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Research Article

PHYTOCHEMICAL SCREENING AND INVESTIGATIONS OF ANTIBACTERIAL ACTIVITIES OF VARIOUS FRACTIONS OF THE ETHANOL LEAVES EXTRACT OF *Moringa oleifera* LAM (*Moringaceae*)

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ABSTRACT
Several reports on the phytochemical screening and antibacterial assays on *M. oleifera* leaves extract have been documented but none of the reports showed the fractionation and separation of the phytoconstituents by solvent extraction and thin layer chromatographic procedures, respectively. The ethanol leaves extract of *M. oleifera* of the family *moringaceae* was investigated for antibacterial activity, preliminary phytochemical analysis, solvent extraction and TLC analysis. These were carried out using standard procedures. The results of the preliminary phytochemical screening revealed the presence of saponins, condensed tannins, flavonoids, terpenoids, steroids, phenolics, alkaloids, phlobatannins, cardiac glycosides and reducing sugars. The TLC separation of the phytoconstituents using chloroform-methanol solvent system resolved the fractionated extract into compounds with R_f values; 0.32, 0.53, 0.54, 0.55, 0.69, 0.89, 0.95 and 0.97. The antibacterial assay results portrayed broad activity spectrum against the test microbes with comparable inhibitory zones by standard antibiotics. The MIC ranged between 10mg/ml and 9Omg/ml for all the organisms. The results from this research have shown the antibacterial potentials of *M. oleifera* implying that the extract could help as a chemotherapeutic agent or might be a lead compound for the development of new potent antibacterial agents.

Keywords: solvent extraction, fractionation, nutraceuticals, TLC, R_f, MBC, MIC.

INTRODUCTION

M. oleifera Lam. is the most cultivated species of a monogeneric family, the *moringaceae* that is native to the sub-Himalayans regions of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree, also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, Baijhan, Mulangay, Sajna or Ben oil tree, was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated in the tropical and subtropical regions.

In the 1940s, a team from the University of Bombay, Travancore University and the Department of Biochemistry at the Indian Institute of Science in Banglore, identified a compound which dissociates into two molecules of benzylisothiocyanate and have antimicrobial activities. However, an in vitro bacterial cultures and observational studies provided a plausible mechanistic underpinning for the plethora of efficacy claims on *M. oleifera*.

In a common sight in rural parts of India, the plant is justifiably described as the miracle tree. It is known as murungai in Tamil, Nadu and Kerala. In East Africa, it is described as mother's best friend *(http//www.dynamicyouth.org/index.php?).* The

plant has an impressive range of medicinal uses with high nutritional value. Different parts of this plant such as the leaves, roots, seeds, barks, flowers, and immature pods contain a quantum of crucial phytoconstituents such as tannins, saponins, alkaloids, steroidal aglycones, reducing sugars, terpenoids, and so on, that act as a cardiac and circulatory stimulants, posses antitumour, antipyretic, anticonvulsant, anti-inflammatory (Kumar, *et al.* 2009), antiulcer, antispasmodic, antidiabetic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antifungal, abortifacient, antibacterial, (Anwar and Rashid, 2007; Ghebremichael, *et al.*, 2005; Lockette *et al*, 2000; Walter *et al*, 2011), antiretroviral, antispeticaemic, antidiarrhoeal, hepatorenal disorders, cardiovascular, gastrointestinal and haematological disorders (Paliwal *et al.*, 2011), anxiety, asthma, bronchitis, cough, diarrhoea, conjunctivitis, cephalgia, arthralgia, psoriasis, semen deficiency, helminthiasis, lactation, pregnancy and diabetes (Nikkon *et al.*, 2003), and they are being employed for the treatment of different ailments in the indigenous system of medicine (Posmontier, 2011; Fahey, 2005; Fakurazi *et al.*, 2008). The plant contains more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than banana, lowers blood sugar in diabetes and lowers blood pressure in hypertension (Fuglier, 1999). This is why *M. oleifera* is praised as nutritional and medicinal cornucopia *(http//www.itdnow.co/benefits).*

Plant Classification (Taxonomy)

Kingdom
Plantae — plants
Subkingdom
Tracheobionta — vascular plants
Superdivision
Spermatophyta — seed plants
Division
Magnoliophyta — flowering plants
Class
Magnoliopsida — Dicotyledons
Subclass
Dilleniidae
Order
Cappareles or Violes
Family
Moringaceae — Horseradish tree
Genus
Moringa Adans — Moringa
Species
M. oleifera Lam — Horseradish tree *(http//www. plants.usda. gov/javalprofile?symbol=moo1).*

