

Effect of extraction methods on physicochemical, nutritional, antinutritional, antioxidant and antimicrobial activity of Moringa (*Moringa oleifera* Lam.) seed kernel oil

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Abstract: The effect of three different extraction methods, namely supercritical CO₂, soxhlet and solvent methods on the yield, efficiency, physico-chemical properties, nutritional, anti-nutritional composition, antimicrobial and antioxidant activities of moringa (*Moringa oleifera* Lam.) seed kernel oil was investigated in this study. Oil extraction for SC-CO₂ were 37.76 g/100g and 98.43%, observed to be higher than those of soxhlet extraction (29.12 g/100g and 76.29%), and significantly lower than solvent extraction (22.12 g/100g and 57.99%). The physico-chemical composition of the oils showed considerable variation among the extraction methods. The SC-CO₂ extracted oil was found to be of superior quality, showing negligible thermal degradation and exhibited significantly ($p < 0.01$) higher nutritional and antioxidant activity and lower anti-nutritional composition than the soxhlet and solvent extracted oils. Oils produced by SC-CO₂ and soxhlet extraction methods had antimicrobial activities higher than solvent extracted oil. SC-CO₂ extracted oil was found to have maximum number of bioactive compounds (14 compounds) followed by solvent (8 compounds) and soxhlet (4 compounds) extracted oil. The results of the study demonstrated that SC-CO₂ would be a promising process for the extraction of moringa seed kernel oil of premium quality.

Keywords: Bioactive compounds, Essential oil, Extraction, Moringa seeds, SC-CO₂

INTRODUCTION

Moringa (*Moringa oleifera* Lam.) is generally known to the age-old world, but only currently it has been found again as a multipurpose tree with an awesome variety of potential uses. Moringa owned to the family “Moringaceae” with genus “*Moringa Adans*” and species “*M. oleifera* Lam”. This species originally comes from North India but now has spread all over the globe in the tropics and subtropics (Enwa *et al.*, 2013). In some parts of the world, *M. oleifera* is called to as the Drumstick tree or the Horse radish tree, Kelor, Marango, Mlonge, Moonga, Mulangay, Nebeday, Saijhan, Sajna or Ben oil tree (Prabhu *et al.*, 2011).

Moringa is an excellent source of nutritional elements reported by Bureau of Plant Industry. Nearly every part of the Moringa plant (leaves, flowers, seeds, roots and bark) can be used as food, medicinal and therapeutic purposes. The seeds can be eaten as such green and roasted; powdered and soaked seeds are used for tea as well as in curries (Anwar *et al.*, 2006). The seeds have powerful coagulative and anti-microbial properties on pathogenic strains of *Escherichia coli*, *Pseudomonas*

aeruginosa, *Streptococcus faecalis*, *Salmonella typhi* and *Shigella dysenteriae* (Oluduro *et al.*, 2010).

Important bioactive compounds present in the moringa seeds have been used for anti-microbial, anti-genotoxic, anti-inflammatory and anti-tumour promoting activities (Prabhu *et al.*, 2011). Excellent source of tocopherols present in the Moringa seed oil, which is considered as the equivalent to olive oil in terms of its chemical properties. It can also be used for edible purpose, rheumatism and gout, purification of blood and enhancing cardiac function (Middleton *et al.*, 2000).

Ben oil is another name for Moringa seed kernel oil, is more stable than canola oil, soybean oil, and palm oil when used for frying of foods (Abdulkarim *et al.*, 2005). Ben oil is blended with sunflower oil and soybean oil enhances the oxidative stability of the mixture (Mani *et al.*, 2007). Comparing the physico-chemical properties, nutritional and antinutritional composition, moringa seed oil is contemplate alternative to olive oil and may be used for human consumption (Nguyen *et al.*, 2011).

