International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.44.53

Research Article
Bactericidal Efficacy of Omega-3 Fatty Acids and Esters Present in *Moringa oleifera* and *Portulaca oleracea* Fixed Oils Against Oral and Gastro Enteric Bacteria

Amal Sabry Othman

Department of Microbiology, Faculty of Applied Medical Sciences, October 6th University, Egypt

Abstract

Background: Medicinal plants are now used as antibacterial compound that is safe for human use and overcome the multidrug resistant phenomenon. **Objective:** This study was done to compare the antibacterial activity of two fixed oils, purslane and MO. **Methodology:** The antimicrobial activity was done using oil diffusion method. The GC-MS analysis was done for the effective oil and protein profile and TEM scanning was done for the highly affected bacteria. **Results:** Purslane oil was the most effective especially against *E. coli*. Protein profile and TEM scanning revealed that the effect was on the outer bacterial membrane with no change in protein profile. The GC-MS analysis revealed that the oil contained Omega-3 fatty acid α-linolenic (ALA, C18:3 n-3); 9, 12, 15-octadecatrienoic acid methyl ester (30.83%). **Conclusion:** These fatty acids are bactericidal compounds that could disrupt the outer bacterial cell membrane and do not affect the internal protein content.

Key words: Purslane, fixed oil, bactericidal, protein profile, GC-MS analysis

Received: June 17, 2016 Accepted: October 19, 2016 Published: December 15, 2016

Citation: Amal Sabry Othman, 2017. Bactericidal efficacy of Omega-3 fatty acids and esters present in *Moringa oleifera* and *Portulaca oleracea* fixed oils against oral and gastro enteric bacteria. Int. J. Pharmacol., 13: 44-53.

Corresponding Author: Amal Sabry Othman, Department of Microbiology, Faculty of Applied Medical Sciences, October 6th University, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Now a days medicinal plants are used for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide. Many have been used as therapeutic alternatives because of their antimicrobial properties[1]. The antimicrobial properties are produced as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of using folk medicine is now increasing in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies[2,3].

Portulaca oleracea (purslane) is reported to be rich in α-linolenic acid and s-carotene and used as a health food for patients with cardiovascular diseases[4]. It contains several types of vitamins and minerals[5], fatty acids[6], glutathione, glutamic acid, aspartic acid dopamine, dopa, coumarins, flavonoids, alkaloids, saponins and anthocyanin[7]. purslane seeds decoction is considered as excellent diuretic. In Africa whole plant is considered as bactericidal in bacillary dysentery, diarrhea, hemorrhoids and used as antidiabetic[8].

A recent study has shown that the flavonoid apigenin isolated from ethanolic extracts of *Portulaca oleracea* L., has antibacterial property and could be used to develop antibacterial drugs[9].

Other study was done in 2015 and revealed that the hydroalcoholic extract of *Portulaca oleracea* leaves and seeds showed different antimicrobial activity[10]. Londonkar and Nayaka[11] showed the antimicrobial and antifungal properties of ethanol extracts of *P. oleracea* on some bacteria and fungi. Bae[12] showed the antimicrobial effect of *P. oleracea* extracts on foodborne pathogens.

Antimicrobial components of *Moringa Oleifera* (MO) have been used against several microorganisms. Aqueous extracts of MO was found to be inhibitory against many pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*[13].

Sayeed *et al.*[14] revealed that the fruit extract of *Moringa oleifera* showed a broad-spectrum antibacterial and antifungal activity.

This study will compare the antibacterial activity of *Moringa olifera* and purslane fixed oils against some gram positive and gram negative pathogenic bacteria, detection of the active compounds by GC-MS analysis and mechanism of action using TEM and protein profile analysis. This may lead to use these healthy natural compounds as antimicrobial agent instead of using hazardous chemical compounds or antibiotics.

MATERIALS AND METHODS

Bacterial strains: Some Gram positive and Gram negative bacterial strains were used (ATCC, US) for examination in this study, *Staphylococcus aureus* (ATCC: 25923), *Staphylococcus epidermidis* (ATCC: 12228), *Streptococcus pyogenes* (ATCC: 19615), *Streptococcus sanguinis* (ATCC: 10556), *Pseudomonas aeruginosa* (ATCC: 27853), *klebsiella pneumoniae* (ATCC: 13883), *Streptococcus pneumoniae* (ATCC: 6305), *Salmonella typhi* (ATCC: 19430), *Escherichia coli* (ATCC: 25922), *Shigella flexneri* (ATCC: 12002). Bacteria were grown on Brain Heart Infusion (BHI) broth medium and maintained on nutrient agar slants[15] at 4°C.

Extraction of *Moringa olifera* and *Purslane* fixed oil
Plant material: Plant materials of *purslane* and *Moringa olifera* were collected from Horbit Village, El-Sharkyea Governorate, Egypt. It was identified, authenticated taxonomically by Dr. Heba El-Gezawy, Pharmacognosy Department, Faculty of Pharmacy, October 6th University. The seed were cleaned, dried under direct sunlight and powdered by a mechanical grinder.

