

Original Article

Antimicrobial activities of *Moringa oleifera* seed and seed oil residue and oxidative stability of its cold pressed oil compared with extra virgin olive oil

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Abstract

Moringa oleifera (MO) seed contains highly valuable substances with an impressive range of medicinal, cosmetic and food uses. MO seed, seed oil and its residue were investigated for antioxidant and antimicrobial activities against selected foodborne microorganisms using disc diffusion and minimum inhibitory concentration. In addition, the physico-chemical properties, fatty acid compositions and oxidative stability of cold pressed MO seed oil in comparison with those of extra virgin olive (EVO) oil were also evaluated and the results indicated that MO residue had the highest antioxidant activity followed by oil and seed, respectively. The extracts of MO seed and residue against *Staphylococcus aureus* showed the maximum inhibition zone of 20.67 and 24.67 mm, respectively, at 100 mg/ml concentration. In comparison, MO seed oil contained a high level of oleic acid (71.87%) to that of EVO oil (78.10%). MO seed oil was much more stable against oxidation than EVO oil based on the oxidative stability index results.

Keywords: antimicrobial activity, moringa oleifera seed, oil, oxidative stability

1. Introduction

Moringa oleifera (MO) Lam. belongs to the mono-generic family, *Moringaceae*, and is widely cultivated in many tropical countries, e.g. Africa, India, Sri Lanka, Ceylon, Thailand, Burma, Mexico, Malaysia and the Philippines (Fahey, 2005). It has been used for treating bacterial infection, fungal infection, inflammation, sexually-transmitted diseases, malnutrition and diarrhea (Arabshahi-Delouee *et al.*, 2009; Fahey, 2005; Farooq *et al.*, 2012; Rahman *et al.*, 2009) for several decades due to its versatile and valuable properties (Tsaknis *et al.*, 1999). Due to beneficial properties of this plant, it has been extensively researched for over the last 10 years (Foidl *et al.*, 2001). Because of high fat content of its seed (35-42% by weight), it can be used as biofuel, vegetable oil, cosmetic,

medicine and animal feed (Farooq *et al.*, 2012; Anwar and Bhanger, 2003; Anwar and Rashid, 2007). Furthermore, the MO seed is also rich in protein (25.5-38.3%) (Abudulkarim *et al.*, 2005; Compaoré *et al.*, 2011; Rahman *et al.*, 2009). It has been reported that polypeptides in seeds are one of the best natural coagulants and can bind to many moieties and possesses antimicrobial properties that can be used for water treatment (Anwar *et al.*, 2007a). The presence of some phytochemical substances especially a short polypeptide found in MO seed extracts was reported to act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Bukar *et al.*, 2010; Suarez *et al.*, 2003).

The synthetic antioxidants are mainly butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), which are used in many food formulations to enhance their oxidative stabilities during storage. Antioxidants derived from natural sources are in-high demand for food applica-

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tions to replace the synthetic antioxidants (Lutterodt *et al.*, 2011). *MO* seed oil is edible and reported to be a rich source of monounsaturated fatty acid, particularly oleic acid (C18:1) (71-74% of total fatty acids) (Ruttarattanamongkol *et al.*, 2014). High-oleic acid oils have been proved to be more resistant toward oxidation (Anwar *et al.*, 2007b). Our previous study suggested that the high oleic acid content of the *MO* oil coupled with its low peroxide value and acidity may qualify the *MO* oil as a natural derived antioxidant in food and cosmetic industries (Ruttarattanamongkol *et al.*, 2014). *MO* seed oil was reported to enhance the oxidative stability of some vegetable oils such as sunflower oil and soybean oil during deep-frying (Anwar *et al.*, 2007b).

There are ongoing efforts to develop new ingredients from underutilized plant seeds. *MO* seed extracts and derivatives can also be used as the natural antimicrobial and antioxidant ingredients in several industries like food, cosmetic and pharmaceutical. In addition, *MO* seed oil has high potential as a possible substitute for imported expensive oil such as olive oil. Hence, this study was conducted to determine: (1) the antioxidant and antimicrobial activities of *MO* seed, seed oil and its residue after oil extraction; and (2) the physico-chemical properties, fatty acid compositions and oxidative stability of cold pressed *MO* seed oil in comparison to those of the commercial extra virgin olive (*EVO*) oil.