Moringa seed kernel oil is conventionally produced by hydro and steam distillation and to lower extent by

solvent extraction method, these extraction methods have more disadvantages (Ali, 1991). Hydro and steam distillation methods are more time consuming processes and high extraction temperatures, causes the degradation of thermal sensitive compounds in the extractant and led to incomplete extraction of oil (Denny, 1988). Unwanted constituents and solvent residues in the final extract are major problems in solvent extraction and it is not possible to obtain solvent free oil. New trends in supercritical carbon dioxide (SC-CO₂) extraction technology have shown promising alternative to conventional extraction methods. SC-CO₂ has been found to produce extracts with waxes, pigments, anti-nutritional constituents free from chemical alterations influenced by temperature, moisture content, and solvent free residues, undesirable compounds such as organic and inorganic salt, sugars, amino acids and tannins (Razazadeh *et al.*, 2008; Da Porto *et al.*, 2009). Extraction methods can have influence on the yield, efficiency, physico-chemical properties, nutritional and antinutritional composition and biological activities of moringa seed kernel oil, particularly antioxidant and antimicrobial activities (Topal *et al.*, 2008; Danh, *et al.* 2011). The objective of this investigation was to study the effect of different extraction methods, namely solvent extraction using methanol, soxhlet extraction with n-hexane and supercritical CO₂ extraction on oil yield, efficiency, physico-chemical properties, nutritional and antinutritional composition and antimicrobial activities of moringa (*Moringa oleifera* Lam.) seed kernel oil to recommend the best method of extraction for quality oil.

MATERIALS AND METHODS

Raw materials: Moringa seeds (PKM-1) were procured from local market in Raichur, Karnataka (India). The shells were removed manually and the kernels were ground by using laboratory hammer mill to obtain fine powder of 0.16 mm particle size (Nguyen *et al.*, 2011).

Solvent extraction: Moringa seed kernel oil was extracted in 150 mL methanol in five hundred milliliter round bottomed flask for 72 h at room temperature (Oluduro *et al.*, 2010).

Soxhlet extraction: The extraction was carried out at 85 °C temperature for 90 min extraction time and n-hexane was used as solvent in Soxhlet apparatus (Malapit, 2010).

SC-CO₂ extraction: Oil was extracted by SC-CO₂ extraction unit (Thar; SFE 500). Cooling of different zones in the 200 mL capacity extraction vessel was done by deionized water at 5 °C. The optimized parameters selected for the study were SC-CO₂ pressure of 200 bar, temperature of 50 °C and dynamic extraction time of 90 min (Liza *et al.*, 2010). Fifty grams of moringa seed kernel powder was placed into the extractor vessel. The flow rates of SC-CO₂ and co-solvent (ethanol) were maintained at 20 and 2 g/min,

respectively (Pradhan *et al.*, 2010). Static extraction process for 30 min was used. The dynamic extraction time (90 min) was started after attaining desired pressure and temperature, by adjusting the exit valve (ABPR-valve) of the SC-CO₂ extraction system. During the static extraction time the sample was allowed to soak in CO₂ and co-solvent in order to equilibrate the mixture at desired pressure and temperature. Carbon dioxide carried the crude extract flooded out of the extraction vessel and then into a collection vessel during the dynamic extraction. After completion of dynamic extraction time carbon dioxide was separated in the collection vessel and then flooded through the vent connected to the fume hood (Palafox *et al.*, 2012).

Extraction yield and efficiency: The extraction yield and extraction efficiency of SC-CO₂ extracted moringa seed kernel oil were determined according to the methods described by Liza *et al.* (2010); Olawale. (2012).

Physico-chemical properties: Density and specific gravity of the extracted oil were determined using the method described by Adejumo *et al.* (2013) and the colour values (*L**, *a** and *b**) were measured by using Hunter lab colourimeter (Cho *et al.*, 2010). The pH, absorbance (at 270 nm) and refractive index were measured according to the procedure explained by Orhevba *et al.* (2013); Ogbunugafor *et al.* (2011). Acid value, saponification value, iodine value and peroxide value of oil samples were determined by using respective standard methods (AOAC, 1990).

Nutritional and anti-nutritional composition: Total phenol, flavonoid, carotenoid, sterol, tocopherol, oleic acid and antioxidant capacity were analyzed according to methods described by Bhatnagar and Krishna, (2013); Ogbunugafor *et al.* (2011); Dauqan *et al.* (2011a); Sabir *et al.* (2003); Dauqan *et al.* (2011b); Nilani *et al.* (2012); Nabasree and Bratati, (2007). Anti-nutritional constituents *viz.*, tannins, saponins and phytates present in the moringa seed kernel oil were determined by the methods described by Inuwa *et al.* (2011), Jaffe, (2003), Anhwange *et al.* (2004) and Lucas and Markaka, (1975).

