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Full Length Research Paper

Assessment of antimicrobial effect of moringa: *In vitro* and *in vivo* evaluation

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This study was carried out to evaluate the antibacterial activity of water and ethanol extracts of *Moringa oleifera* (lam) leaves against major food poisoning reference strains using agar well diffusion method. Ethanolic extract showed greater antibacterial activity than water extract. The highest zones of inhibition were against Gram negative bacteria: *Escherichia coli* O157 ATTC 700728, *E. coli* O78, *E. coli* O26, *Salmonella* Typhimurium ATCC 13311 and *Shigella bodyii* ATCC 9207 with 25, 20, 12, 12 and 11 mm, mean zones of inhibition respectively. Followed by Gram positive bacteria: *Staphylococcus aureus* NCINB 50080 and MRSA ATCC 13565 with 18 and 11 mm mean zones of inhibition. Against *E. coli* O157, ethanolic extracts was more effective than oxytetracycline. *In vivo* study was carried out on 36 growing New Zealand White (NZW*) rabbit fed 0, 0.15, 0.30 and 0.45% *M. oleifera* dry leaves, the rabbit's weight gain, average daily gain (*ADG) and feed conversion efficiency were higher for rabbits fed 0.15 (R_2) and 0.30% (R_3) Moringa than others. Faecal bacterial count revealed that groups R_3 and R_2 showed significantly lower *E. coli*, *S. aureus* and heterotrophic plate count and non-detectable limits of Salmonella than the other groups. Conclusion enhanced supplementations of dry *Moringa* leaves with concentration of 0.15 and 0.30%.

Key words: Moringa leaves, antibacterial, agar well diffusion, bacterial count, rabbit.

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide (Al-Bari et al., 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and add urgency to the search for new infection-fighting strategies (Zy et al., 2005; Rojas et al., 2006). For a long time, plants have been an important source of natural products for health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana et al., 2007). Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. Plants are the cheapest and safest

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alternative sources of antimicrobials (Sharif and Banik, 2006; Doughari et al., 2007a). *Moringa oleifera* (Lam) is the most widely cultivated species of a monogeneric family, which is widely used for treating bacterial infection, fungal infection (Fahey, 2005). *M. oleifera* is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, South Asia, South America and the pacific and Caribbean Islands (Julia, 2008). *Moringa* leaves contain phytochemical compounds which are considered full of medicinal properties (Monica et al., 2010). The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, campfire and many other phytochemicals. It is very important for its medicinal value. Other important medicinal properties of the plant include antispasmodic (Caceres et al., 1992), diuretic (Morton, 1991), antihypertensive (Dahot, 1988), cholesterol lowering (Mehta et al., 2003), antioxidant, antidiabetic, hepatoprotective (Ruckmani et al., 1998), antibacterial and antifungal activities (Nickon et al., 2003). According to World Health Organization (WHO, 2002) and Andy et al. (2008) more than 80% of the world's population rely on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Much research has been done worldwide to identify and study antibacterial compounds found in medicinal plants (Lugman et al., 2007; Silva et al., 2008; Mbosso et al., 2010). According to Rios and Recio (2005) studies, using essential oils or isolated compounds such as alkaloids, flavenoids, sesquiterpenes, lactones, diterpenes, triterpenes and naphtoquinones to test antibacterial effects are necessary to validate the use of a range of popular medicines. The Moringa tree (*M. oleifera*), a phanerogamous plant native to India, has been the object of extensive study due to its multiple uses as raw material in the production of oils, foods, condiments and drugs (Makkar and Becker, 1997). Studies on this plant have revealed promising anti-inflammatory (Ezeamuzie et al., 1996), antifungal (Chuang et al., 2007) and antibacterial (Doughari et al., 2007b) properties. The latter has been attributed to different parts of the plant, such as the leaves, roots, seeds, flowers, frupeel and unripe pods (Anwar et al., 2007).

