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Antibacterial Activities of the Fractions of Methanolic Extract of Maytenus Senegalensis (Lam) Excell (Celastracea) Stem Barks against *Escherichia coli and salmonella typhi*

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Abstract:
Antibacterial activities of the crude extract and pure compounds isolated from Maytenus senegalensis have been reported in literatures, but none of the reported compounds have been assigned to be responsible for the activity. This work evaluated the fractions of methanolic extracts of Maytenus Senegalensis against Escherichia coli, and salmonella typhi. The stem bark of the plant was extracted by cold maceration using methanol. Column Chromatography was carried out on the crude methanol extract using solvents of varying ratios and178 fractions were obtained. Thin layer Chromatography (TLC) analysis of the resulting fractions led to eight combined fractions that were pooled together based on their TLC profile. Antibacterial assay of the crude extracts and the pooled fractions (F'_1-F'_{11} was carried out on the test organization using the agar disc diffusion method. The result showed that the test organizations were both susceptible to the crude extracts and the pooled fractions. The crude extract,fraction F'_6, F'_7, F'_8, F'_9, F'_{10}, F'_{11}had 21±1.0,8±2.0, 16±2.0, 18±2.0, 16±0.5, 14±2.0, 11±1.0, diameter of zone of inhibition on S.typhi while that of E. coliwere 19±1.0, 7±2.0, 15±2.0, 17±2.0, 15±0.5, 13±2.0, 10±1.0 respectively. The results show that the fraction eluted withnon-polar solvents are not active on the test organism while polarfractions have good activities. This further confirmed that water will be good extracting agents for the active metabolites of the plants.

Keywords: *Maytenussenegalensis, Column chromatography, TLC, Antimicrobial activity, and Antibacterial*

1. Introduction

The medicinal value of plants lies in some natural constituents that produce a definite physiological action on human body. Plant-based natural constituents can be derived from any part of the plant like barks, leaves, flowers, roots, fruits and seeds [18]. These natural constituents of plants include alkaloids, flavonoids, saponins, tannins, steroids and phenols.According to World Health Organization (WHO) report of 2001, about 80% of the world's population depends on plants as a source of medicine, especially for millions of people in the rural areas of developing countries[19].These formed the basis of healthcare throughout the world since the earliest days of humanity and are still widely used[13].The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicines[15]. The importance of medicinal plants as well as their importance in pharmaceutical industry has been elucidated by Edeoga, et al [3]. Medicinal plants have constituted the main source of new pharmaceuticals and healthcare products[19][.The search for plants with broad pharmacological activities but low toxicity has increasingly gained importance in recent years. In Nigeria, different plant species are extensively used in traditional medicine to treat different diseases.*M. senegalensis* belong to the celastraceae family andis one of the plant commonly used in North central Nigeria for the treatment of tooth ache, tooth abscesses and mouth infections, gastro-intestinal troubles gastric ulcers, Jaundice and coastal, diarrheaand dysentery (oral interviewed). It is a shrub tree, armed with thorn like branches on which leaves and