Bioactive compounds: Qualitative analysis of bioactive compounds present in the moringa seed kernel oil was analysed by GC-MS (Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS column). Carrier gas used was Helium and the initial temperature of oven was set for 400 °C and held for 3 min and the final temperature of the oven was maintained at 480 °C with rate at 100 °C/ min. 2µL of oil was injected with split mode of 1:50 ratio. Mass spectra were recorded in range of 35-650 amu, with 70 eV electron impact ionization energy. The total running time of 45 min was used for each sample. The volatile compounds present in oil were indicated by comparing the retention times of chromatogram peaks using quadra pole detector with NIST library to correlate the relative retention time indices. Quantitative determinations were made by matching the respective peak areas to TIC areas

from the GC-MS (Mahadkar *et al.*, 2013).

Free radical scavenging activity: Free radical scavenging activity of moringa seed kernel oil against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method reported by Adesugen *et al.* (2008).

Antimicrobial activity: Antimicrobial sensitivity was tested for bacteria and fungi namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Mucor* and *Aspergillus* by using disc agar diffusion technique described by Bauer *et al.* (1966); Cakir *et al.* (2004).

Statistical analysis: Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) software package. The current version of 2015 was used and experimental results were computed for the mean, standard deviation (SD) and standard error mean (SEM). All the experiments were performed at least three times and were subjected to two-way analysis of variance.

RESULTS AND DISCUSSION

Effect of different extraction methods on extraction yield and efficiency of Moringa seed kernel oil: Extraction yield, efficiency and physico-chemical properties of moringa seed kernel oil extracted by three dif-

ferent methods are presented in Table 1. SC-CO₂ extraction recorded highest extraction yield (37.76 g/100g) and efficiency (98.43%), followed by soxhlet (29.12 g/100g and 76.29%), while solvent extraction gave the lowest yield (22.12 g/100g) and efficiency (57.99%). These results were in agreement with the results reported by Danh *et al.* (2011) for extraction of Lavanders essential oil.

The oil yield obtained from solvent extraction method was found lower compared to the SC-CO₂ extraction method. The differences in extraction yield among three methods are significant due to differences in operating conditions (time, pressure, temperature and particulate size) and the constituents present in the oil. Furthermore, SC-CO₂ was driven at a lower temperature than the other methods. In overall, the extraction of Moringa seed kernel oil by SC-CO₂ showed efficient and it could be an excellent alternative to traditional extraction method for achieving higher in terms of yield and extraction efficiency.

Effect of different extraction methods on physico-chemical properties of Moringa seed kernel oil: From the Table 1, it is clearly demonstrated by highest oil density and specific gravity of 0.984 g/cc and 0.998

Table 1. Effect of different extraction methods on yield, efficiency and physico-chemical properties of Moringa seed kernel oil.

| | Extraction methods | | | | |
|--|--------------------|---------|--------------------|----------------|-----------------|
| | Solvent | Soxhlet | SC-CO ₂ | Std. Deviation | Std. Error Mean |
| Extraction yield (g/100g) | 22.13 | 29.12 | 37.76 | 7.830 | 4.520 |
| Extraction efficiency (%) | 57.99 | 76.29 | 98.43 | 20.250 | 11.692 |
| Physical properties | | | | | |
| Density (g/cc) | 0.907 | 0.912 | 0.984 | 0.043 | 0.025 |
| Specific gravity | 0.914 | 0.92 | 0.998 | 0.047 | 0.027 |
| pH | 5.80 | 4.10 | 6.25 | 1.134 | 0.655 |
| Refractive index | 1.415 | 1.461 | 1.487 | 0.036 | 0.021 |
| Absorbance (at 270) nm | 0.02 | 0.022 | 0.027 | 0.004 | 0.002 |
| L* value | 5.87 | 9.06 | 3.40 | 2.838 | 1.638 |
| a* value | -0.93 | -0.75 | -0.54 | 0.195 | 0.113 |
| b* value | 1.11 | 1.87 | 1.08 | 0.448 | 0.258 |
| Chemical properties | | | | | |
| AV (mg of KOH/g of oil) | 3.71 | 3.75 | 2.80 | 0.537 | 0.310 |
| SV (mg of KOH/g of oil) | 188.25 | 188.05 | 185.66 | 1.441 | 0.832 |
| IV (g/mol) | 60.25 | 61.15 | 66.71 | 3.499 | 2.020 |
| PV (meq of O ₂ / kg of oil) | 60.46 | 61.15 | 98.06 | 21.512 | 12.420 |