2. Materials and Methods

2.1 Raw materials

Moringa oleifera Lam. dried seeds with hulls were purchased from Dhevee Greens Corporation Limited (Bangkok, Thailand). They were de-hulled, ground, and sieved through a 1 mm size mesh. The seed powder was kept in air tight containers and stored at 4°C until used. Bertolli® extra virgin olive oil (©2014 Unilever United State of America) was purchased from the local supermarket.

2.2 Chemical reagents

All reagents used were from Merck (Darmstadt, Germany) and Sigma Aldrich (Buchs, Switzerland).

2.3 Oil extraction by Soxhlet Method

For Soxhlet method, the oil was extracted using light petroleum ether (b.p. 40-60°C) in a Soxhlet extractor for 8 h (AOAC, 1984). The solvent residue was removed in an oven at 60°C for 1 h. The seed oil residue was kept in the air tight container and stored at 4°C until used.

2.4 Oil extraction by cold press

The cold press extraction of *MO* seed oil was done using a screw press (Komet Type CA 59 G, IBG Monforts GmbH and Co., Germany) with nozzle number 4 at level

1 speed. The oil was filtered through filter paper (whatman No. 1), then centrifuged at 3000 rpm (2,431 x g) for 3 min to separate the water and residues from the oil. The oil was stored in amber bottles at 4°C until analyzed.

2.5 Antimicrobial assay

The antibacterial activities of three different extracts; 1) cold water extract of *MO* seed powder, 2) cold water extract of *MO* residue after oil extraction by Soxhlet method and 3) *MO* seed oil obtained by Soxhlet method were determined using the diffusion technique of Bauer-Kirby (disk method) according to Lalas *et al.* (2012) and Gortzi *et al.* (2007) with modification. The *MO* seed and residue extracts were prepared by mixing *MO* seed or residue powder (1 mm particle size) in distilled water to achieve 250 mg/ml concentration. The suspension was mixed at room temperature for 20 min and filtered through filter paper (Whatman No.1). The filtrate was used immediately after preparation. For *MO* seed oil extract (~ 36.95% oil content), ethanol was used instead of distilled water. Six bacterial strains were used in this study, among these were two gram-positive, namely *Staphylococcus aureus* (TISTR 1466) and *Bacillus subtilis* (TISTR 008), and four gram-negative, namely *Salmonella typhimurium* (TISTR 292), *Enterobacter aerogenes* (TISTR1540), *Pseudomonas aerogonosa* (TISTR 781) and *Escherichia coli* (780). All tested bacterial strains were obtained from the Thailand Institute of Scientific and Technological Research (TISTR). Chloramphenicol (30 mg/ml), the standard antibiotic, was used to control the sensitivity of the tested bacteria and a control disk (6 mm diameter) without extracts was used as a negative control. The paper disks were soaked in the extracts and then deposited on the surface of the seeded tryptic soy agar (TSA) plates. The plates with the tested organisms at the final concentration of 10^5 - 10^7 cell/ml were incubated for 48 h at 37 °C and the bacterial growth inhibition was observed. Minimal Inhibitory Concentration (MIC) (expressed in mg/ml) against the tested bacterial strains was carried out using the tube dilution technique according to Bukar *et al.* (2010) and Akinyemi *et al.* (2005) with modification. Series of *MO* extract concentrations of 100, 50, 25 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.08 and 0 (control, distilled water) mg/ml were prepared for MIC determinations. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1 ml of standardized inoculum ($1-2 \times 10^7$ cfu/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18–24 h. The experiments were repeated three times and the results (diameters in mm) were expressed as average values with standard deviation.

2.6 Antioxidant activity

The antioxidant activity of *MO* seed powder (1 mm particle size), *MO* seed oil from Soxhlet method and its residue was determined using a DPPH assay. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of

samples was determined according to the method of Turkmen *et al.* (2005) with modification; 2 mg of each sample was vigorously mixed for 1 min in 50 ml of methanol ($\geq 99.9\%$), then filtered using a filter paper No.4. Then, 1 ml of the extract solution was mixed and vigorously shaken with 2 ml of newly prepared 0.1 mM DPPH solution in methanol ($\geq 99.9\%$). The mixture was left to stand in the dark for 60 min. The absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated as $[1 - (\text{absorbance of sample}/\text{absorbance of control})] \times 100$. The determination was carried out in triplicate and reported as mean with standard deviation.

