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Proximate and Physicochemical Characterization of *Moringa stenopetala* Seed Oil Through Soxhlet Extractor in Dawro Zone, Ethiopia

Eliyas Befikadu, Mesfin Bibiso, Camerun Kastro

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ABSTRACT

Background: *Moringa stenopetala* is a fast-growing deciduous cabbage plant for human food and animal feeding in the dry season in Ethiopia's Dawro zone. However, little is known about the proximate and physicochemical parameters of seed oil of *M. Stenopetala* grown in the study area.

Methods: In this study, the proximate composition and physicochemical parameters of oil extracted from *M. stenopetala* seeds were conducted through the Soxhlet extractor as the extracting medium. This study analyzed five selected varieties of *M. stenopetala* seed oils.

Result: The proximate analysis of *M. stenopetala* seed oils revealed that the moisture content ranged from (7.23±0.61%-10.52±0.52%), Oil yield (29.41±1.05%-40.18±0.89%), Ash content (4.91±0.22%-5.77±0.04%), whereas the average crude fiber content was (6.88±0.24% -8.55±0.22%) and crude protein content (26.07±0.62%-30.01±1.31%). The physicochemical parameters of seed oil obtained confirmed that the oils were of good quality to those in the Association of Official Analytical Chemists standards.

Key words: *M. Stenopetala*, physicochemical parameters, proximate composition, Soxhlet extractor.

INTRODUCTION

Moringa stenopetala is a species that belongs to the Moringaceae family. This is represented by a unique genus known as Moringa that holds 14 species, including *M. stenopetala* (Andinet, 2008; Gebregiorgis *et al*. 2011). Referring to their ecological nativities, *M. stenopetala* and *M. oleifera* are often considered the African and Indian Moringa tree, respectively (Temam and Nuredin, 2017). Many Asian and African countries consume the mature seeds of the Moringaceae family in drinks prepared in folk medicine, used as foodstuff spices and cultivated traditionally as cabbage trees and planted as an ornamental tree (Abuye *et al.* 2003; Bishwanath and Tirth, 2019).

M. stenopetala is a drought-resistant traditional medicinal and nutritional plant in Southern Ethiopia. It is extensively distributed in the southwestern part of Ethiopia at an altitude of 1100 to 1800 m above sea level (Desta *et al.* 2011). *M. stenopetala* is domesticated in the lowlands of East Africa and is indigenous to southwestern parts of Ethiopia. It is an on-farm tree that supports approximately high population density. This is a multipurpose vegetable, fruit tree and oil crop have not been tapped concerning its potential (Wakjira *et al.* 2016; Asaminew *et al.* 2018). Information related to the analysis of *M. stenopetala* in the studied sites were scarce. Hence, the present study focused on the proximate and physicochemical characterization of oil through the Soxhlet extraction method from *M. stenopetala* seeds depending on the species, area of cultivation, the solvent used, contact time and the local environmental conditions.

Department of Chemistry, College of Natural and Computational Science, PO Box 138, Wolita Sodo University, Ethiopia.

Corresponding Author: Camerun Kastro, Department of Chemistry, College of Natural and Computational Science, PO Box 138, Wolita Sodo University, Ethiopia.
Email: castro2019came@gmail.com, mefbab2009@gmail.com.

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MATERIALS AND METHODS

Description of the study area

Dawro Zone is located about 500 km far from Addis Ababa, Ethiopia. The geographical position of the zone is found between 6°59′-7°34′ N latitude, 36°.68′ 37°.52′ E longitude and an elevation range from 500 m to 3000 m above sea level (Teshome, 2015).

Apparatus and instruments

Soxhlet extractor with a condenser (Konte, USA), Rotary evaporator (BÜCH Rotavapor R-200, Switzerland), Oven (Selecta, Barcelona, Spain), Water bath SB-651, Japan, Digital weighing balances (Analytical Balance, Sartorius BS 124S, Germany), pH meter (PICO+Lab India), Abbe's refractometer, Reflux condenser, Density bottle, Magnetic

stirrer (Shaker), Heating mantle (Heater), Grinding mill, Ice bath, Kjeldahl flask, Muffle furnace and Sand bath were used for sample preparation and analysis.

Chemicals and reagents

n-hexane, Diethyl ether, Ethanol, Phenolphthalein indicator, NaOH, KOH, HCl, Chloroform, Iodine, Glacial acetic acid, Na_2SO_3, KI, Starch indicator, distilled water, Boiling chips, Kjedahl catalyst, Wij's reagent, NH_4Cl, xylene, NH_4OH were all analytical reagent.