Extraction of fixed oil: First, seeds were cleaned by hand carefully to remove the foreign materials such as other seeds, stones and small stalks, then purslane seed were dried at 50°C for 12 h in an oven and then crushed into powder in a grinder with a size range of 0.55-1.0 mm. Purslane and *Moringa olifera* ground samples were mixed with hexane (1:10, m/V) at (60-80°C) using a soxhlet apparatus. This process of extraction was repeated for 6 h, the hexane distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature[16] of 40±2°C.

Antimicrobial susceptibility tests: Antimicrobial susceptibility testing was done using the disk diffusion method and results were interpreted using the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards (NCCLS) break point criteria[17]. Antimicrobial drugs included penicillin group (amoxil); glycopeptide group (vancocin), aminoglycosides (amikin) and cephalosporin (cephradine and imipenem).

Multidrug resistant strains (MDR) were detected and defined as the non-susceptible strains to at least one agent in three or more antimicrobial categories[18].

Evaluation of antibacterial activity of the fixed oils against bacterial strains: Fixed oils of *Moringa oleifera* and purslane

were examined for their antimicrobial activity against the tested bacteria by agar diffusion method. Dilutions of fixed oils were carried out in DMSO (dimethyl sulfoxide) with concentrations ranging from 10-100% (v/v). Plates were incubated at 35±2°C for 16-20 h. The mean inhibition zone diameter was measured. Each assay was carried out in triplicate.

Preparation of Fatty Acid Methyl Ester (FAME): Seeds (40 g) were dried overnight in hot oven at 50°C and ground into powder with a mortar and pastle, then 0.6 mL of dichloromethane and 4.0 mL of 0.5 N sodium methoxide were added. Acidic catalyzed esterification using the boron triflouride-methanol complex (14% w/v) was added according to the method described by AOAC[19] and Rezanka and Rezankova[20]. The tube was shaken and heated for 30 min at 50°C. The reaction was stopped by adding 5.0 mL of water containing 0.2 mL of glacial acetic acid. The esterified fatty acids were extracted with 3.0 mL petroleum ether (40-600°C). The clear fraction was kept at -20°C until further analysis.

Separation condition of fatty acids on GC/MS

Instrument: The HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector. The FAME in hexane (1 μL) was injected into the column with a split ratio of 100:1. The injector and detector temperature were set at 200 and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}. Separation was carried out on a TR-FAME (Thermo 260 M142 P) (30×0.25 mm ID) with a film thickness of 0.25 μm film (70% cyanopropyl polysilphphenylene siloxane) capillary column. The column temperature was programmed from 100-160 at 2°C min^{-1} and then to 250 at 4°C min^{-1} and finally held at 250°C for 20 min. The weights of the individual FAME were calculated on the basis of their relative peak area compared with that of internal standard and then they were corrected using the corresponding GC response factors for each fatty acid.

Protein profile analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

5x sample buffer: (10% w/v SDS, 10 mM dithiothreitol or β-mercapto-ethanol, 20% v/v glycerol, 0.2 M tris-HCl, pH 6.8 and 0.05% w/v bromophenol blue) should add up to 8 M urea for really hydrophobic proteins.

1x running buffer: (25 mM tris-HCl, 200 mM glycine and 0.1% (w/v) SDS).

Preparing the sample: Mix the protein 4:1 with the sample buffer. Heat the sample by boiling for 5-10 min.

Running the sample on gel: Clamp in your gel and fill both buffer chambers with gel running buffer according to the instructions for your specific apparatus. Pipette the sample into the gel adjusting the volume according to the amount of protein in the sample. Be sure to include a lane with molecular weight standards. Now attach your power leads and run the gel until the blue dye front reaches the bottom with 250 V constant which in a four to twenty percent mini gel needs about 30 min total run time but adjust to the thickness of your gel, the power supply used and the resolution desired. Remove the gel for the power supply and process further-visualize the proteins using coomassie brilliant blue, silver stain or any of the other protein stains[21].

Transmission electron microscope (TEM) examination: Conventional TEM microscopy is frequently selected to visualize the ultrastructural damage on both cell wall and cytoplasm membrane of entire microbes when fixed material can be used[22].

At ultra structural level, a simple negative staining for TEM (JEM-1400 TEM, JEOL-Japan) of bacterial cells can report evidences on the mechanism of membrane disruption by antimicrobial proteins and peptides (AMPPs)[23]. Ultrathin sections obtained by conventional procedures, namely fixation with aldehydes, post-fixation with osmium tetraoxide, dehydration and embedding in epoxy resin, allow the observation of membrane and cytoplasmatic alterations. Treatment with AMPPs can induce several external and internal changes such as membrane bleb, ruffling or detachment, the presence of electro dense dots or fibers, hypodense cytoplasmic release and cell vacuolization[24]. The outer membrane detachment observed is generally related to the extremely high affinity of AMPPs to LPS, the main component of the Gram-negative bacteria cell wall[24].