This study was undertaken to investigate *in vitro*, the potency of aqueous and ethanol extracts of *M. oleifera* (Lam) leaves as antimicrobial agent against some pathogenic bacteria: *Escherichia coli (E. coli)* O26, *E. coli* O78, *E. coli* O157, *Staphylococcus aureus* methicillin resistant *S. aureus* (MRSA) and *Salmonella* Typhimurium. Also, *in vivo* dry powder of *M. oleifera* Lam was studied. The leaves were evaluated as growth promoters and antimicrobial agent against some pathogenic bacteria, including aerobic plate count, *E. coli*, *S. aureus* and *Salmonella*.

MATERIALS AND METHODS

First experiment

Plant collection and preparation

M. oleifera leaves were collected from a private farm of a sandy soil cultivated with Moringa shrubs over an area of 10 feddans (Feddan = 4200 m^2) located in Nubaria province (180 km northern Cairo city). The leaves were washed properly and oven dried at 40°C until the moisture content reached 10-12%. Dry leaves were finally ground using BRAUN house type grinder, sieved (1 mm mesh) and the powder was kept in a well tight polyethylene bags at room temperature until further use.

Preparation of Moringa extracts

The dried powder samples of *Moringa* leaves were extracted with 100% ethanol and distilled water. The extracts were collected three times and filtered through Whatman number 1 filter paper and then concentrated on a rotary evaporator at 45°C as described by Lugman et al. (2011). The sample and solvent mass ratio was 1:2 during extraction. Dry extracts were kept at 4°C till when needed for the assay.

Total phenolic compounds in the extracts was estimated using the Folin Ciocalteu's phenol reagent, where galic was used as a standard. The total carotenoids were spectrophotometrically estimated at 450 nm according to AOAC (1995). For the HPLC, finger print analysis of phenolic compounds (%) using Shimadzu system (Shimadzu Corp. Kyoto, Japan) consisting of the LC-IOAD pumps and injection volume of 20 µl was applied, whereas the separation of compounds was monitored at 280 and 320 nm. The determination of the antioxidative capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The residual H_2O_2 was estimated colorimetrically using Bio-diagnostic Kit according to Blois (2002).

Antimicrobial assay

Preparation of bacterial suspensions

Antibacterial activities were carried out against seven highly pathogenic foodborne pathogenic strains of animal origin, including two Gram positive bacterial reference strains; *S. aureus* (NCINB 50080) and MRSA (ATCC 13565) and five Gram negative bacterial reference strains, including *Salmonella* Typhimurium (ATCC 13311), *S. bodyii* ATCC 9207, *E. coli* O157 (ATTC 700728), *E. coli* O26 and *E. coli* O78 (poultry isolated). Agar well diffusion test (qualitative method). Suspension of bacterial strains was freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 ml of Muller Hinton Broth. The inoculated tubes were incubated at 37°C for 24 h. Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland scale standard was selected for screening of antimicrobial activities. Oxytetracycline 30 µg/ml and Vancomycin 30 µg/ml with MRSA was used as reference drugs.

Agar well diffusion method

The antimicrobial activity of water and ethanolic extract of Moringa

against bacterial strains were evaluated by using the agar-well diffusion method of Katircioglu and Mercan (2006). Hundred mirolitres (µl) of cell culture suspension matching with 0.5 McFarland of target strains were spread onto the plates. For the investigation of the antibacterial activity, 50 µl of extracts, and antibiotic discs of reference drugs were added. Plates were left for 1 h at 25°C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37°C for 24 h. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the strains. For an accurate analysis, tests were run in triplicate for each strain in order to avoid any error.

Second experiment

Rations and feeding experiment

Four batches of rabbit ration each of 100 kg were formulated to contain: 30% alfalfa hay, 25% ground yellow corn, 25% wheat bran, 14% soy bean meal (44%), 3% cane-molasses, 1.5% lime stone, 1% sodium chloride and 0.5% vitamins and mineral mixture (premix of multi vita company-Egypt). Moringa powder dry leaves were added, thoroughly mixed with feed ingredients of each batch at 0, 0.15, 0.30 and 0.45% for R_1 (control), R_2, R_3 and R_4, respectively. Experimental ration were pelleted at 0.3 cm diameter and packed in polyethylene bags until feeding.