Sample collection, preparation, proximate and physicochemical properties of oil

Five *M. stenopetala* seed samples were collected from five selected study sites from February to November 2020 in triplicate. The collected samples were analyzed following standard procedures at Wolaita Sodo and Arbaminch Universities.

The proximate analysis of *M. stenopetala* seed oil was conducted for crude oil extraction, oil yield, moisture content, crude protein, total ash and crude fiber contents. The physicochemical parameters of seed oils were analyzed for color, odor, refractive index, density, specific gravity, free fatty acids, acid value, peroxide value, saponification value, iodine value and pH using recommended analytical procedures.

RESULTS AND DISCUSSION

The proximate composition of *M. stenopetala* seed oil

the proximate composition of seed oil obtained from five different study sites was carried out, and the data were illustrated in Table 1 and Table 2. As presented in Table 1, the percentage of moisture contents of *M. stenopetala* seed oil was ranged between 7.23±0.61% to 10.52±0.52%. Analysis of variance (ANOVA) showed that the moisture content in the Ella Bacho sample was significantly ($p<0.05$) different from the moisture level of other study sites. The highest moisture content found in Ella Bacho was mainly because of its geographical location. The moisture content of oils is an essential factor that affects the yield and quality of the oil extracted. The moisture content of seed oils of this study was significantly ($p<0.05$) higher than the 5.7% reported for *M. stenopetala* by Meta (2018), but the results of this study were within the standard range of 7-11% as reported by AOAC (1990). However, the present data were slightly higher than the 6.1% moisture content reported by Eyassu (2012).

The oil yields of *M. stenopetala* seed ranged from 29.41±1.05% to 40.18±0.89% (Table 1). ANOVA showed that the average oil yields from the five varieties were significantly ($p<0.05$) different. These results may indicate that the oil yield of *M. stenopetala* seeds decreased with an increase in the altitude of the geographical location of the study area. The findings of this study were in agreement with Boukandoul *et al.* (2018). The high oil yield from this site might be accredited to the sandy soil texture and favorable environment for *moringa* growth; results revealed that the *moringa* tree is familiar with sandy soil types.

The total ash content obtained from the present work was ranged from 4.91±0.22% to 5.77±0.04% as shown in Table 1. ANOVA showed that the ash content from Deneba was more significant ($p<0.05$) and the Ella Bacho was significantly ($p<0.05$) lower than ash obtained from other study sites. Ash content is an indicator for essential mineral elements, agreeing with the average ash contents of 4.4-5% for *M. stenopetala* seed oil (Eyassu, 2012) and 4.4- 6.9% for *M. oleifera* seed oil, as revealed by Leone *et al.* (2016).

Table 1: Proximate composition of *M. stenopetala* seed oil (Mean±Std., n = 3, ($p<0.05$).

Variety	Ash content (%)	Fiber content (%)	Moisture content (%)	Oil yield (%)	Protein content (%)
Deneba	5.77^{a}±0.04	8.55^{a}±0.22	7.68^{bc}±0.25	38.20^{b}±0.38	29.75^{a}±0.65
Yello Worbati	5.15^{c}±0.15	7.75^{b}±0.82	8.04^{bc}±0.69	30.22^{cd}±0.33	27.84^{b}±0.39
Lala Ambe	5.03^{dc}±0.06	6.88^{c}±0.24	8.91^{b}±1.12	31.50^{c}±0.57	28.86^{ba}±0.39
Zima Waruma	5.47^{b}±0.04	7.60^{cb}±0.33	$7.23^{c}_{\pm}$0.61	40.18^{a}±0.89	30.01^{a}±1.31
Ella Bacho	4.91±0.22^{c}	7.00^{cb}±0.26	10.52^{a}±0.52	29.41^{d}±1.05	26.07^{c}±0.62
LSD	0.23	0.79	1.27	1.28	1.37
CV	2.36	5.76	8.21	2.08	2.64

LSD = Least significance difference, CV = Coefficient of variation, means with different lower case letters are significantly different.

Table 2: The physicochemical characterization of *M. stenopetala* seed oil (Mean±Std., n = 3).

