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Research Article

COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFECTS OF *Moringa oleifera* AND *Nigella sativa* ON SOME CLINICAL BACTERIAL ISOLATES OBTAINED FROM IGBINEDION UNIVERSITY, OKADA, NIGERIA

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ABSTRACT. Antibacterial susceptibility study of the aqueous, methanol and chloroform extract of *Moringa oleifera* and *Nigella sativa* was carried out to comparatively evaluate the antibacterial effects of these plants against some clinical isolates (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). The susceptibility of the bacterial isolates to the extract of *M. oleifera* and *N. Sativa* were determined by agar well diffusion and minimum inhibitory concentration (MIC) techniques. The findings from this study revealed that *M. oleifera* leaf extract exhibited better antibacterial effects than *N. sativa* seed extract, as indicated by their zones of inhibition and MIC values. The aqueous and chloroform extract of *M. oleifera* exhibited activity only against *S. aureus* and *E. coli* (MIC values of the aqueous and chloroform extract of *M. oleifera* against *S. aureus* and *E. coli* were reported as 3.91 mg/ml and 1.96 mg/ml for *S. aureus*, as well as 7.20 mg/ml and 31.25 mg/ml for *E. coli*), while the methanol extract of *M. oleifera* showed activity against all the three bacterial isolates (MIC values were 31.25 mg/ml, 3.91 mg/ml, and 125 mg/ml for *S. aureus*, *E. coli* and *P. aeruginosa* respectively). Unlike the chloroform extract of *N. sativa* which showed no activity against the three bacterial isolates, the aqueous and methanol extract of *N. sativa* seeds showed some activity only against *S. aureus* with a MIC value of 31.25 mg/ml each. Evidence of the antibacterial effect of *M. oleifera* and *N. sativa*, as revealed in this study, underscores their therapeutic utilization in traditional medicine.

Keywords: *Moringa oleifera, Nigella sativa, Staphylococcus aureus, Escherichia, antibiotic susceptibility*

INTRODUCTION

The richest bioresource of drugs for traditional systems of medicine and nutraceuticals are medicinal plants [1]. Plant products obtained from roots, leaves, seeds, fruits, barks and flowers are the parts of phytomedicines [2]. Mankind has long used medicinal spices and herbs as traditional medicines for different diseases in many parts of the world.

Moringa oleifera is grouped into the Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Genus: *Moringa*, Species: *M. oleifera.* It is widely distributed and naturalized species of a monogeneric family Moringaceae. Horseradish tree, Saijihan, Marango Drumstick tree, Sajna, Kelor, Mulangay, Mlonge and Benzolive are some terms used for *Moringa*. *M. oleifera* is a small native tree that is

indigenous to many regions in South America, Africa, South East Asia, as well as the Pacific and Caribbean Islands [3, 4]. The medicinal usage of *M. oleifera* has been used to treat problems such as skin infection, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera and many other illnesses. It also consists of anti-inflammatory, anti-spasmodic, anti-hypertensive, anti-tumour, anti-oxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol-lowering, renal, anti-diabetic and hepato-protective activities. *Moringa* oil has also found use in the production of skin ointments ever since the Egyptian times and had been claimed to be one of the plants that are very rich in nutrients [4]. Ethanolic extract of leaves, seeds, and flowers of *M. oleifera* against microorganisms have been shown to have antimicrobial activity against organisms such as *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp, *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi A, Staphylococcus aureus, Streptococcus and Candida albicans* have been denoted [5].

Nigella sativa is an indigenous herbaceous plant belonging to the Ranunculaceae family that is more commonly known as the fennel flower plant. This plant, which can grow to a maximum height of about 60 cm, produce black seeds and have blue flowers and finely divided foliage. The black seeds of *N. sativa* are recommended for daily use in Islamic medicine due to its enormous healing potential [2]. In Arabian countries and the Indian subcontinent, *N. sativa* has been used for medicinal and culinary purposes [6].

To further assert the medicinal potential of *M. oleifera* and *N. sativa*, antibacterial susceptibility study of the aqueous, methanol and chloroform extract of these plants was carried out to comparatively evaluate the antibacterial effects of these plants against some clinical isolates (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*).

MATERIALS AND METHODS

Collection and identification of plants

The leaves of the *M. oleifera* plant were collected from Crown Estate Igbinedion University Okada, environment and authenticated by Prof. MacDonald Idu of Plant Biology and Biotechnology (PBB) Department, University of Benin. A voucher specimen of the plant was deposited in the herbarium of the Department. A similar procedure was followed in the authentication of *N. sativa* dried seeds which were purchased from local vendors in Kurmi market at Kano State.