2.7 Physico-chemical properties of cold pressed MO oil and EVO oil

The color (L^*, a^*, b^* value) was measured using the Micro Color (DR Bruno Lange GmbH, Düsseldorf, Germany) while the acid value, free fatty acid (FFA, %), saponification value, peroxide value and iodine value were analyzed using the IUPAC (1992) methods (2.201, 2.202, 2.205 and 2.501, respectively). The determination was carried out in triplicate with standard deviations.

2.8 Fatty acid composition

The fatty acid profiles of cold pressed *MO* seed and *EVO* oil were evaluated by gas chromatography following the method described by Ruttarattanamongkol *et al.* (2014). Gas chromatography analysis was performed on Fisons Gas Chromatography System Model GC8000 with RTX 225 capillary column (30 m length, 0.25 mm inside diameter, ID) using hydrogen as carrier gas at 90 kPa constant pressure. The column was held at 170°C for 2 min and raised to 220°C at 5 °C/min and held for 10 min while the injector and Flame Ionization Detector (FID) temperatures were set to 250°C. The injector was operated in a split mode with 1/50 split rate using an empty glass liner (4 mm ID). The Fatty Acid Methyl Esters (FAME) were identified based on the comparisons of

their retention indices and mass spectra with published data and computer matching. The mass spectra fragmentation patterns were compared with those stored in the mass spectral library NIST05 provided by GC system software. Quantification was performed by area normalization and results were expressed as the percentage of fatty acid methyl esters. The results are the mean of three replications with standard deviations.

2.9 Oxidative susceptibility of cold pressed MO oil and EVO oil

Susceptibility of cold pressed *MO* seed oil to oxidation was evaluated and compared to that of commercial EVO oil using the Rancimat test according to Anwar *et al.* (2003). The test was performed on a Rancimat apparatus 679 (Metrohm Herisau, Switzerland) by measuring the induction period (IP) at 130°C and the conductivity range of 200 $\mu\text{s}/\text{cm}$. Determination of the induction period was based on the conductometric detection of volatile acid. Oxidative Stability Index (OSI) was defined as the oxidation induction time (in hours). The determination was carried out in triplicate and reported as mean with standard deviations.

3. Results and Discussion

3.1 Antimicrobial activity

The antimicrobial activity of the cold water extracted *MO* seed, *MO* seed oil and the cold water extracted *MO* seed residue was determined *in vitro*, using disc diffusion and MIC method against selected pathogenic bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Pseudomonas aerogonosa* and *Escherichia coli*. The bacterial growth inhibition of various *MO* extracts (250 mg/ml) was firstly tested and the result was shown in Table 1. In this study, *MO* extracts were considered active against tested bacterial strains when the zone of inhibition was greater than 6 mm (shown as “-” in

Table 1. Bacterial inhibition of *Moringa Oleifera* (*MO*) seed, seed oil and residues extracts¹

Bacteria	<i>MO</i> seed	<i>MO</i> seed oil ²	<i>MO</i> residue ³
<i>Staphylococcus aureus</i>	-	+	-
<i>Bacillus subtilis</i>	+	+	+
<i>Salmonella typhimurium</i>	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+
<i>Pseudomonas aerogonosa</i>	+	+	+
<i>Escherichia coli</i>	+	+	+

+ = normal growth ; - = > 6 mm inhibition zone

¹250 mg/ml concentration

² Seed oil by Soxhlet extraction

³ Residues after Soxhlet extraction of seed oil

Table 1) according to the general rule for the antimicrobial activities of plant seed extracts (Eilert *et al.*, 1981). The result showed that all *MO* extracts did not show detectable suppression in growth of *Bacillus subtilis*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Pseudomonas aerogonosa* and *Escherichia coli*. The *MO* seed oil was not active against all bacterial strains tested. Similarly, Spiliotis *et al.* (1997) studied the antimicrobial activity of *MO* seed oil on various micro-organisms and found that the oil was not effective against the microbial activity. However, Lalas *et al.* (2012) assessed the antimicrobial activity of the seed oil of *Moringa Peregrina* on various bacterial strains and the oil proved effective against all microorganisms studied. The variation in antimicrobial activity of *MO* seed oil reported by different studies could be attributed to the differences in *Moringa* species and the oil extraction method they used.

