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Original Research Article

Phytochemical Analysis and Antimicrobial Screening of *Psorospermum senegalense* Leaves Extract

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Abstract: The antibacterial activity of the hexane, dichloromethane, etyl acetate and methanol extracts of *psorospermum senegalense* leaf belonging to the family *Hypericaceae*, was determined in vitro, using well diffusion and minimum inhibitory concentration (MIC) determination method against nine human pathogenic bacteria: (klebsiella pneumonia, *Staphylococcus aureus, proteus vulgaris streptococcus pneumonia, vancomycin resistant enterococci, Bacillus subtilis, Escherichia coli, pseudomonas flourescense and streptococcus pyogenes,*) and three human pathogenic fungi (candida tropicalis, candida krusei and *Candida pseudotropicalis*). The hexane, dichloromethane, ethylacetate and methanol extracts displayed a potential antibacterial activity against five out of the nine tested bacteria: (*Staphylococcus aureus,, streptococcus pneumonia, Bacillus subtilis, Escherichia coli, and streptococcus pyogenes,*)and two out of three tested fungi (candida tropicalis, and *Candida pseudotropicalis*)The ethyl acetate extract exhibited the highest zone of inhibition (28 mm) and MIC(3.25mg/ml) against *Streptococcus pyogenes.* The highest inhibition of fungal growth (27mm) and MIC (3.25mg/ml) was recorded against *C. pseudotropicalis* with ethyl acetate extract. The hexane extract exhibited the lowest zone of inhibition (18 mm) and MIC (15mg/ml) against *streptococcus pneumoniae* as well as *C.Krusei and C.pseudokrusei.* Phytochemical analysis showed the presence of carbohydrates, alkaloids Cardiac glycosides, steroids and triterpenes in all the four extracts, tannins and flavonoids were present in the ethyl acetate and methanol extracts. While Saponins and anthraquinones were absent in all the four extracts. The consequences of this investigation suggest that the extracts can be used to discover antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria and fungi responsible for severe illness.
Keywords: psorospermum senegalense, Pathogenic bacteria, Extract, Antibacterial activity, MIC, phytochemical analysis.

INTRODUCTION

During the last 20 years, it has been reported that human infections are increasing at an alarming rate, especially in tropical and subtropical developing countries [1]. This is partly due to the indiscriminate use of anti-microbial drugs and the development of microbial resistance to some of the synthetic drugs [2]. Resistance to most antibiotics occurs through the aegis of extremely efficient enzymes, efflux proteins and other transport systems that often are highly specialized towards specific antibiotic molecules [3]. The fact that microorganisms nowadays tend to develop resistance towards drugs, coupled with the undesirable side effects of certain antibiotics offer considerable potentials for the development of new effective antimicrobial agents; medicinal plants being a prolific source. Various plant extracts possess bacteriostatic and bactericidal effects due to secondary metabolites they contain, namely alkaloids, tannins, flavonoids, and phenolic compounds.

The plant *Psorospermum senegalense spach* belongs to the family *Hypericaceae*. Its local name in Hausa language is 'Huda Tukunya'. It is found in the bush and wooded savanna of the Sandanian zone, recorded only from Senegal to Sierra Leone. The plant is also found in other places like Dakar and Guniea. Local uses reported include that pieces of the plants are attached to millet by the Tenda to discourage bees from nesting on it. The plant enters into a Fula magical treatment to confer protection against evil [4].The plant has a 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 Nigeria decoction of the leaves is used in the treatment of tuberculosis. In

Dakar, a general application is by powder for colic by decoction for gonorrhoea and by maceration for leprosy. In the Ferlo region, a decoction of bark from the main stem and of the roots is added to baths and is made up into draughts for pains in the joints in attacks of fever [5]. In Guinea, the pulped bark and pulped roots is used typically on dermatoses 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. An oil film comes to the surface of this preparation which can be separated off on cooling. This is used externally for skin troubles [4]. Analysis of Guinea material has shown the presence of tannins, sugars and fluorescent anthraquinonic pigment, the last which is toxic and causes photosensitivity and acute irritation to the kidneys and intestine [4]. The plant is also used to treat colics and vaginal discharge. A fumigation of the bark is said to be used to chase demons. The leaves are used as expectorant, fortifier and against nausea. A decoction of the leaves, bark and roots are used in baths and beverages to treat joint pain [6].

