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A Study on Analysis of Physiochemical Characteristics of Moringa, Mango and Leucocephala Seed Oils

Ketemaw Salelign Yazie[1], Babuskin Srinivasan[2] and Ballekallu Chinna Eranna*

[1,2&*]College of Natural Sciences,
Arba Minch University, Arba Minch, Ethiopia, EAST AFRICA.
email:salelgnketemaw@gmail.com ; 2-buski87@gmail.com;* chinnaouct@gmail.com.

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ABSTRACT

The seed oils of *Mangniferaindica (I), Moringa stenopetala (II)* and *Leuceanaleucocephala (III)* were extracted and analyzed for their physiochemical characteristics. The seeds of I, II & III contained 12 %, 30 % and 8 % oil content respectively. The highest oil yield was obtained for Moringa and the lowest was recorded for leucocephala respectively. The oils of I, II & III had respectively, moisture content 6.76, 6.95 and 5.54; ash content 3.13, 5.74 and 4.08; acid value 0.41, 0.37 and 0.64; peroxide value 8.62, 12.37 and 2.65; iodine value 58.5, 68 and 112.7; saponification value 202.3, 182 and 146 respectively. The refractive indexes of all oils were found to be in around 1.45. All the extracted oils were liquid at room temperature and light yellow in color. The properties of oils analyzed showed that these oils can be used as ingredient in Biodiesel, Biopolymers, Bio lubricant, cosmetics and pharmaceutical applications.

Keywords: *Non-edible, extraction, physicochemical, oil yield*.

INTRODUCTION

Fixed oils derived from plants are usually obtained from plant seeds generally referred to as oilseeds. Oilseeds are class of plants in which relatively large amount of lipids are stored in the seed tissue. The amount of lipids found in oilseeds range from 10-35 % for different species respectively. Oil seeds are among most ancient crops domesticated by mankind. Forexample, there is evidence for the cultivation of oil bearing varieties of linseed in the middle east from over 8000 years ago. Over the years there has been spectacular demand for both the oil and oil meals. The significance of oilseeds economically, nutritionally and technologically is enormous. From the earliest times, oilseed products were utilized for a variety of edible and non-edible applications e.g. cooking, body massage, lubrication, cosmetics etc.

During the 20thcentury, the non-edible uses of oilseed products declined substantially due to availability of relatively inexpensive oil derived from fossil fuel reserves. There exists abundant data on the proximate, mineral and other properties of convential oilseeds but not on unconventional oilseeds. However continuous exploration and extraction of oils from the non-conventional oilseeds is expected to checkthe expensive prices of the conventional oils thus making them affordable to people. In this study, three non-conventional oilseeds such as Moringa, mango and leucocephala were investigated for their physiochemical properties.

Non-Edible Vegetable Oils Resources

Non-edible vegetable oils are not suitable for human food due to the presence of some toxic components in the oils. Exploting the production of non-edible oil feed stocks can overcome the problems of food verse fuel, environmental and economic issues related to edible vegetable Palaniswamy, K.P ,**1974**. Moreover, Non-edible crops are expected to use lands that are largely unproductive and those that are located in poverty- stricken areas and in degraded forests. They can also be planted on cultivators’ field boundaries, fallow lands, and in public land such as along railways, roads and irrigation canals.. Non-edible oil plants are well adapted to arid, semi-arid conditions and require low fertility and moisture demand to grow. Moreover, they are commonly propagated through seed or cuttings. Since these plants do not compete with food, seed cake after oil expelling may be used as fertilizer for soil enrichment Azam MM, Waris A, Nahar NM., **2005**.

Advantages of non-edible oils

Preliminary evaluation of several non-edible oil seed crops for their growth feedstock and adaptability.

- They eliminate competition for food and feed. Non-edible oils are not suitable for human food due to the presence of some toxic components in the oils
- The main advantages of non-edible oil are their liquid nature portability, ready availability, renewability, higher heat content, lower sulfur content, lower aromatic content and biodegradability.

Leucaena Leucocephala.