The *MO* seed and residue had a substantial inhibitory effect against *Staphylococcus aureus*. Therefore, *in vitro* antimicrobial activity of *MO* seed and residue against *Staphylococcus aureus* was further investigated. Series of *MO* extract concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.08 and 0 (control, distilled water) mg/ml were prepared for MIC determinations. As shown in Table 2, strong inhibition of *MO* seed and residue extracts against *Staphylococcus aureus* was noticeable. All concentrations of *MO* seed and residue extracts were found to be active against *Staphylococcus aureus* as observed from the 6 mm (0.08 mg/ml) or larger inhibition zone (0.19-100 mg/ml). The maximum inhibition zone of 20.67 and 24.67 mm was obtained against *Staphylococcus aureus* for *MO* seed and residue extracts, respectively, at the maximum concentration of 100 mg/ml. In this study, the water extracts of *MO* seed and residue showed the lowest concentration of inhibitory effect of 0.08 mg/ml as observed from the 6 mm inhibition zone. Chloramphenicol (30 mg/ml), the standard antibiotic, showed a 30 mm inhibition zone, which demonstrated a significantly higher activity against the tested bacteria compared to the *MO* extracts. Bukar *et al.* (2010) reported *Staphylococcus aureus*, *Salmonella typhi* or *Escherichia coli* were sensitive to *MO* seed chloroform and ethanol extracts. Similarly, Saadabi and Abu Zaid (2011) also indicated that aqueous extract of *MO* seed showed a superior antibacterial activity against gram positive bacteria including *Staphylococcus aureus* and *Bacillus subtilis*. It is interesting to note that the presence of types of phytochemicals and their contents in the *MO* extracts dominates the antimicrobial activity of the extracts (Bukar *et al.*, 2010). Solvent extraction involves the distribution of different components between two immiscible phases. Therefore, the type of the solvent used for extraction plays a dominant role on antimicrobial activity of the extract. Ajaiyeoba (2002) and Bukar *et al.* (2010) indicated that *MO* extracts from polar solvent (ethanol and water) extraction were more active than the extracts from non- or less polar solvents such as chloroform. It was stated that the *MO* seed extracted with chloroform showed lower antimicrobial activity compared to *MO* seed extracted with ethanol (Bukar *et al.*, 2010). In relation to the

extracts' phytochemical content, the presence of saponins, alkaloids, tannins and flavonoids was confirmed to enhance the antimicrobial activity of the plants (Bukar *et al.*, 2010; Sing and Bhat, 2003). However, only saponins were detected in the *MO* seed chloroform extract (Bukar *et al.*, 2010). Saadabi and Abu Zaid (2011) also reported that the *MO* seed aqueous extract was more effective than the *MO* seed methanol extract. Nonetheless, their aqueous seed extract possessed higher activity than that presented in our work. This could possibly be attributed to the difference in the extraction method. In their case, the *MO* seed was extracted in water at a much higher temperature (70°C), which is beneficial in extracting more phytochemicals.

3.2 Antioxidant activity

Antioxidant activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging of *MO* seed, *MO* seed oil and its residue is presented in Table 3. The variation in antioxidant activities among samples could be attributed to tocopherol contents, the presence of phenolic compounds and the form of antioxidant compounds (hydrophilic and lipophilic) (Nawirska-Olszańska *et al.*, 2013; Tasioula-Margari and Okogeri, 2001). The result demonstrated that the *MO* seed oil possessed strongest antioxidant ability, followed by *MO* seed and *MO* seed residue, respectively. Our previous study confirmed that α-tocopherol was dominant in the *MO* seed oil. Tocopherols content in *MO* seed oils obtained by Soxhlet extraction, cold press and supercritical fluid extraction methods ranged between 137.9 to 230.3 mg/kg (Ruttarattanamongkol *et al.*, 2014).

