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Original Research Article

Comparative Evaluation of the Antibacterial Activities of Ethanol Leaf Extracts of *Moringa oleifera* (Lamarck) and *Dialium guineense* (Jacqueline)

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Abstract: The antibacterial activities of many medicinal plants have been carried out to assess their potential use in bacteria borne diseases. In this study the antibacterial activities of two medicinal plants, *Moringa oleifera* and *Dialium guineense* as well as their phytochemical contents were carried out and compared. Their leaves were collected separately, dried, pulverized to powder and extracted with ethanol. The phytochemical analysis of *M. oleifera and D. guineense* extracts were carried out using standard methods, while their antibacterial activities were evaluated and compare using some bacterial isolates and by agar well diffusion method. The result of the phytochemical screening showed that both plants possess the same phytochemicals with exemption of steroids which is present only in *D. guineense*, and cardiac glycosides only present in *M. oleifera.* The results of the antibacterial activities are as follows; on *Staphylococcus aureus,* at concentration of 100 mg/ml, *M. oleifera* and *D. guineense* gave inhibition zone diameters (IZD) of 9.5± 0.30mm and 7.5 ±0.14mm respectively, with minimum inhibitory concentration (MIC) of 1.25% and 0.625% respectively. On *Streptococcus pneumonia,* at concentration of 100mg/ml *M. oleifera* and *D. guineense* gave IZD of 8.5± 0.26mm and 8.5 ±0.26 mm respectively, with (MIC) of 0.625% and 1.25% respectively. On *Escherichia coli,* at 100 mg/ml, *M. oleifera* and *D. guineesne* gave IZD of 10.0± 0.34mm and 8.5 ±0.26 mm respectively, (MIC) of 2.5% and 1.25% respectively. On *Pseudomonas aeruginosa* at concentration of 100 mg/ml, *M. oleifera* and *D. guineense* gave IZD of 9.5± 0.32mm and 7.0± 0.09mm respectively. Examination of these results showed that *M. oleifera* is slightly more active than *D. guineesne*.

Keywords: Antibacterial; *Moringa oleifera; Dialium guineense*; comparative.

1. INTRODUCTION

It has been widely observed that traditional medicines and medicinal plants are used in developing countries as therapeutic agents for the maintenance of good health [1], also, it has been shown that increase on the use of medicinal plants in the industrialized countries came from the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies [2]. Bioactive compounds use mainly for medicinal purposes are product of medicinal plants. The action of these compounds may be either by interfering in the metabolism of the microorganisms infecting them or by acting on different systems of living. In whichever way we may look at it the active phytochemicals or bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favor of the host. Based on these, the identification, isolation, purification and characterization of active ingredients of crude extracts of bioactive compound in plants, by various

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analytical methods is important. The antioxidant, antimicrobial and antipyretic effects of the phytochemicals in medicinal plant could be the source of their medicinal properties [3, 4].

Moringa oleifera belongs to the flowering plant family Moringaceae. The family has13 species and they are from tropical and subtropical climates. Their size range from tiny herbs to massive trees. The most widely cultivated species are Moringa oleifera and M. peregrina. Moringa oleifera Lam and are the best known and most widely distributed species of Moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world [5]. The different extracts of the plant had been screened for in vitro antiinflammatory and antioxidant activities [6]. A review of the literature indicated that the presence of quercetin flavonoids [7], sterols [8], tocopherols and alpha and beta carotene and other antioxidants [6] have been reported from the plant. The antibacterial activity of *Moringa oleifera* has been shown in different studies. (Bukar *et al.,* 2010), (Lar *et al.,* 2011), (Bijal and Bhumika, 2015), (Khanitta and Angelika, 2015 and (Bichi and Shehu, 2018) [9-13], had shown the antibacterial effectiveness of the plant extracts on some bacteria isolates.

Dialium guineense Willd., called black velvet tamarind, is common in temperate and tropical areas, a perennial flowering plant and grows in dense savannah forests, shadowy canyons and gallery forests. It naturally grows wild in bushes and forests on moist, sometimes brackish soils across Nigeria and West Africa. In Yoruba land, Nigeria it is called "awin [14]. The antibacterial activities of the plant have also been carried out for instance, Orji *et al.,* 2012 [15], has demonstrated the effectiveness of leaf extract on some bacteria isolates

The aim of this study is to compare the phytochemical contents and the antibacterial activities of the leaf extracts of these two plants on some bacterial isolates.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Instrument and Glassware

Analytical weighing balance, Desiccator, Water bath, Beakers, Conical flask, Ceramic crucible, Whattman, Filter paper, Glass funnels, Measuring cylinder, Syringes, Test tubes, Petri dishes, Universal, Bottles, Sterilizer. Solvents use includes ethanol, dimethyl sulfoxide (DMSO) and distilled water.

