



Research article

Effects of location and extraction solvent on physico chemical properties of *Moringa stenopetala* seed oilMeseret Haile^a, Haile T. Duguma^{b,*}, Gebremedhin Chameno^c, Chala G. Kuyu^b^a Addis Ababa Science and Technology University, Addis Ababa, Ethiopia^b Postharvest Management Department, Jimma University, P.O.Box 307, Jimma, Ethiopia^c Arba Minch University, Sawla Campus, Arba Minch, Ethiopia

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ABSTRACT

This research was initiated to evaluate the physicochemical property of crude oil from *Moringa stenopetala* (M. *Stenopetala*) seed collected from three locations (Damba Gofa, Shelle and Konso) which was extracted with two solvents (hexane and petroleum ether). Physical properties of crude oil varied from 34.8–44.3%, 0.8–0.9, 0.8–0.9 g cm³ and 1.4–1.5 at 40 °C for yield, specific gravity, relative density and refractive index, respectively. The chemical properties of the crude oil varied from 0.1–0.3 mg KOH g⁻¹, 76.5–91.6 g I₂ 100 g⁻¹, 17.6–20.6 mEq kg⁻¹, 154.0–199.3 mg KOH g⁻¹ oil and 0.2–0.4 mg g⁻¹ for Free fatty acid (FFA), iodine value, peroxide value, saponification value and acid value respectively. Two-way interaction effect of all physical and chemical properties showed a significant difference ($p < 0.05$) except the refractive index. The finding indicates that oil extracted with Hexane from Konso had a better quality. The extraction of oil using hexane from M. *stenopetala* seed could be of great importance in edible crude oil production. Further investigation should be done on oxidative stability of crude oil of M. *Stenopetala*.

1. Introduction

There are many underutilized plants in Ethiopia which have the potential to bring a lot of prosperity and most of which has remained unexploited. *Moringa* is one of the underutilized crops by a rural community in southern Ethiopia (Glærum, 2012). The Moringaceae is a single-genus family of oilseed trees with 14 known species (Rashied et al., 2008). It is a crop that tolerates harsh environmental conditions including prolonged drought periods, poor soil and achieving harvest in a short amount of time (Meitzner and prince, 1996; Abuye et al., 2003). Due to its drought tolerance and appear towards the end of the dry season when few other sources of green vegetables are available; it is regarded as a priority crop in Ethiopia to attain food security (Mekonnen, 2003).

M. *stenopetala* grows widely in many parts of the world tropics including Ethiopia. Similar to M. *oleifera*, M. *stenopetala* is currently introduced in several tropical countries for its popular uses but its origins are the southern regions of Ethiopia and Northern Kenya. It is known by different names with different languages such as shiferaw (amharic), halako (Gamo/wolayita), kallanki (Benishangul), Shalchade (Konso), Haleko (Burji) and cabbage tree (English) (Nzikou et al., 2009).

M. *stenopetala* has large edible leaves and seeds and is a multi-purpose

tree of significant economic importance as it has a vital nutritional, industrial and medicinal application (Eyasu, 2012; Abdull et al., 2014; Leone et al., 2016; Boukandoul et al., 2018; Tilahun, 2018). While many parts of M. *stenopetala* trees are deemed useful, the seeds are especially prized for their medicinal powers. The seeds contain edible oil that can be used for cooking and as salad dressings. The leaves and fruits are also consumed as vegetables as they are rich in proteins, essential amino acids, calcium, iron, phosphorous as well as vitamin A and C. In addition to nutritional importance, The medicinal properties of the M. *stenopetala* seed are well documented in the scientific literature (Adegebe et al., 2016; Fahey, 2005; Hsu, 2006; Kasolo et al., 2010) and are further supported by the experiences of generations of traditional Ayurvedic practitioners (Siddique et al., 2010).

Physicochemical properties of M. *oleifera* seeds oil recorded that it had a promising potential application in human nutrition (Gore, 2018). The oil extracted from *Moringa* is known as Ben oil and reported that it has physical and chemical properties equivalent to that of olive oil, contains a large number of tocopherols (Tsaknis et al., 1999) and 70% of oleic acid, an 18 carbon long monounsaturated fatty acid (MUFA). Hence the oleic acid has good oxidative stability compared to polyunsaturated fatty acids (PUFAs), it has been used in the food industry, as it allows for longer

* Corresponding author.