RESULTS

Table 1 showed that all bacteria tested were not multidrug resistant, however 5 (45.5%) strains of them were resistant to vancomycin, 1 (9%) was resistant to amikacin, 1 (9%) for cephradines and 1 (9%) was resistant to amoxil while all of them (100%) were susceptible to Impenenems.

Figure 1 showed that MO fixed oil had very low effect on Gram positive bacteria tested (very small inhibition zone diameter ≤1 cm).

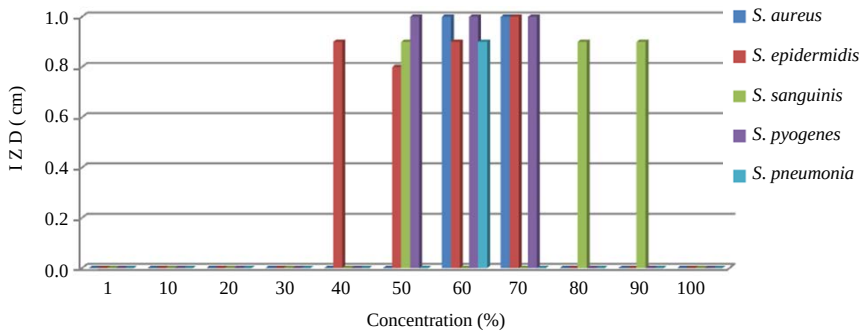

Fig. 1: Effect of *Moringa oleifera* fixed oil on Gram positive bacteria tested

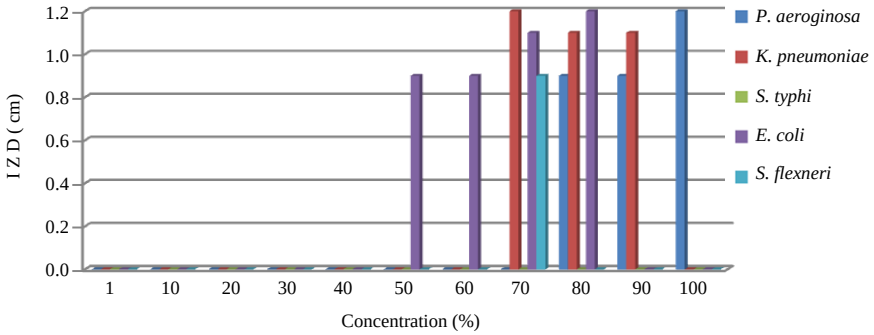

Fig. 2: Effect of *Moringa oleifera* fixed oil on Gram negative bacteria tested

Table 1: Antibiogram of the tested strains to different antibiotics and detection of multi drug resistant ones

Antibiotics	Mean diameter of inhibition zone (mm)				
	Penicillin group	Cephalosporin group		Aminoglycosides group	Glycopeptide group
Bacterial strains	Amoxil	Impenem	Cephradine	Amikacin	Vancomycin
Staphylococcus aureus	32	40	15	28	18
Staphylococcus epidermidis	22	45	32	40	00
Streptococcus pyogenes	30	45	32	30	20
Streptococcus sanguinis	20	30	45	30	00
Streptococcus pneumonia	26	40	40	32	25
Pseudomonas aeruginosa	30	44	33	28	28
Klebsiella pneumoniae	22	18	35	25	18
Salmonella typhi	12	38	28	22	00
Escherichia coli	23	32	30	18	00
Shigella flexneri	28	28	00	32	00

Figure 2 also showed that MO fixed oil had very low effect on Gram negative bacteria tested (very small inhibition zone diameter ≤1.2 cm).

Figure 3 also represented low effect of purslane fixed oil on Gram positive bacteria tested (very small inhibition zone diameter ≤1.2 cm).

Figure 4 illustrated that purslane fixed oil had high effect on *E. coli* at concentrations between 10-70% showing the highest effect at concentration 20%.

The GC-MS showed that purslane contains many fatty acids. It is one of the richest plant sources of It is one of the richest plant sources of Omega-3 fatty acid alpha-linolenic (ALA, C18:3 n-3); 9, 12, 15-octadecatrienoic acid methyl ester (30.83%) (Table 2, Fig. 5).

As *E. coli* was the highly affected bacterial strain in this study, it was chosen to investigate its protein profile before and after treatment and to be scanned by Transmission Electron Microscope (TEM) to see the effect of purslane fixed oil on the protein content and cell membrane structure.

Figure 6 showed no protein profile changes before and after treatment of *E. coli* with purslane fixed oil.