Table 2. Effect of different extraction methods on nutritional and anti-nutritional composition of Moringa seed kernel oil.

| Composition | Extraction methods | | | | |
|-------------------------------------|--------------------|---------|--------------------|----------------|-----------------|
| | Solvent | Soxhlet | SC-CO ₂ | Std. Deviation | Std. Error Mean |
| Nutritional compositions | | | | | |
| Total phenols (mg GAE/g) | 40.12 | 40.18 | 44.71 | 2.633 | 1.520 |
| Total flavanoids (mg RE/g) | 16.82 | 16.9 | 18.25 | 0.804 | 0.464 |
| Total carotenoids (ppm) | 15.26 | 15.72 | 17.06 | 0.935 | 0.540 |
| Total sterols (ppm) | 837.85 | 880.57 | 984.17 | 75.241 | 43.441 |
| Total tocopherols (ppm) | 77.81 | 79.12 | 92.26 | 7.991 | 4.614 |
| Oleic acid (%) | 69.25 | 69.77 | 73.84 | 2.513 | 1.451 |
| Total antioxidants (mg AAE/g) | 37.91 | 38.22 | 36.42 | 0.962 | 0.556 |
| Anti-nutritional composition | | | | | |
| Tannins (%) | 9.10 | 9.12 | 6.13 | 1.721 | 0.993 |
| Saponins (%) | 0.32 | 0.35 | 0.26 | 0.046 | 0.026 |
| Phytate (%) | 11.36 | 11.49 | 3.09 | 4.813 | 2.779 |

Table 3. Effect of different extraction methods on antimicrobial activity of Moringa seed kernel oils.

| Microorganisms | Inhibition zone diameter (mm) | | | | |
|-------------------------------|-------------------------------|---------|--------------------|----------------|-----------------|
| | Oil extraction methods | | | | |
| | Solvent | Soxhlet | SC-CO ₂ | Std. Deviation | Std. Error Mean |
| Gram+ | | | | | |
| <i>Bacillus cereus</i> | 6.51 | 8.56 | 11.52 | 2.519 | 1.454 |
| <i>Staphylococcus aureus</i> | 6.78 | 6.85 | 8.66 | 1.066 | 0.615 |
| Gram- | | | | | |
| <i>Escherichia coli</i> | 0 | 0 | 9.54 | 5.508 | 3.180 |
| <i>Pseudomonas aeruginosa</i> | 0 | 0 | 0 | 0.000 | 0.000 |
| Fungy | | | | | |
| <i>Mucor species</i> | 10.32 | 11.41 | 12.56 | 1.120 | 0.647 |
| <i>Aspergillus species</i> | 8.77 | 9.46 | 12.23 | 1.831 | 1.057 |

Table 4. Effect of different extraction methods on free radical scavenging activity of Moringa seed kernel oils.

| Extraction methods | Concentration of extract (µg/mL) | | | | Std. Deviation | Std. Error Mean |
|--------------------|----------------------------------|-------------------|-------------------|-------------------|----------------|-----------------|
| | 50 | 100 | 150 | 200 | | |
| Solvent | 47.83±0.02 | 49.01±0.01 | 50.12±0.05 | 52.14±0.02 | 1.83308 | 0.91654 |
| Soxhlet | 48.15±0.01 | 52.97±0.04 | 60.12±0.06 | 70.01±0.06 | 9.50293 | 4.75147 |
| SC-CO ₂ | 80.60±0.07 | 82.02±0.06 | 83.28±0.02 | 83.80±0.02 | 1.42785 | 0.71393 |