E-mail address: hailetesfaye840@gamil.com (H.T. Duguma).<https://doi.org/10.1016/j.heliyon.2019.e02781>

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storage and high-temperature frying processing. The higher content of unsaturated fatty acids may present a healthy impact of Moringa seeds oil in human nutrition (Kasolo et al., 2010). Many people in Taiwan or China have been using the seed of Moringa as herbal medicine to treat athlete's foot and tinea (Lalas et al., 2006).

Even though it has been demonstrated to have the potential to be integrated into the marginal, drought-prone farming systems of the country and important food security crop as sources of phytonutrients and raw materials in different chemical industries still it remains underutilized. Ethiopia is importing oil from abroad for chemical, cosmetics and pharmaceutical use. Moreover, peoples of our country use these crops only when there is a serious shortage of food affecting all strata of the population from the poorest to the richest. When there is a serious shortage of food its leaves are consumed by Gofa, Konso, Burji and Gamo tribes as vegetable especially during the dry season (Yisehak et al., 2011). So far from 14 species of Moringa, only Moringa oleifera has been accorded research and development attention.

Unlike *M. oleifera*, very little scientific research project has been conducted on the properties and potential uses of *M. stenopetala* seed oil. As a result, its application in antioxidant, aromatherapy, perfumery, soap and other related industries are limited due to lack of adequate research on the chemical and biological potential of its raw materials. To our knowledge extraction and characterization of physicochemical property of oil from *M. stenopetala* seed has not been reported yet in the study area. Therefore this research focuses on investigating the possibility of extraction and evaluation of physicochemical property of *M. stenopetala* seed oil produced in three locations using two solvent extractions. This could put the mile stone for further research on physicochemical characteristics of *M. Stenopetila* seed oil.

2. Materials and methods

2.1. Description of study site

M. Stenopetala seed was collected from three locations (Damba Gofa, Arba Minch zuriya and Konso) in Gamo Gofa and Segen area Zone, Southern part of Ethiopia, located at a distance of 514 km from Addis

Ababa. Laboratory analyses took place at Arba Minch University Chemistry Department Laboratory and Ethiopian conformity assessment center.

2.2. Sample collection and preparation

The matured pods of similar color were collected from *M. stenopetala* tree (Fig. 1) from two zones in South nation and nationalities of Ethiopia. Gamo Gofa Zone (Gofa and Shelle Districts) and Segen area zone (Konso District). The selection of both zones and Districts were made based on availability and abundance of *M. stenopetala* seed. The seeds were separated from the pods by removing the barks and removed from the pods. Then seeds were cleaned and only those with the uniform overall appearance, size, and shape were selected for this study. The seeds also had extra seed husk which was separated after sun drying to facilitate the separation of husk and seed kernels. Finally, the powder was obtained using grinding mill and kept for further oil extraction experiments.

2.3. Experimental design

Experiments were carried out using completely Randomized Design (CRD) having three *M. stenopetala* producer districts (Demba Gofa, Shelle and Konso) and two extraction solvents (hexane and petroleum ether) in three replicated times.

2.4. Data collected

2.4.1. Extraction and oil yield percentage determination

The oil was extracted following the method described by Adejumo et al. (2013) which uses solvent extraction method using Soxhlet apparatus with the semi-continuous process for the extraction of lipids from foods. Accordingly, grounded Moringa seed samples were placed in porous cellulose thimble and oil were extracted by repeated washing (percolation) with two organic solvents (hexane, and petroleum ether). The flask containing solvent and lipid was removed at the end of the extraction process, then the solvent in the flask was evaporated in Rota vapor and the mass of the oil remaining was measured and the