Table 2. Minimum inhibitory concentration (MIC)¹ of *Moringa Oleifera* (*MO*) seed and residue extracts

Extract concentrations (mg/mL)	Zone of inhibition (mm)	
	<i>MO</i> seed	<i>MO</i> residue ²
100	20.67±0.12	24.67±0.42
50	19.33±0.12	24.00±0.35
25	18.67±0.12	22.67±0.31
12.5	12.33±0.32	17.00±0.52
6.25	13.33±0.25	12.00±0.10
3.12	11.33±0.15	11.67±0.12
1.56	10.33±0.12	10.33±0.06
0.78	10.00±0.10	10.00±0.10
0.39	7.33±0.06	9.33±0.25
0.19	6.00±0.00	9.33±0.06
0.08	6.00±0.00	6.00±0.07
0	0.00±0.00	0.00±0.00
Chloramphical	30.00±0.00	30.00±0.00

¹ Values are presented as mean ± S.D. of triplicated experiments.

² Residues after Soxhlet extraction of seed oil.

Table 3. Antioxidant activity of *Moringa Oleifera* (*MO*) seed, seed oil and seed residue

Sample	DPPH scavenging activity (%)	
	Average	SD
<i>MO</i> seed	23.66	0.36
<i>MO</i> seed oil	30.19	2.98
<i>MO</i> seed residue	14.93	0.22

3.3 Physico-chemical properties, fatty acid compositions and oxidative stability of cold pressed *MO* oil and *EVO* oil

The cold pressed *MO* seed oil was analyzed for its physico-chemical characteristics, fatty acid compositions and oxidative stability index (OSI) and compared to commercial extra virgin olive (*EVO*) oil and the result is presented in Table 4. *MO* seed oil was considerably brighter (higher L* value) and much more yellowish in color (higher b* value)

compared to *EVO* oil. The yellow color of *MO* seed oil is attributed mainly to the natural pigments remaining in the oil during extraction (Anwar and Rashid, 2007). The density of *MO* seed oil (0.93 g/ml) was slightly lower than that of *EVO* oil (0.94 g/ml). The iodine value of *MO* seed oil (68.56 g I₂/100 g oil) was much lower than that of *EVO* oil (87.29 g I₂/100 g oil) indicating a lower unsaturation of the oil. The free fatty acid and acid values of *MO* seed oil were apparently higher than *EVO* oil. This is due to the action of lypolytic enzyme which was enhanced by water contamination in oil during cold press extraction (Lalas and Tsaknis, 2002). The higher acidity of the *MO* seed oil could be due to the higher content of polyunsaturated fatty acids, thereby resulting in the tendency of triglycerides to break down, which further increases the free fatty acid content (Ruttarattanamongkol *et al.*, 2014). Saponification value of *MO* seed oil (121.51 mg KOH/g oil) was slightly lower than those of *EVO* oil (124.86 mg KOH/g oil). The peroxide value of *MO* seed oil (3.33 meq/kg oil) was also significantly lower than that of *EVO* oil (14.09 meq/kg oil). This indicates a lower oxidation of the *MO* seed oil compared to *EVO* oil.

Table 4. Physico-chemical properties, fatty acid compositions and oxidative stability index (OSI) of *Moringa Oleifera* (*MO*) seed oil and extra virgin olive (*EVO*) oil

Properties	<i>MO</i> seed oil	<i>EVO</i> oil
Physical properties		
L*	46.64±0.04	29.10±0.10
a*	1.02±0.12	0.20±0.10
b*	45.61±0.07	6.20±0.20
Density (g/ml)	0.93±0.01	0.95±0.00
Chemical properties		
Iodine value (g I ₂ /100 g oil)	68.56±1.86	87.29±1.24
FFA(%)	1.13±0.00	0.49±0.00
Acid value	2.25±0.02	0.97±0.011
Peroxide value (meq/kg oil)	3.33±0.08	4.09±1.05
Saponification value (mg KOH/g oil)	121.51±2.45	124.86±0.49
Fatty acid (%)		
Palmitic acid (C16:0)	6.25±0.08	11.23±0.12
Palmitoleic acid (C16:1)	1.18±0.04	0.30±0.00
Stearic acid (C18:0)	6.29±0.04	2.56±0.05
Oleic acid (C18:1)	71.87±0.59	76.32±1.50
Linoleic acid (C18:2)	0.50±0.03	6.48±0.21
Linolenic acid (C18:3)	0.20±0.01	0.75±0.02
Arachidic acid (C20:0)	3.85±0.07	0.46±0.01
Eicosenoic acid (C20:1)	2.27±0.03	0.31±0.00
Behenic acid (C22:0)	6.00±0.21	0.16±0.00
Lignoceric acid (C24:0)	1.05±0.07	0.06±0.00
Saturated fatty acids	23.44±0.27	14.65±0.06
Monounsaturated fatty acids	75.33±0.47	78.10±0.95
Polyunsaturated fatty acids	0.70±0.03	7.22±0.04
OSI (h)	36.10±1.06	8.69±1.03