2.1.2 Collection and Preparation of the Plant Samples

Moringa oleifera and Dalium guineense plants were procured from a farmland in Abia State, Nigeria. The leaves of *Moringa oleifera* and *Dalium guineense* were separated out and washed under running water, shade dried at room temperature for 2 weeks, pulverized to powder using a milling machine and stored at room temperature. 75g of each powder sample was extracted by maceration with 725ml of ethanol. Period of extraction was 24 hours. They were filtered using Whatman No 1 Filter paper. Each extract was concentrated and dried using a water bath. The dried samples were stored in a desiccator until the time of use.

2.2 Methods

2.2.1 Phytochemical Screenings

The phytochemical screenings were done according to the method by (Harbone, 1998) [16].

2.2.2 Selection of the Microorganisms

The bacteria strains used were those recommended by the Department of Microbiology in the University of Port Harcourt, Rivers State, Nigeria. The bacterial strains used for this study were *Staphylococcus aureus, Streptococcus pneumonia, Pseudomonas aeruginosa, and Escherichia coli.* All the bacterial strains were grown and maintained in nutrient agar.

2.2.3 Preparation of Extract for Antimicrobial Assay

The concentration of extract was prepared on each occasion by careful weighing and dissolving in a suitable volume of Dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml. The dilutions prepared were 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. The diluent used was DMSO (Dimethylsulfoxide).

2.2.4 Evaluation of Antibacterial Activity

The crude extracts of *Moringa oleifera* and *Dalium guineense* were screened for their antibacterial activity against some Gram-positive and Gram-negative. Agar well diffusion method according to (Cheesbrough, 2006) [17], was used to determine the antibacterial activity of the extracts. 1ml of the already dispersed strain of organism was added into 15ml of nutrient broth in tubes. It was swirled to evenly distribute them and poured into petri dishes. Allowed to solidify on the bench and wells (6mm in diameter) were punctured in the agar medium using sterile stainless cork borer. A Drops of each extract (*M. oleifera* and *D. guineense* respectively) and control (DMSO) was introduced into the wells. Also, this procedure was carried out using their combinations to determine their synergistic effect. Plates were incubated overnight at 37°C. The procedure was carried out in duplicate and the antibacterial activity was interpreted from the size of the diameter of inhibition zone around each well. It was measured and recorded.

2.2.5 Determination of Minimum Inhibitory Concentration (MIC)

A series of culture tubes were prepared, each containing a liquid medium and different concentrations of the antimicrobial agent i.e., 5%, 2.5%, 1.25%, 0.625% and 0.3125%. The tubes were inoculated with the test organism and incubated for 24hrs at 37°C. This procedure was carried out for each of the test organisms. After incubation, the tubes were examined for turbidity (growth). The MIC was determined as the least concentration of the antimicrobial agent that inhibited bacterial growth.

2.2.6 Antibiotic Sensitivity Testing

This was determined using a modified Kirby Bauer`s disc diffusion method (Bauer *et al.,* 1966) [18]. Briefly, the cultures of test bacteria were spread on the Muller Hinton Agar media in petriplates and they were applied on media by swabbing, under laboratory condition. The antibiotic disc (containing eight antibiotics) was gently placed on the agar media at room temperature, plates were incubated at 37°C for 24 hours. This was performed in duplicates and the diameters of the inhibition zones were measured in millimeters.

3. RESULTS

3.1 Result for percentage yield

Table 1: Percentage yield of extracts

Plant	Dry Leaves (g)	Ethanol Extract (g)	Percentage Yield (%w/w)
Moringa oleifera	75	3.42	4.56
Dalium guineense	75	3.35	4.46

3.2 The Result for Phytochemical Screening

Table 2: showing the results for phytochemical screening of the two plants

Plant	Phytochemical content						
M. oleifera	Alkaloids	Saponins	Flavonoids	Anthraquinone	Carbohydrates	Tannin	Cardiac glycosids
D. guineense	Alkaloids	Saponins	Flavonoids	Anthraquinone	Carbohydrates	Tannin	steroids

From table 2 the two plants have almost the same phytochemical content, however, while *Moringa oleifera* has cardiac glycosides, *Dialium guineense* has steroids and that is where they differ in their phytochemical contents.