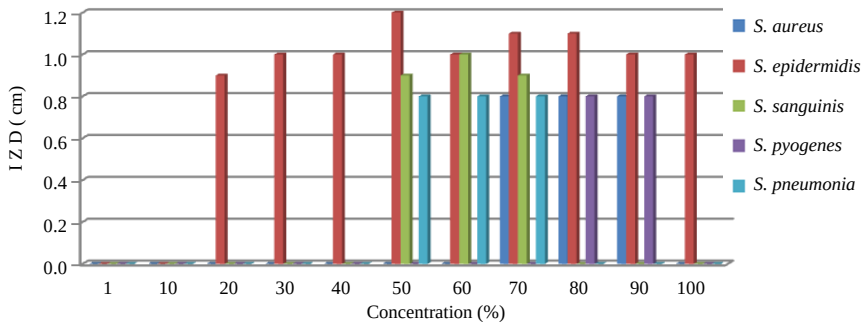

Fig. 3: Effect of purslane fixed oil on Gram positive bacteria tested

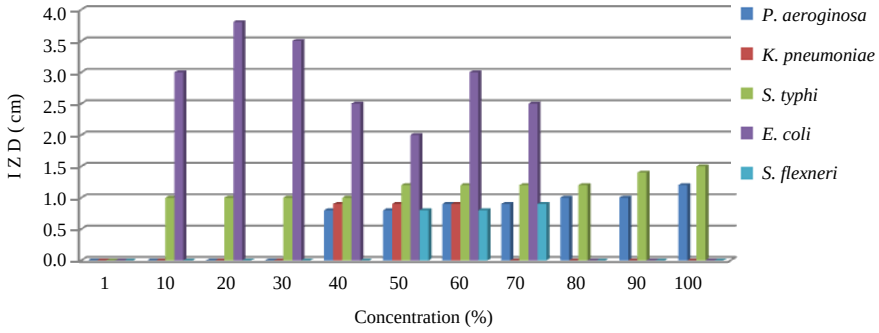

Fig. 4: Effect of purslane fixed oil on Gram negative bacteria tested

Table 2: GC-MS analysis of purslane fixed oil constituents

Peak No.	Compounds	Relative retention time (min)	Percentage
1	Dimethyl-sulfoxide	4.955	0.84
2	2-furancarboxaldehyde, 5-methyl-2-furfural	6.086	6.11
3	Methyl heptyl ketone	7.243	9.62
4	3-(dimethyl-amino) propyl-carbamate	7.694	3.55
5	S-propyl butyl (ethyl) thiocarbamate	7.881	7.16
6	4-methoxycyclohexanone 2-aminomethyl-5-methylamino-1,3,4-oxadiazole	8.415	2.40
7	N-isobutyl-2-oxoimidazolidine-1-car-boxamide	12.297	0.85
8	p-hydroxybenzyl alcohol, 4-methylolphenol	13.236	0.99
9	4-hydroxy-benzeneacetonitrile	14.642	5.42
10	4-hydroxy-benzeneacetonitrile, 4-hydroxy-phthalimidine	14.683	9.22
11	Hexadecanoic acid, methyl ester	18.046	0.52
12	n-hexadecanoic acid	18.513	4.91
13	7-octadecatrienoic acid methyl ester, methyl ester	19.737	2.20
14	6-octadecatrienoic acid methyl ester	20.209	30.83
15	9-octadecatrienoic acid methyl Ester	20.360	7.66
16	Succinic anhydride oleic acid	21.055	1.01
17	2-ethoxy-2,3-dihydro-3,3-dimethyl-benzofuran-5-yl methanesulphonate	23.307	1.30
18	1,2,3-tris(t-butyl)-2-cyclopropene	23.691	0.54

Transmission Electron Microscope showed that the effect of purslane fixed oil on *E. coli* was on the outer cell membrane (Fig. 7b). Figure 7 showed disruption in bacterial cell membrane with releasing of the internal bacterial cell content.

DISCUSSION

Purslane is a very good source of alpha-linolenic acid (Omega-3 fatty acid) which plays an important role in human growth and development and in preventing diseases. Purslane