Thirty six male growing New Zealand White (NZW) rabbits aged five weeks old, with an average body weight of 566.5 g was blocked by weight in four equal groups (nine animals each). Experimental rabbits were housed individually in galvanized metal wire cages equipped with feeding and water troughs. The first group of rabbits was fed on R_1, while the 2nd, 3rd and 4th groups fed on R_2, R_3 and R_4, respectively. Chemical composition of Moringa dry leaves and rabbits feed mixture is given in Table 4.

The feeding experiment was for 56 days, where animals were weekly weighed and feed consumption was individually recorded. During the last week of feeding experiment rectal samples of faeces were individually collected from animals of each group in sterilized sealed nylon bags, where faecal samples were subjected to microbiological examination.

Bacteriological analysis

General purpose nutrient media, enrichment media and other appropriate selective media (all obtained from Oxoid) were employed in the culturing and isolation of selected pathogenic bacteria in the study.

Preparation of media

All dehydrated media were prepared according to manufacturer's instructions. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave at 121°C for 15 min. The sterile media were dispensed or poured into sterilized Petri-dishes and allowed to cool. The sterility of the prepared media was checked by incubation of blindly selected plates at 37°C for 24 h.

Bacterial isolation, identification and enumeration

Ten grams of the fecal sample were weighted in a sterile stomacher bag, and then 90 ml from maximum recovery diluents was added to the sample, the sample was well mixed using stomacher machine. Further serial dilution was done if needed by means of a pipette. 1 ml of the diluent was transferred into two Petri dishes, and then the media were poured into the dishes and were incubated at 37°C for 24-48 h. After incubation period, the counting of the colonies was done using the Stuart Digital colony counter.

Enumeration of total plate count was carried out according to *****EN ISO 4833 (2003) (European Union of international standard 4833: 2003). Ten-fold serial dilution of the bacterial suspension was made. This was done until 10^{-7} dilution was achieved. 0.1 ml was then pipetted from the 10^{-7} dilution onto the surface of each of two Petri dishes containing 15 ml of a solidified and sterile plate count agar (PCA), and then spread evenly with a sterile glass spreader. The plates were then incubated for a maximum of 24 h (including the control plates).

Detection of *Salmonella* was carried out according to modified *ISO 6579 (2002) (International Standard 6579: 2002). Twenty five grams of the fecal sample were weighted in a sterile stomacher bag or flask, and then 225 ml of buffer peptone water was added then 1 ml was plated onto xylose lysine deoxycholate agar (*XLD) plates then incubated at 37°C for 18 h. Typical colonies of *Salmonella* in XLD were red with black center. Biochemical reaction (triple sugar iron agar, lysine iron agar, citrate agar and urea agar) was used for confirmation of *Salmonella* typical colonies.

Enumeration of *E. coli* was carried out according to ISO 16649-2 (2001), 5 ml of violet bile lactose agar medium was added and a 10 ml second layer of the same media was added after the solidification of the agar and then incubated at 44°C for one day. Suspected colonies were confirmed to be *E. coli* by IMViC tests.

Enumeration of coagulase positive *S. aureus* was carried out according to ISO 6888-1 (1999). The fecal sample was prepared as mentioned before and dilution was carried out to 10^{-8}. 1 ml of each dilution was plated onto Baird-Parker agar (Oxoid). Then the plates were incubated aerobically at 37°C/24 h. Black colonies with hallow zone around were confirmed by biochemical including catalase, oxidase, indole, methyl red, Voges Proskauer, Simmon's citrate, urease test, hydrogen sulfide production in triple sugar iron agar medium, sugar fermentation test using different sugars, arginine hydrolysis test, hippurate hydrolysis test, nitrate reduction test, coagulase test were carried out (Quinn et al., 2002).

Statistical analysis

Data of fecal bacterial count were subjected to one-way analysis of variance according to steel Steel and Torrie (1980) applying the general linear model procedure of Statistical Analysis System (*SAS) (2001). Duncan's multiple range tests (1955) was applied to separate significant means.