3.3 Results for Antibacterial Activities

Table 3: Result for antibacterial activities of ethanol leaf extracts of *Moringa oleifera* and *Dialium guineense* on the bacterial isolates

S/No	Test organism	Concentration (mg/ml)	Inhibition zone diameter (IZD) (mm)		
			Moringa oleifera	***Dialium guineense***	**control**
1	*Staphylococcus aureus*	100	9.5± 0.30	7.5 ± 0.14	0
		50	8.3± 0.24	7.2± 0.10	0
		25	7.7± 0.16	7.0± 0.09	0
2	*Streptococcus pneumonia*	100	8.5± 0.26	8.5 ± 0.26	0
		50	8.0± 0.13	8.0± 0.13	0
		25	7.7± 0.15	7.5± 0.15	0
3	*Escherichia coli*	100	10.0± 0.34	8.5 ± 0.26	0
		50	8.9± 0.29	8.0± 0.13	0
		25	8.1± 0.20	7.9± 0.19	0
4	*Pseudomonas aeruginosa*	100	9.5± 0.32	7.0± 0.09	0
		50	7.9± 0.18	6.4± 0.05	0
		25	0.0± 0.00	0.0± 0.00	0

From the result in table 3 above, *Moringa oleifera* gave the highest activity at concentration of 100 mg/ml on Escherichia coli with inhibition zone diameter of 10.0± 0.34 mm, while *Dialium guineense* gave highest activity at concentration of 100 mg/ml on *Escherichia coli* and *Streptococcus pneumonia* with inhibition zone diameter of 8.5 ± 0.26 mm.

Table 4: The minimum inhibitory concentration (MIC) of *Moringa oleifere* and *Dialium guineense* on the bacterial isolates

S/No	Test organism	Minimum inhibitory concentration (%)	
		Moringa oleifera	*Dialium guineense*
1	*Staphylococcus aureus.*	1.25	0.625
2	*Streptococcus pneumonia*	0.625	1.25
3	*Escherichia coli*	2.5	1.25
4	*Pseudomonas aeruginosa.*	0.0	0.0

From table 4, we have the highest minimum inhibitory concentration of 2.5% of *Moringa oleifera* on *Escherichia coli*, while *Dialium guineense* has the highest minimum inhibitory concentration of 1.5% on *Streptococcus pneumonia* and *Escherichia coli.*

Table 5: Result for antibiotic Sensitivity testing against bacterial isolates.

S/No	Antibiotics	Inhibition zone diameter (IZD) (mm)			
		Staphylococcus aureus	*Streptococcus pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	Gentamicin	15	12	19	19
2	Cefixime	27	19	20	19
3	Cefuroxime	22	30	29	17
4	Ceftazidime	29	20	21	19
5	Ciprofloxacin	21	20	30	33
6	Nitrofurantoin	18	23	20	20
7	Augmentin	21	15	18	15
8	Ofloxacin	22	16	13	17

From the result on table 5 on the antibiotic sensitivity testing, it is clear that all the antibiotic where sensitive to the test organisms. However, cefuroxime and ciprofloxacin were more active than the rest of the antibiotics used.

DISCUSSION

A lot of issues have been expressed about the increasing prevalence of pathogenic microorganisms, which are resistant to the recent antibiotics that have been produced in the last three decades [19]. Antimicrobials from plants have enormous therapeutic potential as they can be used with lesser side effects that are often associated with synthetic antimicrobials. M. oleifera for instance is a very important plant, with impressive range of medicinal uses and high nutritional value [20].

Phytochemical Screening

Examination of the phytochemical results showed the presence of Alkaloids, saponin, flavonoids, anthraquinone, Carbohydrate, Cardiac glycosides and Tannin in *Moringa oleifera.* This is similar to that reported buy Ojiako, 2014 [21], with the exemption of cardiac glycoside and carbohydrate. The leaves of *M. oleifera* have also been known to contain a number of phytochemicals such as flavonods, saponins, tannins and other phenolic compounds that have antimicrobial activities [22-24].