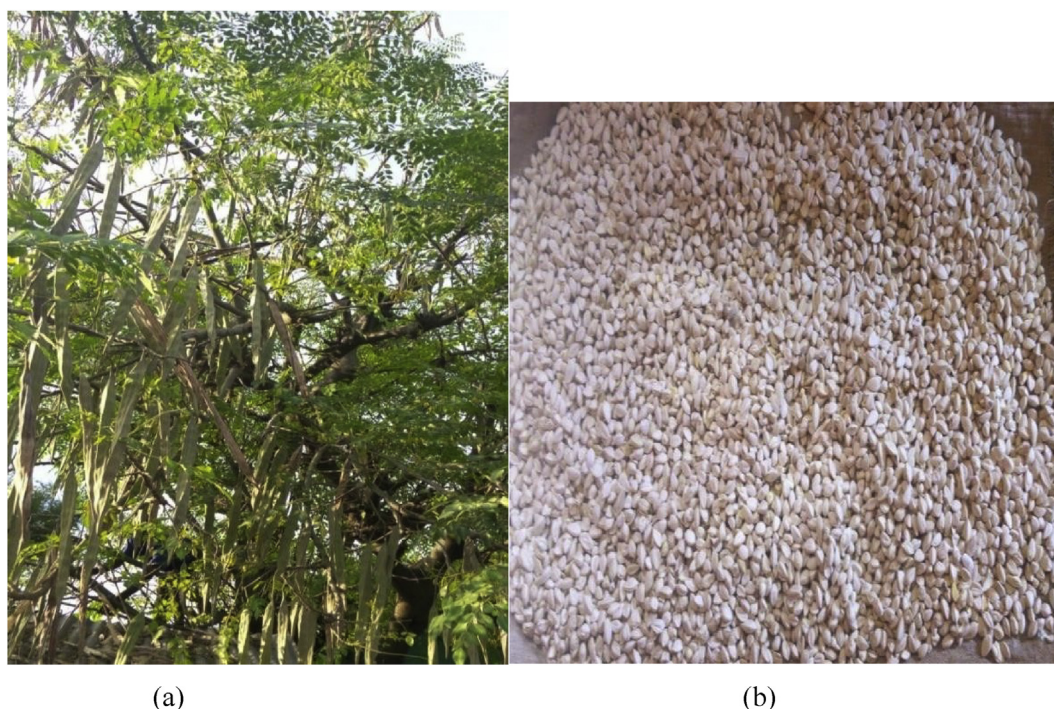


Fig. 1. *M. stenopetala* tree and matured pods (a) and *M. Stenopetala* seed (b) collected for the experiment.

percentage oil yield was calculated using the following formula:

$$\% \text{ oil yield} = \frac{\text{Weight before extraction} - \text{Weight after extraction} \times 100\%}{\text{Weight of sample before extraction}}$$

2.4.2. Physical property determination

Density, specific gravity and refractive index of extracted oil were determined using standard analytical methods of AOAC (2000) (method 969.18)

2.4.3. Chemical property determination

Saponification and peroxide value were determined following the method described by Akpan et al. (2006). Iodine value was determined using titration following the method described by Garcia (2010) and calculated using the following equation.

$$\% \text{ Iodine Number} = \frac{(B - A) \times 0.00317 \times 0.001269 \times 100}{\text{Weight of sample}}$$

where; B = Volume consumed by Blank, A = Volume consumed by Sample, 1ml of 0.025 N₂S₂O₃ = 0.00317g.

Peroxide value was determined following the method described by Farooq et al. (2006) and expressed as the percentage of free fatty acid calculated as oleic acid. The dish containing 5g of the oil sample was accurately weighed, poured into a conical flask and re-weighed, thus obtaining the actual weight of the oil taken. Fifty milliliters of hot neutral alcohol was added with a few drops of phenolphthalein and shaken vigorously. The solution was titrated with 0.5 M sodium hydroxide (NaOH) solution with constant shaking until the pink color remains constant. From the quantity of 0.5M alkali used, the percentage of acid present calculated, stating the result in terms of oleic acid (Farooq et al., 2006).

2.4.3.1. Determination of acid value. Acid value was determined using titration following the method described by Gatar et al. (2012) and calculated using the following equation

$$\text{Acid Value} = \frac{56.1 (V * N)}{m}$$

where: V=Volume of the KOH solution used N= Normality of KOH m = Mass of oil sample.

