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Comparative Evaluation of the Antibacterial Effects of *Nigella sativa* and *Moringa oleifera* on some Clinical Bacteria Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DEG and MUED designed the study, wrote the protocol, and wrote the manuscript. Authors DEG and FA managed the analyses of the study and performed the statistical analysis. Authors FA and ECA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In this study, the antibacterial effect of the n-hexane, methanol, and ethanol extracts of two traditional medicinal plants, *Nigella sativa* and *Moringa oleifera* was carried out to comparatively assess the antibacterial efficacy of these plants against some selected multi-drug resistant diarrheagenic bacterial isolates such as *Escherichia coli, Salmonella typhi, Shigella* spp, *Enterobacter aerogenes, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*.
Methodology: The inhibitory effects of extracts were assessed using disk diffusion method at six different concentrations i.e. 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml, and 400 mg/ml. The minimum inhibitory concentrations and minimum bactericidal concentrations of the plant seed extracts were determined by the micro-broth dilution technique. Synergism and antagonism studies were similarly done to determine the combined effects of both *N. sativa* and *M. oleifera* seed oil. Further, resistant bacteria were subjected to sodium dodecyl sulphate treatment for plasmid curing and continued with the antibiotic susceptibility test.

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Results: The oil extracts of *N. sativa* were found to be more effective than that of *M. oleifera* (at MIC and MCC of 50 mg/ml and 100 mg/ml respectively). It was observed that the plasmids were cured in all the *E. coli* isolates. Among the pathogens, *E. coli* was highly susceptible to both seed oils.
Conclusion: Higher antibacterial activity was observed when the extracts were used in combination, showing a strong synergistic effect against all the pathogens tested.

Keywords: Nigella sativa; Moringa oleifera; antimicrobial agents; plasmid curing; multi-drug resist ance, antibacterial.

1. INTRODUCTION

From time immemorial, man depended on plants as medicine. The plant kingdom represent a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years [1].

The use of plant product as medicines could be traced as far back as the beginning of human civilization. The earliest mentioned medicinal used plant in Hindu culture is found in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository human knowledge. The active compounds isolated, have provided leads in the development of several lifesaving drugs, which are in use today [2]. Two of such plants are *Nigella sativa* and *Moringa oleifera.*

The daily increasing microbial resistance to several conventional antimicrobial agents necessitates the quest for cheap, affective, readily available and non-toxic products of natural origin. The need therefore to establish the efficacy of the seeds oil against some drug resistant strains of diarrheagenic bacteria has become vital, especially in the poor resource areas of Lokoja.

The study aim at justifying the claims in folkloric medicine on the antibiotic potential of *Nigella sativa* and *Moringa oleifera* and to determine the antibacterial activities of *N. sativa and M. oliefera* organic extracts against multi drug-resistant diarrheagenic bacteria.

2. MATERIALS AND METHODS

2.1 Plant Samples and Test Organisms

The seeds of *Nigella sativa* were obtained from the market in Kano, Kano State while the *Moringa Oleifera* seeds were collected from Professor Rev. Sr M.U.E Dibua’s garden in Nsukka, Enugu State. Both seeds were authenticated by Mr. Raphel Ozioko in Department of Botany, Biological sciences Faculty, University of Nigeria, Nsukka. Multi-drug resistant Gram negative bacteria isolated from diarrheal children age 0-5 years, were obtained from Prof. Rev. Sr Dibua’s laboratory The organisms include; *Escherichia coli*, *Salmonella typhi, Shigella* spp, *Klebsiella pneumonia, Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Resistance break points were those recommended by the Clinical and Laboratory Standards Institute, and was reported as sensitive or resistant based on break points.

2.2 Preparation of Plant Samples for Extraction

The dried seed samples were cleaned thoroughly to remove dirt, stones and deteriorated seeds. It was then crushed and ground to fine powder using mechanical machine. Both powdered sample were stored differently in clean and dry polythene bag until use.

2.3 Extraction of the Oil Using Soxhlet Extraction Process

The seeds were extracted using the Soxhlet Extraction Process described by [3] as follows: A quantity (300 ml) of the organic solvent was poured into a round bottom flask equipped with a Soxhlet apparatus and condenser. Ten grams of the sample was placed inside the extractor. The Soxhlet extraction was then carried out at 60°C which was allowed to continue for 1 hour. At the end of extraction, the oil-solvent mixture was dried in the oven and cooled in the dessicator. Further extraction was carried out until the oil was fully extracted from the seeds.

2.4 Phytochemical Analysis of the Extracts

The phytochemical screening was carried out using the methods described by [4]. The

phytochemical analyses of the oil extracts were done to verify the presence of terpenoids, flavonoid, tannins, glycosides, saponins, steroids and alkaloids. These secondary metabolites are the bioactive substances responsible for the antimicrobial activities [5].

2.5 Preparation of Crude Seed Oil Extracts and Impregnated Paper Disks

A 2g amount of the oil extracts was weighed out, and then dissolved in 5 ml of dimethyl sulphoxide (DMSO). Subsequently, each solution was serially diluted two-fold to obtain 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml concentrations and stored in the refrigerator at 4^0C.