Preparation and extraction of the plant materials

The leaves of *M. oleifera* and seeds of *N. sativa* were separately washed with distilled water and air-dried for two weeks. They were subsequently pulverized before maceration with absolute methanol, chloroform and distilled water. The powdered plant samples were weighed and poured into extraction tanks and 2,500 ml of each solvent was measured and separately added to the contents of the extraction tanks. The tanks were tightly covered and allowed to stand with periodic agitation. After 24 hours for the aqueous extract and 48 hours for the methanol and chloroform extract, the suspensions were then filtered with Whatman No.1 filter papers. The filtrates obtained were dried at a controlled temperature of 40 ^{o}C. The yields were deduced and extract stored in sterile bottles at 4 °C for further analysis [7].

Identification of the test organisms

The test organisms used for this work were collected from Igbinedion University Teaching Hospital (IUTH), Okada and University of Benin Teaching Hospital (UBTH), Benin-city, Edo State, Nigeria. They were *S. aureus, P. aeruginosa,* and *E. coli.* The test organisms were identified using standard microbiological methods which involved colonial morphology, Gram's staining and biochemical reactions [8].

Preparation of the different concentrations of the extract

Sterile bijou bottles were used to prepare eight different concentrations (by serial double dilution technique) of the different plant extract to evaluate their antibacterial effects. The concentrations were 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, 7.82 mg/ml, 1.95 mg/ml, and 0.98 mg/ml prepared for antibacterial susceptibility testing.

Screening of plant extract for susceptibility testing

Each plant extract was subjected to antibacterial assay against the test organisms. The screening was done with the agar well diffusion technique as previously prescribed [9].

Determination of minimum inhibitory concentration (MIC)

The MIC of each extract is the lowest concentration of the extract that will inhibit the visible growth of the test organisms after overnight incubation. The MICs observed were recorded in mg/ml [10].

Statistical analysis

Descriptive statistics of the datasets, as well as Levene test of homogeneity and unpaired Student's t-test, were performed with the IBM Statistical Packages for Social Sciences (SPSS), version 22.0.

RESULTS AND DISCUSSION

Performance of the extraction protocol

The yield and percentage yield of aqueous, methanolic and chloroform extract of *M. oleifera* leaves are presented in Table 1. A 120 g aqueous extract of the *M. oleifera* leaves gave a yield of 72.3g (60.3 %), while a 110 g methanolic extract gave a yield of 53.4 g (48.5 %), and a 130 g chloroform extract gave a yield of 31.8 g (24.5 %). Statistical analysis showed that there was no significant difference ($p = 0.292$; $\alpha = 0.05$) between percentage yields of aqueous and methanol extract. However, there were significant differences between methanol and chloroform extract ($p = 0.005$; $\alpha = 0.05$) as well as between aqueous and chloroform extract ($p = 0.005$; $\alpha = 0.05$).

Table 2 represents the yield and percentage yield of aqueous, methanol and chloroform extract of *N. sativa* seeds. A yield of 521 g (86.8 %) was obtained from a 600 g aqueous extract of *N. sativa*; 530 g methanol extract gave a yield of 159 g (30.0 %), while a yield of 218 g (39.6 %) was derived from 550 g chloroform extract. Statistical analysis indicated that there was no significant difference ($p = 0.23$; $\alpha = 0.05$) between the percentage yield of methanol and chloroform extract. However, there were significant differences between the aqueous and methanol extract ($p = 0.000$; $\alpha = 0.05$), as well as between the aqueous and chloroform extract ($p = 0.000$; $\alpha = 0.05$).

Table 1. *Yields of the aqueous, methanol and chloroform extract of Moringa oleifera leaves*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Aqueous	120	72.3	60.3[a]
Methanol	110	53.4	48.5[a]
Chloroform	130	31.8	24.5[b]

Key: g = gram, % = percentage, values with the same letter (a) showed no significant difference while values with different letters (a, b) showed significant difference.

Table 2. *Yields and Percentage Yields of the aqueous, methanol and chloroform extract of Nigella sativa seed*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Aqueous	600g	521g	86.8[a]
Methanol	530g	159g	30[b]
Chloroform	550g	218g	39.6[b]

Key: g = gram, % = percentage, values with the same letter (a) showed no significant difference while values with different letters (a, b) showed significant difference.

Antibacterial susceptibility assay

The mean zones of inhibition of *N. sativa* and *M. oleifera* extract against *S. aureus* are presented in Table 3. Except for the chloroform extract of *N. sativa* which exhibited no activity for all the test concentrations of the extract (1.95 to 250 mg/ml) against *S. aureus*, both the aqueous and methanol extract of *N. sativa* and *M. oleifera*, as well as the chloroform extract of *M. oleifera*, exhibited a varying degree of activity against *S. aureus*. The most potent activity against *S. aureus* was exhibited by the chloroform extract of *M. oleifera*.