2.5. Statistical analysis

Data analysis was conducted using Minitab version 16 and analysis of variance (ANOVA) to determine the significant differences in physico-chemical properties of samples. All diagnostic tools were tested prior to data analysis and indicated that the residuals of all parameters were normally distributed. Differences between the sample means were conducted using Tukey's test at $\alpha = 0.05$ level for parameters showed a significant difference.

3. Result and discussion

3.1. M. stenopetala seed oil yield and physical property

Table 1 shows the range of values for oil yield and physical properties of M. stenopetala seed crude oil as affected by production locations and extraction solvents. The minimum and maximum values of yield, specific gravity, relative density, and refractive index value vary from 34.8–44.3, 0.8–0.9, 0.8–0.9 and 1.4–1.5 respectively. Oil yield, specific gravity and relative density of M. stenopetala seed oil were significantly ($P < 0.05$) affected by production locations and extraction solvents.

The lowest oil yields (34.8%) was observed in M. stenopetala seed collected from Gofa district and extracted with hexane, while the highest

Table 1

Oil yield and physical properties of Moringa stenopetala seed crude oil.

Extraction solvent	Locations	Oil Yield (%)	Specific gravity	Relative density (gml ⁻¹)	Refractive index
Hexane	Gofa	34.8 ^c	0.9 ^a	0.9 ^a	1.4 ^a
	Shelle	42.3 ^{ab}	0.8 ^b	0.9 ^a	1.4 ^a
	Konso	42.3 ^{ab}	0.9 ^a	0.9 ^a	1.4 ^a
Petroleum Ether	Gofa	35.3 ^{bc}	0.8 ^b	0.8 ^b	1.4 ^a
	Shelle	44.0 ^a	0.8 ^b	0.9 ^a	1.5 ^a
	Konso	44.3 ^a	0.9 ^a	0.9 ^a	1.5 ^a

Results are mean values of triplicate determination and means with the different letters across the column are significantly different ($p < 0.05$).

oil yield (44.3%) was observed in moringa seed collected from Konso and extracted with petroleum ether even though it's statistically similar with oil obtained from Shelle district. The oil yield obtained in this study is similar with the result reported by Essayas (2012) who reported the M. stenopetala oil yield in the range of 39.3–44.5%. Variation in oil yield with location and extraction solvent might be attributed to the diversity of natural habitats, agro climatic and constraints ripening stage. Different authors reported significant oil yield variation among Moringa seed varieties. Oil yield of (49.8–54.3%) recorded for M. peregrine (Somali et al., 1984; Tsaknis 1999) and (40.5–44.9%) for M. stenopetala (Lalas et al., 2003). Moreover, it could be due to the variation among capacity of solvent used to extract the oil from the seed. Sayyar et al., (2009) stated that n-hexane as a more preferable solvent for extraction of Jatropha seed oil as compared to petroleum ether, the extraction yield with n-hexane is about 1.3% more than that of petroleum ether (47.3% and 46.0% wt. respectively) under similar conditions. Banat et al. (2013) also obtain the highest oil yield by n-hexane. The range of oil content (34.8–44.3%) of M. stenopetala seeds in the present analyses was found to exceed those of cotton seed (15.0–24.0 %) and soybean (17.0–21.0 %) and comparable with those of safflower (25.0–40.0 %). The high oil yield allows the possibility of economic exploitation which Results in lower operation costs compared to some other oil seeds.

The Specific gravity of M. stenopetala seed oil was found to be in the range of 0.80–0.90gml⁻¹. The maximum specific gravity (0.9%) was observed in moringa seed collected from Konso district regardless of extraction solvent although it is not significantly different from Gofa district extracted with hexane. The obtained value for specific gravity is in agreement with the value recorded for M. Oleifera seed oil (0.9050) by FAO/WHO (2009) international standard for edible oil cited in (Adegbe et al., 2016).