flowers grow (flowers are hermaphrodite with five sepals and petals)[6]. *M. senegalensis* has a wide variety of habitat ranging from open woodland, wooded grassland, bush land, scrubs and riverine area[8].It is use in the treatment of diabetes and other ailments in Nupeland, North Central Nigeria[6][1]. The leaves of *M. senegalensis* are traditionally used in West Africa for the management of cancer and other disorders associated with inflammation[5].*M.senegalensis* leaves are also used for treatment of diarrhea and intestinal worms in calf, dog bites[10]; respiratory ailments, and tuberculosis[11]. The decoction of the root bark of the plant is widely used in Sudan and other African countries in the traditional medicine to treat malaria [14]. The methanolic and water extracts of *M. senegalensis* has shown moderate inhibitory effects against HIV-1 protease[7]. Acetone extracts of *M.senegalensis* is active on *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus*with minimum inhibitory concentration value of 0.32 to 0.33 mg/ml[13]. Another study by Sosa et al[16] demonstrated the topical anti-inflammatory properties of *M.senegalensis* root extract and, in particular those obtained with lipophillic solvents (n-hexane and chloroform); this activity was due to the presence of maytenoic acid, which was found to be an anti-inflammatory triterpene twice as active as the NSAID (Non-steroidal anti-inflammatory drug) indomethacin and only three times less active than hydrocortisone [16]. Ghazi et al., reported the isolation the following compound-:(-) – 4-methylepigallocatechin 5- O-β-glucopyranoside, (+) – 4-methylepigallocatechin 3- O-β-glucopyranoside, and (-) – 4-epicatechin (4β-8) (-) – 4-methyl epigallocatechin from the methanol extract of the stem-bark of *Maytenus senegalensis*[22].Also, Festus et al., reported the isolation of sixteen compounds from the methanol leave extract namely-: (6S,9S) 9,10-dihydroxy-4,7-Z-megastigmadien-3-one, mayselignoside A and maysefurapyranone A, (+) lyoniresinol, (-) isolariciresinol, dihydrodehydrodiconiferyl alcohol, (-) epicathechin, (+) gallocatechin, (-) epigallocatechin, procyanidin β-2,3-dihydrokaempferol 3-O-β-D-glucopyranoside, quercetin 3-O-β-D- glucopyranoside, kaemferol 3-O-β-D-xylopyranoside, 3,5-dimethylgallate and benzoyl maleic acid[5]. All the compounds elucidated by Festus et al; were tested for cytotoxicity against mouse lymphoma cell line (L5178Y), only (-) epigallocatechin was able to showed high cytotocixity and completely inhibited cell growth at the investigated dose of 10 µg/mL. In a similar work, Sami, et al., reported the isolation of (20α)-3- hydroxyl-2-oxo-24-no-friedela-(10),3,5,7-tetraen-carboxylic acid-(29)-methyl ester (pristimerin) [14] from the leaves of the plants. All these compound that were isolated were not tested against all the bacteria that were susceptible to the crude extracts. The work was aimed at testing antibacterialactivities of the column chromatography fractions/compounds of *M.senegalensis* which can be use forthe formulation of herbal medicines.

2. Methodology

2.1. Collection of Sample

The plant material (*M. senegalensis*) was collected in Jing village of Pankshin Local Government Area of Plateau State, Nigeria around March, 2014. The plant material was originally identified and authenticated by a taxonomic ethno-botanist from the Federal College of Forestry Jos, Plateau State, Nigeria, where the voucher specimen was deposited.

2.2. Sample Preparation

The plant material (stem-bark) was air dried at room temperature under shade, and pulverized with mortar and pestle. The powder was packaged into polyethylene bags and stored in dry and well- ventilated room until used. Extraction was done by cold maceration.The dried powdered plant material(500g) was soaked in methanol for 24 hours with constant shaken at intervals to enhance the extraction. The process was repeated until extraction was completed (this was indicated by a faint coloured marc). The extract was sieved and filtered using Whattman filter paper size No.1.Concentration of the extract (filtrate) was carried out using a BuchiRotavapour R-114 at 40 °C. The crude extract (dried concentrate) was stored in labeled sterile screw capped sample bottles at 5 °C in the refrigerator until when required for used.

2.3. Column Chromatography/ Fractionation

Fractionation of the crude methanolic extract of the plant was carried out using a column (24/40 England). The end of the column was blocked with cotton wool and the wet method of column packing was employed using n-hexane. Adsorption of the extract was carried out using 1:3 w/w of extract to adsorbent ratio. The extract (15g) was dissolved with little mixture of ethyl acetate and methanol, the solution so form was adsorbed on the silica gel (80-120) mesh size and was smeared on an open porcelain mortar to enhance evaporation of the solvent. After the solvent, has evaporated, it was carefully ground to powder. The adsorbed extract was added to the top of the packed column and elution was carried out using solvent/solvent mixtures in order of their increasing polarity. Starting with 100% hexane, the polarity was increase with ethyl acetate (5% to 100%) and methanol (5% to100%). During the elution, 100ml of the eluates were collected into 250 ml beakers which resulted to 178 fractions. The different fractions collected were examined for their homogeneity by TLC and those fractions containing the same TLC profile were pooled together. The eluting solvent was then allowed to evaporated at room temperature to isolate the organic compound(s) which were then stored in sample bottles for the microbial test.

2.4. Thin Layer Chromatography (TLC) Analysis

TLC was carried out on the crude extract using different solvent systems (Hexene:ethyl acetate 4:1, 2:1, 1:1 and ethylacetate: methanol 9.5 :0.5, 9:1,4:1).Development of the chromatograms were done in a closed tank in which the atmosphere had been saturated with the eluent vapour by lining the tank with filter paper.Also, during the fractionation process, TLC was also carried out to examine the homogeneity of the different fractions collected using the various solvent system. Visible bands were marked with pencil under Uv/visible light before either viewing under iodine crystals or spraying with H_2SO_4 and heating for optimal colour development[11].R_f values were calculated for all the TLC carried out and the results are as presented in the table1.