Study Objectives

It is the purpose of this research work;

- To identify the phytochemicals present in the ethanolic leaves extract of *M.oleifera*
- To fractionate the phytoconstituents in the crude extract using solvent or liquid — liquid extraction.
- To separate the phytoconstituents using thin layer chromatography (TLC).
- To validate its folkloric medicinal use in bacterial chemotherapy by carrying out an in vitro antibiotic assay to simulate the phytochemicals' antibacterial potentials.
- To assess the efficacy of the ethanol extract in the therapy of urinary tract infections, respiratory tract infections and septicaemia.
- To suggest directions for further clinical research that could be carried out by local investigations in the developing countries.
- To highlight claims from the tradomedicinal lore that would benefit from further rigorous scientific evaluation.

MATERIALS AND METHOD

Reagents used -

Table 3: List of the reagents used and their manufacturers

Chemical/Reagents	Manufacturers
• Ferric chloride	Lab Tech Chemicals
• Iodine	Fisher Scientific Company, Chemical, Manufacturing Division, Fair Lawn, New Jersey, USA.
• Potassium iodide	Lab Tech Chemicals
• Sodium Carbonate	May and Baker Nigeria Limited and BDH Chemicals Ltd., Poole, England.
• Sodium hydroxide	Labtech Chemicals
• Sodium hydrogen carbonate	East Anglia Chemicals
• Crystal violet	BDH Chemicals Ltd., Poole, England.
• Copper sulphate	BDH Chemicals Ltd., Poole, England.
• Chloroform	BDH Chemicals Ltd., Poole, England.

• Ethanol	BDH Chemicals Ltd., Poole, England.
• Acetic anhydride	BDH Chemicals Ltd., Poole, England.
• Methanol	BDH Chemicals Ltd., Poole, England.
• Ethanoic acid	BDH Chemicals Ltd., Poole, England.
• Sulphuric acid	Fisons Plc., Scientific Equipment Division, Liughborough, LEII Org., England.
• Hydrochloric acid	May and Baker Laboratories, England.
• Distilled water	Pharmaceutical Chemistry Laboratory, Delsu, Abraka.
•Dimethyl sulphoxide	Kermel Laboratories, China.

2.2 Apparatus/Equipments

- Test tubes
- Measuring cylinders
- Glass stirrer
- Pyrex beakers
- Conical flasks
- Volumetric flasks
- Fisher Scientific heating mantle
- Superfit rotary evaporator
- Hot air oven
- Inoculating loop
- Autoclave
- Separating funnel
- Burchner`s funnel
- Whatman`s funnel
- Thin Layer Chromatographic plates

Collection and Authentication of Plant Materials

The *M. oleifera* Lam leaves were obtained from Agbor in Ika North-East Local Government Area, Delta State, Nigeria, on March 26, 2012. A specimen of the plant was authenticated by the Pharmacognosy and Tradomedicine Department, Faculty of Pharmacy, Delta State University, Abraka, Delta State and some of the plant seedlings were deposited at the Pharmacognostic Garden of the afore-mentioned institution.

Preparation of Plant Extracts

The leaves were washed with water, air-dried for two weeks and then pulverised using an electric mill. 300 grams of the powdered plant materials was macerated with a- 1000 ml (1 litre) solution of 70% v/v Ethanol for 2 days with intermittent shaking. The percolates were then filtered with Whatman's No. 1 filter paper and the filtrate was concentrated *in vacuo* at 40°C under reduced pressure using a rotary evaporator. The concentrated extract was stored at 4°C until it was used.

Preliminary Phytochemical Analysis

Preparation of Extract for Phytochemical Screening

5 grams of the extract was dissolved to 100 ml with distilled water. This was shaken thoroughly prior to preliminary phytochemical screening.

Phytochemical Screening

Qualitative phytochemical screening of the crude extract was performed according to Parekh and Chanda (2007) to identify the various active chemical constituents. Alkaloids were detected by Mayer's reagent, Dragendorrf's reagent and Wagner's reagent, while for cardiac glycosides, the Keller-Killiana test was carried out. Steroids were detected using the Salkowski's test and Liebermann-Burchard test. The frothing and emulsion tests were used to detect the presence of saponins. Terpenoids were detected using the Salkowski's reaction, reducing sugars using Fehling's reaction and tannins` detection was done using Ferric chloride solution.