were recorded in SC-CO₂ followed by soxhlet extraction method (0.912 g/cc and 0.920), whereas the lowest of 0.907 g/cc and 0.914 were recorded in solvent extraction method. According to Adejumo *et al.* (2012), the density and specific gravity of oil decreased as the temperature increased. This might be due to the fact that the oil seeds frequently lose some of its characteristics when exposed to higher temperatures, resulting decline in its density and specific gravity in case of solvent (85°C) and soxhlet (85°C) extraction methods on the other side, in case of SC-CO₂ extraction method the density and specific gravity of oil increased due to lower extraction temperature (50°C) used. The values of specific gravity of the oil samples were within the range of 0.90-1.16 for edible oil given by FAO/WHO, (2009). These values were in good agreement with the results reported by Belewu *et al.* (2010); Tint and Mya, (2009) for *Jatropha curcas* seed oil. SC-CO₂ extraction yielded the oil of nearly neutral pH value (6.25), followed by solvent extraction shown slightly acidic (5.80) and soxhlet extraction highly acidic pH (4.10). This might be due to the enzymatic activity of oil at higher temperature (Wang *et al.*, 2010). The lowest refractive index was obtained for solvent extraction (1.415), followed by soxhlet extraction (1.461) and the highest value of 1.487 was obtained in SC-CO₂ extracted oil.

The maximum colour value *L** of the oil was found to be 3.40 for SC-CO₂ followed by soxhlet (9.06) and solvent extraction (5.87). The colour values of *a** were observed to be -0.54 (SC-CO₂), -0.75 (Soxhlet) and -0.93 (Solvent extraction). Similarly *b** values obtained 1.08 for SC-CO₂, 1.87 for soxhlet and 1.11 for solvent. Similar trends have been reported for SC-CO₂ extracted ginseng seed oil (Orhevba *et al.*, 2013). The colour values were found minimum in SC-CO₂ extracted oil compared to the other two extraction methods. This might be due to higher temperature of 85 °C applied in

soxhlet extraction that lowered the specific gravity thereby the color of the oil. These values are in agreement with the earlier findings of Rahman *et al.* (2012); Orhevba *et al.* (2013). The color intensity of the vegetable oils depends mainly on the presence of pigments such as chlorophyll, which is effectively removed during the degumming, refining and bleaching process. For edible and domestic purposes the vegetable oils with minimum values of color index are more suitable (Anwar *et al.*, 2006).

The chemical properties of oil are the most important properties that determine the original conditions of the oil. Acid and peroxide values are valuable indices for the oil quality measurement. The iodine and saponification values indicate the degree of unsaturation of fatty acids in the oil. The acid and saponification values of the SC-CO₂ extracted oil were significantly lower (185.66 mg of KOH/g of oil) than the soxhlet (188.05 mg of KOH/g of oil) and solvent (188.25 mg of KOH/g of oil) extracted oil. Similarly, more significant differences found in iodine and peroxide values of SC-CO₂ oil compared to soxhlet and solvent extracted oil. The acid values of SC-CO₂ extracted Moringa seed kernel oil was found within the range specified by FAO/WHO (2009).

Effect of extraction methods on nutritional and anti-nutritional composition in Moringa seed kernel oil:

Moringa oleifera seed kernel oil extraction had been studied by several authors viz, Mani *et al.* (2007); Nguyen *et al.* (2011); Palafox *et al.* (2012); Premi and Sharma (2013), Zhao and Zhang (2013). However, none of these investigations reported the detailed the comparison of oil obtained by SC-CO₂ and other conventional extraction methods for moringa seed kernel oil. Quantitative analysis was made by observing the nutritional and anti-nutritional composition of seed kernel oil, and the results are depicted in Table. 2. It is observed from the results that the total phenols (44.71

Table 5. Effect of different extraction methods on retention of bioactive compounds in Moringa seed kernel oil.