RESULTS AND DISCUSSION

In vitro antimicrobial activities of *Moringa* leave extracts

In this study, the ethanolic leaf extract of *M. oleifera* was found to produce respectively, the highest zones of inhibition (25, 20, 12, 12, 11 mm) against all the Gram negative bacteria (*E. coli* O157 ATTC 700728, *E. coli* O78 (poultry isolate), *E. coli* O26 (poultry isolate), *S.* Typhimurium ATCC 13311 and *S. bodyii* ATCC 9207). The zones of inhibition for the Gram positive bacteria, *S. aureus* NCINB 50080 and MRSA ATCC 13565 were 18 and 11 mm, respectively. Some zone of inhibition reach more than one and a half to twice more effective than

Table 1. Some phytochemical compounds and antioxidants capacity of water and ethanolic extracts of *Moringa* leaves.

Item	Water extract	Ethanolic extract
Total carbohydrates (%)	26.76±1.58	23.43±1.45
Total chlorophyll (g/kg)	6.54±0.34	7.54±0.65
Total carotenoids (mg/kg)	823±54	1567±151
Total phenols (mg/g)	6.01±0.14	41.35±0.47
Total flavonoids (mg/g)	17.61±0.09	22.56±0.59
Total antioxidant capacity (µg/ml)	0.17±0.01	0.35±0.02

Table 2. Chemical composition of *Moringa* dry leaves and rabbits feed mixture.

Item	Moringa leaves	Feed mixture
Moisture (%)	10.23	10.00
Dry matter composition (%)		
Crude protein	31.06	19.11
Crude fiber	9.81	8.29
Ether extract	11.34	2.76
Soluble carbohydrates	29.31	54.02
Ash	18.48	15.82

Chemical composition was determined according to A.O.A.C. methods (1995).

Table 3. *In vitro* evaluation of water and ethanolic extracts of *Moringa* on pathogenic microorganisms under study when compared with reference drugs.

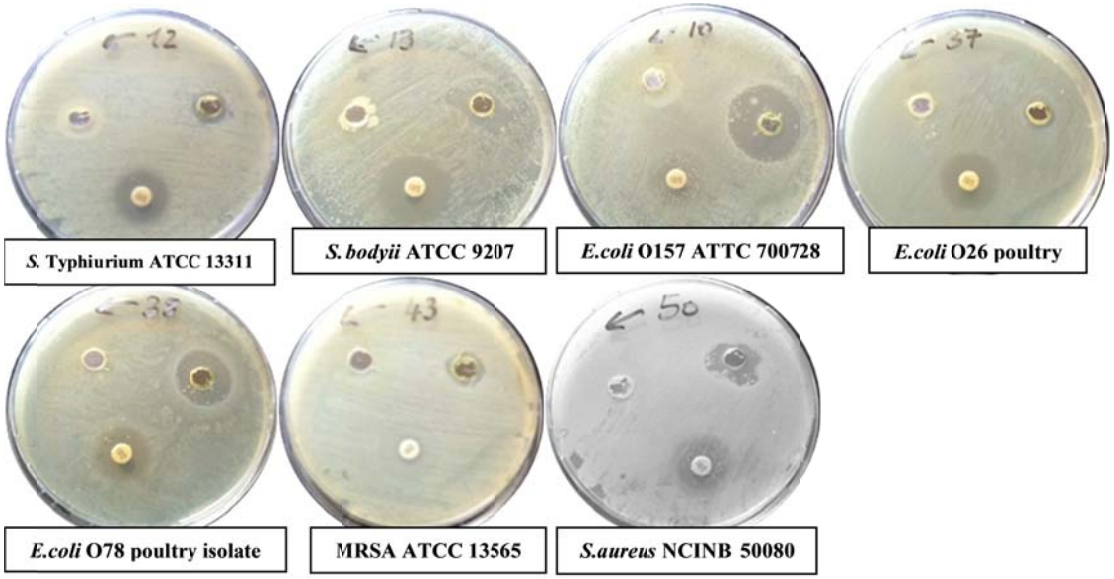
Strain	Aqueous extract	Ethanolic extract	Oxy-tetracycline OT 30 µg/d	Vancomycin VA 30 µg/d
Gram Negative Bacteria				
S. Typhimurium ATCC 13311	-ve	12	16	ND
S. bodyii ATCC 9207	-ve	11	22	ND
E. coli O157 ATTC 700728	-ve	25	13 static	ND
E. coli O26 (poultry isolate)	-ve	12	18	ND
E. coli O78 (poultry isolate)	-ve	20	16	ND
Gram Positive Bacteria				
MRSA ATCC 13565	-ve	11	ND	10/20s
S. aureus NCINB 50080	-ve	18	16	ND

reference antibiotic such as Oxytetracycline (30 µg/disc) as in the case of *E. coli* O157 ATTC 700728, where antibiotic showed zone of bacteriostatic effect reaching 13 mm diameter while *M. oleifera* ethanolic extracts show zone of inhibition reaching 25 mm. On the contrary, water extracts do not show any hindrance effect on the tested bacteria as shown in Table 3.

