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RESEARCH PAPER

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Phytochemical Studies, Antimicrobial and Antituberculosis Evaluation of Extracts from the Stem Bark of *Psorospermum senegalense* (Spach)

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ABSTRACT

Psorospermum senegalense which is traditionally used in the treatment of tuberclosis (TB) in Northern Nigeria was investigated. Phytochemical analysis revealed the presence of flavonoids, carbohydrates, cardiac glycosides, steroids, triterpenes, alkaloids, tannins and saponins. Preliminary antimicrobial studies, showed that the ethyl acetate (EA) extract showed the highest zone of inhibition (ZI) ranging from 25 to 31 mm on the test organism. The hexane (HE), dichloromethane (DCM) and methanol extract showed ZI ranging from 18-21, 20- 27 and 20-24 mm on the test organism respectively. The results of the minimum inhibitory concentration (MIC) determination shows that EA inhibited all the test organism at a low concentration of 7.5 mg/mL except for Pseudomonas aeruginosa (PA) and Candida albican (CA) which showed an MIC of 15 mg/mL. On Mycobacterium bovis (BCG) an MIC of 2.5 mg/mL was observed for EA, while other extracts did not show activity.

Keywords: Psorospermum senegalense, phytochemical, antimicrobial, antituberculosis activity, Mycobacterium bovis and BCG.

INTRODUCTION

Tuberculosis remains a major public health concern with over 2 billion people currently infected, 8.6 million new cases per year, and more than 1.3 million deaths yearly[1]. The current drug regimen combination for tuberculosis consists of, rifampicin, isoniazid ethambutol and pyrazinamide, administered over a period of six months [2,3]. Although the success rate of this treatment is high, the utility of this regimen is limited by the issues of compliance, which has resulted in the rise of resistant strains to some or all of the first- and second-line antibiotics [4]. These strains, known as multidrug resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) strains of*Mycobacterium tuberculosis* (*M. tb*), have very bad disease outcomes [5]. Similarly, nearly all drugs used for the treatment of tuberculosis are associated with adverse side effects on the human organism. These have place a demand for the search of new classes of drugs with less side effect, low toxicity and shorter regime of treatment.

In the present investigation we report our finding on the anti-TB studies of the leave extracts of *Psorospermum senegalense,* which belongs to the *Hypericaceae* family. *Psorospermum senegalense 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, leprous and syphilitic conditions. In northern Nigeria decoction of the leaves is used in the treatment of tuberculosis [6]. In Guinea, the pulped bark and pulped roots is used typically on dematoses generally and a decoction of leafy twins 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 [6]. 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 [6].

In the current investigation carried out, a screening of the methanol, ethyl acetate, and dichloromethane and hexane extracts of stem bark of *Psorospermum senegalense* against pathogenic bacteria, fungi and *mycobacterium bovis* is done in order to detect new sources of antimicrobial and antituberculosis agents.

MATERIALS AND METHODS

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.

Preliminary Phytochemical Screening

The extracts were subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods [7,8]. The metabolites tested for included: carbohydrates, tannins, saponnis, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

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 labelled. 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 Fluoconazole) 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,Candidatropicalis,Candida krusei* and *Candida albicans*. The cork and bore diffusion method of [9], 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 ^{o}C. About 5 discrete colonies were asceptically 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 ^{o}C, while the fungi were incubated at 34^{o}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 [10]. 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 ^{o}C for 18h. Minimum inhibition
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^{o}C for bacteria and 34^{o}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 microwell plates were employed for the determination of antimycobacterial activity of the extracts as described by [11]. 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 microwell 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^{o}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.

RESULTS AND DISCUSSION

Phytochemical screening (Table 1) of the crude methanol, ethyl acetate, dichloromethane and hexane extracts revealed the presence of carbohydrates, cardiac glycosides, alkaloids, tannin, flavonoids, Saponins, anthraquinones, steroids and triterpenes. These could be responsible for high antimicrobial activity and antituberculosis activity demonstrated by the plant extracts and also validates the claim by the traditional healers in the treatment of several ailments. The antimicrobial sensitivity test of the leave extracts of *Psorospermum senegalense* showed that the extracts have moderate to good activity.The Determination of zone of inhibition (ZI) showed inhibition ranging from 18-22 mm (HE), 20-27 mm (DCM), 25-31 mm (EA) and 20-24 mm (ME) against the entire test organisms.