The fatty acid composition of *MO* seed oil and *EVO* oil is shown in Table 4. Both oils contained more than 70% total unsaturated fatty acids; the major fatty acid was oleic (C18:1). Based on its fatty acid profile, *MO* seed oil was considered as a high-oleic oil (>70%). Similar fatty acid profile of *MO* seed oil obtained from supercritical fluid extraction (Nguyen *et al.*, 2011; Zhao and Zhang, 2013) or by solvent and cold press extraction methods (Anwar and Rashid, 2007; Ghazali and Mohammed, 2011; Lalas and Tsaknis, 2002; Nguyen *et al.*, 2011; Manzoor *et al.*, 2007; Zhao and Zhang, 2012) was also reported. In comparison, *EVO* oil showed that *MO* seed oil had higher palmitoleic acid (C16:1), stearic acid (C18:0), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0) and total saturated fatty acids and lower polyunsaturated fatty acids. This finding is in agreement with those reported by Tsaknis (1998) and Lalas and Tsaknis (2002). Several reports indicated that oils rich in oleic acid are more stable to oxidative rancidity and stable during frying (Abudulkarim *et al.*, 2005; Anwar *et al.*, 2007b; Mohammed *et al.*, 2003; Warner and Knowlton, 1997). More importantly, oleic acid enriched diets have been reported to reduce the risk of coronary heart disease (Mohammed *et al.*, 2003; Anwar *et al.*, 2007b).

The susceptibility to oxidation of *MO* seed oil and *EVO* oil was characterized by Rancimat method under accelerated aging temperature of 130°C. The induction period is the time taken to reach the point of inflection on the conductivity versus time curve and is defined as the oxidative stability index (OSI) (Lutterodt *et al.*, 2011). The result in Table 4 demonstrates that the OSI value of the *MO* seed oil was approximately 4.15 times higher than that of *EVO* oil. It was stated that the polyunsaturated fatty acid (PUFA) content, degree of unsaturation and α -, γ - and δ -tocopherols of oils play an important role in oxidative stability of oils (Abuzaytoun and Shahidi, 2006; Lutterodt *et al.*, 2011). Moreover, oils with higher degree of unsaturation generally had lower oxidative stability (Uluata and Özdemir, 2012). The higher degree of unsaturation and linoleic acid content (more prone to oxidation) of *EVO* oil resulted in its lower OSI value in comparison to *MO* seed oil. Our previous study showed that the α -tocopherol content of cold pressed *MO* seed oil (137.8 mg/kg) was noticeably higher than that reported for olive oil (15-88 mg/kg) (Lalas and Tsaknis, 2002; Ruttarattanamongkol *et al.*, 2014; Tsaknis, 1998). Additionally, it was also stated that tocopherols play an important role on the resistance to oxidation of oils (Lalas and Tsaknis, 2002).

4. Conclusions

The *MO* seed, seed oil and its residue after Soxhlet extraction were evaluated for their antimicrobial activity against six pathogenic bacterial strains. *MO* seed and residue extracts proved to be active against *Staphylococcus aureus* with the minimum concentration inhibition of 0.08 mg/ml, while *MO* seed oil did not show detectable suppression in growth of bacterial strains tested. However, further studies are needed

to evaluate other biological activities of *MO* seed and its residue to identify and characterize the active components that are responsible for their antimicrobial activity. Cold pressed *MO* seed oil contained high monounsaturated to saturated fatty acids ratio with high oleic acid content comparable to *EVO* oil. *MO* seed oil was less susceptible to oxidation than *EVO* oil as shown by its higher OSI value. *MO* seed oils could be an acceptable substitute for highly monounsaturated oils such as olive oil. It may have a potential role as a new source of health-promoting diets with high oxidative stability.

