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PHYTOCHEMICAL COMPONENTS, ANTISTAPHYLOCOCCAL ACTIVITY AND ACUTE TOXICITY OF METHANOL CRUDE EXTRACT OF *MORINGA OLEIFERA* ROOT BARK

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ABSTRACT

This study was to evaluate the phytochemical components, antimicrobial activity and acute toxicity of methanol extract of *Moringa oleifera* root bark. The Cinical isolates were characterized as *S. aureus* isolates and further identified as MRSA by disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). MRSA confirmation was done using Oxoid® DR0900 penicillin binding protein (pbp2') latex agglutination test kits. Pulverised *Moringa oleifera* root bark was defatted with n-hexane to yield hexane fraction (HEF). The dried marc was extracted with methanol using Soxhlet extractor to obtain methanol crude extract (ME). Qualitative phytochemical analyses of the extract were carried out using standard procedures. The antimicrobial activities of ME was evaluated on the MRSA, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were evaluated. The preliminary acute toxicity and sub-acute toxicity of ME were evaluated. Statistical analysis was done with ANOVA followed by Duncan post Hoc test using SPSS v 17 software. Latex agglutination test confirmed 39 strains of the clinical isolates to be MRSA. The *S. aureus* isolates were resistant to vancomycin at 30 µg/ml and sensitive to the extract at ME: MIC (3.0 ± 0.1 to 5.0 ± 0.5 mg/ml) and MBC (3.0 ± 0.1 to 6.0 ± 0.5 mg/ml). Phytochemical analysis of the extract showed the presence of alkaloids, glycosides, steroids, terpenoids, flavonoids, saponins, tannins, resins, reducing sugars, proteins, fats and oil and carbohydrates. The oral acute toxicity test showed the LD_{50} of ME as 3663.96 mg/kg, with no significant change ($P > 0.05$) in the hematological, serum biochemical parameters and weight of the rats.

KEYWORDS: Phytochemical, components, antistaphylococcal, activity, acute, toxicity, methanol, extract.

INTRODUCTION

Moringa oleifera has an impressive range of medicinal uses with high nutritional value and medicinal benefits. Different parts of Moringa contain a profile of important minerals and are good source of protein, vitamins, beta-carotene, amino acids and various phenolics. Moringa provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol.

Moringa roots have antibacterial activity[1] and are reported to be rich in antimicrobial agents. These are reported to contain an active antibiotic principle, pterygospermin, which has powerful antibacterial and fungicidal effects. A similar compound is found to be responsible for the antibacterial and fungicidal effects of its flowers.[2] The root extract also possesses antimicrobial activity attributed to the presence of 4- α-L-rhamnosyloxybenzyl isothiocyanate.[3] The aglycone of deoxy-niazimicine [N-benzyl, S-ethyl thioformate] isolated from the chloroform fraction of an ethanol extract of the root bark was found to be responsible for the antibacterial and antifungal activities.[4] The bark extract has been shown to possess antifungal activity,[5] while the juice from the stem bark showed antibacterial effect against *Staphylococcus aureus.*[6] The fresh leaf juice was found to inhibit the growth of microorganisms [*Pseudomonas aeruginosa* and *Staphylococcus aureus*], pathogenic to man.[7]

Moringa oleifera has also been reported to exhibit other diverse activities. Aqueous leaf extracts regulate thyroid hormone and can be used to treat hyperthyroidism and exhibit an antioxidant effect.[8] A methanol extract of *M. oleifera* leaves conferred significant radiation protection to the bone marrow chromosomes in mice.[9] Moringa leaves are effective for the regulation of thyroid hormone status.[9] A recent report showed that *M. oleifera* leaf may be applicable as a prophylactic or therapeutic anti-HSV [Herpes simplex virus type 1] medicine and may be effective against the acyclovir-resistant variant.[10] The flowers and leaves also are considered to be of high medicinal value with antihelmintic activity.[10] An

infusion of leaf juice was shown to reduce glucose levels in rabbits.[11] *M. oleifera* is coming to the forefront as a result of scientific evidence that Moringa is an important source of naturally occurring phytochemicals and this provides a basis for future viable developments. Different parts of *M. oleifera* are also incorporated in Kumar *et al.*[12] various marketed health formulations. The seeds have specific protein fractions for skin and hair care. Two new active components for the cosmetic industry have been extracted from oil cake Purisoft® consists of peptides of the Moringa seed. It protects the human skin from environmental influences and combats premature skin aging. With dual activity, antipollution and conditioning/strengthening of hair, the *M. oleifera* seed extract is a globally acceptable innovative solution for hair care.[13,14]