The nativity of the plant is Asian and African continents. It is referred as Aleyitate in Arbaminch, Ethiopia. It belongs to fabaceae family. It is an ornamental plant. The collection of literature studies mainly mentions that the shrub is used for pharmaceutical purposes and treatment of cancer Anti diabetic and antioxidant properties of alkaloids from *Leucaenaleucocephala* Amit Suhane, *et al*, 2012. This opens the possibility of extraction of oil from the seeds of the plant for further investigation Agarwal AK, 2007.

Fig.1. *Leucaenaleucocephala* seeds& plant

Fig. 2*Moringa stenopetala* seeds

Moringa Stenopetala

Moringa Stenopetala belongs to the family Moring cease and is often referred to as the African 'Moringa Tree' because it is native only to Ethiopia and northern Kenya.Piyush S et *al*, 2007 Though it does grow in many other parts of the old- and new-world tropics, it is not as widely known as its close relative to *Moringaoleifera*AtanuAdhvaryu,2006 The M. stenopetala tree, locally called aleko, grows widely in southern Ethiopia, mainly in the Keffa, GamoGofa, DebubOmozones and in Konso and Dherashe areas. In the Arbaminch and Wollayta areas, the local people cook the leaves of the M. stenopetala tree. The people of Konso use the tree not only for food but, also as a medicine and they cultivate large areas around their villages M. Lovell, *et al*, **2006**. The leaves and roots of *M. stenopetala* are used as a cure for malaria, stomach problems and diabetes Shashidhara, Y.M. **2010** and the seeds are used in some areas to clear muddy water. There are no reports on the uses of the seeds or the oil of *M. stenopetala* in Ethiopia. This would make it a potential feedstock for the production of biodiesel and bio-lubricants, since it does not compete with food production.

Table1 Scientific classifications of *Leucaenaleucocephala*

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideae
(unranked):	Mimosoid clade
Genus:	*Leucaena*
Species:	*L. leucocephala*

Table. 2&3 Scientific classification of Moringa , Mango

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Brassicales
Family:	Moringaceae
Genus:	*Moringa*
Species:	*M. oleifera*

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Anacardiaceae
Genus:	*Mangifera*
Species:	*M. indica*

Mangniferaindica- Mango

Mangoes (*Mangniferaindica L.*) are one of Thailand's most economically important fruit. It is also widely being cultivated in Ethiopia. Particularly the new variety which has been used as raw material for many canned fruit products. Only the mango flesh is utilized by these factories, resulting in a vast amount of mango seeds being discarded as waste. Mango seed consists of a tenacious coat enclosing the kernel. The seed content of different varieties of mangoes ranges from 9% to 23% of the fruit weight (Dhingra, *et al*, **1985)** and the kernel content of the seed ranges from 45.7% to 72.8%. Mango kernel contain almost 15 wt% of oils (H.A., A. Anwar,**1976)**. Interest in the development of uses for this waste material has stimulated studies on the use of the mango seed kernels for animal feed and extraction of oil from mango kernel for use as cocoa butter substitute fats Gaydou, E.M. ,1984.

Figure. 3*Mangnifera indica* seeds

Statement of the Problem

The rapid utilization of edible vegetable oils and fossil fuels for industrial and home applications has recently been of great concern because of the declining resources of these oils. As the demand for vegetable oils for food has increased tremendously in recent years, it is impossible to justify the use of these oils for fuel use purposes such as biodiesel production and other industrial applications. Moreover, these oils could be more expensive to use as fuel.

Hence, the contribution of non-edible oils such as *Leucaenaleucocephala*, *Moringastenopetala* & *Mangniferaindica* can be investigated as a potential source of Biodiesel & Bio-lubricant and other industrial applications.

Scope of the Study

Non-edible vegetable oils are not suitable for human food due to the presence of some toxic components in the oils. Production of non-edible oils feed stocks can over- come the problems of food verse fuel, environmental and economic issues related to edible vegetable oil. More over non-edible oil feedstock development could become a major poverty alleviation program for the rural poor apart from providing energy security in general and to rural areas in particular and upgrading the rural non-farm sector.