| Extraction method | Compound analyzed | Molecular formula | Mol. Wt. (In grams) | Retention time | % Area of peak |
|--------------------|--|--|---------------------|----------------|----------------|
| Solvent | Cycloheptasiloxane | C ₁₄ H ₄₂ O ₇ Si ₇ | 518 | 13.509 | 2.66 |
| | Cyclooctasiloxane | C ₁₆ H ₄₈ O ₈ Si ₈ | 592 | 16.148 | 1.35 |
| | Hexadecanoic acid | C ₁₇ H ₃₄ O ₂ | 270 | 18.651 | 2.85 |
| | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 19.097 | 18.16 |
| | 9,12-Octadecadienoic acid, | C ₁₉ H ₃₄ O ₂ | 294 | 20.262 | 1.65 |
| | Octadecanoic acid | C ₁₈ H ₃₆ O ₂ | 284 | 20.961 | 5.32 |
| | 9-Octadecenoic acid | C ₁₈ H ₃₄ O ₂ | 282 | 22.457 | 1.01 |
| | Eicosanoic acid | C ₂₀ H ₄₀ O ₂ | 312 | 22.702 | 0.65 |
| Soxhlet | Tetradecanoic acid | C ₁₄ H ₂₈ O ₂ | 228 | 17.031 | 4.23 |
| | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 19.086 | 29.69 |
| | Octadecadienoic acid | C ₁₉ H ₃₄ O ₂ | 294 | 20.653 | 42.75 |
| SC-CO ₂ | Octadecanoic acid | C ₁₈ H ₃₆ O ₂ | 284 | 20.942 | 8.35 |
| | Oxiraneethanol | C ₈ H ₁₆ O ₄ | 176 | 8.188 | 1.12 |
| | Nonanoic acid | C ₁₀ H ₁₈ O ₃ | 186 | 12.755 | 1.59 |
| | 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane | C ₁₈ H ₅₂ O ₇ Si ₇ | 576 | 14.63 | 13.51 |
| | Propanoic acid | C ₁₆ H ₃₀ O ₄ | 286 | 15.498 | 2.35 |
| | Cyclooctasiloxane | C ₁₆ H ₄₈ O ₈ Si ₈ | 592 | 16.917 | 5.4 |
| | Hexahydropseudoionone | C ₁₃ H ₂₆ O | 198 | 18.583 | 0.35 |
| | Z-5-Octadecen-1-ol acetate | C ₂₀ H ₃₈ O ₂ | 310 | 18.635 | 0.53 |
| | Heptasiloxane | C ₁₄ H ₄₄ O ₆ Si ₇ | 504 | 18.722 | 1.42 |
| | Hexadecanoic acid | C ₁₇ H ₃₄ O ₂ | 270 | 19.35 | 15.29 |
| | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 19.73 | 9.82 |
| | 9,12-Octadecadienoic acid | C ₁₉ H ₃₄ O ₂ | 294 | 20.952 | 11.15 |
| | Phytol | C ₂₀ H ₄₀ O | 296 | 21.217 | 33.07 |
| | Octadecanoic acid | C ₂₂ H ₄₄ O ₄ | 372 | 21.601 | 3.94 |
| | 11,14-Eicosadienoic acid | C ₂₁ H ₃₈ O ₂ | 322 | 21.748 | 0.47 |

mg GAE/g) extracted by SC-CO₂ was the highest among the total phenols obtained from other two extraction methods. Total flavonoids and total carotenoid in SC-CO₂ extracted oil were 18.25 (mg RE/g) and 17.06 (ppm), respectively, whereas, soxhlet extracted oil had 16.9 mg RE/g of total flavonoids and 15.72 ppm of total carotenoids and 16.82 mg RE/g and 15.26 ppm were found in solvent extracted oil. These results are in contrast with the findings of Oliveira *et al.* (2013) who reported similar results on supercritical fluid extraction of oil from passion fruit seeds and its processing residue (cake), whereas similar variation in TPC from 21 to 28 mg of GAE/ g extract has been reported. The occurrence of higher flavonoids in moringa oil would increase the economic health prospective of the oil as the earlier discovery suggested that flavonoids carry antioxidant activity through scavenging or chelating activity and play inhibitory role in cancer and heart disease (Ogbunugafor *et al.*, 2011). Total carotenoids content obtained from SC-CO₂ extraction (15.26 ppm) was more compared to soxhlet extraction (15.75 ppm).

It was observed that the essential oil extracted using SC-CO₂ method exhibited a higher amount of total sterols (984.17 ppm), whereas soxhlet and solvent ex-

tracted oil had lower values of 880.57 ppm and 837.85 ppm, respectively. The extraction methods differed significantly for total tocopherols and it was noted that the SC-CO₂ method yielded oil with a total tocopherols of 92.26 ppb followed by soxhlet extraction (79.12 ppb) and solvent extraction (77.81 ppb). These results are comparable with the earlier findings reported by Tsaknis *et al.* (1999); Palafox, (2012) reported for *Moringa oleifera* variety mbololo seed oil.