Dialium guineense showed the presence of Alkaloids, saponin, flavonoids, anthraquinone, Carbohydrate, tannin, and steroids. This is similar to the result obtained by Osuagwu *et al.,* 2013 [25] with the absence of anthraquinone and carbohydrate. Gideon *et al*., 2013 [26], showed that flavonoids, alkaloids, tannins and saponins are present in the leaf and bark of *D. guineense* of ethanolic and aqueous extract. However, this study showed the presence of steroids and carbohydrate in addition.

Comparing the two plants, the phytochemical contents are very much similar with alkaloids, saponin, flavonoids, anthraquinone and carbohydrates been present in both plants, however, while cardiac glycosides are present in *Moringa oleifera,* and not in *Dialium guineense,* steroids are present in *Dialium guineense*. In these two is the phytochemical content differ in both plant.

Antibacterial activities

The activities of the *Moring oleifera* on the bacterial isolates used showed that the extracts are active against the four bacterial isolates used namely *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa,* at concentration of 100 and 50mg/ml. however, at concentration of 25mg/ml, the extract is not active against *Pseudomonas aeruginosa,* but active on all the other bacteria isolates. This result is support by Bukar *et al.,* 2010 [9] which showed that the extract is sensitive to most of the organisms used in this study.

The activities of the *Dialium guineense* ethanol leaf extract showed that it active against all the bacteria isolates used in this study just like *Moringa oleifera* at concentration of 100 and 50mg/ml, but at 25mg/ml, like *Moringa oleifera* it was not active against Pseudomonas aeruginosa, but still active on the other bacterial isolates. The fact that the extract is active against *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa* is partially supported by Osuagwu *et al.,* 2013 [25], which showed that the ethanol leaf extract is active against *Pseudomonas aeruginosa*, but little activities against *E. coli* and *Staphylococcus aureus.*

Comparing the antibacterial activities of the two plant, it is clear they have relatively the same antibacterial action on the bacterial isolates used in this study. However, a closer look at the result showed that *Moringa oleifera* ethanol leaf extract has more activity on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at concentration of 100mg/ml, with inhibition zone diameters of 9.5± 0.30mm, 10.0± 0.34mm and 9.5± 0.32mm respectively than those of *Dialium guineense* with inhibition zone diameters of 7.5 ± 0.14mm, 8.5 ± 0.26mm and 7.0± 0.09mm respectively at the same concentration. The same activity trend is also obtained on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* with respect to other concentration. On the other hand, the activities of the two extracts on *Streptococcus pneumonia* are relatively the same at concentration of 100mg/ml and 50mg/ml with inhibition zone diameter of 8.5± 0.26mm and 8.0± 0.13mm respectively. However, at concentration of 25mg/ml *Moringa oleifera* gave more activity than *Dialium guineense* with inhibition zone diameters of 7.7± 0.15mm and 7.5± 0.15mm respectively. The activity of both extracts at concentration of 25mg/ml on *Pseudomonas aeruginosa* showed zero activities.

Examination of the minimum inhibitory concentration (MIC) of the two extracts showed that the occurred in the range of 0.625% to 2.5% in *Moringa oleifera* and 0.625% to 1.25% in *Dialium guineense*. However, there was no inhibition by *Pseudomonas aeruginosa* for concentration range of 0.3125 to 5% used in determining its MIC and thus the MIC could not be determined. However, in comparison, the results of the activities of the ethanol leaf extracts of the two plants, showed very close level of activities against the bacteria isolates used.

Examination of the results on the antibiotic susceptibility test carried out, it was observed that Ceftazidime had the highest activity against *Staphylococcus aureus*, Cefuroxime had the highest activity against *Streptococcus pneumoniae* and Ciprofloxacin had the highest activity against *Escherichia coli* and *Pseudomonas aeruginosa.* On the average, cefuroxime and ciprofloxacin were more active than the rest of the antibiotics used on the test organisms used in this study. However, when compare their activities to those of the two plant extracts, they gave higher performance. On the other hand, isolation of the pure active agents from the plants may give more activities at lower concentrations.

CONCLUSION

The results from the above study showed that the ethanol leaf extracts of the two plants, *Moringa oleifera* and *Dialium guineense* have appreciable antibacterial activities against the bacteria isolates use, and if well developed, could be used as natural antibiotics in treating infections caused by *S. aureus*, *Streptococcus pheumoniae, Escherichia coli* and *Pseudomonas aeruginosa*.

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