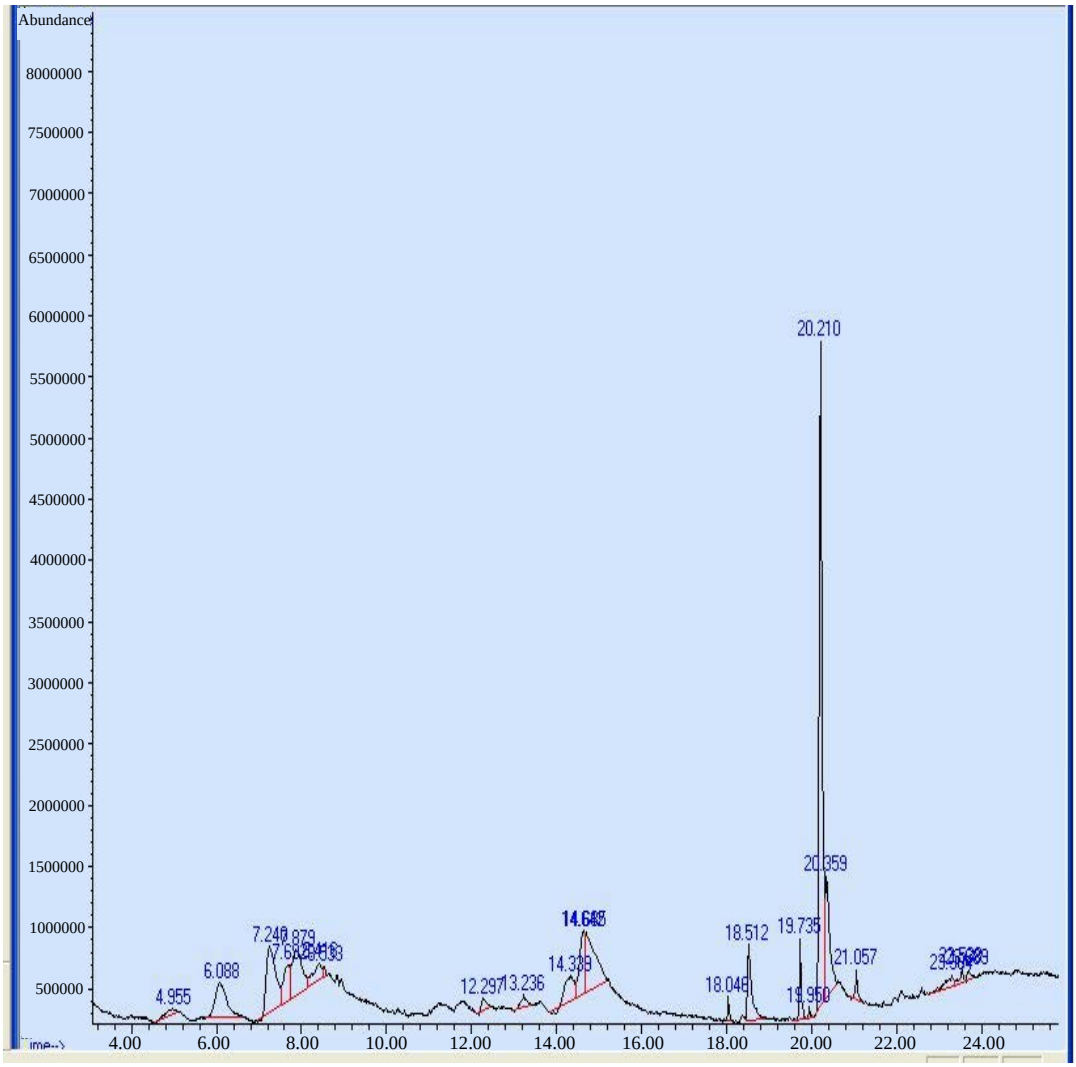

Fig. 5: Total chromatogram for GC-MS analysis of purslane fixed oil constituents

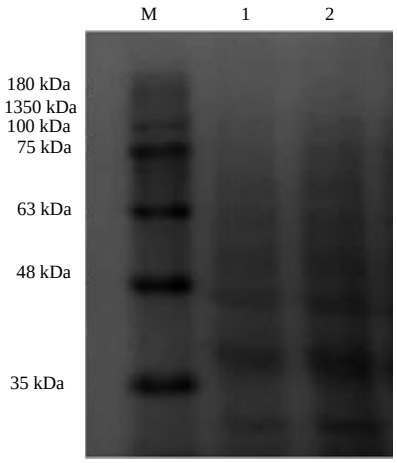

Fig. 6: Protein profile for *E. coli* before and after treatment with purslane fixed oil