Solvent Extraction (Stas-Otto Liquid-Liquid Separation) of the Crude Extract

5 grams of the crude extract was heated with l00 millilitres (ml) of 3M Hydrochloric acid solution under reflux for 3 hours. 30 m1 of the hydrolysed extract was measured into a clean separating funnel. 20 m1 of chloroform was added and the mixture was shaken to facilitate the preferential partitioning of the phytoconstituents into the solvents. Then, another 20 ml of chloroform was added and the elution of the chloroform phase was done. This was labeled the CHLOROFORM PHASE.

To the aqueous phase in the funnel was added 15 ml of 10%w/v sodium hydroxide solution to basify it. 20 ml of Chloroform was added to the basified extract and the elution of the chloroform extract was done. This was labeled Fraction D and the sodium hydroxide extract portion was labeled fraction E.

To the chloroform phase was added 20 ml of 8%w/v Sodium bicarbonate solution to extract the strongly acidic compounds. The chloroform portion was eluted into a beaker. The sodium bicarbonate extract was labeled fraction A. 15 ml of 10%w/v Sodium hydroxide solution was added to the chloroform phase and the separating funnel was shaken. The chloroform portion was eluted and it was labeled fraction C. while the sodium hydroxide portion was labeled fraction B.

The diagrammatic representation of the solvent extraction procedure as described by Olaniyi,

A.A. and Ogungbamila, F.O., (1998), is shown below:

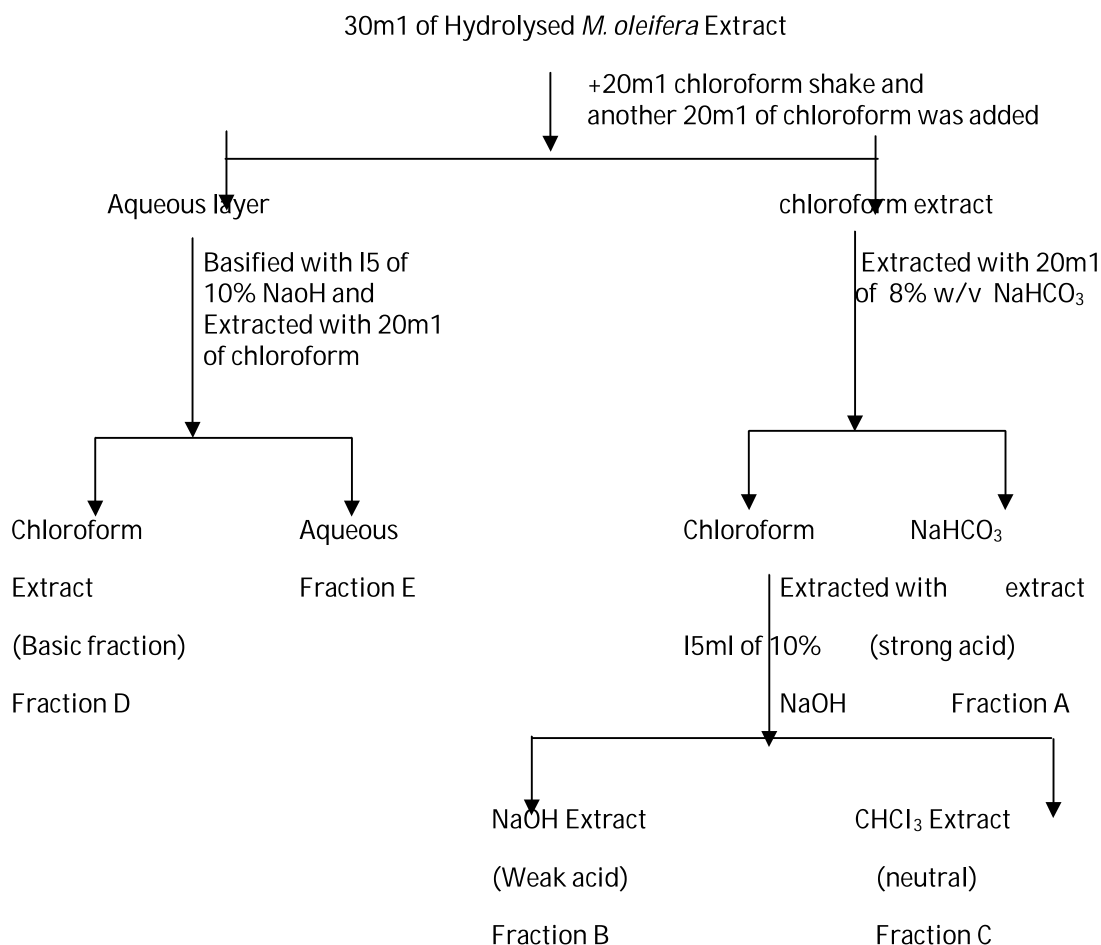

Fig . 4: Schematic Representation of Solvent Extraction of *M. oleifera* Leaves Extract

Phytochemical screening of the different fractions- Fraction A, Fraction B, Fraction C, Fraction D and Fraction E, were carried out according to the afore-mentioned methods.

Thin Layer Chromatographic Analysis

Preparation of the Solvent System

30 m1 of chloroform was measured into a beaker. 70 m1 of methanol was added and they were mixed thoroughly. The chloroform-methanol solvent system was transferred into a clean chromatographic developing tank and covered for 1 hour. The solvent system was allowed to saturate the developing tank with its vapour to ensure equilibration.

Preparation of TLC Plates for Development

2 cm was measured from one end of the thin layer chromatographic plate. This was taken as the point of origin. Each fraction of the extract was spotted along the 2 cm mark, 2 cm apart from one another using a narrow capillary tube. The procedure was repeated to increase the concentration of the fractionated extract. The spots were allowed to dry before they were placed into the developing tank containing saturated vapour of the solvent system. The TLC plates were developed until the solvent front got to the predetermined mark (10.4cm) on the TLC plate. The plates were then viewed using a ultra-violet lamp and the various R_f values of the spots were calculated using the formula stated below as described by Evans W.C. (2009)

$$Rf = \frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent front}}$$

Antibacterial Assay

Test Micro Organisms

Four clinical strains of human pathogenic bacteria made up of *E. coli*, *K. oxytoca*, *S. saprophyticus* and *P. aeruginosa*, were used for the antibacterial assay. The organisms were obtained from the laboratory stock of the Hospitals Management Board, Central Hospital, Agbor, Delta State.

Antimicrobial Agents (Control Antibiotics):
Ofloxacin was used as the positive control for the antibacterial assay.

Media
Mueller Hinton Agar
Mueller Hinton Nutrient Agar
Nutrient Broth (Titan Biotech LTD, Bhiwadi-301019, Rajasthan, India.).

Preparation of Media
Mueller Hinton Agar (MHA)
3.8 grams of MHA was properly homogenised in l00ml of distilled water using a water bath maintained at 60°C. It was then dispensed into conical flasks and the flasks were corked properly before placing into the autoclave for sterilization at 121°C for 15 minutes.
The Mueller Hinton Nutrient Agar and nutrient broth were prepared using the afore-mentioned procedure.

Identification of Test Organisms
Colonies of the clinical isolates were subcultured on nutrient agar to restore normal growth before examination. The organisms were identified using microscopy (by Gram staining procedures), cultural growth examination and biochemical tests (BPC, 1994).

Preparation and Standardization of Bacterial Cultures
The identified clinical isolates were subcultured on nutrient agar slant and stored at 4°C in a refrigerator until they were used. A loopful of the test organisms was inoculated unto a nutrient agar plate and incubated at 36±1°C for 24 hours. Discrete colonies on the 24-hour culture plate were collected using a sterile wire loop and inoculated into a 5ml sterile nutrient broth and incubated for 30 minutes. The test tubes were shaken thoroughly and the turbidity of the bacterial suspension were adjusted by comparing it with the 0.5 Mcfarland standard to obtain 10^6 cfu/ml bacterial culture.

Preparation of Extract for Antibacterial Assay
30mg of the crude extract was dissolved with 1ml of 50% v/v DMSO and efficient stirring of the solution was ensured. The same procedure was repeated using 60mg and 90mg of the crude extract.