2.5. Preparation of Medium

Muller Hinton Agar was the medium of choice. Muller Hinton Agar (38 g; Biotech UK) medium was suspended in one liter (1L) of distilled water, and brought to boil to dissolve the medium completely before sterilizing by autoclaving at 121 °C for 15 minutes. The medium was later dispensed into 90 mm sterile agar plates (10 mls each) and left to set. The agar plates were incubated for 24 hours at 37 °C to confirm their sterility. When it was observed that no growth had occurred after 24 hours, the plates were considered sterile.

2.6. Preparation of McFarland Standard

Exactly 0.5 McFarland equivalent turbidity standard was prepared by adding 0.5 ml of 1% Barium chloride solution ($BaCl_2.2H_2O$) to 99.5 ml of 1% sulfuric acid solution and mixed thoroughly. A small volume of the turbid solution was transferred to cap tube of the same type that was used to prepare the test inoculum. This was then stored in the dark at room temperature. Exactly 0.5 McFarland gives equivalent approximate density of bacteria 1 to 2 x 10^8 (CFU)/mL [2][17].

2.7. Inoculum Preparation

The Growth method of inoculation was used to prepare the inoculums. Three to five well – isolated colonies of the test organisms were individually scooped with the help of a loop. The growth was transferred into a tube containing 5ml of prepared bacteriological peptone water. The broth culture was incubated at 35 ±2 °C until it achieves the turbidity of the 0.5McFarland standard (usually two to six hours) [2][17].

2.8. Test Organisms

The Clinical isolates (*Escherichia coli* and *Salmonella typhi*)were collected from stock cultures of Central Diagnostic Laboratory, Nigeria Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The isolates were characterized and maintained on DCA culture at 4°C prior to use.

2.9. Preparation of Extract Concentrations

The Extract (both the crude and the various pooled fractions obtained during column chromatography) were prepared at varying concentration ranging from 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.825 mg/ml, 3.912 mg/ml, and 1.956 mg/ml using DMSO as the solvent for dissolution.

2.10. Susceptibility Testing

The Sensitivity of the test organisms, *Escherichia coli* and *Salmonella typhi* to the crude extract and the various fractions of the plant extract were carried out using Kirby-Bauer Antimicrobial susceptibility (Agar disc diffusion method) [17].

Antimicrobial disks were prepared by punching Whattman filter papers with a paper punching machine and the disks were autoclaved at 160 °C for 15 mins to sterilize them. Thereafter, the disks were impregnated with the various prepared concentrations of the extracts by the use of micropipettes. The disks are place in a petriplates separately and the extracts added using the micropipettes one after the other according to their prepared concentrations until the disks absorb the extracts [17].

A sterile swap was used to inoculate evenly the test specie on the surface of the medium. The plates were allowed to dry for about 5 mins. Flame sterilized forceps were used to place the impregnated disk on the surface of the agar. The disks were placed at equal distances apart on the surface of the agar. The disks were also gently pressed onto the surface of the agar using a sterile forcep or wire loop. The plates were inverted and incubate for 24 hours at 37 °C. Using caliper, the diameter of the zone of inhibition(s) was measured for each extract used.

3. Results and Discussion

Thirty-eight point seven four grams (38.74 g) of the crude extract was recovered; it's chocolate in colour. Fifteen grams (15g) of the extracts was subjected column chromatography and thin Layer Chromatography. The fractions/compounds were evaluated for antimicrobial activities of against the test organisms and the results are shown in the tables 1-4. The result of this work indicates that the Methanolic extract of the stem bark of *M. senegalensis* contain various metabolites as in indicated by the different R_f values table 2. The pooled fractions (F'_5, F'_8, F'_{10}, F'_{11}) have single R_f values while the others pooled fraction (F'_1, F'_2, F'_3, F'_4, F'_6, F'_7, F'_9)has two to four R_f values. These R_f value(s) signifies the characteristics of a compound and show that those pooled fractions with single Rf values are pure compounds. The TLC for the pooled fractions F'_5, F'_8, F'_{10}, F'_{11} were run using three different solvent system and all have a single Rf values. These implies that these fractions are single compounds. The sharp melting of the pooled fractions (F'_5 = 295 ^oC, F'_8 = 201 ^oC, F'_{10} = 195 ^oC, F'_{11} = 193^oC) further confirmed the purities of the fractions.