The phytochemical screening and scabicidal activity of the leaves of *psorospermum senegalense* have been reported by Lawal and Abu sa-eed (2013) [5], Jansen *et al.;* in 2008 [7] reported the In vitro antiplasmodial activity of ethnobotanically selected plants from Burkina Faso, in this research the best antiplasmodial results were obtained with *Psorospermum senegalense* dichloromethane extract. Micheal *et al.;* in 2010 [8] also reported the *in vitro* anticonvulsant properties of psorospermum senegalense.

The objective of this research was to evaluate the potential of this plant extracts and phytochemicals on standard microorganism strains which were isolated from hospital.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant material was collected fresh from Zaria, Nigeria in September, 2013. Taxonomical identification was done at the Herbarium of the Biological Sciences Department, A.B.U, Zaria, Nigeria and its voucher specimen with number 900206 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 was percolated 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 vacuum at 40˚C using rotator evaporator and later subjected to air drying to give dried crude extracts.

Phytochemical screening

The hexane, dichloromethane, ethyl acetate and the methanol extracts were subjected to phytochemical screening using standard techniques. The metabolites tested for included, carbohydrates, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

Test for carbohydrates (Molisch's test)

The four crude extracts (1g respectively) were dissolved in about 5 ml of distilled water and heated in a water bath. The solution was filtered. To the filtrate, four drops of Molisch's was added. 3 ml of concentrated sulphuric acid was carefully added to the mixture from the side of the test tube to form a lower layer. A purple color appeared at the interface on all the extracts.

Test for tannins (ferric chloride test)

The four crude extracts were respectively dissolved in 10 ml of the distilled water and shaken vigorously for about 30 seconds. It was allowed to stand. A honey comb like structure was observed in the ethyl acetate which lasted for more than 30 minutes.

Tests for flavonoids (ammonical silver nitrate test)

The four crude extracts (0.5 g) were respectively dissolved in 5ml of distilled water. Four drops of the solution of ammonical silver nitrate were added. The ethyl acetate and the methanol extracts gave a yellowish – brown color while the other two showed no coloration. On heating it was observed that the color turned brownish black.

Test for anthraquinones (Bronstrager's test)

The four extracts (0.5g) were respectively dissolved in 10 ml of chloroform and shook properly and filtered. 5 ml of 10% of ammonia solution was added to the filtrate and stirred. There was no coloration observed in any of the extracts.

Test for cardiac glycoside (kella-killanitest)

The four crude extract (0.5 g) were respectively dissolved in 5 ml glacial acetic acid containing traces of ferric chloride in a test tube was held at an angle of 45˚ and 1 ml of concentrated sulphuric acid was added carefully down the side. All the four samples showed purple ring colour at the interface.

Test for steroids and triterpenes (leiberman-burchards test)

Acetic anhydride (5 ml) was respectively added to 5 ml of each of the four extracts in a test tube. 1 ml of concentrated sulphuric acid was added carefully down the side of the test tube. A pink colour appeared in the chloroform, ethyl acetate and methanolic extracts which later changed into a blue green colour. The petroleum ether extracts showed no coloration

Test for alkaloids

Few drops of Mayer's reagent were added to about 0.5 g each of the four crude extracts in a test tube. There were was no precipitate

Antimicrobial Screening

Antimicrobial activities of the hexane, dichloromethane, ethyl acetate and methanolic extracts were determined using some pathogens.