Table 1. Phytochemical screening of the extracts of *psorospermum senegalense.*

Metabolites	HE	DCM	EA	ME
Carbohydrate	+	+	+	+
Cardiac glycoside	+	+	+	+
Tannins	-	-	+	+
Saponins	-	-	-	-
Flavonoids	-	+	+	+
Anthraquinones	-	-	-	+
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	+	+	+	+

Key: + = present, - = absent, HE = hexane extract, DCM = dichloromethane extracts, EA = Ethyl acetate extracts, ME = Methanol extracts

The ethyl acetate extract had the highest zone of inhibition of 31 mm against *Bacillus subtillis*. The results of the minimum inhibitory concentration (MIC) showed that EA fraction

inhibited the growth of all test organisms at a low concentration of 7.5 mg/mL, except *Pseudomonas aeruginosa* and *Candida krusei* which had MIC of 15 mg/mL. Higher MIC values were observed for DCM (15 mg/mL), HE and ME fraction all showed MIC at 15 to 30 mg/mL. The microorganisms were bactericidal at a higher concentration; EA (MBC/MFC; 15-30 mg/mL), DCM (MBC/MFC; 30-60 mg/mL), ME and HE (MBC/MFC; 60 mg/mL). Antituberculosis evaluation of the 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.

Table 2. Determination of Zones of Inhibition (mm) of the extracts on test organisms.

Test Organisms	HE	DCM	EA	ME	CFX	FCZ
Staphylococcus aureus	20	25	28	20	35	-
Bacillus Subtilis	21	27	31	22	37	-
klebsiella Pneumoniae	21	24	29	22	40	-
Proteus mirabilis	18	22	27	20	35	-
Salmonella typhi	-	-	-	-	42	-
Shigella dysenteriae	22	26	30	24	35	-
Pseudomonas aeruginosa	20	23	26	0	35	-
Candida albicans	21	24	28	20	-	35
Canadida krusei	18	20	25	20	-	37
Canadida tropicalis	-	-	-	-	-	32
Canadida stellatoidea	-	-	-	-	-	34

Key: DCM = Dichloromethane, EA = Ethylacetate, ME = Methanol,HE = Hexane, CFX = Ciprofloxacin, FCZ = Fluoconazole, - = no activity.

Table. 3 Minimum Inhibitory Concentration (MIC) of the extracts (mg/ml).

Test Organisms	HE	DCM	EA	ME
Staphytococcus aureus	15	15	7.5	15
Bacillus Subtilis	15	7.5	7.5	15
klebsiella Pneumoniae	15	15	7.5	15
Proteus mirabilis	30	15	7.5	30
Shigella dysenteriae	15	15	7.5	15
Pseudomonas aeruginosa	15	15	15	15
Candida albicans	15	15	7.5	15
Canadida krusei	30	15	15	30

CONCLUSION

The result of this investigation shows that *psorospermum senegalense* stem bark extracts can be used to discover antituberculosis and antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria and fungi responsible for severe illness. The current study recommends bioactivity guided isolation and purification of lead compounds for antimycobacterial and antimicrobial activity from the plant species.

Table 4. Minimum bactericidal/fungicidal concentration (MBC/MFC) of the extract (mg/ml).

Test Organism	HE	DCM	EA	ME
Staphytococcus aureus	60	30	15	60
Bacillus subtilis	60	30	15	60
klebsiella pneumonia	60	30	15	60
Proteus mirabilis	60	60	30	60
Shigella dysenteriae	60	30	15	60
Pseudomonas aeruginosa	60	30	30	60
Candida albicans	60	30	15	60
Canadida krusei	60	60	30	60

Table 6. Results of the Anti-tuberculosis activities of the extracts and standard drug.

	Concentration (µg/mL)				
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
Rifampicin	+	+	+	+	+

Key: NA = No activity; + = MIC

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