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References

- Abudulkarim, S.M., Long, K., Lai, O.M., Muhammad, S.K.S. and Ghazali, H.M. 2005. Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. *Food Chemistry*. 93, 253-263.
- Abuzaytoun, R. and Shahidi F. 2006. Oxidative stability of flax and hemp oils. *Journal of the American Oil Chemists' Society*. 83, 855-861.
- Ajaiyeoba, E. 2002. Phytochemical and antibacterial properties of *Parkia biglobosa* and *P. bicolor* leaf extracts. *African Journal of Biomedical Research*. 5, 125-129.
- Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. 2005. Screening of crude extracts of six medicinal plants used in South –West Nigerian unorthodox medicine for antimethicillin resistant *S. aureus* activity. *BMC Complimentary Alternative Medicine*. 5(6), 1-7.
- Anwar, A., Latif, S., Ashraf, M. and Gilani A.H. 2007a. *Moringa oleifera* : a food plant with multiple medicinal uses. *Phytotherapy Research*. 21, 17-25.
- Anwar, F., Bhanger, M. and Kazi, T. 2003. Relationship between rancimat and active oxygen method values at varying temperatures for several oils and fats. *Journal of the American Oil Chemists' Society*. 80, 151-155.
- Anwar, F. and Bhanger, M.I. 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperature regions of Pakistan. *Journal of the American Oil Chemists' Society*. 51, 6558-6563.
- Anwar, F., Hussain, A.I., Iqbal, S. and Bhanger, M.I. 2007b. Enhancement of the oxidative stability of some vegetable oils by blending with *Moringa oleifera* oil.

Food Chemistry. 103, 1181-1191.

Anwar, F. and Rashid, U. 2007. Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. Pakistan Journal of Botany. 39, 1443-1453.

AOAC. 1984. Official Methods of Association of Official Analytical Chemists. (W. Horwitz, ed.). Association of Official Analytical chemists (AOAC), Washington, DC.

Arabshahi-Delouee, S., Aalami, M. and Urooj, A. 2009. Drumstick (*Moringa oleifera* L) leaves: a potential source of natural lipid antioxidants. Journal of Food Process Engineering. 1-13.

Bukar, A., Uba, A. and Oyeyi, I. 2010. Antimicrobial profile of *Moringa Oleifera* Lam. Extracts against some food-borne microorganisms. Bayero Journal of Pure and Applied Sciences. 3, 43-48.

Compaoré, W.R., Nikiéma, P.A., Bassolé, H.I.N., Savadogo, A., Mouecoucou, J., Hounhouigan, D.J. and Traoré S.A. 2011. Chemical composition and antioxidative properties of seeds of *Moringa oleifera* and pulps of *Parkia biglobosa* and *Adansonia digitata* commonly used in food fortification in Burkina Faso. Current Research Journal of Biological Sciences. 3, 64-72.

Eilert, U., Wolters, B., Nahrestedt, A. 1981. Planta Medica. 42, 55-61.

Fahey, J.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees Life Journal. 1, 5-19.

Farooq, F., Rai, M., Tiwari, A., Khan, A.A., Farooq, S. 2012. Medicinal properties of *Moringa oleifera*: an overview of promising healer. Journal of Medicinal Plants Research. 6, 4368-4374.

Foidl, N., Makkar, H.S., Becker, K. 2001. The potential of *Moringa oleifera* for agricultural and industrial uses. In: "The Miracle Tree/ The Multiple Attributes of *Moringa*" (Ed. Lowell J Fuglie). CTA. USA.

Ghazali, H.M., Mohammed, A.S. 2011. *Moringa (Moringa oleifera)* seed oil: composition, nutritional aspects, and health attributes. In Nuts & seeds in health and disease prevention (Chapter 93). Elsevier Inc. pp 787-793.

Gortzi, O., Lalas, S., Tsaknis J., Chinou I. 2007. Evaluation of the antimicrobial and antioxidant activities of *Origanum dictamnus* extracts before and after encapsulation in liposome. Molecules. 12, 932-945.

IUPAC. 1992. International Union of Pure and Applied Chemistry Commission on Oils, Fats and Derivatives. Standard methods for the analysis of oils, fats and Derivatives (7th eds.). Blackwell Scientific Publications, Oxford, U.S.A.