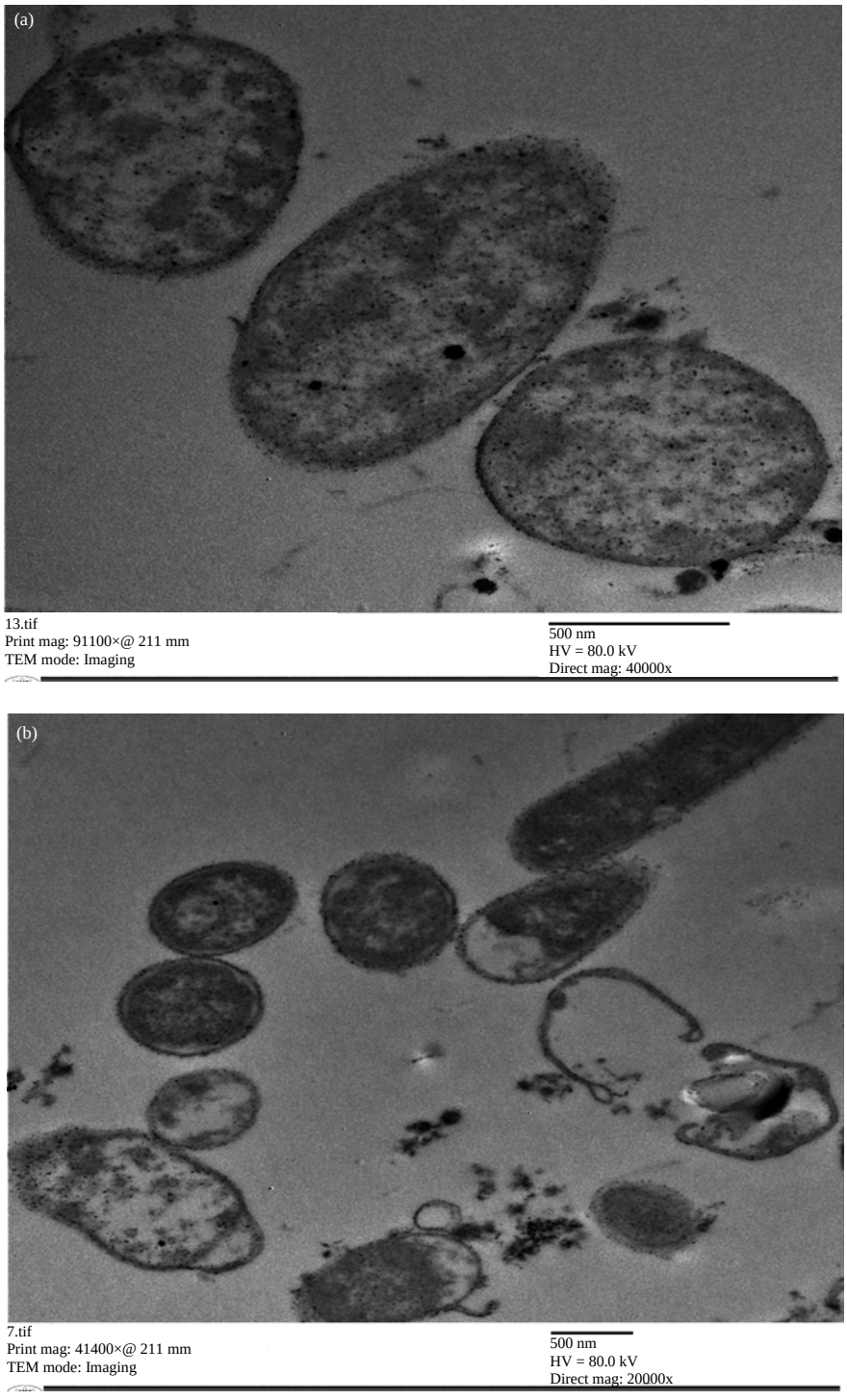

Fig. 7(a-b): TEM for the effect of purslane fixed oil on *E. coli*, *E. coli* before treatment and (b) *E. coli* after treatment

has been shown to contain five times higher Omega-3 fatty acids than spinach. Omega-3 fatty acids belong to a group of polyunsaturated fatty acids essential for human growth, development, prevention of numerous cardiovascular diseases and maintenance of a healthy immune system[25].

This study compared the activity of two fixed oils against some gram positive and Gram negative bacteria. The study showed that purslane was more efficient than MO showing the higher effect against *E. coli* especially at low concentrations (20%). This result was in agreement with

some researches. Zhao *et al.*[26] in their study found that different additive dosages of *P. oleracea* extracts in daily ration would bring different effects on cecal *Lactobacillus* and *E. coli*. Shuai Peng and his colleagues in their study also showed that purslane seed oil could affect *E. coli* and other Gram positive cocci[27]. The same was done on methanol extract of *Portulaca oleracea* and showed high activity against both Gram positive organisms *Bacillus subtilis* and *Staphylococcus aureus* and only active against one Gram-negative bacteria namely: *Pseudomonas aeruginosa*[28].

In contradiction to the current result, it has been reported that crushed seed extract of *Moringa oleifera* had bactericidal activity against *Staphylococcus pyogenus* and *Pseudomonas aerogenosa*[29]. Harvey[30] also reported that pterygospermin, a bactericidal and fungicidal compound contained in an aqueous extract made from seed of *Moringa oleifera* was effective against *Staphylococcus aureus* as the antibiotic neomycin.

The present study also indicated that all the strains were resistant to all dilutions of *Moringa olifera* fixed oil this was in agreement with other study done by examining aqueous methanolic extract and fixed oil of MO against some bacteria and viruses showing varying degree of antimicrobial activity was observed ranging from sensitive for *B. stearothermophilus* to resistant for *P. aeruginosa*[31-33,9]. Also another study was done comparing relative antimicrobial activity of seed extracts against bacteria (*Pasturella multocida*, *E. coli*, *B. subtilis* and *S. aureus*) and fungi (*Fusarium solani* and *Rhizopus solani*) revealed that *P. multocida* and *B. subtilis* were the most sensitive strains and their activity was influenced by cations (Na^+, K^+, Mg^{2+} and Ca^{2+})[13].

Nayaka *et al.*[9] showed the antibacterial activity of hydroalcoholic extract of some parts of *P. oleracea* by five pathogenic bacteria, including *Salmonella typhimurium* and *P. mirabilis*. The highest inhibition zones for *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogenes* were 14.56 ± 0.21 and 11.68 ± 0.13 mm, respectively[34]. Londonkar and Nayaka[11] studied the ethanol extract of the aerial parts of *P. oleracea* and showed its inhibitory effect against strains of *S. aureus*, *K. pneumonia*, *Bacillus cereus* and *Aspergillus fumigates*. Bae[12] showed that the ethyl acetate extract of *P. oleracea* had the highest antimicrobial activity against *S. aureus* and *Shigella dysenteriae*.