Collection and preparation of microbial culture

The pure clinical bacteria and fungi isolates of *vancomycin Rest enterococci, Staphylococcus aureus, streptococcus pyogenes, Bacillus Subtillis, Streptococcus* pneumoniae, *Klepsiella pneumoniae, Escherichia coli proteus vulgaris and pseudomonas fluorescense,*) and three human pathogenic fungi (candida tropicalis, candida krusei and *Candida pseudotropicalis)*were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. All the microbes were screened for purity and were maintained in slants of nutrient agar for bacteria and slants of sabouroud dextrose agar for fungi.

Preparation of solution of plant extracts for antimicrobial screening test

Well diffusion method was used for the determination of the antimicrobial activities of the extracts. Each crude extracts was weighed out and dissolved in 10 ml of Dimethyl sulphoxide (DMSO) to obtain a concentration of 10 mg/ml. This was the initial concentration used to check the antimicrobial activities of the extracts.

Media preparation

Muller Hinton and Sabouroud dextrose agar were the media used as the growth media. They were prepared according to the manufacturer's instruction, boiled to dissolve and were sterilized at 121˚C for 15 minutes. The media were cooled to 45˚C and 29 ml of the sterilized media were poured into sterilized petri dishes. The media were and allowed to cool and solidify and then cooled.

Determination of microbial activities of the various extracts

The media prepared above were inoculated with 0.1 ml standard inoculums of the test organisms. The inoculums were spread evenly over the surface of the medium by the use of a sterile swab. The agar plates were seeded with the test organisms and the sabouroud dextrose agar with the fungi. The inoculated plates were incubated at 37 °C for 24 hrs for the bacteria and at 30 °C for 1 – 7 days for the fungi. Standard cork borer of 6 mm diameter was used to cut a well at the centre of each inoculated plate and 0.1 ml of the solution of the extracts was introduced into each well. The plates were then incubated at 37˚C for 24 hours for the bacteria and at 25˚C for 24 hours for the fungi. They were observed after the periods of incubation for zone of inhibition of growth. The zones were measured with a caliper and the values were recorded in millimetres.

Determination of minimum inhibitory concentrations (MIC) of the extracts

The minimum inhibitionory concentration of the extracts was carried out on the test organisms using the broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions. Mc-farlands turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and was dispensed into test tubes and the microorganisms were then inoculated and incubated at 37 ˚C for 6 hours. Dilution of the test microorganisms in the normal saline was performed until the turbidity marched that of the Mac farlands scale by visual comparison. At this point the microorganisms had a concentration of about 1.5 x 10^8 cfu/ml. Two fold serial dilutions of the extracts in the broth were performed to obtain the concentration of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 ml respectively. The Initial concentration was obtained by dissolving 0.1 g of the extracts in 100 ml of the sterile broth. Having obtained the different concentrations of the extract in the broth, 0.1 ml of the standard inoculum of the test microorganism in the normal saline was then inoculated in to the different concentrations. Incubation was made at 37 °C for 24 hrs after which each broth was observed for turbidity. The lowest concentration of the extract in the broth which showed no turbidity was recorded as the Minimum Inhibition Concentration (MIC).

Minimum Bactericidal Concentration/ Minimum Fungicidal Concentration

The minimum bactericidal concentration/minimum fungicidal concentrations (MBC/MFC) were carried out to determine if the test microbes were killed or only their growth was inhibited. Mueller-Hinton agar was prepared and sterilized at 121 ˚C for 15 minutes, poured into Petri dishes and were allowed to cool and solidify. The content of the MIC in

the serial dilution was sub - cultured onto the prepared medium and incubation was done at 37 ˚C for 24 h. Thereafter each plate of the medium was observed for colony growth. The value obtained in the plate with lowest concentration of the extracts without colony growth was recorded as the MBC/MFC.