The relative density of M. stenopetala seed oil was found to be in the range of 0.80–0.90gml⁻¹. Similar values were recorded for all treatments in terms of relative density except the lowest value (0.8gml⁻¹) recorded for seed collected from Gofa district and extracted with petroleum Ether. The relative density observed in this study is in better agreement with the value recorded (0.903 gml⁻¹) by Ejigu et al. (2008) for M. stenopetala seed oil. It's also consistent with the finding of Meta (2018) who reported the average density of M. stenopetala oil 0.907.

The refractive index (RI) of oil is related to the degree of saturation and distinctive for each type of oil and is indicative of oil purity. The refractive index of the oil was in the range of 1.4–1.5. There was no significant difference among treatments in terms of refractive index. The refractive index of the M. stenopetala seed oil recorded in this study is comparable with reports of different authors, Manzoor et al. (2007) reported the RI of M. oleifera oil of 1.4648 and Anwar et al. (2006) reported 1.4581.

3.2. Chemical properties

Table 2 shows the range of values for the chemical property of M. stenopetala seed crude oil as affected by production locations and extraction solvents. The minimum and maximum values of FFA, iodine

Table 2Chemical properties of *Moringa stenopetala* seed crude oil.

Extraction solvent	Locations	FFA (mgKOHg ⁻¹)	Iodine value (g I/100g)	Peroxide value (meqkg ⁻¹)	Saponification Value (mgKOHg ⁻¹)	Acid value (mgKOHg ⁻¹)
Hexane	Gofa	0.2 ^{ab}	87.9 ^{ab}	18.5 ^{ab}	175.7 ^{ab}	0.4 ^a
	Shelle	0.3 ^a	83.8 ^{ab}	20.6 ^a	192.3 ^a	0.4 ^a
	Konso	0.1 ^b	80.0 ^{ab}	17.6 ^b	199.3 ^a	0.3 ^{ab}
Petroleum Ether	Gofa	0.1 ^b	91.6 ^a	18.9 ^{ab}	159.0 ^b	0.3 ^{ab}
	Shelle	0.2 ^{ab}	76.5 ^b	19.1 ^{ab}	154.0 ^b	0.2 ^b
	Konso	0.1 ^b	84.3 ^{ab}	18.5 ^{ab}	177.0 ^{ab}	0.2 ^b

Results are mean values of triplicate determination and means with the different letters across the column are significantly different ($p < 0.05$).

value, peroxide value, saponification value and acid values varies from 0.1–0.3, 76.5–91.6, 17.6–20.6, 154.0–199.3 and 0.2–0.4 respectively. The chemical properties of the oil showed significantly ($P < 0.05$) influenced by the interaction effect of extraction solvents and production locations.

Free fatty acid value varied from 0.1 to 0.3 and the maximum free fatty acid was recorded from a sample collected from Shelle district and extracted with hexane solvent. The minimum free fatty acid content was obtained from seed collected from Konso and extracted with hexane solvent. There is a significant difference among treatments in terms of free fatty acid contents of the *M. stenopetala* seed crude oil. Free Fatty Acids are produced by the hydrolysis of oils and fats and it is not desired in the oil meant for consumption. It measures the extent to which glycerides in the oil have been decomposed by lipase action. The free fatty acid recorded in this study is moderately low and indicated that the oil from *M. stenopetala* seed can be used for human consumption.

The Iodine Number gives a measure of the average degree of unsaturation of oils and fats: the higher the iodine value, the greater the number of C=C double bonds. The highest iodine value (91.6 g I/100g) was recorded from seed collected from Gofa and extracted using petroleum ether and the lowest iodine value (76.5 g I/100g) was recorded from seed collected from shelle and extracted using petroleum ether. The recorded iodine values are in line with the value reported by Lalas et al. (2003) (65.8 g I/100 g) and that of iodine value reported by Ejigu et al. (2008) (69gI/100g). The result indicated that *M. stenopetala* oils are relatively stable to oxidation when compared to, sunflower (125–140gI/100g), linseed (170–180gI/100g) and soybean oil (128–143gI/100g) (Gunstone, 2009). Aremu et al. (2006) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity. A similar observation was also stressed by Aremu et al. (2006) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity. The variation observed among treatments could be due to mineral contents of the soil of different location where the plants are grown and the extraction solvent used.