Antibacterial activity of water and ethanol extracts of *M. oleifera* Lam. leaves in Egypt were evaluated. Agar well diffusion method was applied to be used in this study. The ethanolic extract has greater antibacterial activity than the corresponding water extracts (Plates 1 to 7).

Growth performance

Body weight gain, average daily gain (ADG) and feed conversion efficiency of rabbits fed on experimental ration



Plates 1. Water extract, antibiotic reference drug and ethanolic extract in sequence anticlockwise with the arrow.

Table 4. Growth performance of rabbits fed rations with different supplements of *M.oleifera* dry leaves.

Item	Experimental rations				
	R_1 (0%)	R_2 (0.15%)	R_3 (0.30%)	R_4 (0.45%)	SEM
Animal No.	9	9	9	9	-
Duration period	---------- 56 days ----------				
Initial weight, g	560	573	571	562	24.07
Final weight, g	2024^b	2192^a	2219^a	1997^b	62.01
Body weight gain, g	1464	1619	1648	1435	64.66
Average daily gain, g	26.14^b	28.91^a	29.43^a	25.63^b	1.15
Dry matter intake, g	148^b	146^b	155^a	144^b	2.74
Feed Conversion (g. intake/g. gain)	5.66^c	5.05^a	5.27^b	5.62^c	0.11

a, b and c: Means with different superscripts in the same row are significantly different at ($P<0.05$) SEM=standard error of means.

are given in Table 4. Total weight gain and ADG of rabbits fed on 0.15 and 0.30% Moringa leaves ration were higher than those fed 0% (control) and 0.45% Moringa (R1 and R4) with no significant difference ($P<0.05$) between the two groups. A similar trend was observed for the feed conversion efficiency (g DM intake/g weight gain) where the rabbits fed on R2 and R3 diet performed better (5.05 and 5.27, respectively). Body weight gain of rabbits fed Moringa was 5.66 and 5.62, respectively. Body weight gain of rabbits fed Moringa supplemented rations relative to those fed control (R_1) was higher by 10.6, 12.5 and 2.0% with rations R_2, R_3 and R_4, respectively. Several previous studies reported that the inclusion of Moringa leaves in the diets of rabbits was associated with better weight gain (Nuhu, 2010; Adeniji and Lawal, 2012; Dougnon et al., 2012). None of these studies mentioned any adverse effect of Moringa leaves on the growth rate with increasing level of supplementation. However, Ewuola et al. (2012) found that rabbits that fed diets containing 0, 5, 10 and 15% Moringa (MOLM) has lower ADG with increasing Moringa substitution level being 6.8, 5.4, 6.5 and 3.8 with diets containing 0, 5, 10 and 15% Moringa leaves. There is no rigid explanation for the adverse effect of feeding o.45% Moringa ration on weight gain of rabbits in this study. It might hold true that, Moringa leaves which are known for their high contents of essential amino acids, vit A, B, C and E, antioxidant and antimicrobial compounds could play a role as a growth promoter at certain supplementation level. While its high contents of phenols,

Table 5. Pathogenic bacterial count in faecal samples of rabbits fed different *M. oleifera* leaves supplements.

Feeding groups	Heterotrophic plate count ($\times10^6$)	Salmonella ($\times10^4$)	*E. coli* ($\times10^5$)	*S. aureus* ($\times10^2$)
Control (0% M)	28.33±0.96[a]	2.56 ±0.57[b]	2.47±0.11[b]	30.00±1.63[a]
Group2 (0.15% M)	2.50±0.82[b]	ND	1.70±0.17[b]	1.83±0.09[b]
Group 3 (0.30% M)	1.47±0.19[b]	ND	ND	1.40±0.08[b]
Group 4 (0.45% M)	30.67±0.55[a]	5.67 ±1.64[a]	7.46±0.51[a]	28.00±0.82[a]

All samples were counted in 1 g faecal sample except *Salmonella* in 25 g sample. Each value is mean of two pooled faecal samples from each group. ND = non-detectable; a, b: means within the same raw with different superscripts are significantly different at ($p<0.05$).

tannins, alkaloids and cumarins might accumulate in the body, causing inhibition of feed utilization or decomposition of some antimicrobial compounds.