The crude extracts and fractionation of *Maytenus senegalensis's* stem bark have antimicrobial activity. The extracts have widest zones of inhibition against *Salmonella typhi* followed by *Escherichia coli.* These differences in zones of inhibition may be directly related to the susceptibility of each organism to the crude extract and the various fractions obtained by column chromatography.

The result of the antimicrobial susceptibility test indicates that the crude extract show high activity on both the test organisms, having the highest zone of inhibition of diameter 21±1mm at 500mg/ml on *Salmonella typhi* while *Escherichia coli* is susceptible with a zone diameter of 19±0.5mm at 500mg/ml. The lowest concentration of 1.956mg/ml have zone of inhibition of diameter of 7±1.0mm and 9±1.2mm on *E. coli* and *S. typhi* respectively. Pooled fraction F'_1, F'_2, F'_3, F'_4, having more than one R_f values showed no activity with all the prepared concentrations on the test organisms. F'_5 with a single Rf values shows no activity at all concentration on all the test organism. These fractions were eluted with the solvents system of hexane: ethyl acetate (100:0) to ethyl acetate: hexane (90 :10). $F_{6'}$

having three $R_f$values (0.34,0.45,0.63) has zone of inhibition diameter with a value of 7±2 mm and 2±2.0 mm at the highest concentration of 500 mg/ml and lowest of 15.625mg/ml for *Escherichia coli* while *Salmonella typhi* has 9±1.3 mm and 4±2.0mmzone of inhibition diameter respectively. The zone of inhibition increase from F'7 to F'8 and gradually started decreasing from F'9 to F'11 as shown in table 3 and 4.$F_{8'}$ with a single Rf values has the highest susceptibly among all the pooled fractions with *Salmonella typhi* having zone of inhibition diameter of 20±1 mm at 500 mg/ml while *Escherichia coli* have zone of inhibition diameter of 17±2 mm at 500 mg/ml.Also, at a lower of 1.956 mg/ml Salmonella *typhi* having zone of inhibition diameter of 5±1.2 mm while *Escherichia coli* have zone of inhibition diameter of 3±1.2 mm. It's great to note here that at these lowest concentrations of 1.5625 mg/ml for crude extracts have activities on the test organisms, their zone of inhibition diameter is both greater than 5mm. F'_8 also, has zone of inhibition diameter are greater than 5mm. The result show that the extracts have activity on the tested organisms, which confirm the use of the plants by healer for the treatment diarrhea.

4. Conclusion

From the foregoing discussion, it may be concluded that the crude methanolic extract of *M. senegalensis* and some of its pooled fractions obtained by column chromatography are susceptible to *Escherichia coli* and *Salmonella typhi.* The crude extract and column guided fractions $F_{6'}$, $F_{7'}$, $F_{8'}$, $F_{9'}$, $F_{10'}$, and $F_{11'}$ have zone of inhibition diameters greater than or equal to 6mm and as mention earlier, these inhibition value signifies susceptibility to the test organism.

More so, of all these fractions, not all of the fractions show zones of inhibition, while some of the fractions show significant zone of inhibition, fractions $F_{8'}$, F_{10}, and F_{11}which contain single constituent show the best zone of inhibition. inhibition on *S. typhi* while that of *E. coli* were 19±1.0, 7±2.0, 15±2.0, 17±2.0, 15±0.5, 13±2.0, 10±1.0 respectively. The results show that the fraction eluted withnon-polar solvents are not active on the test organism while polarfractions have good activities. The two tested organisms are common cause of gastro-intestinal tract infections. It's of no surprise that many traditional medical practitioners and people interviewed mention the plant as a remedy for gastro-intestinal disorder and dysentery.

S/No	Solvent systems			
	Hexane (%)	**Ethylacetate (%)**	**Methanol (%)**	**Fractions**
1.	100	0	0	F_1 - F_4
2.	95	5	0	F_5 - F_{16}
3.	90	10	0	F_{17} – F_{28}
4.	80	20	0	F_{29} – F_{39}
5.	70	30	0	F_{40} – F_{48}
6.	60	40	0	F_{49} – F_{50}
7.	50	50	0	F_{51} – F_{65}
8.	40	60	0	F_{66} – F_{68}
9.	30	70	0	F_{69} – F_{70}
10.	20	80	0	F_{71} – F_{83}
11.	10	90	0	F_{84} – F_{86}
12.	0	100	0	F_{87}– F_{104}
13.	0	95	5	F_{105} – F_{106}
14.	0	90	10	F_{107} – F_{115}
15.	0	80	20	F_{116} – F_{139}
16.	0	70	30	F_{140}– F_{152}
17.	0	60	40	F_{153} – F_{161}
18.	0	50	50	F_{162} – F_{170}
19.	0	40	60	F_{171} – F_{178}