Using a punching machine, 6mm diameter disk was cut off clean sterile Whatman's No. 1 absorbent filter paper. The disks were sterilized by autoclaving for 15 minutes. The 6mm diameter sterile disks of absorbent paper were impregnated with varying concentration of the extracted oils.

The impregnated disks were then left to dry for hours in the incubator at 37°C, after which the disks were transferred back into the reagent bottle and stored in the refrigerator at 4°C. Sterility test was carried out by taking random samples of the prepared disks and placed aseptically on sterile Muller- Hinton Agar. The disks were then aseptically placed evenly on the surface of the agar and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37^0C for 18 - 24 hr to determine bacterial growths.

2.6 Antibacterial Activity of the Seed Oil Extracts

The antibacterial activity of the n-hexane, methanol and ethanol oil extracts of the plant seeds were evaluated against the multi-drug resistant clinical isolates using agar disc-diffusion method according to Kirby Bauer [6]. The breakpoints used were those recommended by the National Committee for Clinical Laboratory Standards on guidelines for susceptibility testing [7].

The inocula of the test organisms were prepared by transferring 3 to 4 colonies of the cultures (18 hours old) into 9 ml of sterile Mueller Hinton Broth and incubated at 37°C for 6 to 7 hr. The bacterial cultures were adjusted with 0.5 McFarland turbidity standard and streaked evenly onto the Mueller Hinton agar plate with sterile cotton swab. The seeded plates were left for drying for 3 to 5 minutes, and the appropriate oil-impregnated discs were placed on the agar using a sterile forceps and were gently pressed down to ensure contact. Then the plates were incubated in an upright position at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition in mm (including the 6 mm disk) near the agar surface and the results were recorded. A reading of 6 mm meant no zone of inhibition. The end point was taken as complete inhibition of growth as determined by the naked eye. Positive control (Ciprofloxacin, 30 µg disc) and negative control (DMSO) were also included for each experiment.

A strain is said to be sensitive if the diameter is >13 mm and resistant if it is <6 mm. It is said to be intermediate for inhibition diameter within this range [2].

2.7 Determination of the Synergistic Antibacterial Effect of Both Seed Oil Extracts on the Test Isolates

Disc diffusion technique, as described by National Committee for Clinical laboratory Standards, [7] was used to determine the combined effects of both *N. sativa* and moringa seed oil. Sterile Whatman No. 1 filter paper discs containing varying concentrations of both seed oil were used. The bacterial isolates were grown in liquid media for 18 hours. Each culture was diluted to 10^5cfu/ml and was used to flood the surface of Muller-Hinton agar plates in triplicate and the plates were dried. The prepared discs were placed in such a manner that the inhibitory circles would just touch each other tangentially. The plates were then incubated at 37°C for 18 - 24 hours. The zones of inhibition produced by each seed oils were measured in three different directions around the disc and the mean diameter was recorded. The zone of inhibition produced due to the individual and mutual effects of both plant seed oil were recorded. Data were analyzed as follows: (i) indifference, when both the zones of inhibition remain unaffected; (ii) antagonism, when the zones of inhibition receded and assumed kidney shape; (iii) synergism, when there was enlargement of zones at the site of meeting the inhibition zones of both seed oil extracts.

2.8 Determination of Minimum Inhibitory Concentration (Mic)

A broth dilution method as described by National Committee for Clinical Laboratory Standard [7] was adopted in the determination of MIC. Two-fold serial dilutions of the reconstituted oil extract (400 mg/ml) were made in nutrient broth to achieve a concentration range of 3.125- 400 mg/ml. A 0.1 ml suspension of the test organism was inoculated into 1 ml of each concentration of the oil extract in duplicates. A tube containing nutrient broth only was seeded with the test organism as described above to serve as control. All culture tubes were incubated at 37°C for 24 hr. Growth was scored visually by the turbidity of the culture. The tube with the least concentration of the seed extracts without growth (turbidity) after incubation was taken and recorded as the Minimum Inhibitory Concentration.

2.9 Determination of Minimum Cidal Concentration (Mcc)

A 0.1 ml of the cell suspensions from the tubes showing no growth were sub-cultured onto oil extract-free liquid medium incubated at 37^{0}C for 18-24 hr and examined for bacterial growth. The tubes that showed no growth at this stage was further sub-cultured onto nutrient agar plate and incubated at 37^{0}C for 18-24 hr. The lowest concentration of oil extract that showed no growth on the plate after 18-24 hr was taken as the Minimum Cidal Concentration.