Peroxide values measures the content of hydroperoxides in the oil (Kapoor et al., 2009). Its low value indicates high resistance to oxidation. The peroxide value of *M. stenopetala* crude oil of the current finding ranged from 17.6 to 20.6 meqkg⁻¹ with a different location and extraction solvent used. Peroxide value of crude oil of *M. stenopetala* seed showed a significant difference between location and extraction solvent. The minimum peroxide value was recorded at Konso and extracted with hexane and the maximum was recorded at Shelle and extracted with hexane. The recorded peroxide values were high in this study and could be due to the oil is not refined and because of high temperature during extraction that leads to fatty acid oxidation. A similar study was reported, oxidation of fatty acids at high temperature and exposure to oxygen during the extraction process high peroxide value (Allen and Hamilton, 1994).

The Saponification values of the oils were ranged from 154 to 199.3mgKOHg⁻¹ with different locations and extraction solvents. The maximum value (199.3) was recorded from a sample collected from Konso and extracted with hexane solvent and the minimum value was recorded *M. stenopetala* from Shelle and extracted with a petroleum

solvent. The value obtained for the oil of *M. stenopetala* (154–199.3 mg KOHg⁻¹ oil) in this study was comparable with those reported by Anwar and Bhangar (2006) (186 mgKOHg⁻¹oil), Anwar et al. (2006) (184 mg KOHg⁻¹oil), and Manzoor et al. (2007) (179 mg KOHg⁻¹oil) in the oil of *M. oleifera* cultivated in Pakistan. A similar result was also reported by Rekha and Leema (2017) that the saponification value for the *Moringa Oleifera* seed oil was 164. Since saponification value is an indicator of molecular weight or size as a function of the chain lengths of the constituent fatty acid (Tilahun, 2018), the value of around 195 indicates that oil contains mainly fatty acid of high molecular mass while the oils having high saponification value (around 300) have mainly fatty acids of low molecular mass and are useful for soap making (Boukandoul et al., 2018). The present result indicated that oil from *M. stenopetala* seed contains fatty acid of high molecular mass and mainly useful for consumption rather than soap making.

The acid value of *M. stenopetala* seed oils was ranged from 0.2 to 0.4 mg KOHg⁻¹ and it is within acceptable acid value limit for edible oil which is less than 1 (Sampson, 2005). The low-level acid values being viewed as a testimony to the freshness of the crude oil and that show that the oil is not degraded from the processing stage (Ochigbo and Paiko, 2011). This could be attributed to the presence of natural antioxidants in the seeds such as vitamins C and A as well as other possible phytochemicals like flavonoids. The value of acidity (0.2–0.4 mg KOHg⁻¹) obtained in this study was considerably lower than *M. oleifera* oil from Pakistan which is 0.8 (Anwar and Rashid, 2006). The low acid value obtained in result indicated that, *M. stenopetala* seed oil could be acceptable for edible applications.

4. Conclusion

M. stenopetala is one of the underutilized plants in Ethiopia which have the potential to bring a lot of prosperity. Oil seed collected from all locations were showed almost similar physicochemical property with literature values of commercially sold oils for human consumption. From treatment evaluated, oil extracted with Hexane from Konso has better physicochemical properties. Furthermore, physicochemical properties of *moringastenopetala* seed oil in this study indicates high iodine and saponification value which implies high unsaturated to saturated fatty acids ratio and might be an acceptable substitute for high saturated oils such as olive oil and palm oil. The current finding demonstrated that the oil has great potential to be utilized as a source of new vegetable oil for human consumption and its full potential should be exploited. Generally, the quality of the oil obtained in this study is comparable with other commercial edible oil. In the search for alternative oil sources, particularly those rich in unsaturated fatty acids, *Moringa stenopetala* seed oil has valuable promise. Therefore, the production of useful oil from its seed could be of great economic importance in substituting foreign currency for importing oil for Ethiopia. Further investigation is needed on refining of *M. Stenopetala* seed crude oil and characterizing of fatty acid.

Declarations

Author contribution statement

Haile T Duguma: Conceived and designed the experiments;

Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Meseret Haile: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Gebiremedhine Chameno, Chala G. Kuyu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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