Antihypertensive compounds thiocarbamate and isothiocyanate glycosides have been isolated from the acetate phase of the ethanol extract of moringa pods.[15] The cytokinins have been shown to be present in the fruit. A new *O*-ethyl-4-(ά-L-rhamnosyloxy)benzyl carbamate together with seven known bioactive compounds, 4(ά- L-rhamnosyloxy)-benzyl isothiocyanate3, niazimicin4, 3-*O*-(6′-*O*-oleoyl-D-glucopyranosyl)-β sitosterol,[15,16] β-sitosterol-3-*O*-D-glucopyranoside, niazirin, β -sitosterol and glycerol-1-(9-octadecanoate)have been isolated from the ethanol extract of the *Moringa* seed.[16] Lately, interest has been generated in isolating hormones/growth promoters from the leaves of *M. oleifera*. Nodulation of black-gram (*Vigna munga* L.) has been shown to increase vigorously with the application of an aqueous-ethanol extract of *M. oleifera* leaves, although the nature of the active ingredient is still unknown. *Moringa* leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids.[16,17] The high concentrations of ascorbic acid, oestrogenic substances and β-sitosterol,[18] iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β -carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine present in *Moringa* leaves and pods make it a virtually ideal dietary supplement.[17,18]

The composition of the sterols of *Moringa* seed oil mainly consists of campesterol, stigmasterol, β-sitosterol, avenasterol and clerosterol accompanied by minute amounts of 24 methylenecholesterol, campestanol, stigmastanol and isoavenasterol. The sterol composition of the major fractions of *Moringa* seed oil differs greatly from those of most of the conventional edible oils. The fatty acid composition of *M. oleifera* seed oil reveals that it falls in the category of high oleic oils (67.90%–76.00%) among the other component fatty acids. *Moringa oleifera* is also a good source of different tocopherols the concentration of those is reported to be 98.82–134.42, 27.90–93.70, and 48.00– 71.16 mg/kg, respectively.[19] Isolated nitrile glycosides (niaziridin & niazirin) from the leaves, pods and bark of *Moringa oleifera* by reverse phase HPLC. Fourty four compounds from the essential oil isolated from the leaves of *Moringa oleifera* by GC-MS analysis.[20]

There is hope for combating the revenge mechanism of bacteria called resistance, through proper and adequate research into mechanisms of resistance, development of antimicrobials with specific target of resistant genes and advance study of biotechnology of natural products.

MATERIALS AND METHODS

Materials

Clinical isolates: The clinical isolates used include *Staphylococcus aureus*, methicilin resistant *Staphyloccocus aureus*, from Bishop Shanahan Hospital, Nsukka, University of Nigeria Teaching Hospital, Ituku/Ozalla Enugu state and Federal Medical Centre Abakaliki, Ebonyi State.

Media: Nutrient Agar (Fluka) Sigma Aldrich UK, Mueller-Hinton Agar (MHA), Oxoid Ltd, England, Mannitol Salt Agar (MSA), Oxoid Ltd, England.

Reagents: PBP2a or PBP2' test kit dr0900a lot. no. 130422. Oxoid Ltd, Japan, staphylase test kit dr0595a., (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24, UK), Oxoid antimicrobial susceptibility test discs. Hydrogen peroxide (H_2O_2), Dimethylsulfoxide (DMSO), distilled water, silical gel, (Titan Biotech Ltd, India). 0.5 McFarland turbidity standard.