The oleic acid content varied in *M. oleifera* essential oil extracted by different methods. It was found that the SC-CO₂ extracted oil possessed more oleic acid with a value of 73.84% as compared to other two methods namely, soxhlet extraction (69.77%) and solvent extraction (69.25%). Corn, sunflower and canola oils are reported as high oleic acid vegetable oils and have enough oxidative stability during frying of foods. Oleic acid rich oils have low saturated fatty acid levels, therefore Moringa seed kernel oil with high oleic oils could be a healthier substitute for partially hydrogenated vegetable oils (Petukhov *et al.*, 1999; Abdulkarim *et al.*, 2009 and Dinesha *et al.*, 2016).

A wide range of total antioxidants were observed in different extraction methods. Oil obtained by SC-CO₂ extraction method (36.42 mg AAE/g) contained higher

amounts than soxhlet (38.22 mg AAE/g) and solvent (37.91 mg AAE/g) extracted oil. These results indicating the occurrence of large quantities of total antioxidants in moringa seed kernel oil have not been reported previously. These values are in good agreement with the findings of Ogbunugafor *et al.* (2011). Higher total antioxidant capacity of SC-CO₂ extracted oil might be due to higher solubility power of SC-CO₂ compared to other two solvents. The hexane extracts did not exhibit antioxidant activity by DPPH and the same results have been reported by Oliveira *et al.* (2013).

The results showed that the lowest amounts of anti-nutritional contents namely, tannin, saponin and phytate in the extracted oil were found to be the highest in SC-CO₂ extracted oil (6.13, 0.26 and 3.09%) followed by soxhlet method (9.12, 0.35 and 11.49%) and solvent method (9.10, 0.32 and 11.36%). These findings are in agreement with the results of previous studies reported by Mbah *et al.* (2012) on effect of cooking methods on antinutritional contents of *Moringa oleifera* seeds. From the findings of this study, SC-CO₂ extracted oil contained lower concentrations of the analyzed anti-nutritional factors compared to soxhlet and solvent extraction methods due to higher pressure applied in SC-CO₂ extraction method and hence dissolution of anti-nutritional constituents in the SC-CO₂ was higher than other two solvents (Couto *et al.*, 2009). Hence, SC-CO₂ extracted moringa oil conformed lower amount of anti-nutritional composition and it might be recommended as cooking oil by blending with other edible oils.

Effect of different extraction methods on antimicrobial activity of Moringa seed kernel oil: Moringa seed kernel oil presented excellent antibacterial and antifungal activities against the test organisms as shown in Table 3. Diameter zone of inhibition (DZI) for gram-positive bacterium viz., *Bacillus cereus* and *Staphylococcus aureus* was found higher in SC-CO₂ (11.52 and 8.66 mm) followed by soxhlet (8.56 and 6.85 mm) and solvent (6.51 and 6.78 mm) extracted oil. Similar results have been reported by Bukar *et al.* (2010); Talreja, (2010). Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) did not show DZI. DZI was not observed for the Moringa seed kernel oil extracted by all the three methods where as DZI of 9.54 mm for *Escherichia coli* was noticed in SC-CO₂ extracted oil.

Results revealed that, among the bacteria, moringa seed kernel extract was effective against gram positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus*, whereas it was not effective against gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. It might be due to variation in the peptidoglycon layer present in the cell wall of gram negative bacterial cell (Bukar *et al.*, 2010). Fungal pathogens like *Mucor species* exhibited more DZI

(10.32, 11.41 and 12.56 mm) and *Aspergillus species* exhibited lower DZI (8.77, 9.46 and 12.23 mm).

Moringa seed kernel extract had strong inhibitory effect against *Bacillus cereus*, *Staphylococcus aureus*, *Mucor species* and *Aspergillus species*. Whereas, it showed less effective against *E. coli* and *P. aeruginosa*. This suggested that, the SC-CO₂ extracted moringa seed kernel extract might be used for treatment of infections caused by these organisms except *E. coli* and *P. aeruginosa*, which were found to be resistant to the activity of the SC-CO₂ extracted moringa seed kernel extract (Abalaka *et al.*, 2012).

The antimicrobial activity of *M. oleifera* seed extract might be due to the presence of an phytochemicals. Bukar *et al.* (2010) and Suarez *et al.* (2003) identified, the presence of a short polypeptide named 4 (α -L-rhamnosyloxy) benzyl- isothiocyanate in moringa seeds. The peptide might act directly on microorganisms and might result in growth inhibition by destroying the cell membrane (Abalaka *et al.*, 2012).