The chloroform extract of *M. oleifera* showed the highest activity even with activity at 1.95 mg/ml. A MIC of 31.25 mg/ml was respectively reported for aqueous and methanol extract of *N. sativa* and methanol extract of *M. oleifera*. Statistical analysis showed no significant difference between the activities of the aqueous and methanol extract of *N. sativa* ($p = 0.276$; $\alpha = 0.05$) against *S. aureus* as well as between those of *N. sativa* aqueous extract and *M. oleifera* methanolic extract ($p = 0.199$; $\alpha = 0.05$). However, the methanol extract of *N. sativa* was significantly different ($p = 0.031$; $\alpha = 0.05$) from those of *M. oleifera*.

The mean zones of inhibition of *N. sativa* and *M. oleifera* extract against *P. aeruginosa* are presented in Table 4. Of the six extract assayed, only the methanol extract of *M. oleifera* (MMO) exhibited activity against *P. aeruginosa* with an MIC value of 125 mg/ml.

Table 3. *Mean zones of inhibition of Nigella sativa and Moringa oleifera leaf extract against Staphylococcus aureus*

Extract	Concentrations (mg/ml) of extract and their mean zones of inhibition ± standard error (mm)							
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.63mg/ml	7.82mg/ml	3.91mg/ml	1.95mg/ml
ANS	29.0±0.9	26.5±0.5	19.8 ±0.7	12.7 ±0.5	NA	NA	NA	NA
MNS	18.8±0.7	20.2±0.3	17.8±0.5	15.3±1.0	NA	NA	NA	NA
CNS	NA	NA	NA	NA	NA	NA	NA	NA
AMO	27.2±0.1	25.8±0.7	22.7±0.8	18.0±1.1	20.5±0.8	22.3±0.5	16.7±8.4	NA
MMO	30.0±0.3	28.5±1.2	26.7±0.6	21.8±1.1	NA	NA	NA	NA
CMO	31.0±0.4	28.0±1.1	27.3±0.9	26.1±0.8	24.3±1.4	22.1±0.9	18.0±0.5	13.0±0.6

Key: ANS=Aqueous extract of *Nigella sativa*, MNS=Methanol extract of *Nigella sativa*, CNS=Chloroform extract of *Nigella sativa*, AMO=Aqueous extract of *Moringa oleifera*, MMO=Methanol extract of *Moringa oleifera*, CMO=Chloroform extract of *Moringa oleifera*, NA = No Activity; mm = millimeter.

Table 4. *Mean zones inhibition of Nigella sativa seed and Moringa oleifera leaf extract against Pseudomonas aeruginosa*

Extract	Concentrations (mg/ml) of extract and their mean zones of inhibition ± standard error (mm)							
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.63mg/ml	7.82mg/ml	3.91mg/ml	1.95mg/ml
ANS	NA	NA	NA	NA	NA	NA	NA	NA
MNS	NA	NA	NA	NA	NA	NA	NA	NA
CNS	NA	NA	NA	NA	NA	NA	NA	NA
AMO	NA	NA	NA	NA	NA	NA	NA	NA
MMO	28.0±0.3	25.3±0.4	NA	NA	NA	NA	NA	NA
CMO	NA	NA	NA	NA	NA	NA	NA	NA

Key: ANS=Aqueous extract of *Nigella sativa*, MNS=Methanol extract of *Nigella sativa*, CNS=Chloroform extract of *Nigella sativa*, AMO=Aqueous extract of *Moringa oleifera*, MMO=Methanol extract of *Moringa oleifera*, CMO=Chloroform extract of *Moringa oleifera*, NA = No Activity; mm = millimeter.

Table 5 represents the mean zones of inhibition of *N. sativa* and *M. oleifera* extract against *E. coli*. All the extract of *N. sativa* exhibited no activity, while all the extract of *M. oleifera* exhibited a varying degree of activity against *E. coli*. Statistical analysis indicated a significant difference ($p = 0.028$; $\alpha = 0.05$) between the aqueous and methanolic extract of *M. oleifera*; while no significant difference ($p = 0.025$; $\alpha = 0.05$) was observed between the aqueous and chloroform extract of *M. oleifera*, as well as between the methanol and chloroform extract of *M. oleifera* ($p = 0.430$; $\alpha = 0.05$).