Lalas, S., Gortzi, O., Athanasiadis, V., Tsaknis, J. and Chinou, I. 2012. Determination of antimicrobial activity and resistance to oxidation of *Moringa peregrine* seed oil. Molecules, 17, 2330-2334.

Lalas, S. and Tsaknis, J. 2002. Characterization of *Moringa oleifera* seed oil variety "Periyakulam 1". Journal of Food Composition and Analysis. 15, 65-77.

Lutterodt, H., Slavin, M., Whent, M., Turner, E. and Yu, L. (L.). 2011. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chemistry. 128, 391-399.

Manzoor, M., Anwar, F. and Iqbal, T. 2007. Physico-chemical characterization of *Moringa concanensis* seeds and seed oil. Journal of the American Oil Chemists' Society. 84, 413-419.

Mohammed, A.S., Lai, O.M., Muhammad, S.K.S., Long, K. and Ghazali, H.M. 2003. *Moringa oleifera*, potentially a new source of oleic acid-type oil for Malaysia. Investigating in Innovation. 137-140.

Nawirska-Olszańska, A., Kita, A., Biesiada, A., Sokół-Lętowska, A., Kucharska, A.Z. 2013. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. Food Chemistry. 139, 155-161.

Nguyen, H.N., Gaspillo, P.D., Maridable, J.B. and Malaluan, R.M. 2011. Extraction of oil from *Moringa oleifera* kernels using supercritical carbon dioxide with ethanol for pretreatment: optimization of the extraction process. Chemical Engineering and Processing. 50, 1207-1213.

Rahman M.M., Sheikh M.M.I., Sharmin S.A., Islam M.S., Rahman M.A., Rahman M.M. and Alam M.F. 2009. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. Against some human pathogenic bacteria. Chiang Mai Universit Journal of Natural Sciences. 8, 219-227.

Ruttarattanamongkol, K., Siebenhandl-Ehn, S., Schreiner, M. and Petrasch, A. 2014. Pilot-scale supercritical carbon dioxide extraction, physico-chemical properties and profile characterization of *Moringa oleifera* seed oil in comparison with conventional extraction methods. Industrial Crops and Products. 58, 68-77.

Saadabi, A. M. and Abu Zaid, I.E. 2011. An *in vitro* antimicrobial activity of *Moringa oleifera* L. seed extracts against different groups of microorganisms. Australian Journal of Basic and Applied Sciences. 5, 129-134.

Singh, B. and Bhat, T.K. 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. Journal of Agricultural and Food Chemistry. 51, 5579-5597.

Spiliotis, V., Lalas, S., Gergis, V. and Dourtoglou V. 1997. Comparison of antimicrobial activity of seeds of different *Moringa oleifera* varieties. Pharmaceutical and Pharmacological Letters. 7, 39-40.

Suarez, M., Entenza, J.M., Dorries, C. 2003. Expression of a plant -derived peptide harbouring water-cleaning and antimicrobial activities. Biotechnology and Bioengineering. 81, 13-20.

Tasioula-Margari, M., Okogeri, O. 2001. Isolation and characterization of virgin olive oil phenolic compounds by

HPLC/UV and GC-MS. *Journal of Food Science*. 66, 530-534.

Tsaknis, J. 1998. Characterisation of *Moringa peregrine* Arabia seed oil. *Grasas y Aceites*, 49, 170-176.

Tsaknis, J., Lalas S., Gergis, V., Dourtoglou, V., Spiliotis, V. 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. *Journal of American Chemical Society*. 47, 4495-4999.

Turkmen, N., Sari, F. and Veliogly, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry*. 93, 713-718.

Uluata, S., Özdemir, N. 2012. Antioxidant activities and oxidative stabilities of some unconventional oilseeds. *Journal of American Oil Chemists Society*. 89, 551-559.

Warner, K., Knowlton, S. 1997. Frying quality and oxidative stability of high-oleic corn oil. *Journal of American Oil Chemists Society*. 74, 1317-1321.

Zhao, S. and Zhang, D. 2013. A parametric study of supercritical carbon dioxide extraction of oil from *Moringa oleifera* seeds using a response surface methodology. *Separation and Purification Technology*. 113, 9-17.