Effect of different extraction methods on free radical scavenging activity in Moringa seed kernel oil:

Free radical scavenging activity of different extraction methods for moringa seed kernel oil is depicted in Table 4. Free radical scavenging activity was more in SC-CO₂ extracted moringa oil (80.60±0.07, 82.02±0.06, 83.28±0.02 and 83.80±0.02 at 50,100,150 and 200 µg/mL) than the solvent (47.83±0.02, 49.01±0.01, 50.12±0.05 and 52.14±0.02 at 50,100,150 and 200 µg/mL) and soxhlet extracted oil (48.15±0.01, 52.97±0.04, 60.12±0.06 and 70.01±0.06 at 50,100,150 and 200 µg/mL). The percentage inhibition of DPPH radicals increased with increasing concentration of oil. This might be due to the quenching of proton free radicals and decrease in absorbance of DPPH-oil mixture, and the results are in agreement with the findings of Asokkumar *et al.* (2009). This is in accordance with previous discovery that suggested the flavonoids carry antioxidant activity through scavenging or chelating have deterrent role in cancer and heart disease (Middleton *et al.*, 2000). Consequently, the significance of the antioxidant components present in the moringa seed kernel oil used to maintain health. Moringa seed kernel essential oil could be the future trend moving towards using foods as medicine in controlling various chronic diseases.

Effect of different extraction methods on retention of Bioactive compounds in Moringa seed kernel oil:

The comparison between the major bioactive compounds present in the essential oils obtained by different extraction methods shown in Table 5. From these results, it is seen that the retention of bioactive compounds in oil was influenced by the extraction method used. The oil obtained by solvent extraction method showed the presence of Cycloheptasiloxane (2.66%), Cyclooctasiloxane (1.35%), Hexadecanoic acid (2.85%), n-Hexadecic acid (18.16%), 9,12-

Octadecadienoic acid (1.65%), Octadecanoic acid (5.32%), 9-Octadecenoic acid (1.01%) and Eicosanoic acid (0.65%). Soxhlet extracted oil represented four compounds viz., Tetradecanoic acid (4.23%), n-Hexadecanoic acid (29.69%), Octadecadienoic acid (42.75) and Octadecanoic acid (8.35%), whereas SC-CO₂ extracted oil showed highest number of compounds viz., Oxiraneethanol (1.12%), Nonanoic acid (1.59%), 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane (13.51%), Propanoic acid (2.35%), Cyclooctasiloxane (5.40), Hexahydrodropseudoionone (0.35), Z-5-Octadecen-1-ol acetate (0.53), Heptasiloxane (1.42), Hexadecanoic acid (15.29), n-Hexadecanoic acid (9.82), 9,12-Octadecadienoic acid (11.15), Phytol (33.07), Octadecanoic acid (3.94) and 11,14-Eicosadienoic acid (0.47%). This higher bioactive compounds retention in SC-CO₂ extracted oil might be due to the lower temperature of extraction and higher solubility of SC-CO₂. Results are comparable with the findings reported for bioactivity of *Moringa oleifera* by Vijay and Samrot, (2010).

Conclusion

Significant variation in the extraction yield, efficiency, physico-chemical composition, nutritional, anti-nutritional and bioactivity was observed in the *Moringa* seed kernel oil obtained by different extraction methods. The oil yield and efficiency obtained from SC-CO₂ was higher compared to that of the solvent extraction (37.76 g/100g and 98.43 %), but much higher than the soxhlet extracted oil (29.12 g/100g and 76.29 %). Additionally, the bioactive components present in the SC-CO₂ extracted oil was observed to be not thermally amended (14 compounds) as contrast to the soxhlet (4 compounds) and solvent extracted oils (8 compounds). The antimicrobial activity of SC-CO₂ extracted oil against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Mucor species* and *Aspergillus species* were higher than that of the other two extraction methods. Ultimately, SC-CO₂ extracted oil had the elevated antioxidant activity (83.80±0.02 µg/mL) compared to conventional extraction methods. In overall, SC-CO₂ extraction method found to be a promising technology for extracting oil from moringa seed kernels with budding approaches in food as a preservative with antimicrobial and antioxidant activities.

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