Table 5. *Mean zones of inhibition of Nigella sativa seed and Moringa oleifera leaf extract against Escherichia coli*

Extract	Concentrations (mg/ml) of extract and their mean zones of inhibition ± standard error (mm)							
	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.63mg/ml	9.82mg/ml	3.91mg/ml	1.95mg/ml
ANS	NA	NA	NA	NA	NA	NA	NA	NA
MNS	NA	NA	NA	NA	NA	NA	NA	NA
CNS	NA	NA	NA	NA	NA	NA	NA	NA
AMO	25.3±0.4	19.8±0.5	23.6±0.8	18.2±0.3	12.8±0.5	11.8±0.2	NA	NA
MMO	27.5±0.3	29.1±0.7	29.1±1.1	28.0±0.3	25.0±0.3	21.0±0.4	17.0±0.6	NA
CMO	26.1±0.4	26.8±0.5	18.6±0.8	19.5±0.2	NA	NA	NA	NA

Key: ANS=Aqueous extract of *Nigella sativa*, MNS=Methanol extract of *Nigella sativa*, CNS=Chloroform extract of *Nigella sativa*, AMO=Aqueous extract of *Moringa oleifera*, MMO=Methanol extract of *Moringa oleifera*, CMO=Chloroform extract of *Moringa oleifera*, NA = No Activity; mm = millimeter.

The MIC of the plant extract against the three test organisms is presented in Table 6. The chloroform extract of *N. sativa* showed no activity at all the varying concentrations of the extract used in the present study. Aqueous and methanol extract of *N. sativa* showed activity only against *S. aureus* with MIC value of 31.25 mg/ml. There was no significant difference between the aqueous and methanol extract of *N. sativa* ($p = 0.71$; $\alpha = 0.05$). The aqueous and chloroform extract of *M. oleifera* showed activities against only *S. aureus* and *E. coli*, while the methanol extract of *M. oleifera* showed activity against all the three isolates. The only chloroform extract of *M. oleifera* had the lowest MIC value of 1.95 mg/ml against *S. aureus*.

It has also been reported that chloroform extract of *N. sativa* showed no activity against the three bacteria examined [6]. Aqueous extract of *N. sativa* seeds also showed no activity against *S. aureus* but activity against *E. coli* at 25 mg/ml [2].

Table 6. *Minimum inhibitory concentrations (MICs) of aqueous, methanol and chloroform plant extract against the isolates*

Extracts	MIC (mg/ml) of the extract and their mean zones of inhibition ± standard error (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
ANS	31.25 (12.7 ±0.5)	NA	NA
MNS	31.25 (15.3±1.0)	NA	NA
CNS	NA	NA	NA
AMO	3.91 (16.7±8.4)	7.82 (11.8±0.2)	NA
MMO	31.25 (21.8±1.09)	3.91 (17±0.6)	125 (25.3±04)
CMO	1.95 (13±0.6)	31.25 (19.5±0.2)	NA

Key: ANS=Aqueous extract of *Nigella sativa*, MNS=Methanol extract of *Nigella sativa*, CNS=Chloroform extract of *Nigella sativa*, AMO=Aqueous extract of *Moringa oleifera*, MMO=Methanol extract of *Moringa oleifera*, CMO=Chloroform extract of *Moringa oleifera*, NA = No Activity; mm = millimeter.

In this study, the aqueous and chloroform extract of *M. oleifera* showed activity against *S. aureus* and *E. coli*. This was in agreement with the report of Vinoth *et al*. [11]. The methanol extract of *M. oleifera* also showed activity against all the three bacterial isolates used in this study. Besides, *M. oleifera* showed more antibacterial activity than *N. sativa* against the three bacterial isolates used in this study. Also, the methanol extract of *M. oleifera* was the only extract with activity against all three bacterial isolates used in this study.

The methanol extract of *M. oleifera* had been reported to contain some phytochemical constituents such as tannin, phenol, flavonoid, and saponins [5, 12, 13]. Phenolic acid toxicity to micro-organisms is mediated by the mechanism of enzyme inhibition, substrate deprivation, and formation of cell wall complex [14]. Tannins mechanism of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes; as well as their ability to cause substrate deprivations, and formation of cell wall and metal ion complexes [14]. The biological activities of saponins range from antimicrobial, antimalarial, antiplasmodial, antiviral to the antitumor activities [15, 16].

In this study, there was no significant difference in the percentage yield of the aqueous extract of *M. oleifera* in comparison with the methanol extract. However, the aqueous extract of *M. oleifera* showed activity against *S. aureus* and *E. coli* but not *P. aeruginosa*. Therefore, methanol was the best solvent for extraction in this study which was in agreement with the report of Javed *et al*. [17].

CONCLUSION

M. oleifera leaf extract showed more activity than *N. sativa* seed extract. Aqueous, methanol and chloroform were used for extraction, with the methanol extract of *M. oleifera* exhibiting more antibacterial activity than the others concerning the three

bacterial isolates used in this study. Also, this study shows that *M. oleifera* leaves has a better antibacterial effect than *N. sativa* seeds. There is a need to carry out advanced research to isolate the bioactive compounds responsible for *M. oleifera* impressive antibacterial activity.

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