2.10 Plasmids Curing of Resistant *E. coli* Isolates

Resistant *E. coli* isolates were selected and subjected to plasmid curing following the method described [8]. Overnight broth culture was inoculated into 4.5 ml nutrient broth. 0.5 ml of 10% sodium dodecyl sulphate was added and incubated for 48 hours at 37°C. 0.5 ml of the broth was added into a freshly prepared 4.5 ml nutrient broth which was incubated for another 18 - 24 hr at 37°C. After which post plasmid antibiotic susceptibility testing using the oil extracts was carried out on the *E. coli*. Confirmation of curing was done by disc diffusion assay using the standard antibiotics and 100 mg/ml concentration of the plant seeds oil extracts. The reason for this second susceptibility test is to check if the curing agent has removed the resident plasmid present in the resistant isolates.

2.11 Statistical Analysis

Results obtained were reported as mean ± standard deviation. One way analysis of variance (ANOVA) was done using SPSS software version 25.0 for Windows. Significant level was set at P-value of 0.05.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of the Seed Oil Extracts

Different phytoconstituents have been identified in both plant seed oil extracts, as revealed in Table 1. N-hexane was seen to be more efficient in extracting the phytochemicals in both plant seeds while the ethanolic and methanol extracts showed abundance of flavanoids (+++) and tannins (+++) respectively. *N. sativa* oil extracts therefore, contains in abundance, all analysed phytochemicals while *M. oliefera* contains moderately all the tested phytochemical.

The results of the disc diffusion tests of the plant seeds extracts were outlined in Fig. 1–6. Varying concentrations of the seed oil extracts showed significant differences ($p<0.05$) in their efficacy against all the tested isolates. The results of the disc diffusion tests revealed that n-hexane oil extracts of *N. sativa* seeds have higher antibacterial activity against all isolates compare to the methanolic and ethanolic oil extracts. This implies that the n-hexane oil extract of *N. sativa* seeds has a high potency and can be better used for treatment of diarrhoeagenic bacteria. The n-hexane oil extracts showed significantly higher inhibitions against the tested isolates at 50 mg/ml to 400 mg/ml concentration and least inhibition at 25 mg/ml concentration. A higher zone of inhibition was observed on *E. coli.* It is evident from the zone of inhibition that n-hexane oil extracts of higher concentrations had better efficacy on tested isolates in comparison to the oil extracts from methanol and ethanol.

The bacteria isolates were all susceptible to the methanolic and n-hexane oil extracts of *M. oleifera* at 100 mg/ml and 200 mg/ml concentration respectively. This showed that at concentrations lower than 200 mg/ml, the tested bacteria isolates were resistant to the n-hexane oil extracts of *M. oleifera* which on the other hand were susceptible to that of *N. sativa* n-hexane oil extract. However, the ethanol oil extract of

M. oleifera showed significant inhibition on the bacterial isolates at the highest concentration (400 mg/ml), that is, the highest inhibition was observed at concentration of 400 mg/ml.

Table 2 showed the result of the Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCC) of the plant seeds oil extracts for *Escherichia coli, Salmonella* species, *Shigella* species, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Escherichia coli showed the MIC of 50 mg/ml for both methanolic and n-hexane oil extracts of *N. sativa* and 100 mg/ml for the ethanolic oil extract. The trend was observed for other isolate except for *Pseudomonas aeruginosa* which showed a MIC of 200 mg/ml for both n-hexane and methanol oil extracts and 400mg/ml for the ethanol oil extract.

Higher values of MICs were observed for *M. oleifera* oil extracts to all MDR isolates. However, *E. coli* demonstrated MIC at a concentration lower than that of other tested bacterial isolates (100 mg/ml) which is the lowest concentration of the oil extract which inhibited bacterial growth resulting in visually clear tubes after 18-24 hr incubation. The result also revealed that the MICs of *M oliefera* oil extracts were generally higher than those of *N. sativa* oil extracts. The least activity was recorded for ethanol oil extracts of all the seeds oils.

Table 1. Phytochemical analysis of *Nigella sativa* and *Moringa oleifera* seed oil extracts

Test for constituents	N-hexane oil extract		Ethanol oil extract		Methanol oil extract	
	NS	MO	NS	MO	NS	MO
Alkaloid	++	+	++	+	++	++
Steroid	++	++	+	+	+++	++
Terpenoid	++	++	+	+	+++	++
Flavonoid	+++	++	+++	++	+++	++
Glycoside	++	+	+++	+	++	++
Fat and oil	+++	+++	++	++	+++	++
Saponin	++	+	+	++	+++	++
Tannin	+++	++	+++	++	+++	++
Reducing sugar	++	+	+	+	+++	+

Key: - = absent + = low in abundance ++ = moderate in abundance
+++ = high in abundance NS: Nigella sativa MO: Moringa oleifera