MATERIALS AND METHODS

Sample Collection

All the samples needed for the study was collected from Arba Minch region, Ethiopia. Study site. The project work was carried out in Department of chemistry laboratory, College of Natural Sciences, Arba Minch University, Abaya Campus.

Materials

Mangniferaindica, *Moringastenopetala* and *Leucaenaleucocephala* seeds were collected and cracked to obtain the kernels. The kernels were then ground in a food grinder to reduce the article size to a maximum diameter of 700 mm as measured by a sieve, sealed in a plastic container and stored in a refrigerator until extraction. The storage conditions assured eliminating effects of oxygen and humidity and to avoid oxidation of the dried seed powder during storage time. KI, $Na_2S_2O_3$, KOH, NaOH, phenolphthalein, HCl, starch indicator, $I_2$and Bromide (Zn Br) as well as Galactial acetic acid was supplied by AMU, Abaya campus IN Organic LAB. The seed ofMoringaSetenopetalasamples was assayed from February to March 2018fromArba Minch (southern region, Ethiopia). The oil from Moringastenopetala was extracted properly. The seed, before extraction, was air-dried for 1 week.

Methods

Mechanical Stirrer Method

Mechanical stirring machine consisted of temperature controller to control temperature within the range of 0- 100º C and speed controller - for controlling speed of stirrer in terms of revolution per minute (rpm). In this machine hot plate functions as a heating source to maintain the temperature of the solution. The beaker was placed on the hot plate then vegetable seed powder (60 g) was introduced into it at the beginning. The reaction started when hexane of 300 ml was poured into the beaker and continuous stirring with mild heating was carried out for 8 hrs and allowed to cool for 30min, the mixture decanted to another beaker and subjected to rotary evaporator as result the oil and the reagent n-hexane separated.

Stage of separation of the oil

An evaporation process was applied to the mixture of oil and solvent in order to remove one from the other. The process was developed in a rotary evaporator (IKA-WERK HB 4 basic, Germany). The oil product was weighted in a scale to calculate the oil mass percentage of extraction by means of equation 1:

$$\%\ \text{yield of bio oil conversion} = (\text{total weight of oil}/\text{total weight of powder})^{*}100 \qquad (1)$$

Refractive Index,

Refractive index also called index of refraction is a measure of the bending of a ray of light when passing from one medium into another. It can also be defined as a dimensionless, number that describes how light or any other radiation propagates through the medium. In this research work, the refractive index was determined using the refractometer (Erma hand refractometer) as suggested YOON, S.and KIM, S. It has range of 0-32 % A drop of the oil was placed on the surface of the refractometer and the reading was taken. The method used for the determination of density and refractive index (at 40 °C) was adapted from AOAC Method 969.18) (AOAC, 1990).

Smoke Point

Smoke point of selected oils was determined in open lab with lots of fresh air flow. About 10-20 ml of oil was taken in a beaker and heated until it starts giving out smoke at this point smoke point was noted using digital thermometer. Smoke point was determined according to the method described by *British Standards Methods of Analysis* (1976) (BS 684: Section 1.8).

Saponification Value

Saponification value of an oil or fat is the number of potassium hydroxide required to neutralize the fatty acid resulting from the complete hydrolysis of 1 g of sample. In determining the saponification value of the acid, 2 g of oil or fat was weighed into a flask.

Twenty-five (25) ml alcoholic potassium hydroxide solution was poured into the conical flask containing the oil, and it was attached to reflux condenser. This was heated through boiling water bath for 1 hour with random shaking. After one hour, 1 ml of the phenolphthalein solution was added and titrated with the standard hydrochloric acid. This procedure was repeated for each of the five samples. Then the blank (that is potassium hydroxide solution without oil) was titrated using the same procedure. The saponification value was then determined through equation 1.

$$\text{Saponification Valu} = (b - a)\frac{28.05}{w} \qquad (1)$$

Where as “a” is titrate of sample, “b” is titrate of the blank and “w” is the weight of sample.