In contrast to these results a relative study comparing the antibacterial andantifungal efficacy of MO steam distillate observed more inhibition for *E. coli* followed by *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa* and *B. subtilis.* In case of fungi, *Aspergillus niger* was strongly inhibited followed by *Aspergillus oryzae*, *Aspergillus terreus* and *Aspergillus nidulans*[35].

In the present study purslane fixed oil was analyzed by GC-MS to detect the active antibacterial ingredient that could affect *E. coli*. It was found that Omega 3 was the most prevalent compound (30.83%).

Omega 3 and oleic acids are of polyunsaturated fatty acid which are known for their inhibitory effect on Gram negative bacteria compared to saturated fatty acids. They are incorporated to the outer cell membrane and increase membrane permeability so the concentration gradient necessary between the organism and its environment may be dissipated resulting in death of the organism[36]. This may interpret why there was no change in protein profile of *E. coli* before and after treatment with purslane oil while the TEM showed disruption of the outer cell membrane and releasing of all internal cell content.

CONCLUSION

Plant oil extract has antibacterial activity. It contains unsaturated fatty acids that have bactericidal effect. This study showed that purslane fixed oil was more effective than MO's. *E. coli* was the highly affected bacterial strain. Omega 3 and oleic acids are of polyunsaturated fatty acids that present in purslane oil and has bactericidal activity by disrupting the outer cell membrane. Using of natural compounds can reduce the phenomenon of antimicrobial resistance.

ACKNOWLEDGMENT

The author thanks deeply Prof. Mohammed Abdulla Hussein, Biochemistry Department, Faculty of Pharmacy, October 6th University, for his grateful help and support.

REFERENCES

1. Bugno, A., M.A. Nicoletti, A.A.B. Almodovar, T.C. Pereira and M.T. Auricchio, 2007. Antimicrobial efficacy of *Curcuma zedoaria* extract as assessed by linear regression compared with commercial mouthrinses. Braz. J. Microbiol., 38: 440-445.
2. Vijaya, K. and S. Ananthan, 1997. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. J. Altern. Complement. Med., 3: 13-20.
3. Dilhuydy, J.M., 2003. [Patients' attraction to Complementary and Alternative Medicine (CAM): A reality which physicians can neither ignore nor deny]. Bull. Cancer, 90: 623-628, (In French).