Determination of Antibacterial Susceptibility
The agar well diffusion method was used to screen the antibacterial activity of the plant extract and it was performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1999) with minor modification under aseptic conditions. Bacterial suspension was diluted with sterile physiological solution (0.9% sodium chloride solution) to 10^6 cfu/ml (turbidity = McFarland Standard 0.5). Agar wells were borne on the already set agar plates using a sterile cork borer. Bacterial suspension was swabbed uniformly on the surface of the MHA plate with a sterile swab streaker and the inoculums was allowed to dry for 5 minutes. 30 mg/ml of plant extract was meticulously placed into the MHA well. This procedure was repeated using 60 mg/ml and 90 mg/ml. 50%v/v Dimethyl sulphoxide (DMSO) solution was used as negative control while the standard antibiotic Ofloxacin, was used as positive control. The inoculated plates were allowed to stand for 10 minutes to allow for diffusion extract and then incubated at 37°C for 24 hours in an incubator. The diameters (mm) of the inhibition zones were measured (diameter of agar well 6.5 mm being included) using a transparent measuring millimetre ruler. The study was performed in triplicate and the results were expressed as means±standard deviation of the three parallel measurements.

Determination of the Minimum Inhibitory Concentration (MIC) Of The Plant Extract
The determination of the MIC was carried out using ten concentrations; 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml and 100 mg/ml.
1 ml of 1 mg plant extract was measured into a sterile petri dish. 19 ml of MHA was then added and the mixture was swirled to enhance efficient miscibility of the agar and the extract. Upon setting of the agar-extract plates, standardized bacterial inoculums was streaked over the surface using a sterile swab streaker. The same procedure was repeated using 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml and 100 mg/ml. Two agar plates were also prepared, one containing sterile MHA only while the other plate contained MHA and the bacterial inoculums. The plates were allowed to stand for 10 minutes on a workbench to allow for the diffusion of the extract and they were then incubated at 37oC for 24 hours. The study was performed in triplicates and the least concentration of the extract that showed no visible growth was taken as the MIC of the extract.

Determination of Minimum Bactericidal Concentration (MBC) of Plant Extract
The minimum bactericidal concentration was determined by subculturing loopful of the MIC culture plate that did not support bacterial growth unto a Mueller Hinton nutrient agar plates and incubating further at 37°C for 24 hours. The concentration of the extract that caused the complete absence of growth or clearance was taken as the MBC.

Statistical Analysis
All experiments were performed in triplicates and the diameter zones of inhibition data obtained were expressed as mean ± standard deviation. The statistical analysis was performed using the analysis of variance (ANOVA). The P-values < 0.05 were considered significant.

Result
Yield

Weight of powdered leaves 300 grams

Weight of extract obtained 35.5 grams

$$\text{Extract Yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of powdered leaves}} \times \frac{100}{1}$$

$$= \frac{35.5}{300} \times \frac{100}{1}$$

$$= \mathbf{11.83\%}$$

The extraction of 300 g of the pulverized *M. oleifera* leaves using 2 litres of 70%v/v Ethanol gave a yield of 11.83%.

Preliminary Phytochemical Screening
Tables 4a and 4b show the results of the preliminary phytochemical screening of the crude extract and the hydrolysed leaves extract of *M. oleifera.* The results unfolded the presence of flavonoids, terpenoids, alkaloids, saponins, reducing sugars, phenols, steroidal aglycones, condensed tannins, phlobatannins, and cardiac glycosides in the crude ethanolic extract while the terpenoids and cardiac glycosides were absent in the ethanolic extract heated under reflux with 3N Hydrochloric acid for 3 hours.

Table 4a: Preliminary Phytochemical Screening Results of the Crude Extract From *M. oleifera* Leaves

S.No	Phytochemical	Inference
1.	Alkaloid	++
2.	Terpenoid	+
3.	Flavonoid	+
4.	Saponin	++
5.	Steroid	+
6.	Cardiac glycoside	+
7.	Condensed tannins	+ +
8.	Ellagitannins	-
9.	Phlobatannins	+
10.	Reducing sugars	+ +
11.	Phenolics	++

Table 4b: Phytochemical Screening Results of the Fractionated *M. oleifera* Extract Obtained by solvent Extraction

S.No	Phytochemical	Fractions				
		A	B	C	D	E
1	Alkaloid	-	-	-	++	-
2	Terpenoid	-	-	-	-	-
3	Flavonoid	-	+	-	-	-
4	Saponin	-	-	-	-	+
5	Steroid	-	-	+	-	-
6	Cardiac glycoside	-	-	-	-	-
7	Phenolics	-	++	-	-	++
8	Tannins	++	-	-	-	-
9	Phlobatannins	-	-	-	+	-
10	Reducing Sugars	-	++	-	-	++