In vivo antimicrobial activities of *Moringa* extracts

Faecal bacterial count as shown in Table 5, revealed that group R_3 (0.30% M) and group R_2 (0.15% M) showed the least bacterial count, which was significantly lower than that in the other two groups; control group R_1 (0% M) and group R_4 (0.45% M). Groups R_2 and R_3 showed non-detectable limits of *Salmonella* when compared with control group R_1 and group R_4. *E. coli* count showed that group R_3 was free of *E. coli*, group R_2 count was $1.70\pm0.17\times10^5$ which was significantly lower than control group R_1 and group R_4 with count reaching $2.47\pm0.11\times10^5$ and $7.46\pm0.51\times10^5$, respectively. Groups R_2 and R_3 showed low *S. aureus* count of $1.83\pm0.09\times10^2$ and $1.40\pm0.08\times10^2$ respectively, which were significantly lower than the control group R_1 ($30.00\pm1.63\times10^2$) and group R_4 ($28.00\pm0.82\times10^2$). The same finding was observed in indicated heterotrophic plate count where groups R_2 and R_3 showed a low bacterial count; $2.50\pm0.82\times10^6$ and $1.47\pm0.19\times10^6$ respectively, which were significantly lower than the control group R_1 ($28.33\pm0.96\times10^6$) and group R_4 ($30.67\pm0.55\times10^6$).

Antibacterial activity of water and ethanol extracts of *M. oleifera* Lam. leaves in Egypt were evaluated. Agar well diffusion method was applied to be used in this study. The ethanolic extract has greater antibacterial activity than the corresponding water extracts (Plates 1 to 6).

In our investigation, highest zones of inhibition were found in the leaf ethanolic extract against Gram negative bacteria under investigation: *E. coli* O157 ATTC 700728, *E. coli* O78 (poultry isolate), *E. coli* O26 (poultry isolate), *S.* Typhimurium ATCC 13311 and *S. bodyii* ATCC 9207 with the zone of inhibition equals 25, 20, 12, 12, 11 mm, respectively.

Agar well diffusion test against Gram positive bacteria, including *S. aureus* NCINB 50080 and MRSA ATCC 13565 show zone of inhibition equals 18 and 11 mm, respectively. A zone of inhibition was more than one and a half to twice as much effective as known antibiotic oxytetracycline (30 µg/disc) as in the case of *E. coli* O157 ATTC 700728 w zone of bacteriostatic effect of 13 mm diameter while, *M. oleifera* ethanolic extracts show zone of inhibition reaching 25 mm. On the contrary, aqueous extract does not show any hindrance effect on the tested bacteria shown in Table 3 and Plate 1. Many authors have reported antimicrobial activities of plant extracts on food-borne pathogens (Moreira et al., 2005; Afolabi, 2007; Kotzekidou et al., 2007; Atiqur and Sun 2009), which indicates the possibility of use of plant extract as sanitizers and preservatives. The antimicrobial activity of the extracts tested, reveal bioactivity on wide range of pathogenic and toxigenic organisms liable to cause food-borne illnesses such as *E. coli, S. aureus, P. aeruginosa, S. typhi* and *S*. Typhimurium (Bukar et al., 2010).

This result is because in the traditional method of treating a bacterial infection, a decoction of the plant parts or boiling the plant in water is employed, whereas, according to the present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Nair et al. (2005). Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt et al., 1994; Afolayan and Meyer, 1995). Vinoth et al. (2012) reported that the ethanolic extract was active against *S. typhi* and *S. aureus* whereas the aqueous extract exhibited an inhibitory effect on *S. aureus* only, and proved that the ethanol extract was more active than the aqueous extract against *S. typhi*.