RESULTS

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

Table 2: Antimicrobial Sensitivity test of the various extracts against test organisms

TEST ORGANISMS	DCM	EA	MEOH	HEXANE
Vancomycin Rest enterococci	**R**	**R**	**R**	**R**
Staphylococcus aureus	**S**	**S**	**S**	**S**
Streptococcus pyogenes	**S**	**S**	**S**	**S**
Bacillus Sitibilis	**S**	**S**	**S**	**S**
Streptococcus Pneumoniae	**S**	**S**	**S**	**S**
klebsiella Pneumoniae	**R**	**R**	**R**	**R**
Escherichia coli	**S**	**S**	**S**	**S**
Proteus Vulgaris	**R**	**R**	**R**	**R**
Pseudomonas flourescense	**R**	**R**	**R**	**R**
Candida tropicalis	**S**	**S**	**S**	**S**
Candida pseudotropicalis	**S**	**S**	**S**	**S**
Candida krusei	R	R	R	R

Key: S= Sensitive; R = Resistance, HE = hexane extract, DCM = dichloromethane extracts, EA = Ethyl acetate extracts, ME = Methanol extracts

Table 3 Determination of Zones of Inhibitory (mm) of the extracts on test organisms

TEST ORGANISMS	DCM	EA	MEOH	HEXANE
Vancomycin Rest enterococci	0	0	0	0
Staphylococcus aureus	25	27	24	20
Streptococcus pyogenes	24	28	22	20
Bacillus Sitibilis	27	31	25	22
Streptococcus Pneumoniae	22	27	20	18
Klebsiella Pneumoniae	0	0	0	0
Escherichia coli	25	27	22	19
Proteus Vulgaris	0	0	0	0
Pseudomonas flourescense	0	0	0	19
Candida tropicalis	24	26	21	18
Candida pseudotropicalis	23	27	20	18
Candida krusei	0	0	0	0

Key: DCM – Dichloromethane, EA = Ethylacetate, MeOH = Methanol, HEX = Hexane

Table: 4 Minimum Inhibitory Concentration (mg/ml) of the extracts against the test organisms

Test Organisms	DCM	EA	MeOH	HEXANE
Staphylococcus aureus	**7.5**	**3.25**	**7.5**	**7.5**
Streptococcus pyogenes	**7.5**	**3.25**	**7.5**	**7.5**
Bacillus Sitibilis	**3.25**	**3.25**	**7.5**	**7.5**
Streptococcus Pneumoniae	**7.5**	**3.25**	**7.5**	15
Escherichia cocci	**7.5**	**3.25**	**7.5**	15
Candida tropicalis	**7.5**	**7.5**	**7.5**	15
Candida pseudotropicalis	**7.5**	**3.25**	**7.5**	15

Key: DCM – Dichloromethane, EA = Ethylacetate, MeOH = Methanol, HEX = Hexane

Table 5: Minimum bactericidal/fungicidal concentration of the extract against the test microbes (mg/ml)

Test Organisms	DCM	EA	MeOH	HEXANE
Staphylococcus aureus	**15**	**15**	**15**	**30**
Streptococcus pyogenes	**15**	**15**	**30**	**30**
Bacillus Sitibilis	**15**		**15**	**30**
Streptococcus Pneumoniae	**30**	**15**	**30**	**30**
Escherichia cocci	**15**	**15**	**30**	**30**
Candida tropicalis	**15**	**15**	**30**	**30**
Candida pseudotropicalis	**15**	**15**	**30**	**30**

Key: DCM – Dichloromethane EA = Ethylacetate MeOH = Methanol HEX = Hexane

Table 6: Antimicrobial Sensitivity test of standard drugs (positive controls)

TEST ORGANISMS	Ciprofloxacin	Sparfloxacin	Fluconazole
Vancomyan Rest enterococci	R	S	R
Staphylococcus aureus	S	S	R
Streptococcus pyogenes	S	S	R
Bacillus Sitibilis	S	S	R
Streptococcus Pneumoniae	S	S	R
Websiella Pneumoniae	S	S	R
Escherichia cocci	S	S	R
Proteus Vulgaris	R	S	R
Pseudomonas flourescense	R	S	R
Candida tropicalis	R	R	S
Candida pseudotropicalis	R	R	S
Candida krusei	R	R	S