Solvents: Methanol, (Sigma Aldrich, U.K), n-hexane, (Sigma Aldrich, U.K), ethyl acetate, (Sigma Aldrich U.K), dichloromethane (Sigma Aldrich U.K).

Equipment: Test tubes, Petri-dish, pipette, micropipette, microcentrifuge tube, measuring cylinder, flat bottom flask, Soxhlet extractor, glass chromatographic column, autoclave, refrigerator, cotton wool, weighing balance, foil, sterile loops and swabs, incubator, antibiotic disc dispensers.

Animals: Wister strain albino rats of both sexes weighed between 80 -150 g which were bred in the Department of Pharmacology University of Nigeria Nsukka, Ethical committee of the department certified the use of the animals. The animals were housed in standard environmental conditions with 12 h light-dark cycle. The animals were divided into extract treated groups and the control groups. All the animals were fasted for 12 h, but were allowed free access to water, before commencement of the experiments.[21]

Methods

Collection, authentication and processing of plant materials

The root of *Moringa oleifera* was collected from Nsukka Local Government Area, Enugu State, Nigeria. The plant

materials were identified and authenticated by a Botanist at the Biological Science Department, University of Nigeria, Nsuka. Confirmation of taxonomic identity of the plants was achieved by Mrs. Immanuela Udoma by comparison with voucher specimens kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, and use of documented literature.[22] The plant materials were air-dried in the laboratory for four weeks. The dried samples were grinded to coarse powder with a mechanical grinder; the powdered was stored for future use.

Extraction of root extract

The pulverized root of *Moringa oleifera* (3 kg) was defatted with 10 litres of n-hexane by cold maceration overnight. The marc was dried and extracted with 20 litres of methanol for 4 hours using Soxhlet extraction technique to yield methanol crude extract (ME) using established standard procedures by Harborne, Iwu, Trease and Evans.[20,22,23]

Qualitative phytochemical analysis

Methanol crude extract (ME) and its fractions of the *Moringa oleifera* root bark was subjected to phytochemical tests using established standard procedures by Harborne, Iwu, Trease and Evans.[20-23]

Standardization of inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4 ^{o}C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of barium sulphate solution.[21]

Characterization of the clinical isolates

A total of 2,372 clinical isolates of microbes were obtained from hospitalized patients admitted between September 23 2012 and June 21, 2013 from 3 different hospitals in the South-East region of Nigeria. The hospitals were: Bishop Shanahan Hospital, Nsukka, University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu State and Federal Medical Centre, Abakaliki, Ebonyi State. These isolates were taken from urine, sputum, open wound, abscess, ear and nasal swab in the orthopedic and intensive care departments of the hospitals. The distribution of the isolates based on their sources are: sputum (376), urine (327), absces swab (466), open wound swab (762), ear/nasal swab (441).

Latex agglutination procedure: For each supernatant to be tested, one circle of the test card was labeled '**T**' for testing with Test Latex and another with '**C**' for Control Latex. The latex reagent was properly mixed by inversion several times and a drop of test Latex or Control Latex was added to each labeled circle accordingly. 50µl of supernatant was placed on the Test circle and the Control circle and mixed thoroughly with the latex with the aid of the provided sterile plastic mixing stick. The mixing was done for three minutes and observed for agglutination under normal lighting conditions. The results of the Test and Control reactions were recorded before disposing the reaction card safely into disinfectant.

Determination of MIC and MBC of the extracts on MRSA clinical isolates

Preparation of stock solution

Stock solution (50 mg/ml) of the plant extracts were prepared by dissolving 1250 mg extracts and fractions in 25 ml of sterile water and DMSO (dimethyl sulfoxide) diluted in 1:5 (DMSO:Water). The DMSO is an organic solvent that aid the dissolution of organic substance that will not dissolve easily in water alone. The water and DMSO dilution was carried out to avoid the interference of DMSO with original activity of the extract.[24]

25 ml of stock is prepared to allow for 5 ml overage. Dimethyl sulfoxide has been shown to improve the efficiency of fungicides, to possess anti-inflammatory effects, as well as additional non-specific biological effects and it is for this reason that control experiments were conducted in all cases to account for additive effects, if any.[24,25]