Key:
+ + = Abundant
+ = Moderately Present
- = Absent

Thin Layer Chromatographic Analysis

The solvent system — chloroform: methanol (3:7) resolved fraction A into one component, fraction B into two components, fraction C into one component, fraction D into two components and fraction E into three components. This is shown in table 5 below:

Table 5: The Retention Factor Values of the Fractionated *M. oleifera* Leaves Extract

Fraction	R_f Values of Components		
A	0.55	-	-
B	0.69	0.95	-
C	0.53	-	-
D	0.89	0.97	-
E	0.32	0.69	0.95

Antibacterial Assay

Sensitivity Test (Susceptibility Test)

Table 6 shows the result of the antibacterial activity of *M. oleifera* leaves extract on the test microorganisms. The positive control (ofloxacin) had the highest zone of inhibition (34mm) on *E. coli* while the negative control (50% v/v DMSO) did not cause any inhibition.

Table 6: Antibacterial Activity of *M. oleifera* Crude Ethanolic Leaves Extract

	Diameter Zone of Inhibition (mm)				
	Plant Extract			Ofloxacin	50% DMSO
	30mg	60mg	90mg		
E. coli	12	17	14	34	-
S. saprophyticus	16	15	14	27	-
P. aeruginosa	11	12	16	32	-
K. oxytoca	16	19	17	30	-

Table 7 shows the result of the MIC screening of the ethanolic extract. The result revealed that the MIC ranged from 10 mg/ml to 90 mg/ml for all the organisms except *K. oxytoca* which was resistant at the various concentrations used.

Table 7: MIC of *M. oleifera* Ethanolic Leaves Extract

	Concentration of Crude Ethanolic Extract (mg/ml)									
	10	20	30	40	50	60	70	80	90	100
E. coli	+	+	-	-	-	-	+	+	+	+
S. saprophyticus	-	-	-	-	-	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+	+	+	-	-
K. oxytoca	+	+	+	+	+	+	+	+	+	+

The result of the MBC screening revealed that the MBC of the ethanolic leaves extract on *S. saprophyticus*, *E. coli* and *P. aeruginosa* were 50mg/ml, 60mg/ml and 90mg/ml respectively but there was no MBC on *K. oxytoca* at the various concentrations used.

Table 8: MBC of *M. oleifera* Leaves Extract

Organism	MBC
E. coli	60mg/ml
S. saprophyticus	50mg/ml
P. aeruginosa	90mg/ml
K. oxytoca	-

Discussion of Findings

Due to several intricacies of modern or the orthodox antibiotics, there has been a significant shift towards alternative therapy and herbal remedies. Antibiotic screening on natural products obtained from *M. oleifera* used in the Complementary and Alternative Medicine (CAM) is a major thrust of research and development. Therefore, in a bid to discover new antimicrobials that would be effective against multi-drug resistant microbial strains, phytochemical screening and investigations into the antibacterial profile of the ethanolic leaves extract of *M. oleifera* were carried out. It is suggested that this plant drug would have enormous health benefits with little untoward effects that is common with the synthetic drugs. During the course of this research, investigations have afforded many phytochemicals with promising antibacterial properties.

The extraction of the pulverized *M. oleifera* leaves with 70% v/v ethanol gave a yield of 11.83%. The preliminary phytochemical screening of the 5% w/v extract revealed the presence of terpenoids, cardiac glycosides, alkaloids, phenols, phlobatannins, flavonoids, saponins, condensed tannins and reducing sugars but cardiac glycosides and terpenoids were absent in the ethanolic extracts heated under reflux at 100°C with 3M hydrochloric acid for 3 hours. The absence of these phytochemicals in the hydrolysed extract might be attributable to the acid hydrolysis of the functional groups in the phytoconstituents.

Phytochemical screening is based on colour formation, precipitate formation, interfacial film formation, frothing or emulsion production. Alkaloids contain one or more nitrogen on their heterocycles which may be primary (mescaline), secondary (Nicotine) or tertiary (Quinine). Alkaloidal reagents are presumed to react with the nitrogen on the heterocycles resulting in coloured precipitate formation. Tannins contain phenolic hydroxyl groups which are reduced by reducing agents such as ferric chloride solution resulting in a blue-black, brown or red precipitate formation. Reducing sugars such as rhamnose produce a deep blue or green coloration on addition of Fehlings solution due to the reduction of copper sulphate present in Fehling's reagent to copper (II) oxide. Non-reducing sugars do not convert copper sulphate to the corresponding copper (II) oxide. This forms the basis for the colour change. Saponins form colloidal solutions on hydrolysis in water, hence, they foam upon shaking. Saponins may be neutral or acidic on the basis of the structural moiety. Foam formation is based on saponification, a reaction involving soap formation which serves as the emulgent. This might be the basis for the emulsion test.