Key: S= Sensitive R = Resistance

Table 7: Determination of Zones of Inhibitory (mm) of the standard drugs (positive controls)

TEST ORGANISMS	Ciprofloxacin	Sparfloxacin	Fluconazole
Vancomyan Rest enterococci	0	35	0
Staphylococcus aureus	37	41	0
Streptococcus pyogenes	35	37	0
Bacillus Sitibilis	41	40	0
Streptococcus Pneumoniae	36	38	0
Websiella Pneumoniae	40	37	0
Escherichia cocci	32	35	0
Proteus Vulgaris	0	32	0
Pseudomonas flourescense	0	30	0
Candida propicalis	0	0	35
Canada pseudotropicalis	0	0	32
Canachia tropicalis	0	0	34

Drug Concentration
Ciprofloxacin = 5mg/ml
Sparfloxacin = 5mg/ml
Fluconazole = 5mg/ml

Table 6: Anti-tuberculosis activity of the extracts

	P.senegalense				**Rifampicin**
Extract Concentration (mg/ml)	HE	DCM	EA	ME	
5	NA	NA	+	NA	+
2.5	NA	NA	NA	NA	+
1.25	NA	NA	NA	NA	+
0.625	NA	NA	NA	NA	+
0.3125	NA	NA	NA	NA	+

Key: NA = No activity; + = Activity

DISCUSSION

The antimicrobial sensitivity test revealed that all the extracts tested exhibited moderate to good antibacterial and antifungal activity against five out of the nine tested bacteria and two out of three fungi tested. The ethyl acetate extracts showed the highest activity against *Staphylococcus aureus,, streptococcus pneumonia, Bacillus subtillis, Escherichia coli, streptococcus pyogenes,* candida tropicalis, and *Candida pseudotropicalis*) with a zone of inhibition of -27,-27, -31, -27, -28,-26, and -27 mm respectively (Table3). and minimum inhibitory concentration (MIC) of 3.25mg/ml for all the pathogenic organism above except *Candida tropicalis* which had MIC of 7.5mg/ml The minimum bactericidal/fungicidal concentration (MBC/MFC) was found to be 15 mg/ml against the entire test microorganism (Table 5) which tested positive except bacillus subtillis which had MBC of 7.5mg/ml. The hexane extracts showed the least activity against *Staphylococcus aureus,, streptococcus pneumoniae, Bacillus subtillis, Escherichia coli, streptococcus pyogenes,* candida tropicalis, and *Candida pseudotropicalis*) with a zone of with zone of inhibition of -20, -18, -22,-19, -20, -18 and -18 mm, respectively (Table 3). And MIC of 15 mg/ml for, *streptococcus pneumoniae, Escherichia coli, candidatropicalis*, and *Candida pseudotropicalis*) and MIC of 7.5mg/ml for *Staphylococcus aureus, Bacillus subtillis and streptococcus pyogenes.* The MBC/MFC for the hexane extract was 30 mg/ml, and at this concentration, the extract exhibited activity against all the test microorganism except *klebsiella neumonia, proteus vuLgaris, vancomycin resistant enterococci, pseudomonas flourescense and candida krusei* which none of the extracted tested was active against.

The phytochemical screening of the leaf psorospermum senegalense showed the presence of carbohydrates, alkaloids Cardiac glycosides, steroids and triterpenes in all the four extracts, tannins and flavonoids, were present in the ethyl acetate and methanol extracts. Saponins and anthraquinones were absent in all the four extracts (Table 1).

CONCLUSION

The consequences of this investigation suggest that psorospermum senegalense leave extracts can be used to discover antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria and fungi responsible for severe illness.

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