Acid Value

The acid value of an oil/fat is the number of potassium hydroxide required to neutralize the free acids resulting from the complete hydrolysis of 1g of the sample. In determining the acid value, 25 ml diethyl either with 25 ml ethanol was mixed and 1.0 ml of 1.0 % phenolphthalein solution was neutralized and was titrated with 0.1 ml sodium hydroxide solution then 4 g of the oil in the neutralized solvent mixture was dissolved and titrated with 0.1 ml sodium hydroxide solution. The acid value was therefore determined using equation (2).

$$\text{Acid value} = \text{Titration (ml)} \times \frac{5.61}{\text{wieght}} \qquad (2)$$

Peroxide Value

The concentration of peroxides in oil gives an indication of the extent of spoilage. The oil was treated with potassium iodide in an organic solvent. The peroxide liberated the iodine from potassium iodine. The iodine was titrated with standard thiosulfate.

In determining the peroxide value of the acid, the test was carried out in subdued daylight and 2 g of oil was weighed into a clean dry boiling tube, 1 g of powdered potassium iodide and 20 ml solvent mixture was added to the oil that was inside the boiling tube. Then, it was placed in a boiling water bat for about 60 seconds. After which the contents of water was poured into it and the washing was added to the titration flask containing 20 ml potassium iodide solution.

The tube to be used was washed twice with 25 ml portion of water and the washings were added to the titration flask. Finally, it was titrated with 0.002 ml thiosulfate, using starch as indicator. The blank determination was carried in the same manner as test sample but **without oil**. The peroxides value was therefore determined using equation (3).

$$\text{Peroxide} = \frac{2(a-b)}{w} \qquad (3)$$

Where as “a” is titrate value for the sample, “b” titrate value for the blank and “w” weight of sample.

Iodine Value

2 grams of oil samples was weighed in250mLconical flasks and then25mL of carbon tetra chloride was added to each oil sample and content was mixed well. 25mL of Hanus reagent was added to the solution, swirled for proper mixing, and kept in the flask in dark for half an hour. After standing, 15mL of potassium iodide solution was added and then 100mL of distilled water was added into the mixture and 1mL starch indicator solution was added to the sample solution. Then, liberated iodine was titrated with 0.01N of sodium thiosulphate solution; then, at the end, blue color was formed and then disappeared after thorough shaking. The blank determination was carried in the same manner as test sample but **without oil**. The iodine value was estimated using the following formula (4):

$$\text{Iodine value} = \frac{(b-a) \times N \times 1.269 \times 100}{W},$$

Where $b$is blank titer value, $a$is sample titer value; $N$is normality of thiosulphate, and$W$is weight of sample.

RESULTS AND DISCUSSION

Extraction & Yield of oil

Moringa, Mango & L. leucocephala seeds were collected and allowed to dry in hot air oven for around 5-6 hrs at 70°C. Then all the samples were powdered using high speed laboratory blendor. After blending, the powder has been sieved and oil was extracted as per method described above. The yield of each oil has been calculated and represented below.

Yield of Moringastenopetela = 18 g/ 60 g x 100 = 0.3 x 100= 30 %

Yield of Mangniferaindica (Mango seed) = 7.6 g/60g X 100=0.126 x 100=12.6%

Yield of Leucaena*leucocephala =4.8 g/60g*100=0.08*100= 8%*

Table. 4 Physicochemical properties of mango seedkernel oil

Property	Value
Total yield of oil- dry matter (%)	12.6±0.10
Moisture content of seed (%)	6.76±0.04
Ash content (%)	3.13±0.03
Acid value (mg KOH/g oil)	0.41±0.03
Peroxide value (mg/g oil)	8.62±0.04
Iodine value (mg/100g oil)	58.54±1.6
Saponification value (mg KOH/g oil)	202.36±2.5
Refractive index	1.475
State at ambient/room temperature	Liquid
Color	Yellow
Specific gravity	0.915
Smoke point	178+0.02