Previous studies conducted by Rahman et al. (2009) showed that ethanol extract of fresh *M. oleifera* leaves has extensive antibacterial effect against all tested Gram-negative bacteria (*S. shinga, P. aeruginosa, S. sonnei, Pseudomonas* spp.) and some Gram-positive bacteria (*B. cereus, B. subtilis, S. lutea, B. megaterium*) and their respective zones of inhibition were 17.5±0.34, 21.21±0.05, 21.50±0.08, 21.25±0.1.3 and 16.25±0.04, 20.23±0.56, 19.50±0.21, 20.50±0.04 mm, respectively. Cáceres et al. (1991) found *M. oleifera* leaf extracts can inhibit the growth of *S. aureus* and *P. aeruginosa*. Likewise, in a study by Valsaraj et al. (1997) evaluating the

antibacterial effect of 78 plants used in India to treat infectious diseases, *P. aeruginosa* and *S. aureus* were inhibited by extracts of Moringa peel. Mandal and Mandal (2011), Paul et al. (2011), Prabhll et al. (2011) and Suarez et al. (2003) found Moringa extracts produced bacteriostatic and bactericidal effects on *S. aureus*. Djakalia et al. (2011) stated that *Moringa* leaf extract have antimicrobial activities. They inhibit the growth of *S. aureus* in the feed and rabbits intestines. Bukar et al. (2010) reported that *M. olifera* leaf ethanolic extract had the broadest spectrum of activity of the test bacteria. The results showed that activity against four bacterial isolates *Enterobacter* spp. (7 mm), *S. aureus* (8 mm), *P. aeroginosa,* (7 mm) and *E. coli* (7 mm) were sensitive at a concentration of 200 mg/ml. While Shigella spp. and *S.* Typhi were not sensitive at all concentrations used. Also, Napoleon et al. (2009) reported *Enterobactor* spp., *S. aureus, P. aeruginosa, S.* Typhi and *E. coli* to be sensitive to ethanol, chloroform and aqueous extract of *M. olifera* leaf at a concentration of 200 mg/l.

The results of the present work totally oppose the findings of investigations of many authors who observed resistance of *E. coli* to *Moringa* extract which matches findings from a study of the antibacterial properties of Indian plants showing Moringa extracts to be ineffective against *E. coli* (Bhawasar et al., 1965). Also, Rajendrhan et al. (1998) reported *E. coli* to be resistant to Moringa extracts.

On the other hand, the present study suggests that *M. oleifera* Lam. leaves used contain bio-components whose antibacterial potentials are highly comparable with that of the antibiotic Oxytetracycline against all Gram-negative and Gram-positive bacteria tested. The activity of the plant against both Gram-positive and Gram negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007). Today, most pathogenic organisms are becoming resistant to antibiotics (Chandarana et al., 2005). Moringa leaves have been reported to be a good source of natural antioxidants such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000). To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *M. oleifera* Lam. could become promising natural antimicrobial agents with potential applications in the pharmaceutical industry for controlling the pathogenic bacteria. Doughari et al. (2007b) observed inhibition halos up to 8 mm when challenging *Salmonella* with aqueous and ethanolic Moringa leaf extracts. The authors attributed the antibacterial effect to the presence of saponine, tannic, phenolic and alkaloid phyto constituents. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

Moringa roots have antibacterial activity (Rao et al., 2001) 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 (Rao, 1957). The root extract also possesses antimicrobial activity attributed to the presence of 4- α-L-rhamnosyloxybenzyl isothiocyanate (Eilert et al., 1981). 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 (Nickon et al., 2003). The fresh leaf juice was found to inhibit the growth of microorganisms, *P. aeruginosa* and *S. aureus* (Caceres et al., 1992).

Conclusion

In conclusion, ethanolic *Moringa* leaves extract were capable of inhibiting the growth of Gram- negative and positive bacteria. *M. oleifera* leaves at concentration of 0.15-0.30% can treat common pathogenic bacterial infections and could be used for preventing and treating malnutrition and to enhance growth rate. There is need to carry out more pharmacological studies to support the use of *M. oleifera* as a medicinal plant.

Conflict of interest

The author(s) have not declared any conflict of interests.

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