4. Liu, L., P. Howe, Y.F. Zhou, Z.Q. Xu, C. Hocart and R. Zhang, 2000. Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties. J. Chromatogr. A, 893: 207-213.
5. Boroushaki, M.T., M.H. Boskabady and F. Malek, 2004. Antitussive effect of *Portulaca oleracea* L. in guinea pigs. Iran. J. Pharmaceut. Res., 3: 187-190.
6. Xin, H.L., Y.F. Xu, X.Q. Yue, Y.H. Hou, M. Li and C.Q. Ling, 2008. Analysis of chemical constituents in extract from *Portulaca oleracea* L. with GC-MS method. Pharmaceut. J. Chin. People's Liberat. Army, 24: 133-136.
7. Peksel, A., I. Arisan-Atac and R. Yanardag, 2006. Antioxidant activities of aqueous extracts of purslane (*Portulaca oleracea* Subsp. *sativa* L.). Ital. J. Food Sci., 18: 295-308.
8. Sultana, A. and K. Rahman, 2013. *Portulaca oleracea* Linn: A global panacea with ethnomedicinal and pharmacological potential. Int. J. Pharm. Pharmaceut. Sci., 5: 33-39.
9. Nayaka, H.B., R.L. Londonkar, M.K. Umesh and A. Tukappa, 2014. antibacterial attributes of apigenin, isolated from *Portulaca oleracea* L. Int. J. Bacteriol. 10.1155/2014/175851
10. Mousavi, S.M., G. Bagheri and S. Saeidi, 2015. Antibacterial activities of the hydroalcoholic extract of *Portulaca oleracea* leaves and seeds in Sistan Region, Southeastern Iran. Int. J. Infect., Vol. 2. 10.17795/iji-23214
11. Londonkar, R. and H.B. Nayaka, 2011. Phytochemical and antimicrobial activities of *Portulaca oleracea* L. J. Pharm. Res., 4: 3553-3555.
12. Bae, J.H., 2004. Antimicrobial effect of *Portulaca oleracea* extracts on food-borne pathogens. J. Food Sci. Nutr., 9: 306-311.
13. Saadabi, A.M. and I.E. Abu Zaid, 2011. An *in vitro* antimicrobial activity of *Moringa oleifera* L. seed extracts against different groups of microorganisms. Aust. J. Basic Applied Sci., 5: 129-134.
14. Sayeed, M.A., M.S. Hossain, M.E.H. Chowdhury and M. Haque, 2012. *In vitro* antimicrobial activity of methanolic extract of *Moringa oleifera* Lam. fruits. J. Pharmacogn. Phytochem., 1: 94-98.
15. Cappuccino, J.G. and N. Sherman, 1995. Microbiology Lab Manual. Benjamin-Cummings Publishing Co., USA., Pages: 477.
16. Hussein, M.A., N.A. Gobba and M.H. El Bishbishy, 2014. Composition, *in vitro* antioxidant and antitumor properties of essential oil from the seeds of *Moringa oleifera*. Int. J. Pharma Sci., 4: 532-540.
17. CLSI., 2004. Performance standards for antimicrobial susceptibility testing; 14th informational supplement. M100-S14, National Committee for Clinical Laboratory Standards, Wayne, PA., USA.
18. Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli and M.E. Falagas *et al*., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect., 18: 268-281.
19. AOAC., 1984. Official Methods of Analysis. 14th Edn., Association of Analytical Chemists Inc., Arlington, USA.
20. Rezanka, T. and H. Rezankova, 1999. Characterization of fatty acids and triacylglycerols in vegetable oils by gas chromatography and statistical analysis. Analytica Chimica Acta, 398: 253-261.
21. Suardana, I.W., K.J.P. Pinatih, N.L.K.A. Ratnawati and D.A. Widiasih, 2013. Protein profile analysis of *Escherichia coli* O157:H7 from human and animals origin. Int. J. Curr. Microbiol. Applied Sci., 2: 204-214.
22. Hammer, M.U., A. Brauser, C. Olak, G. Brezesinski, T. Goldmann, T. Gutsmann and J. Andra, 2010. Lipopolysaccharide interaction is decisive for the activity of the antimicrobial peptide NK-2 against *Escherichia coli* and *Proteus mirabilis*. Biochem. J., 427: 477-488.
23. Torrent, M., S. Navarro, M. Moussaoui, M.V. Nogues and E. Boix, 2008. Eosinophil cationic protein high-affinity binding to bacteria-wall lipopolysaccharides and peptidoglycans. Biochemistry, 47: 3544-3555.
24. Torrent, M., B.G. de la Torre, V.M. Nogues, D. Andreu and E. Boix, 2009. Bactericidal and membrane disruption activities of the eosinophil cationic protein are largely retained in an N-terminal fragment. Biochem. J., 421: 425-434.
25. Uddin, M.K., A.S. Juraimi, M.S. Hossain, M.A.U. Nahar, M.E. Ali and M.M. Rahman, 2014. Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid and antioxidant attributes. Scient. World J. 10.1155/2014/951019
26. Zhao, X.H., X. He, X.F. Yang and X.H. Zhong, 2013. Effect of *Portulaca oleracea* extracts on growth performance and microbial populations in ceca of broilers. Poult. Sci., 92: 1343-1347.
27. Peng, S., W. Dai, H. Yu, Y. Wang, X. Wang and S. Sun, 2014. Antibacterial activity of aqueous and ethanolic extracts of *Portulaca oleracea* L. and taraxacum mongolicum against pathogenic bacteria of cow mastitis. Int. J. Applied Res. Vet. Med., 12: 210-213.
28. Bakkiyara, J. and S. Pandiyaraj, 2011. Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. Int. J. Pharm Bio Sci., 2: 484-491.
29. Suarez, M., M. Haenni, S. Canarelli, F. Fisch and P. Chodanowski *et al*., 2005. Structure-function characterization and optimization of a plant-derived antibacterial peptide. Antimicrob. Agents Chemother., 49: 3847-3857.
30. Harvey M., 2005. *Moringa* leaf powder-The world's greatest unknown supplement. http://www.articlecity.com/articles/health/article_3610.shtml
31. Anwar, F., M. Ashraf and M.I. Bhanger, 2005. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. J. Am. Oil Chem. Soc., 82: 45-51.

32. Fahey, J.W., 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. Trees Life J., Vol. 1.
33. Guevara, A.P., C. Vargas, H. Sakurai, Y. Fujiwara and K. Hashimoto *et al*., 1999. An antitumor promoter from *Moringa oleifera* Lam. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 440: 181-188.
34. Jabeen, R., M. Shahid, A. Jamil and M. Ashraf, 2008. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak. J. Bot., 40: 1349-1358.
35. Kekuda, T.R.P., N. Mallikarjun, D. Swathi, K.V. Nayana, M.B. Aiyar and T.R. Rohini, 2010. Antibacterial and antifungal efficacy of steam distillate of *Moringa oleifera* Lam. J. Pharmaceut. Sci. Res., 2: 34-37.
36. Thompson, L., A. Cockayne and R.C. Spiller, 1994. Inhibitory effect of polyunsaturated fatty acids on the growth of *Helicobacter pylori*: A possible explanation of the effect of diet on peptic ulceration. Gut, 35: 1557-1561.