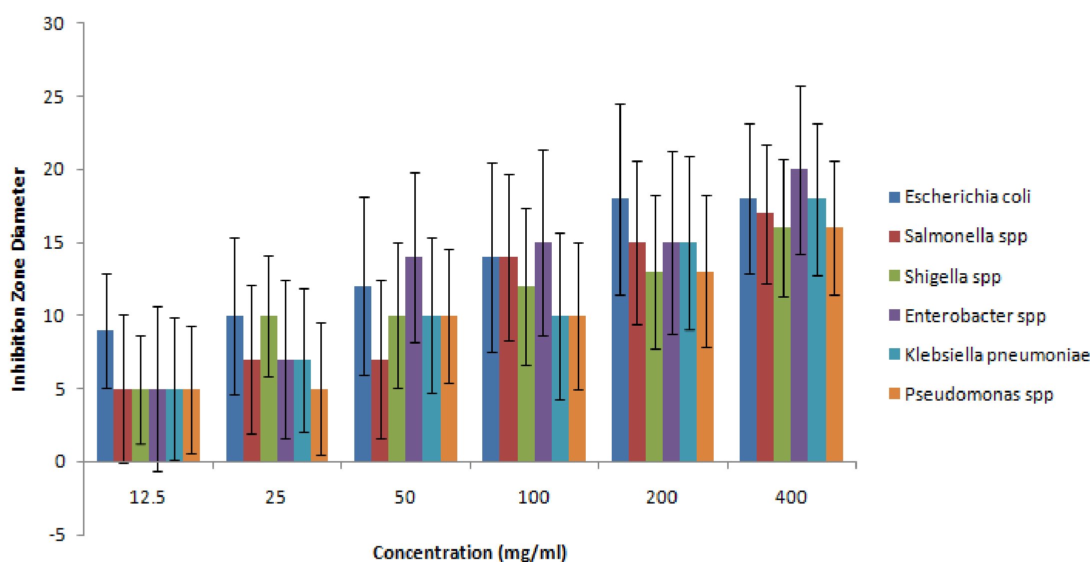

Fig. 1. Antibacterial activity of n-hexane oil extract of *Nigella sativa* on bacterial isolates

The Minimum Cidal Concentration (MCC) of *Escherichia coli, Salmonella* species*, Shigella* species, *Enterobacter aerogenes* and *Klebsiella pneumoniae* for the n-hexane and methanolic oil extracts of *N. sativa* was 100 mg/ml. That of the ethanol oil extracts was 200 mg/ml. This was the lowest concentration, from which there was no bacterial growth during MIC determination that was sub-cultured onto oil extract free nutrient agar.

All concentrations of the ethanol oil extracts of *M. oleifera* were not cidal to the tested isolates indicating that ethanol had the least antibacterial activity compare to n-hexane and methanol Table 3.

Table 2. Minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC) of the *Nigella sativa* oil extracts on bacterial isolates

Bacterial isolates	n-hexane extract		Methanol extract		Ethanol extract	
	MIC	MCC	MIC	MCC	MIC	MCC
Escherichia coli	50	100	50	100	100	200
Salmonella species	50	100	100	100	100	200
Shigella species	50	100	100	100	100	200
Enterobacter aerugenes	50	100	100	100	100	200
Klebsiella pneumoniae	50	100	100	100	100	400
Pseudomonas aeruginosa	200	200	200	200	400	400

Table 3. Minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC) of the *Moringa oleifera* oil extracts on bacterial isolates

Bacterial isolates	n-hexane extract		Methanol extract		Ethanol extract	
	MIC	MCC	MIC	MCC	MIC	MCC
Escherichia coli	100	200	100	200	200	-
Salmonella species	200	200	100	100	200	-
Shigella species	200	200	200	400	400	-
Enterobacter aerugenes	200	400	200	400	200	-
Klebsiella pneumoniae	200	200	200	400	200	-
Pseudomonas aeruginosa	400	400	200	400	-	-

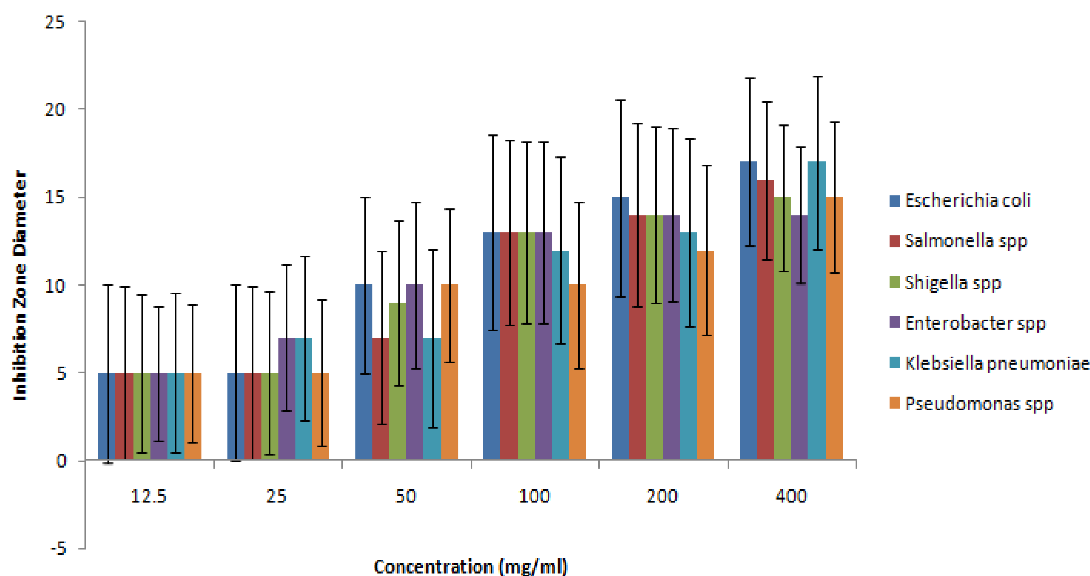

Fig. 2. Antibacterial activity of methanolic oil extract of *Nigella sativa* on the bacterial isolates