Naturally occurring glycosides in the presence of mineral acids undergo hydrolysis into sugars and aglycones. The glycone moiety is reduced by the ferric chloride solution in the presence of an acid giving a characteristic colour formation. However, steroids contain keto functional groups which are reduced by sulphuric acid in the salkowski reaction. This reduction forms the basis for the colour change.

Many reviews and articles reporting the antibacterial activities of flavonoids (Pretorious J.C., 2003; Khan *et al.*, 2003), anthraquinones (Cowan *et al.*, 2000), polyphenols and phenols (Hatano, *et al.*, 2000; Funatoyawa, *et al.*, 2004), and tannins (Tominaga *et al.*, 2002), have been published in recent years. Several phenolic compounds have been identified and isolated from plants and they have shown promising

bacterial inhibiting properties against specific and broad spectrum of cultured as well as clinical bacterial strains including Methicillin-Resistant Staphylococcus aureus (MRSA), and multi-drug resistant bacteria. The presence of alkaloids such as moringine, moringinine and spirachin, as well as pterygospermin have been shown to demonstrate antibacterial activity (Gianina *et al.,* 2003).

Alkaloids, phenols, flavonoids and glycosides have a number of biological activities and strong antibacterial potentials (Robbers et al., 1996). Alkaloids have exhibited promising activity against *H. pylori* (Hadi and Bremner, 2001) and a number of other bacterial strains (Sinha *et al.,* 2001; Saeed and Sabir, 2001; Khan *et al.,* 2001; Kren and Martinkova, 2001; Similarly, a few glycosides have presented with antibacterial activities The antibacterial potential of terpenoids have been documented. Terpenoids are bioactive molecules which are a part of plants' defence mechanisms as phytoprotectants (Morrissey and Osbourn, 1999).

The fractionation of the phytochemical compounds in the plant extract under study using solvent extraction procedures was also carried. This afforded five fractions — A, B, C, D and E. On phytochemical screening of fraction A (strong acid), tannins were identified. Screening of fraction B gave positive results with flavonoids, reducing sugars and phenolics and fraction C (neutral portion) indicated the presence of steroidal aglycones. Fraction D (basic fraction) gave positive result with alkaloids, and phlobatannins, while fraction E (aqueous portion) showed the presence of saponin, phenolics and reducing sugars, as described in table 4b above.

Thin layer chromatographic analysis using chloroform-methanol solvent system resolved fraction A into one component with R_f value 0.55, fraction B into two components with R_f values 0.54 and 0.95, fraction C into one component with R_f value 0.53, fraction D into two components with R_f values 0.89 and 0.97, and fraction E into three components with R_f values 0.32, 0.69 and 0.95 as shown in table 5 above.

The plant extract under study had varied extents of antibacterial activities which were concentration-dependent. The extract had the largest diameter zone of inhibition on *E. coli* and *K. oxytoca* at 60 mg/ml as seen in table 6 above. Also, it was observed that an increase in concentration of extract resulted in diminished activity on *S. saprophyticus* while reverse was the case with *P. aeruginosa.* The antibacterial activities of the extract suggest that the plant could be of relevance in the treatment of infections caused by these organisms.

Ofloxacin, the control antibiotic had greater diameter zone of inhibition on the test microbes than that of plant extract. However, there was no significant difference ($P>0.05$) between the diameter zones of inhibition of the extract and the control antibiotic. The statistical analytical method utilized in comparing the diameter zones of inhibition between the plant extract and the control antibiotic is the analysis of variance (ANOVA).

Studies have shown that the antimicrobial potential of *M. oleifera* leaves extract may be attributable to the presence of an array of phytochemicals. Following structural elucidation using mass spectroscopy and nuclear magnetic resonance spectroscopic procedures, the presence of a short polypeptide named 4-(alpha-L- rhamnosyloxy) benzylisothiocyanate was investigated. It was argued that the peptide might act directly on microbes and result in growth inhibition by disrupting cell membrane synthesis, a mechanism of action similar to the Beta Lactam and cephalosporin antibiotics, or the inhibition of the synthesis of essential enzymes (Bukar *et al.*, 2010; Suarez *et al.,* 2003).