Varieties	Color property	Odor property	RI at 40°C	P (g/cm^3)	SG at (30°C)
Deneba	Pale yellow	Odorless	1.464^{a}±0.001	0.88^{a}±0.005	0.90^{ba}±0.01
Yello worbati	Pale yellow	Odorless	1.464^{a}±0.001	0.85^{b}±0.003	0.90^{ba}±0.03
Lala Ambe	Pale yellow	Odorless	1.464^{a}±0.001	0.86^{b}±0.002	0.89^{ba}±0.02
Zima Waruma	Pale yellow	Odorless	1.464^{a}±0.001	0.88^{a}±0.012	0.92^{a}±0.02
Ella Bacho	pale yellow	Odorless	1.464^{a}±0.001	0.85^{b}±0.002	0.87^{b}±0.008

RI= Refractive index, P= Density, SG= Specific Gravity, Std.= Standard Deviation, LSD= Least Significance Difference, CV= Coefficient of Variation. Means with a different letter are significantly different and means with the same letters are not significantly different.

The crude fiber content of *M. stenopetala* oils ranged between 6.88±0.24%-8.55±0.22%, as reported in Table 1. The ANOVA showed that the crude fiber content obtained from Lala Ambe was significantly lower. The fiber content obtained from Deneba was significantly higher than the other study sites. The crude fiber content of the present finding was within the standard range (≤12%) as reported by AOAC (1990). The crude fiber above 12% indicates a high level of undigested cellulose (Saeed and Shola, 2015). Therefore, the current findings show that all samples have low levels of undigested cellulose.

According to the current findings, the crude protein contents of *moringa* seed oil agreed with the result of (Anwar and Bhanger, 2003). The crude protein content obtained in this study wasranged between 26.07±0.62%-30.01±1.31%, as indicated in Table 1. ANOVA also showed that the crude protein content obtained from Ella Bacho was significantly ($p<0.05$) lower, and the one obtained from Deneba and Zima Waruma was significantly ($p<0.05$) higher than the other study site.

Physicochemical Characterization of *M. stenopetala* seed oil

The color and odor of physicochemical properties of *M. stenopetala* seed oils were presented in Table 2. The extracted oil yields were liquid at normal condition, and they were pale yellow color with an odorless characterization. These findings agreed with the pale yellow seed oil reported for *M. stenopetala* (Anwar and Bhanger, 2003; Lalas *et al.* 2003).

The refractive index of the seed oil in all varieties was 1.464±0.001 (Table 2). This finding was consistent with the results of (Campas-Baypoli *et al.* 2014; Manzoor *et al.* 2007). However, the refractive index value somewhat varied to those of *M. stenopetala* oils of 1.453 from Kokwa Island (Lalas *et al.* 2003). The above differences might be due to variations in oxidation stability, physical state and fatty acid compositions.

The density of seed oil obtained in this study was in the range of 0.85±0.002 g/cm^3-0.88±0.012 g/cm^3 (Table 2). ANOVA also showed that the oil density from Deneba and Zima Waruma was more significant than the density of seed oil from Lala Ambe and Yello Worbati. The present findings of the average density of seed oils were in close agreement with the corresponding value of 0.907 g/cm^3 for *M. stenopetala* seed oil (Meta, 2018).

The specific gravity of the extracted oil from *M. stenopetala* seed was ranged from 0.87±0.008 to 0.92±0.02, as presented in Table 2. ANOVA showed that the specific gravity of seed oils obtained from Zima Waruma was significantly higher and Ella Bacho was considerably lower than other varieties in the study sites. Orhevba *et al.* (2013) and Omosuli *et al.* (2017) reported 0.86±0.01 for *M. oleifera* seed oils, which agrees with the specific gravity of *M. stenopetala* seed oils of the present study.

The free fatty acids of oil obtained from *M. stenopetala* seeds are presented in Table 3. ANOVA showed that the free fatty acid of Ella Bacho was significantly higher than the free fatty acid of other study sites. Similarly, the free fatty acids of Deneba, Yello Worbati, Lala Ambe and Zima Waruma were not significantly ($p<0.05$) different.

A high free fatty acid value of moringa seed oil of 1.38 mg KOH/g was associated with a high deterioration rate of the oils (Manzoor *et al.* 2007). The lower the values of free fatty acids, the greater the oxidative storage stability. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (Hasan *et al.* 2016).

The acid value of *M. stenopetala* seed oil ranged from 1.09±0.03 to 1.77±0.09 mg KOH/g (Table 3). ANOVA showed that the acid values of Deneba, Yello Worbati, and Zima Waruma were non-significant. This finding was consistent with the results of Andinet (2008) and Hasan *et al.* (2016). The low acid value in oil is a sign that the oil could have a better shelf life and protect against its rancidity and oxidation. This could be ascribed to natural antioxidants in the *M. stenopetala* seeds, such as vitamins C and A (Aremu *et al.* 2015). The high acid value of oil showed that the oil might not be suitable for human consumption. Thus, the entire oils obtained in the present study were considered good consumer consumption because the values were in line with the standard range ≤4 of AOAC (1990).