Synergistic interactions of *N. sativa* and *M. oleifera* were presented in Table 4. Remarkable increase in bioactivity was numerically recorded against all tested bacteria isolates as average inhibition zone value was increased from 15 mm theoretical value to 22 mm as compared to the potency recorded when the two plant seed oil extracts were tested independently. An appreciable synergistic action was observed against *E. coli* while moderate synergistic action was observed against *Enterobacter aerogenes* isolates, and a weak synergism was observed among *Pseudomonas aeruginosa* isolates. No antagonistic action was observed. Synergy was seen in 100% isolates of *E. coli, Salmonella* species and *Shigella* species followed by 80% in isolates of *Enterobacter aerogenes*, 61% of *Klebsiella pneumoniae* and 58% in isolates of *Pseudomonas aeruginosa*.

Table 5 showed the Plasmid Curing Analysis of MDR *E. coli* isolates using 10% SDS with 80% of the selected multi-drug resistant (MDR) *E. coli* isolates successfully cured. The cured organism showed sensitivity against the plant seeds oil extracts. It was observed that the ethanolic oil extract of *M. oleifera* was weakly reactive on the plasmid cured MDR *E. coli* whereas the n-hexane and methanol oil extracts produced an interestingly wide zone of inhibition. There was significant difference in the antibacterial susceptibility pattern before and after curing indicating correlation between plasmid and drug resistance in the isolates of *E. coli*.

Tables 6 and 7 showed the result of sensitivity test before and after plasmid curing on selected MDR *E. coli*.

Table 4. Synergistic interactions of n-hexane oil extract of *Nigella sativa* with *Moringa oleifera*

Organism	Combination (100 mg/ml/ disc) mm	Synergistic action on percent isolates
E. coli	23±01	100
Salmonella species	22±01	100
Shigella species	22±02	100
Enterobacter aerugenes	20±01	80
K. pneumoniae	19±01	61
P. aeruginosa	23±01	58

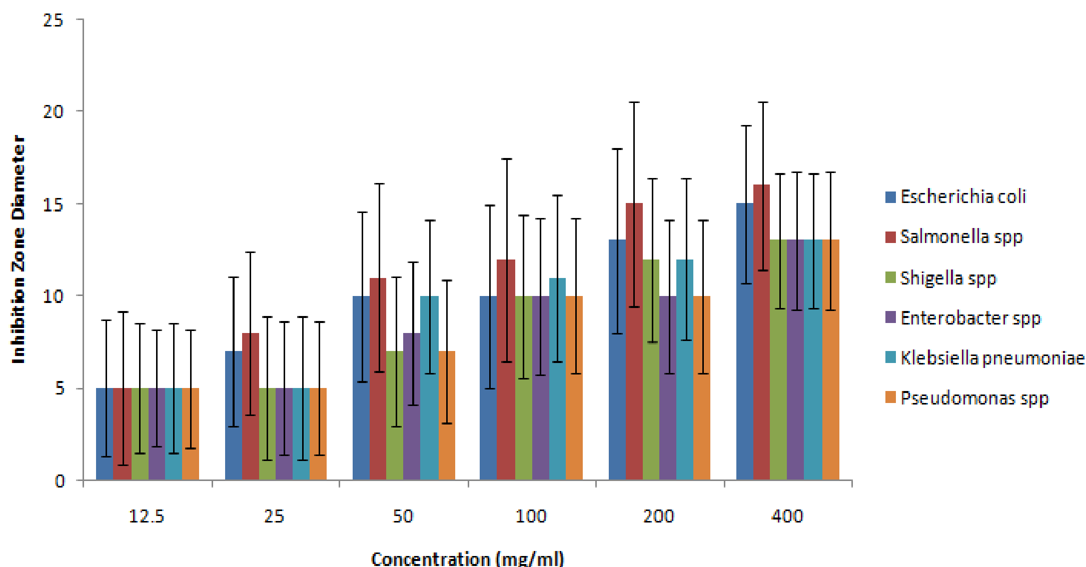

Fig. 3. Antibacterial activity of ethanolic oil extract of *Nigella sativa* on the bacterial isolates