Table. 5 Physicochemical propertiesof*Moringa stenopetela*oil

Property	Value
Total yield of oil- dry matter (%)	30±0.8
Moisture content of seed (%)	6.95±0.12
Ash content (%)	5.74±0.04
Acid value (mg KOH/g oil)	0.37±0.02
Peroxide value (mg/g oil)	12.37±0.06
Iodine value (mg/100g oil)	68±0.23
Saponification value (mg KOH/g oil)	182±2.1
Refractive index	1.458
State at ambient/room temperature	Liquid

Color	Yellow
Specific gravity	0.912
Smoke point	180+0.01

Table 6: -Physicochemical propertiesof L.*leucocephalaseed*oil

Property	Value
Total yield of oil- dry matter (%)	8±0.04
Moisture content of seed (%)	5.54±0.03
Ash content (%)	4.08±0.06
Acid value (mg KOH/g oil)	0.64±0.05
Peroxide value (mg/g oil)	2.65±0.04
Iodine value (mg/100g oil)	112.74±0.06
Saponification value (mg KOH/g oil)	146±1.12
Refractive index	1.453
State at ambient/room temperature	Liquid
Color	Yellow
Specific gravity	0.908
Smoke point (%)	187.92±0.42

*Among the oils extracted from seeds***,** theoil *Yield of Leucaena* **leucocephala** (8%) and *Mangniferaindica (Mango seed) (12.6%) which was* similar observations with Literature reported (Tsaknis, ***et al.*** 2002) and theoil ***Yield difference,*** may be because of the increased ability of the polar solvent to overcome forces that bind lipids within the sample matrix. Better yield performance has also been observed for Moringa stenopetala seed oil (30%). Such variation in oil content across habitat and species might be attributable to the variety of plant, environmental and geological conditions of the regions and the extraction methods used(***Brajendra K*** et al. 2009).

Physical and Chemical Parameters of Oil

The acidity, iodine, Refractive index**,** Ash value, peroxide, Moisture value and Saponification values are the major characterization parameters for oil quality. The mango kernel and other two seed oils were found to be light yellow in color. All oils were liquid at room temperature. The specific gravityof all oils was around 0.9 and these value falls within the report found in the literature (Palaniswamy, K.P *et al*. 1974).

Moisture Value

The moisture content (on dry weight basis) of Moringa and Mango seeds were found to be 6.95 % and 6.76 % respectively. For *L. leucocephala* seeds5.54 % the lowest value was recorded.It was reported that the moisture contentaffected the oil extraction operation. The low moisturecontentcaused brittleness and the higher moisture contentcaused plasticizing effects, which reduced the level of compression (Lakshminarayana, G, 1983). This may be due to the hardness of the seeds.

Ash Value

Ash content was determined according to the ISO method 749 (Anon., 1977). Two grams of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 550°C until constant mass was achieved. The results suggested that the *Moringastenopetala* seed oil ***(***5.74±0.04***)*** provided highest Ash value followed by***L. leucocephala***seed oil (4.08±0.06) and Mango seed oil (3.13±0.03).This result indicated that moringa seed contains more amount of minerals when compared to other seeds taken in this study. The values recorded fell within the ranges reported in the literature (Hanna, Xu. et *al 2007*).

Acid Value

The acid value was a measure of total acidity of the lipid, involving contributions from all the constituent fatty acids that make up the glyceride molecule (Liu Y, et al, 2009). The results suggested that the highest acid value was recorded for *L. leucephala* seed oil(4.08mg KOH/g oil), whereas the other two seed oils such as Moringa and mango found to contain low acid values (0.37 & 0.41mg KOH/g oil). It can be seen that most of the values fall within the ranges published in the literature (Singh SP, 2010).

Peroxide Value

Generally; the peroxide value should be less than 10 mg/g oil in the fresh oils (McGinley, L. 1991). The results showed that the peroxide values varied widely in the extracts, ranging from ***2.65-12.37*** mg/g oil for the different seed oils. (Ojeh, 1981) reported that oils with high peroxide values are unstable and easily become rancid. Here the results suggested that *Moringa stenopetala* seed oil was reported to contain high peroxide value, followed by Mango seed oil (8.62 mg/g oil) so these oils become rancid easily. It can be seen that most of the values fall within the ranges published in the literature(Jumat S al., 2010).And the seed L**. *leucocephala***oil contained low peroxide values and they are not easily oxidizable. Mechanism can be represented as follows.