Preparation of extract concentrations for agar dilution for MIC and MBC determination

Twenty ml volume of Muller Hinton Agar (MHA) was used in 9 cm Petri dishes for agar dilution MICs. Dilution schemes using formula $C_{E1}V_{E1}= C_{E2}V_{E2}$ are given in Tables 1.[25]

C_{E1} = Stock concentration of the extract and fractions = 50 mg/ml

V_{E1}= Volume of the extract and fractions in the agar dilution = to be determined

C_{E2} = Concentration of the extract and fraction in agar dilution (1 mg/ml – 10 mg/ml)

V_{E2} = Volume of reaction mixture in MHA plate = 20 ml.

Table 1: Extract concentrations in agar dilution method.

S/N	C_{E1}(mg/ml)	V_{E1} (ml)	C_{E2} (mg/ml)	Volume of MHA (ml)	V_{E2} (ml) Volume of reaction mixture
1	50	4.00	10	16.00	20
2	50	3.60	9	16.40	20
3	50	3.20	8	16.80	20
4	50	2.80	7	17.20	20
5	50	2.40	6	17.60	20
6	50	2.00	5	18.00	20

7	50	1.60	4	18.40	20
8	50	1.20	3	18.80	20
9	50	0.80	2	19.20	20
10	50	0.40	1	19.60	20

Determination of MIC of methanol extract and fractions

By means of a sterile calibrated micro pipette, 0.002 ml of the MRSA clinical isolates suspension was streaked with a sterile loop on the surface of the MHA and allowed for 10 minutes for complete absorption of the inoculum by the medium. The plates were incubated in an inverted position at 37^0c for 24 hr before taking the results. The least concentration that inhibits the growth of the organism is taken as the MIC (minimal inhibitory concentration). The control plate without antimicrobial agents was also incubated.[25]

Determination of MBC of methanol extract

The value of MBC is an extension of MIC. The agar plates showing no growth in the MIC tests were used for the determination of the MBC. Discs were cut from the agar plate of the MIC concentration and two preceding concentrations and transferred into the corresponding containers of the fresh Muller Hinton broth (recovery medium). The media were also incubated at 35^oC for 48hrs. At the end of incubation the media were observed for any visible growth or turbidity. The absence of growth in the recovery medium is evidence of total cell death. The minimal concentration of the antimicrobial agent that produces total cell death is taken as the MBC.[21,24,25]

Acute Toxicity Study

Acute oral toxicity study was performed in accordance to OECD guidelines (Orga- nization of Economic Cooperation and Development) 423 guide-line (Acute toxic class method).[21] The acute oral toxicity of the extracts was determined using Miller and Tainter method.[26] Toxicity assay was performed in male albino Wistar rats (80-150g), the rats were randomly divided into 6 groups with 6 animals in each group. The animals were kept fasting overnight provided only water, after which the methanol extract of the roots was administered orally with increasing doses (50 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg, 5000 mg/kg, 10 g/kg body weight) by intra-gastric tube to determine the safe dose. The control group (group 7) was treated with orally administered distillated water (2 ml/kg) only. The animals were observed continuously for 1h, then frequently for 4h and later at the end of 24 h for general behavioral, neurological and autonomic profile.

Sub-acute toxicity study

The animals were divided into four groups with control, given daily oral graded doses of the extracts 50 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg bodyweight.[21] The experiment was carried out for 21 days, with oral administration of methanol extract. At the end of 21 days, the animals were deprived of food overnight and sacrificed by cervical decapitation for hematological, liver and biochemical parameters. This experiment was repeated thrice for confirmation of results.

Statistical analysis

Results were expressed as mean ± SD and differences between sets obtained were determined using ANOVA followed by Duncan post Hoc Test with the use of SPSS v 17 software. Differences were considered significant at $p < 0.05$.

RESULTS

Table 2: Results of phytochemical analysis of the Methanol extracts.