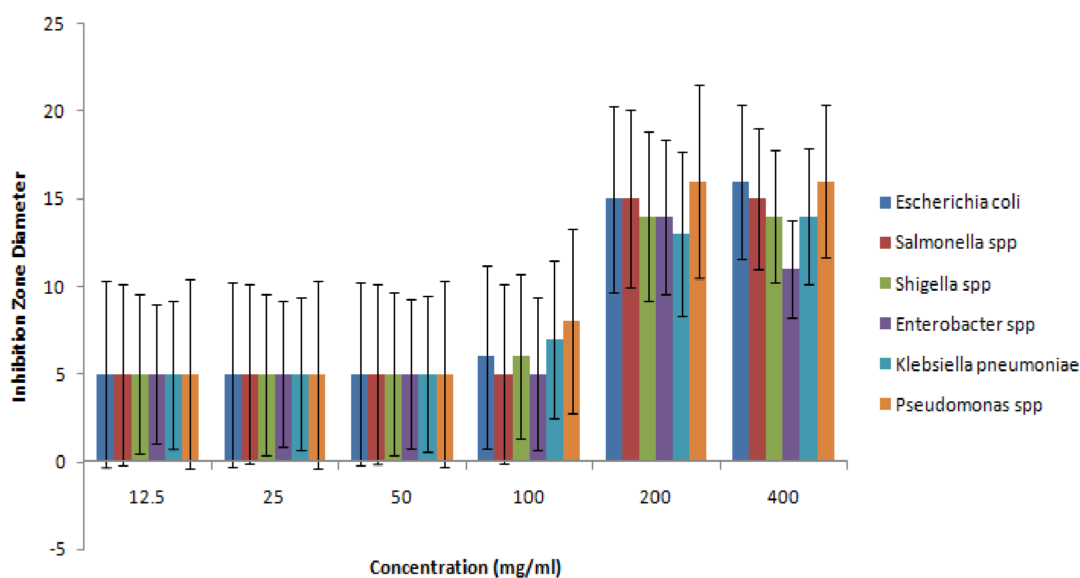

Fig. 4. Antibacterial activity of n-hexane oil extract of the *Moringa oleifera* on the bacterial isolates

Table 5. Plasmid curing analysis of MDR *E. coli* isolates using 10% SDS

Test antibiotics	Number of organisms resistant (pre-curing)	Number/percentage cured	Number/percentage resistant (post-curing)
Ceftazidime	10	10 (100.00)	0 (0)
Cefuroxime	10	10 (100.00)	0 (0)
Gentamicin	10	8 (80.00)	2 (20.00)
Cefixime	10	10 (100.00)	0 (0)
Augmentin	10	5 (50.00)	5 (50.00)

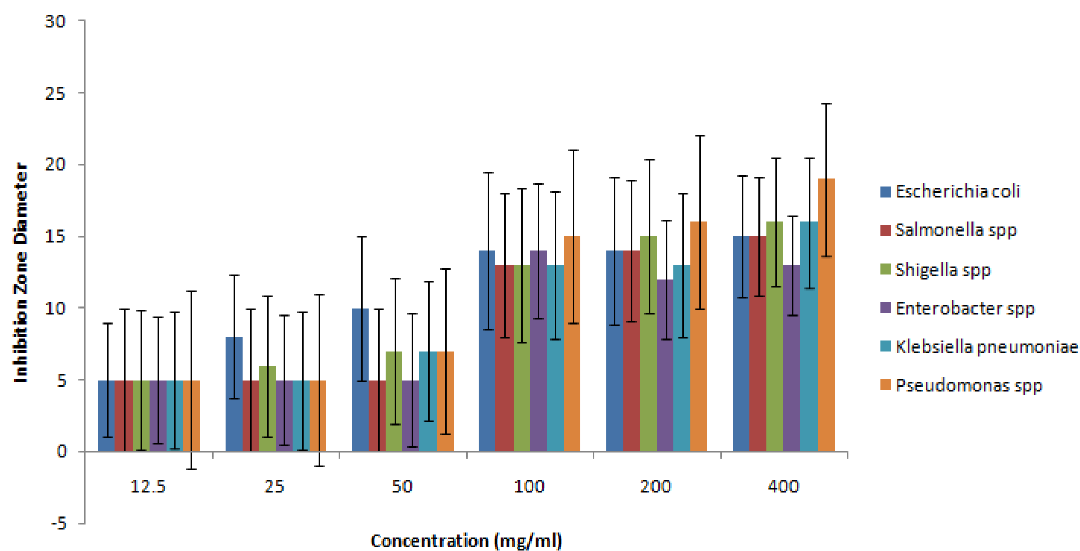

Fig. 5. Antibacterial activity of methanolic oil extract of *Moringa oleifera* on the bacterial isolates