Table 3: Characterization of pH, free fatty acids, acid value, peroxide value, saponification value and iodine values (Mean±Std., n = 3) of *M. stenopetala* seed oils.

Varieties	FFA (mg. KOH/g)	AV (mg. KOH/g)	PV (meq/kg)	SV (mg. KOH/g)	IV (gI_2/100g)	pH value
Deneba	1.05^{b}±0.03	1.77^{a}±0.09	9.29^{ba}±0.49	173.23^{a}±7.03	71.23^{b}±1.94	6.83^{ba}±0.07
Yello Worbati	1.19^{b}±0.06	1.62^{a}±0.12	8.67^{b}±0.35	172.89^{a}±3.58	69.10^{b}±1.23	6.95^{ba}±0.15
Lala Ambe	1.10^{b}±0.03	1.09^{b}±0.03	9.61^{a}±0.86	178.53^{a}±1.46	78.53^{a}±1.40	6.65^{b}±0.07
Zima Waruma	1.06^{b}±0.04	1.72^{a}±0.046	9.39^{ba}±0.19	176.27^{a}±3.02	78.93^{a}±3.15	7.04^{a}±0.19
Ella Bacho	1.39^{a}±0.18	1.20^{b}±0.16	8.53^{b}±0.43	179.67^{a}±1.53	68.57^{b}±0.67	6.86^{ba}±0.38
LSD	0.16	0.18	0.93	7.08	3.42	0.38
CV	7.79	6.83	5.64	2.21	2.56	3.04

FFA= Free fatty acid, AV= Acid value, PV= Peroxide value, SV= Saponification value, IV= Iodine value, LSD= Least Significance Difference, CV= Coefficient of Variation. The means with different letters are significantly different, and all means with the same letter are not significantly different.

Oils peroxide value obtained from *M. stenopetala* seeds was ranged between 8.53±0.43 meq/kg and 9.61±0.86 meq/kg (Table 3). ANOVA showed that the peroxide values of Lala Ambe were significantly highest and the peroxide value of Ella Bacho was the lowest. The oil having a higher percentage of peroxide from the standard is unstable and grows rancidity quickly. A report by Meta (2018) indicated that oil with increased susceptibility to auto-oxidation was due to moisture or trace elements. However, the present result has some variation. This may be due to the difference in the variety of plants, cultivation, climate, extraction method used and the nature of solvent applied.

The saponification value of oil from this study was found between 172.89±3.58 mg KOH/g to 179.67±1.53 mg KOH/g of oil, as shown in Table 3. This finding was in agreement with a report of Meta (2018) for *M. stenopetala* seed oils. According to the ANOVA, the mean of saponification value of entire findings of the sites was nonsignificant (pÂ0.05).

The iodine value of the oil obtained from the current finding was ranged from 68.57±0.67g I_2/100 g to 78.93±3.15 g I_2/100 g, as shown in Table 3. ANOVA showed that the iodine values of Lala Ambe, Zima Waruma, and Deneba were significantly not different. The iodine values obtained from the extracted seed oil of Ella Bacho and Yello Worbati of the present study were significantly ($p<0.05$) higher than palm oil of 50–55g I_2/100 g and significantly lower than corn oil (103-135 g I_2/100 g) standards of Kenya vegetable oil (Chebet *et al*. 2016; Hasan *et al*. 2016).

The pH value of the oils was ranged from 6.65±0.07-7.04±0.19 as presented in Table 3. ANOVA showed that the pH value of Zima Waruma was significantly ($p<0.05$) higher, and the pH value of Lala Ambe was significantly lower. The results obtained for the entire seed oil varieties indicated that the pH values were significantly ($p<0.05$) lower than the pH of previous literature, 7.53 for *M. stenopetala* (Meta, 2018). The pH of the present sample was indicative of the presence of a reasonable quantity of free fatty acids. High concentrations of free fatty acids are undesirable in vegetable oils because they can reduce the palatability and shelf-life of the oil (Babatunde and Bello, 2016).

CONCLUSION

The essential oil extracted from *M. stenopetala* seeds collected from the study area was ranged from 29.41-40.18% with unique characteristics. A significant percentage of proximate composition was recorded for the selected seed oils analyzed. The low moisture content obtained in the entire finding determines that *M. stenopetala* seed oils were effective and may not need drying before extracting or being stored under comfortable temperature. Physicochemical parameters in the oil indicated good quality attributes of edible oils falling within the standard limits of AOAC.

Conflict of interest: None.

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