Chemical constituent	Test	Crude methanol extract
Alkaloids	Dragendorff's reagent, Mayer's reagent, Wagner's reagent	++++
Glycosides	Fehling's solution I and II	+ +
Steroids	General Test	+ +
Terpenoids	General Test	+
Flavonoids	Ammonium Test, 1 % Aluminium Chloride solution Test.	+ +
Saponins	Frothing Test, Emulsion Test, Fehling's Test	+ +
Tannins	Ferric chloride Test, Lead Acetate Test	+ +
Resins	Precipitation Test, Colour Test	++
Reducing Sugar	Fehling's solution I and II	++
Proteins	Millon's Test, Xanthoproteic Reaction Test, Picric Acid Test, Biuret Test	+++
Fats and Oil	General filter paper Test	++
Carbohydrate	Molisch's	+++

Key:
(-): Not present.
(+): Present in small concentration.

(++): Present in moderately high concentration.
(+++): Present in very high concentration.
(++++): Abundantly present.

Table 3: MIC and MBC of methanol crude extract in mg/ml.

S/N	Clinical isolates	MIC	MBC	S/N	Clinical isolates	MIC	MBC
1	SP4	4.3 ± 0.5	5.0 ± 0.5	21	EN390	4.3 ± 0.5	5.3 ± 1.1
2	SS8	3.3 ± 0.5	5.3 ± 1.1	22	SS310	3.3 ± 0.3	4.6 ± 0.5
3	AB20	3.3 ± 0.5	4.0 ± 0.5	23	OW417	3.0 ± 0.3	4.3 ± 0.3
4	SP22	4.6 ± 0. 5	4.3 ± 0.5	24	AB570	3.3 ± 0.1	3.6 ± 0.5
5	OW30	4.0 ± 0.5	4.6 ± 0.5	25	OW578	3.3 ± 0.5	4.3 ± 1.0
6	SS33	4.3 ± 0.5	5.3 ± 1.0	26	AB600	3.0 ± 0.5	5.3 ± 1.0
7	EN35	4.0 ± 0.5	5.3 ± 0.5	27	OW620	3.3 ± 0.3	5.0 ± 0.5
8	OW36	3.0 ± 0.1	4.6 ± 0.5	28	SP651	3.3 ± 0.3	4.3 ± 0.5
9	EN38	3.3 ± 0.1	5.3 ± 0.3	29	OW819	3.0 ± 0.1	4.3 ± 0.5
10	SS42	3.3 ± 0.3	4.6 ± 0.5	30	EN831	3.0 ± 0.3	4.6 ± 1.0
11	OW53	3.3 ± 0.5	4.6 ± 0.5	31	AB841	3.6 ± 0.5	4.6 ± 0.5
12	SS57	4.3 ± 0.5	5.0 ± 1.0	32	OW940	4.0 ± 0.5	5.6 ± 0.3
13	AB61	3.3 ± 0.3	4.3 ± 0.5	33	OW947	4.3 ± 0.3	5.3 ± 0.3
14	EN62	3.3 ± 0.5	4.6 ± 0.3	34	AB1009	3.6 ± 0.3	5.6 ± 1.0
15	OW123	3.3 ± 0.5	5.3 ± 1.0	35	OW1104	4.0 ± 0.5	5.6 ± 0.5
16	EN127	4.0 ± 0.3	4.3 ± 0.5	36	SP1172	4.3 ± 0.3	5.3 ± 1.0
17	OW154	4.3 ± 0.5	5.3 ± 0.5	37	OW1420	3.6 ± 0.1	4.3 ± 0.3
18	AB187	4.3 ± 0.3	5.6 ± 1.1	38	OW1827	3.6 ± 0.3	5.0 ± 0.5
19	EN208	5.0 ± 0.5	5.3 ± 0.5	39	AB1956	4.6 ± 0.5	5.6 ± 1.1
20	SS235	4.6 ± 0.3	5.3 ± 0.5				

Values were expressed as Mean ± SD, N = 3
Key:
SP: Sputum
SS: Skin swab
AB: Abscess
OW: Open wound
EN: Ear/Nasal

Preliminary evaluation of toxicity of methanol crude extract and n-hexane extract fraction

The evaluation of acute toxicity and toxicity indices of methanol crude extract and n-hexane extract fraction was carried out to ascertain the safety of the extract root bark. The results are presented in the tables below.