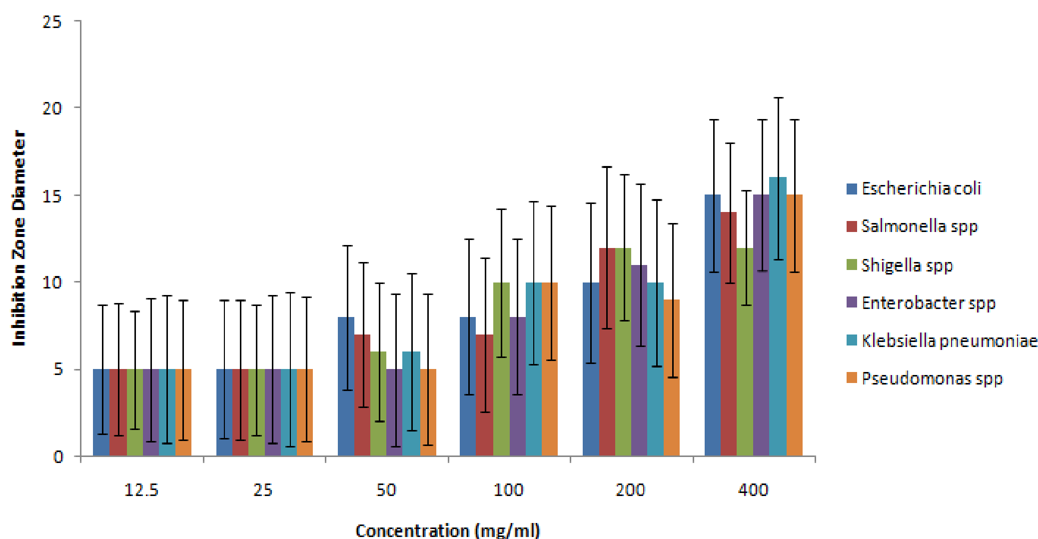

Fig. 6. Antibacterial activity of ethanolic oil extract of the *Moringa oleifera* on the bacterial isolates

Table 6. Result of sensitivity test with *N. sativa* oil extracts before and after plasmid curing on *E. coli*

MDR *E. coli* isolates	Zone of inhibition (mm) at 400 mg/ml before plasmid curing			Zone of inhibition (mm) at 400 mg/ml after plasmid curing		
	n-hexane extract	Methanol extract	Ethanol extract	n-hexane extract	Methanol extract	Ethanol Extract
1	10	7	6	24	20	15
2	7	6	6	20	16	10
3	10	6	6	20	22	15
4	8	6	6	25	18	14
5	8	10	6	21	20	17
6	6	8	6	10	16	17
7	9	6	6	6	7	6
8	8	8	6	23	15	13
9	8	6	6	22	17	13
10	8	7	6	20	13	12

4. DISCUSSION

The abundance of different identified phytoconstituents varies in both plant seeds oil extracts. These differences could be due the strength of each solvent (n-hexane, methanol and ethanol) in extracting the bioactive components. In this present study, terpenoids, flavonoid, tannins, glycosides, saponins, steroids and alkaloids were tested and detected in both *N. sativa* and *M. oleifera* oil extracts. Similar findings reported the presence of saponin and alkaloids in *Nigella sativa* oil and alkaloids, phenolics, flavonoids and tannins in ethanolic extract of *Moringa oleifera* oil respectively [9]. The study is also in analogy with other researchers who also reported presence of alkaloids, tannins, saponins and flavonoids in *M. oleifera* oil extracts [10,11]. Flavonoid has been shown to possess antimicrobial properties [12]. Other researchers had reported that tannins, saponins, flavanoids and terpenoids occur in the Moringa seed oil extracts [13].

The crude n-hexane, methanol and ethanol oil extracts of the *N. sativa* and *M. oleifera* were tested for their antibacterial activity against multi drug resistant diarrheagenic bacterial isolates. The results revealed that both seeds oils extracts (*N. sativa* and *M. oleifera*) produce antimicrobial activity against the tested organisms. These results were in agreement with the reports of

other researchers, showing significant antibacterial activity of *N. sativa* against several Gram negative bacteria [14,15]. From past studies, *N. sativa* and *M. oleifera* have the immense medicinal value. The antibacterial activities of these seeds oils extracts have been demonstrated by several research groups. Previous study on *N. sativa* and *M. oleifera* crude oil extracts and phytoconstituents also supports the fact that plant is active against various pathogens. Findings from this work reveal that *N. sativa* seed oil extract had both bactericidal and bacteriostatic activity on the tested isolates at varying concentrations.

The higher resistance of these Gram-negative bacteria to external agents has been earlier documented, and it is attributed to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergents and hydrophilic dyes. Results of the present study are consistent with the literature data reported [16].

The ethanol oil extract of *M. oleifera* had less effect on the test organisms at the various concentrations used, but there was appreciable antimicrobial activity demonstrated by the methanol oil extract. A similar research conducted showed that *M. oleifera* crude extracts had no activity against *E. coli,* showing disagreement with our findings [17]. Our findings confirm other reports on the antibacterial activity of *M. oleifera* seed extracts [18]. It was reported that the ethanol oil extracts of Moringa seeds have high antibacterial activity against *Salmonella typhi,* while the aqueous extract had low activity against the same organism [13]. Another study showed that both methanol and n-hexane extracts of *M. oleifera* displayed antimicrobial activity against *S. typhi,* even though it was resistant to the ethanol extract [19]. However, *M. oleifera* extracts had low efficacy values on *E. coli* when compared to the standard antibiotics as previously reported [20]. Aney *et al.* found an extract of *M. oleifera* seeds to be as effective as several antibiotics against *E. coli* [21]. According to Shekhar *et al.* crude ethanol extract of *M. oleifera* tested against *E. coli*, *S. typhi*, *V. cholera, Shigella dysentriae* and *Pseudomonas pyocyaneus,* showed activity against *E. coli* at reduced extract concentrations [22].