Step – 1 Peroxide + KI + H+------I_2

Step – 2 I_2 (purple) + 2Na2S2O3 -------Na2S4O6 + 2NaI (colorless)

Iodine Value

It is used to determine the unsaturation of oils and in assessing the stability of oil in industrial applications (Xu*et al*., 2007). Iodine value was measured according to the method as given by (Pearsons, 1976). The results suggested that the ***L. leucocephalaseed*** oils ***(112.74 mg/g)*** has highest iodine value followed by *Moringa stenopetala* seed oil (68 mg/g) and Mango seed oil (58.54mg/g). It can be seen that most of the values fall within the ranges published in the literature (Feria, M. J, *et al* 2011).The lowest iodine value, which reflect its characteristic of seed oil such as higher resistance to oxidation, longer shelf life and higher quality. The differences in iodine values between oil samples maybe due to the different fatty acid compositions.

Saponification Values

The number of potassium hydroxide required to neutralize the fatty acid resulting from the complete hydrolysis of 1 g of the sample. The saponification value is a useful tool for the evaluation of the chain length (molecular weight) of fatty acids occurring in the triacylglycerol's in oil. The lower saponification value indicates a very high content of low molecular weight triacylglycerol. Saponification value was determined according to AOCS Method Cd 3-25 described in Allen and Marvin (1982). The results suggested that the Saponification value was highest for Mango seed oil (202.36mg/g) followed by the *Moringa stenopetala* seed oil (182 mg/g)and ***L. leucocephala seed oil(146 mg/g)and*** values recorded fell within the range reported in the literature (Pendyala, *et al*2010).

Refractive Index Determination

Also called index of refraction, is a measure of the bending of a ray of light when passing from one medium into another. It can also be defined as a dimensionless, number that describes how light or any other radiation propagates through the medium. The results suggested that the refractive index of all seed oils were recored to be around 1.45. The viscosity of the oil produced with Moringa stenopetala was the highest compare with other two seed oil, possibly because of the water that was bound in the oil during extraction. It can be seen that most of the values fall within the ranges published in the literature (Quinchia L.A, et al, 2010)

Smoke Point ^{O}C

The smoke point of an oil increases as free fatty acid content decreases. Heating an oil produces free fatty acid and as heating time increases, more free fatty acids are produced, thereby decreasing smoke point. Heating increases peroxide value of oils therefore oils with more free fatty acids will have greater peroxide value. Smoke point (according to the method described by British Standards Methods of Analysis. BS 684: Section 1.8) were measured. The results suggested that the ***L. Leucocephala seed*** oil (187.92±0.42) was reported to contain high, Smoke point followed by Moringa seed oil (180+0.01) and Mango seed oil(178+0.02).This provides a useful characterization of its suitability for frying. It can be seen that most of the values fall within the ranges published in the literature (Moharram, *et al*, 1982).

CONCLUSION AND RECOMMENDATIONS

In this study, oils were extracted from three non-conventional sources such as moringa, mango and leucocephala and their physicochemical properties were analyzed. The properties of the oils enumerated in this work, falls within the standard as recommended by ASTM D6751 and make some of them good substitutes to conventional oils. We therefore suggest that potential of the non-conventional seed oils should be tapped for both domestic and industrial uses. Based on the physiochemical properties of oils examined in this work, we believed that it should be profitable to tape oils from these non-conventional resources to supplement the ever increasing demand on conventional oils and use the abundant availability of the oilseeds to expand the economic horizon of the countries where the weather permits growth of oilseeds. The properties of oils analyzed showed that these oils can be used as ingredient in Biodiesel, Biopolymers, Biolubricant, cosmetics and pharmaceutical applications. In future more studies are needed to be undertaken to specifically analyze and study the properties according to the respective industries. However, our work has addressed the potential of non-conventional oil sources.

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