Acute toxicity test of methanol crude extract

The acute toxicity test result of methanol crude extracted is as presented in table 24.

Table 4: Acute toxicity test of methanol crude extract.

Group	Dose mg/kg	N/D	% Mortality	Observation Period (h)	Symptoms of Toxicity
1	50 mg	6/0	0	24	No toxic symptoms
2	250 mg	6/0	0	24	No toxic symptoms
3	500 mg	6/1	16.6	24	Slow movement and dullness of animals
4	1000 mg	6/2	33.3	24	Weak and less active
5	5000 mg	6/2	33.3	24	Marked behavioral change, restlessness and gradual death
6	1 g	6/4	66.6	24	restlessness and gradual death
7 (control)	2 ml distilled water	6/0	0	24	No change observed

N = no. of rats, D = no. of Deaths

The LD_{50} is determined from probit vs Log Conc. graph.

As presented in Table 4, the result of the acute toxicity test (LD_{50}) of the extract was determined from probit vs Log Conc. graph to be 3663.96 mg/kg.

It is obvious from figure 1, that methanol extract of the root bark of *moringa oleifera* is safe for human consumption at a dose below the LD_{50}, with reference to LD50 values recommended by the organisation of Economic Co-operation and Development (OECD, Paris,

France) (Walum, 1998) are as follow: very toxic, <5 mg/kg; toxic, >5<50 mg/harmful, >50<500 mg/kg; no label, >500<2000 mg/kg.[21,27]

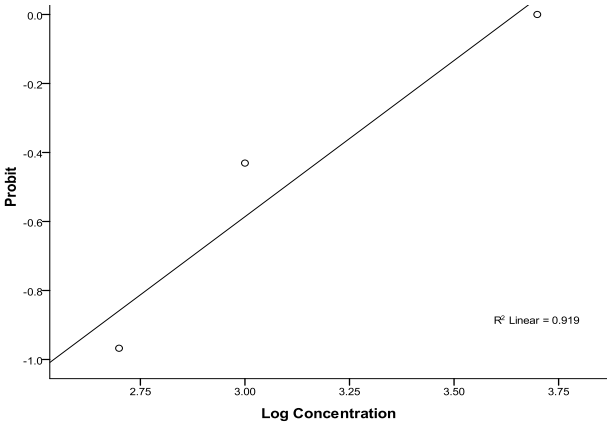

Fig. 1: Determination of LD50 value of Methanol crude extract in rats.

LD_{50} = 3663.96 mg/kg

Determined from probit analysis onSPSS v17

LD50 values recommended by the organisation of Economic Co-operation and Development (OECD, Paris, France)[27] are as follow: very toxic, < 5 mg/kg; toxic, > 5 < 50 mg/harmful, > 50 < 500 mg/kg; no label, > 500 < 2000 mg/kg.[21,27]

Sub-acute toxicity study of the methanol crude extract

The sub-acute toxicity test results are presented in Table 25, to check the effect of the extract on the liver, kidney and blood cells.

Table 5: Effects of the graded doses on haematological parameters of rats.

Parameters	Control (A)	50 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	1000 mg/kg (E)
PCV%	44.6 ± 0.6	42.2 ± 0.9	45.3 ± 0.3	45.1 ± 0.2	46.2 ± 0.4
RBC%	7.1 ± 0.9	7.3 ± 0.2	7.40 ± 0.5	7.4 ± 0.5	7.5 ± 0.5
WBC%	13.2 ± 0.1	13.9 ± 0.1	13.75 ± 0.1	13.8 ± 0.2	14.2 ± 0.2
MCV%	51.2 ± 0.2	51.1 ± 0.1	49.1 ± 0.1	48.8 ± 0.2	50.2 ± 0.1
PLT%	409.1 ± 8.1	409.0 ± 7.5	406.22 ± 8.1	399.1 ± 8.2	388.40 ± 8.2

Values were expressed as Mean ± SD, N = 6

Table 6: Effects of the graded doses on biochemical parameters of rats.

