterpene isolated from Indigofera longifolia were reported to be active against TB. <sup>[3,4]</sup> reported the anti-TB activity of a diterpenes isolated from Calceolaria pinnifolia. The structural modification of Ursolic acid at the C-3 position

to cinnamate-based esters resulted in enhanced anti-TB activity as reported by. <sup>[5]</sup>

In the present investigation we report our finding on the anti-TB studies of *Psorospermum senegalense*, which belongs to the Hypericaceae family. It is found in the bush and wooded savanna of the Sudanian zone, recorded only from Senegal, Sierra Leone, Dakar and Guniea. Local uses reported include general usage in Senegal for all skin infections. A bark decoction of the root is used in washes and bathes for common dermal troubles and for herpes, eczema, leprosy and syphilitic conditions. In northern Nigeria decoction of the leaves is used in the treatment of tuberculosis. In Guinea, the pulped bark and pulped roots is used typically on dematoses generally and a decoction of leafy twigs is given by draught as a diuretic and febrifuge. A filtrate from a prolonged boiling of the leaves is deemed in Senegal to alleviate respiratory trouble and is taken to treat leprosy. An oil film comes to the surface of this preparation which can be separated off on cooling. This is used externally for skin troubles. The plant is also used to treat colics and vaginal discharge. The leaves are used as expectorant. <sup>[6]</sup>

In the present paper, we describe the bioactivity guided isolation and structural determination of a triterpene  $\alpha$ -amyrin ( $3\beta$ -hydroxy-urs-12-en-3-ol) from the ethyl

acetate soluble fraction of the stem bark of *Psorospermum senegalense* and its anti-TB activity.

## Experimental

### Plant material

The plant material was collected fresh from Zaria, Nigeria in September, 2013. Taxonomical identification was done at the Herbarium Department of the Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and its voucher specimen with number 014 deposited there. The plant was air-dried under shade, segregated and pulverized by mechanical pounding using wooden mortar and pestle. The pulverized plant material was stored away from moisture until needed.

### Extraction of plant materials

The pulverized plant material (500g) was carefully weighed and macerated with 95% methanol for two weeks. The extract was decanted, filtered and labeled. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. The combined extract was partitioned with hexane, dichloromethane and ethylacetate. The extracts were concentrated in vacuo at 40°C using a rotatory evaporator and later subjected to air drying to give dried crude extracts.

### Antimicrobial studies

The antimicrobial activities of the HE, DCM, EA and ME extracts and standard drugs (Ciprofloxacin, Sparfloxacin and Fluconazole) were determined using microbial strains and fungi obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria (ABUTH); *Shigella dysenteriae*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Candida tropicalis*, *Candida krusei* and *Candida albicans*. The cork and bore diffusion method of<sup>[7]</sup> was used to determine the antimicrobial activity of the test compounds. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24h at 38 °C. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter Cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the test extracts. The plates were incubated for 24h at 38 °C, while the fungi were incubated at 34°C for 48h. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibition zone (mm) produced by the test compounds.

### Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentrations (MIC) were determined for the extracts using micro broth dilution

method in accordance with.<sup>[8]</sup> Serial dilution of the least concentration of the extracts that showed activity were prepared using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 38 °C for 18h. Minimum inhibitions Concentrations (MIC) were recorded as the lowest concentrations of the compounds showing no visible growth (turbidity) in the broth.

### Minimum Bactericidal Concentration (MBC/MFC)

The minimum bactericidal and minimum fungicidal concentration were determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar (OXOID) plates and incubated at 38°C for bacteria and 34°C for fungi for 48h. The MBC/MFCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

### Antituberculosis studies

Sterile 96 micowell plates were employed for the determination of antimycobacterial activity of the extracts as described by.<sup>[9]</sup> About 100 mg of each extract was transferred into a sterile bottle, dissolved with 0.5 mL dimethylsulphoxide (DMSO) and 0.5 mLdistill water. The extracts were further diluted (1:10) in 7H9 Middlebrook broth to give 10 mg/mL concentration. Into each of the 96 micowell plates was transferred 50 µL of sterile 7H9 broth starting from well 2 to 12. To each of the first wells was added 100 µL of 10% DMSO in sterile media (prepared by dispensing 0.1 ml of DMSO into 9.9 mL of 7H9 broth as control), 100 µl of 25 µg/mL solution of rifampicin (standard) and 100 µL of each plant extract. Using a multi-channel pipette, 50 µL was carefully removed from well 1 and added to well 2, mixed thoroughly by pipetting up and down four times, and the process continued to well 11 from which 50 µL was withdrawn and discarded.