Ample evidence of individual plant extracts as antimicrobial agent are available, but research on combined effect of two or more plants is very limited [23]. The aspect of synergistic mechanisms becomes the apparent strategy employed by the plants as observed in this study. Robust combinational effect were observed, when the two seeds oils were used in combination. It is well known that most of the independent plant-derived extracts possess weak potency against pathogenic bacteria compared to antibiotics. Hence the combination of plant extracts that demonstrate improved efficacy for killing the drug resistant microbes may form a strong basis for control of such pathogens. There seems to be a

Table 7. Result of sensitivity test with *M. oleifera* extracts before and after plasmid curing on *E. coli*

MDR *E. coli* isolates	Zone of inhibition (mm) at 400 mg/ml before plasmid curing			Zone of inhibition (mm) at 400 mg/ml after plasmid curing		
	n-hexane extract	Methanol extract	Ethanol extract	n-hexane extract	Methanol extract	Ethanol extract
1	6	8	8	16	16	13
2	6	6	6	7	17	20
3	6	6	6	13	15	12
4	6	6	6	12	15	14
5	6	6	6	13	15	17
6	6	6	6	17	16	16
7	6	6	6	6	9	6
8	6	6	6	16	12	15
9	8	7	6	14	18	15
10	6	7	6	15	16	12

double attack of both agents on different target sites of the bacteria which lead to a synergistic effect. Screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds and some plants have provided good indications of these potentials for use in combination with other plants extracts. However, to our knowledge there is no data available on combination effects of *N. sativa* and *M. oleifera* seed oil.

For *N. sativa* oil extracts, the minimum inhibitory concentration (MIC) is highest for the n-hexane and methanol oil extracts on *Pseudomonas aeruginosa* (200 mg/ml), and lowest for the *E. coli, Salmonella* species, *Shigella* species, and *K. pneumonia* (50 mg/ml). While the MIC of *M. oleifera* oil extracts was generally lower than those of *N. sativa* oil extracts. The least activity was recorded for the ethanol extracts of all the seeds oils.

In order to check the involvement of plasmid in the resistance to antibiotics observed in some isolates, plasmid curing was done using sodium dodecyl sulphate (SDS) which was administered to bacterial populations in sub-lethal doses (that is 10%). This led to the elimination of plasmid DNA without harming the bacterial chromosome and thus the bacteria maintain the ability to reproduce and generate offspring. The results show similar trend with those obtained earlier on [24], which showed the presence of plasmid in all isolates that were resistant to at least one antibiotic. This was also in line with the previous research findings on the virulence factors, plasmid profiling and plasmid curing analyses of multi-drug resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species isolated from patients with acute Otitis media [25,26]. Probably, the cured plasmids may be responsible for mediating some or all of the expressed resistances of the microorganisms.

Bacteria employ an extensive repertoire of plasmid, transposons and bacteriophages to facilitate the exchange of resistance and virulence determinants among and between species. As a result, the opportunity for rapid emergence of high-level resistance even in the absence of direct selection by specific antimicrobial pressure abound [27]. In bacteria, the acquisition of resistance may be due to chromosomal mutations or through plasmids that are often capable of transfer from one strain of organism to another, even across the species barrier [28]. The process of transfer and acquisition of resistance determinants among microorganisms is a natural, unstoppable phenomenon exacerbated by the abuse, overuse and misuse of antimicrobials in the treatment of human illness and in animal husbandry, aquaculture and agriculture [29].

5. CONCLUSION

All the evaluated plant seeds extracts were found to be active against the multi-drug resistant Gram-negative bacteria. The tested phytochemical constituents were also detected in both *N. sativa* and *M. oleifera* seed extracts.

Robust combinational effect were observed, when the two seeds oils were used in combination. Plasmids were present in greater proportions of the treated multi-drug resistant bacteria. The plasmid curing led to the elimination of plasmid DNA. The cured plasmids may be responsible for possibly mediating some of the expressed resistances of the microorganisms.

We recommended the evaluation of the exact plant-extract ratio at which the interaction is maximal between the two plants extracts. A wider study with increase in the number of drugs, increase number of clinical isolates especially diarrheagenic isolates and the identification of the effective compounds in the crude extracts are also necessary in order to establish the mode of action against the diarrhea isolates and the mechanism of synergy, which is fundamental to development of pharmacological agents to treat such diseases using medicinal plants. There is a need to carry out advanced research to isolate the bioactive compounds responsible for *N. sativa* impressive antibacterial activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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