Parameters	Control (A)	50 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	1000 mg/kg (E)
BILURIBIN	1.60 ± 0.01	1.63 ± 0.02	1.61 ± 0.01	1.60 ± 0.02	1.58 ± 0.01
CREATININE	0.73 ± 0.01	0.69 ± 0.01	0.71 ± 0.01	0.69 ± 0.01	0.69 ± 0.01
ALT	34.11 ± 0.02	33.17 ± 0.02	33.02 ± 0.15	31.40 ± 0.15	32.60 ± 0.02
ALP	129.4 ± 0.15	129.88 ± 0.15	129.31 ± 0.21	130.11 ± 0.22	132.11 ± 0.20
AST	63.1 ± 0.1	66.1 ± 0.2	64.41 ± 0.2	63.22 ± 0.2	66..53 ± 0.2

Values were expressed as Mean ± SD, N = 6
Key:
AST: Aspatate amino transferace
ALTS: Alanine amino transferace
ALP: Alkaline phosphate.

As shown in Tables 5 and 6, the results of the effect of the methanol extract at graded doses below the LD_{50} on the haematological and biochemical parameters of the rat further confirms the safety of the extracts for human consumption. There was no significant difference (P > 0.5) when the values of the parameters were compared with the control rat group. This shows that the extract had no effect on the liver, kidney and blood of the rat at these doses.

Effects of graded doses of methanol crude extract on body weights of rats

Table 7: Effects of graded doses methanol crude extract on body weights of rats.

Parameters	Control	50 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Weight before extract administration (g)	95.3 ± 4.2	110.2 ± 6.2	115.3 ± 8.2	120.4 ± 9.2	150.7 ± 8.2
Weight after 21days (g)	150 ± 10.3	155 ± 9.2	145 ± 11.4	135 ± 12.5	95 ± 8.2
% weight difference	57.89 %	53.46 %	38.09 %	35 %	Weight lost 3 %

Values were expressed as Mean ± SD, N = 6

As shown in Table 27, there was general increase in the body weight of the animals except at 1000 mg/kg were some of the animals in the group showed little weekness and later recovered. This may explain the little drop in weight at the dose of 1000 mg/kg with little behavioral change

DISCUSSION

In the present study it was observed that out of 58 clinical isolates of *S.aureus* tested for resistance, 39 isolates were confirmed MRSA and 19 MSSA prevalence of 32.75 % and MRSA 67.24 % using PBP2[1] latex agglutination test. It is believed that the high percentage of MRSA is due to long stay of the patient in the hospital as they are vulnerable and easily infected by MRSA of different clonal structure or hetrogenous MRSA especially when hospitalized for long period.[28] The specimen used for this study was collected from hospitalized patient for over six months. Increasing antibiotic resistance in major *S. aureus* clones intensifies precautionary policies for public health care systems. In this study, we have shown the upward trend of MRSA probably due to dissemination of resistance by clonal spread and horizontal transfer of *mec* A genes and regulatory sequences. Our study suggest that there are VISA strains from some of the hospitalized patients that serve as reservoir for the transmission of this MRSA and it has been reported that vancomycin resistance has the potential to become a widespread problem in both MRSA and MSSA strains.[28]

CONCLUSIONS

The efficacy of many antibiotics is being threatened by the emergence of microbial resistance to existing chemotherapeutic agents because of their indiscriminate and inappropriate use.[29] The use of some antibiotics is associated with side effects, including allergy, immune suppression, and hypersensitivity.[29]

There is an increasing awareness that many components of traditional medicine are beneficial while others are harmful, hence WHO encourages and supports countries to identify and provide safe and effective remedies for use in the public and private health services (Sofowora).[29] Considering the toxicity studies and the antistaphylococcal activities of the Methanol crude extract of the root bark, it is recommended that Moringa oleifera root bark be considered as an effective, save herbal remedy for a resistant infection caused by MRSA,

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