### Inoculation

The 5-7 day old culture of BCG monitored on UV spectrophotometer at 650 nm (OD 0.2-0.3) was diluted 1/1000 by adding 50 µL cell culture to 50 mL 7H9/ADC medium, where 50 µL of diluted culture was inoculated to all wells of the plate. The plates were incubated at 30°C for 7 days and after incubation stained with tetrazolium dye for growth/inhibition of organisms. The column number of the row at which no apparent growth was seen was recorded as activity.

### Spectral data

The compound (P2) appeared as a white solid. The 1H NMR (CDCl<sub>3</sub>, 400 MHz) δ 3.2 (1H, t), 0.7 (1H, t), 5.2 (1H, t); <sup>13</sup>C NMR(CDCl<sub>3</sub>, 100MHz): δ 38.9 (C-1), 28.0 (C-2), 79.1 (C-3), 38.8 (52.7), 18.3 (C-6), 33.0 (C-7), 42.7 (C-8), 47.9 (C-9), 38.8 (C-10), 24.2 (C-11), 125.8 (C-12), 145.1 (C-13), 47.6 (C-14), 27.2 (C-15), 28.1 (C-16), 33.0 (C-17), 55.2 (C-18), 42.0 (C-19), 40.9 (C-20), 30.6 (C-21), 46.5 (C-22), 23.6 (C-23), 21.2 (C-24), 15.6

(C-25), 16.9 (26), 24.2 (C-27), 29.7 (C-28), 17.1 (C-29), 23.3 (C-30)

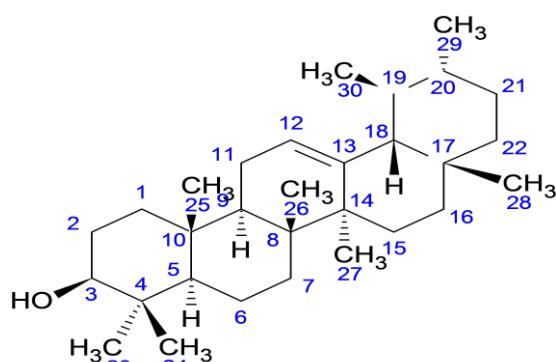


Figure 1: Structure of P2 ( $\alpha$ -amyrin).

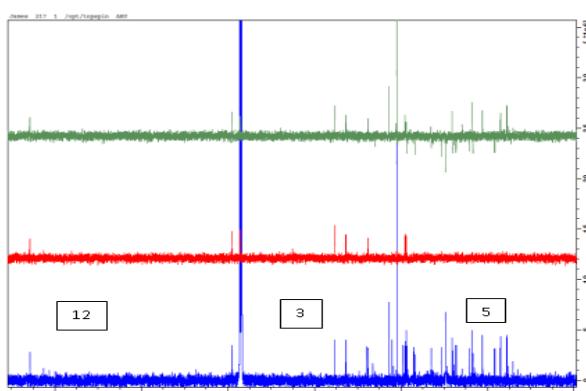


Figure 2: DEPT and decouple  $^{13}\text{C}$ -NMR spectra of P2.

## RESULTS AND DISCUSSION

Antituberculosis evaluation of the crude extracts (table 1) reveals that only the ethyl acetate extract had activity with MIC of 2.5 mg/mL against *Mycobacterium bovis*, while other solvent fractions were not active. This activity demonstrated by ethyl acetate fraction showed that the plant had potential that can be explored in the search for anti-TB drug and so was subjected to column chromatography which led to the isolation of a triterpene  $\alpha$ -amyrin (P2).