Table 1: Showing thevarious solvent systems used and the fractions obtained from the Column Chromatography

S/No.	Pooled fractions	RF values	Solvents systems	Yields (g)
1.	F'1	0	Hexane: EtoAC (9:1)	0.20
2.	F'2	0.70, 0.80,0.86	Hexane: EtoAC (4:1)	1.13
3.	F'3	0.61,0.70,0.80,0.86	Hexane: EtoAC (2:1)	2.06
4.	F'4	0.61,0.70,0.80,0.86	Hexane: EtoAC (1:2)	1.13
5.	F'5	0.58	EtoAC:MeOH(9.5:0.5)	0.01
6.	F'6	0.34,0.45,0.63	EtoAC: MeOH(9:1)	1.11
7.	F'7	0.85,0.95	EtoAC:MeOH(9:1)	0.15
8.	F'8	0.81	EtoAC:MeOH(4:1)	0.02
9.	F'9	0.66, 0.76	EtoAC:MeOH(4:1)	0.12
10.	F'10	0.71	EtoAC:MeOH: H2O(4:4:1)	0.01
11.	F'11	0.79		0.02

Table 2: Rf values of the various Pooled fractions using different solvent ration

EtOAC =Ethyl acetate, MeOH=methanol

Concentration (mg/ml	Crude extract	F'1	F'2	F'3	F'4	F'5	F'6	F'7	F'8	F'9	F'10	F'11
500	19±0.5	-	-	-	-	-	7±2.0	15±2.0	17±2.0	16±0.5	14±0.5	15±1.0
250	18±1.0	-	-	-	-	-	6.5±1.0	13±1.0	15±1.1	14±1.0	12±1.0	14±0.7
125	17±1.5	-	-	-	-	-	6±0.8	12±1.0	14±0.6	13±0.5	10±1.5	11±1.0
62.50	15±1.3	-	-	-	-	-	4±1.0	10±1.3	12±0.4	±0.4	9±1.3	9±1.3
31.25	13.5±0.7	-	-	-	-	-	4±1.0	9±0.7	11.5±0.	10.5±0.7	7.5±0.7	8.5±2.0
15.625	11±1.0	-	-	-	-	-	2±2.0	7±1.0	9±1.0	8±1.0	5±1.0	7±1.0
7.823	9±0.5	-	-	-	-	-		5±1.0	8±0.5	5±0.5	4±0.5	6±0.5
3.912	8±1.2	-	-	-	-	-		4±1.2	4±1.0	3±1.2	-	3±1.2
1.956	7±1.0	-	-	-	-	-		-	3±1.2	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Sensitivity pattern of the crude extracts and column chromatography pooled fractions against Escherichia coli

Concentration (mg/ml	Crude extract	F'1	F'2	F'3	F'4	F'5	F'6	F'7	F'8	F'9	F'10	F'11
500	21±1.0	-	-	-	-	-	9±1.3	16±2.0	18±2.0	16±0.5	14±0.5	11±1.0
250	20±0.8	-	-	-	-	-	8±2.0	14±1.0	17±1.1	14±1.0	12±1.0	10±0.7
125	19±1.3	-	-	-	-	-	7±1.4	13±1.0	15±0.6	13±0.5	10±1.5	9±1.0
62.50	17±2.0	-	-	-	-	-	6±1.0	11±1.3	12±0.4	±0.4	9±1.3	8±1.3
31.25	16.5±1.5	-	-	-	-	-	5±1.0	9±0.7	11.5±0.	10.5±0.7	7.5±0.7	7.5±2.0
15.625	14±0.6	-	-	-	-	-	4±2.0	7±1.0	9±1.0	8±1.0	5±1.0	6±1.0
7.823	13±1.0	-	-	-	-	-		5±1.0	8±0.5	5±0.5	4±0.5	4±0.5
3.912	11±0.	-	-	-	-	-		4±1.2	6±1.0	3±1.2	-	3±1.2
1.956	9±1.2	-	-	-	-	-		-	5±1.2	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Sensitivity pattern (zone of inhibition) of the crude extracts and column chromatography pooled fractions against Salmonella typhi

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