The MIC obtained shows that different concentrations were effective against some of the test organisms. The most susceptible organisms to the antibacterial activity of the extract was *E. coli* while the least susceptible was *P. aeruginosa. K. oxytoca* was resistant at the various concentrations utilised. The MBC determined revealed that at a concentration of 50-90mg/ml bactericidal effect was observed.

The mechanism of action by which the phytochemical constituents of *M. oleifera* exert their antibacterial activity might be attributable to bacterial enzyme inhibition such as the sortase inhibitory effect, DNA replication, bacterial toxin action (Fakai *et al.*, 2002) and causing the lysis of bacterial cells. It had been suggested that pterygospermin acts by the inhibition of the transaminase enzyme(*http://www.atgcchecker.com/pubmed/20341204)* and through cell membrane perturbations. This, when coupled with the action of Beta lactams on the transpeptidation of the bacterial cell wall could lead to an enhanced antimicrobial effect of the combinations.

Antimicrobial peptides probably interact with cellular membranes in two stages. Firstly, cationic amino acids are attracted by negative charges such as phospholipoidal groups on the surface. Secondly, hydrophobic acid and positively charged patches of the peptides interact with the aliphatic fatty acids and

anionic components respectively. This induces membrane destabilizaton and bacteria are thought to be killed by the leakage of cytoplasmic contents, loss of membrane potential, change of membrane permeability, lipid distribution, the entry of peptides and the occlusion of anionic cell components or the actuation of autolytic enzymes.

Tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes (Kamba and Hassan, 2010). Saponins might act by altering the permeability of cell walls and hence exert toxicity on all organised tissues. They exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Moyo *et al.*, 2012). It was suggested that polyphenols such as gallic acids act possibly by binding to bacterial dihydrofolate reductase (DHFR) enzymes, inhibition of supercoiling activity of E. coli bacterial gyrase by binding to the ATP binding site of gyrase B and binds to bacterial DNA thereby inducing topoisomerase IV enzyme-mediated DNA cleavage and growth inhibition (Sakharkar *et al.*, 2010).

Summary

The phytochemical screening and investigation into the antibacterial potential of the ethanolic leaves extract of *M. oleifera* Lam leaves showed or highlighted the antibiotic spectra of the plant extract under assay, suggesting a promising lead as an alternative antibiotic and it yielded scientific support to their use in traditional Ayurvedic medicine. The plant extract was found to exhibit comparable antibacterial activity with the standard antibiotic - Ofloxacin against some clinical isolates. This antibacterial potential was stated to be attributable to the presence of an array of bioactive principles such as alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, phenols, steroids, saponins and reducing sugars.

The separation of the phytoconstituents in the extract was carried out using thin layer chromatography following fractionation by the Stas-Otto solvent extraction procedures.

CONCLUSION

The results obtained from the preliminary phytochemical screening and investigations into the antibacterial potentials of the ethanolic extract from *M. oleifera* Lam leaves revealed the presence of an array of bioactive principles called phytochemicals whose antibacterial potentials were comparable with those of the standard antibiotics - Ofloxacin, a DNA gyrase inhibitor against the Gram positive and Gram negative bacterial strains tested. The antibacterial potential of the extract under assay may be indicative of the presence of an array of broad spectrum bioactive principles.

In concluding, *M. oleifera* Lam leaves extract could be a promising naturally occurring antibacterial agent with potential applications in the pharmaceutical industry for controlling the pathogenic bacterial infections such as urinary tract infections (UTI), respiratory tract infections and Septicaemia caused by the test microbial strains used in this research.

Recommendation

Based on the current research, promising chemotherapeutic moieties such as *M. oleifera* extract should be subjected to further advanced studies such as in vitro assays, clinical trials, and descriptive toxicological assays such as those for teratogenicity, carcinogenicity and mutagenicity, the later being simulated using the AME'S TEST. Candidate compounds identified from the extract under study should be analysed retro-synthetically in a bid to identifying and elucidating the readily available and/or accessible lead or starting materials for the pharmaceutical industry. For the future of chemotherapy, the pharmacokinetics, mechanisms of action and resistance should be taken into account in the appraisal of novel antimicrobials such as *M. oleifera* Lam leaves extract.

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