The triterpene P2 isolated was found to exhibit antimicrobial as well as antituberculosis property and Steroids and Triterpenes have been reported to have antibacterial properties.<sup>[10]</sup>

Compound P2 was isolated as a white amorphous solid which gave a positive result to the Salkowski's test for steroid/triterpenes. Its  $^1\text{H}$ -NMR spectrum revealed three regions typical of the triterpenoidal nucleus at 0.5 ppm to 2.5 ppm representing overlapping methyl, methylene and methine protons; an oxymethine proton at 3.2 ppm assigned to the position 3 of triterpenoidal nucleus and a single unsaturated proton signal at 5.22 ppm. The  $^{13}\text{C}$  NMR spectrum of compound P2 revealed a total of 30 carbon signals, 8 of which were methyl signals, 9 were methylene carbon signals, 7 were methine and there were

6 quaternary signals, these data is typical of  $\alpha$ -amyrin.<sup>[11,12,13]</sup> The signal between 10.9 ppm and 55.6 ppm represent a region of overlapping methyl, methylene and methine carbon atoms, an oxymethylene carbon signal at 79.0 was typical of position C-3 of  $\alpha$ -amyrin , and finally the unsaturated carbon signals at  $\delta$ 125.8 and  $\delta$ 138.0 ppm were assigned to two carbon olefinic system. These NMR data are very similar to the data for  $\alpha$ -amyrin and a comparison with data reported for  $\alpha$ -amyrin showed good agreement.<sup>[11,12,13]</sup> Therefore, the structure of compound P2 was determined to be  $\alpha$ -amyrin (Figure.1 & 2).

Antimicrobial evaluation of P2 (Table 2) reveals the highest zone of inhibition was 28 mm against *S. dysenteriae*, with MIC of 62.5 $\mu\text{g}/\text{mL}$  other organism were inhibited at a higher concentration (125  $\mu\text{g}/\text{mL}$ ). The compound P2 was also bactericidal at a low concentration of 125  $\mu\text{g}/\text{mL}$  against *S. dysenteriae* and 250  $\mu\text{g}/\text{mL}$  against the other organism. Anti-TB evaluation of P2 showed MIC of 125  $\mu\text{g}/\text{mL}$  against BCG, as compared to the crude ethyl acetate fraction (MIC: 2500  $\mu\text{g}/\text{mL}$ ). These results clearly point to the fact that the plant *P.senegalense* has potential that can be explored in the search for antibacterial and anti-TB drug from nature.

Table 1: Results of the Anti-tuberculosis activities of the extracts, P2 and standard drug

Extract/drug	2500	1250	625	125	62.5
HE	NA	NA	NA	NA	NA
DCM	NA	NA	NA	NA	NA
EA	+	NA	NA	NA	NA
ME	NA	NA	NA	NA	NA
P2	+	+	+	+	NA
Rifampicin	+	+	+	+	+

Key: NA = No activity; + = MIC

Table 2: Results of the Antimicrobial activities of P2.

Test Organism	ZI (mm)	MIC ( $\mu\text{g}/\text{ml}$ )	MBC/MFC ( $\mu\text{g}/\text{ml}$ )
MRSA	-	-	-
VRE	23	125	250
Staphylococcus aureus	24	125	250
S.feacalis	24	125	250
Bacillus Subtilis	26	125	250
Pseudomonas aeruginosa	-	-	-
Enterobacter sp	24	125	250
P. retgeris	25	125	250
Shigella dysenteriae	28	62.5	125
Candida stellatoidea	-	-	-
Candida pseudotropicalis	-	-	-
Candida albican	25	125	250

Key: - = No activity; ZI= Zones of Inhibition MIC= minimum inhibitory concentration, MBC= Minimum

Bactericidal Concentration, MFC= Minimum Fungicidal Concentration.

## CONCLUSION

This is the first report of an antituberculosis investigation of *P.senegalense*. The finding of antituberculosis activity in this plant is unique as it has not been reported before, though antimicrobial activity of the isolated compound have been reported. This report validates the claims of curing tuberculosis and other infectious diseases, synergistic coupling of the compound with antimicrobial agents could improve therapeutic efficiency in the face of rising bacterial resistance, however this needs further investigation.

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