J. Myanmar Acad. Arts Sci. 2018 **Vol. XVI. No. 1B**

EXTRACTION AND CHARACTERIZATION OF OIL FROM MEZE SEEDS (*MADHUCALONGIFOLIA*)

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Abstract

In this research work, meze seed oil was extracted from meze seeds (*Madhuca longifolia*). Meze seeds were collected from Simehtun village near the vicinity of Yadanabon University, Amarapura Township, Mandalay Region. Firstly, the characteristics of meze seed kernelssuch as moisture content, ash content, protein content, crude fiber content and crude fat content were investigated. The phytochemicals and elemental composition present in meze ~~seeds~~ kernels were also investigated. Meze seed oil was extracted by two different methods; with expeller and by using different solvents such as 95% ethanol, Special Boiling Point (SBP 62/82) and petroleum ether, followed by either simple or vacuum distillation. Among these solvents, petroleum ether is the most suitable to get the highest oil yield. The effect of volume of solvent and extraction time on yield of extracted oil was also studied .The most suitable extraction time is 4 hours for each solvent respectively. Extracted meze seed oil was identified by FT-IR spectroscopy. The physico-chemical properties such as specific gravity, refractive index, colour, relative density, moisture, acid value, saponification value, unsaponifiable matter, iodine value and peroxide value of extracted meze seedoils were also determined. The fatty acids composition of meze seedoil such as 25.913% palmitic acid, 19.330% stearic acid, 43.716% oleic acid, 9.824% linoleic acid and traces of other fatty acids was revealed by Gas Chromatography Analysis.

Keywords: meze seed oil,expeller, SBP 62/82, petroleum ether

Introduction

Mahua (*Madhuca longifolia)* is an important economic plant, growing throughout the tropical and subtropical region. It is a native plant of India, Sri Lanka and also Myanmar (tropical region). Myanmar name is Meze or Myintzuthaka. It is a fast growing tree that grows up to approximately 20 meters in height, possesses evergreen or semi-evergreen foliage with wider and round canopy. It belongs to the family Sapotaceae. *Madhuca longifolia*

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have tremendous therapeutic and potential use but due to unawareness of people it is not fully utilized.(http:// www. grow-tree.com, Why Tree).

Its flowers appear with new leaves between February and April and fruits ripening begin from May to August. Fruits (berries) are ovoid or sub-globose, generally 2.5 to 5 cm long, greenish in colour but they turn reddish-yellow or orange when ripe and fleshy with few seeds (Mishraet al. 2013).

The seeds have been used to produce oil usually by using mechanical expression method, solvent extraction method, or both methods. Mechanical pressing is the most common method for oil extraction which includes of different types of press such as hydraulic press, screw press and rolling press. Solvent extraction is a method which is able to extract over 98% oil. However, this method has its own disadvantages such as the necessary equipment is high in cost, the process is quite dangerous in correlation with fire and explosion and the solvent used, requires specific process before conducting the next process (International Food Research Journal 18 (4) 2011).

Mahua seed oil (semisolid at ambient temperature) is used for the care of the skin, used for the manufacture of soaps, detergent and as a vegetable butter. It can also be used as fuel oil. The seed fat has emulsion property so it is mostly used as an emulsifying agent in few pharmaceutical industries. It is generally applied as massage oil in many parts of India as it is good to moisturize skin (http:// www. grow-tree.com, Why Tree).

Thus, the objectives of this study is to compare the different extraction methods of meze (Mahua) seed oil and, to give information of different characteristics of meze seed oil depending on the extraction methods.

Materials and Methods

Materials

Mature meze seeds were collected from Simehtun village near the vicinity of Yadanabon University Campus, Amarapura Township, Mandalay Region from August to October for a particular year. Best Oil Press machine was used to express the meze seed oil. For solvent extraction, three types of solvents such as 95% ethanol(BDH, Analar grade, boiling point of 78-80ºC), SBP (62/82) (boiling point of 60-80ºC) and petroleum ether (boiling point of 35-60ºC)were used to extract meze seed oil.

Methods

Pretreatment of Meze Seeds

The collected meze seeds were dehulled by hand in order to obtain the inner kernels. The seed kernels were then sun-dried to reduce the moisture content (in the range of 4-6%)for 2-3 days. Then they were kept tightly in tin or plastic bags at room temperature (27-30 °C) and ready to extract the oil.

Extraction of Meze Seed Oil by using Expeller

The dried meze seed kernels were crushed using expeller (Best Oil Press Machine with Heater ,Model No.02, Taiwan made)between 45-50ºCto obtain meze oil and oil cake residues separately. The pressed oil was settled and filtered to remove the residual solids.

Extraction of Meze Seed Oil by Using Different Solvents

Solvent Extraction

For solvent extraction, dried meze seed kernels were subjected to grinding in the grinder for reduction of size from lump form to coarse particles. Further grinding was done by recycling coarse particles to produce the pulverized seed kernels (size of -10 mesh). About 5 g of pulverized dried meze seed kernels and 200 mL of 95% ethanol were used to extract the meze seed oil at the boiling point of solvent. Extraction time for about 4 hr was used to obtain the maximum yield of oil.

Distillation

The miscella obtained from the solvent extraction was distilled (either simple or vacuum)to remove the solvent. Simple distillation was carried out at the boiling point of each solvent. The conditions used for vacuum distillation were 60°C and 530 mmHg for 95% ethanol, 50°C and 420 mmHg for SBP (62/82), 30ºC and 480-500 mmHg for petroleum ether. The traces of solvent in the oil were driven off by keeping in the oven for about 1 hr at 110 °C. The oil extracted was accurately weighed.

The same procedure was done with250, 300,350,400mL of 95% ethanol respectively. Similarly, extraction of meze seed oil was also conducted with SBP (62/82) and petroleum ether at their boiling points as described above.

Effect of the volume of solvent and that of extraction time on the yield of extracted meze oil was also studied.

Methods of Identification and Analysis

Physico-chemical properties, phytochemical characteristics and elemental composition of meze seed kernel were firstly investigated. The functional groups present in the extracted meze oil was identified by Fourier Transform Infrared (FT-IR) spectrum. To evaluate the quality of meze seed oil such as specific gravity, refractive index, colour, relative density, moisture, acid value, saponification value, unsaponifiable matter, iodine value and peroxide value were determined and compared with the commercial oil. Gas Chromatography analysis was also conducted to find out the fatty acid composition of meze seed oil.

Results and Discussion

The physico-chemical properties such as moisture, ash, protein, crude fibre, crude fat and carbohydrate content of Myanmar meze seed kernels are shown in Table(1). It is seen that the percentage of crude fat is the highest in Myanmar meze seed kernel and it is also higher than literature values. Its Protein content is significantly different from the literature values. From the results of phytochemical investigations shown in Table(2), it can be seen that meze seed kernel contains alkaloids, flavonoids, glycosides, phenolics, reducing sugar, tannin, saponin, carbohydrate and α - animo acid except starch. Elemental composition of meze seed kernels analyzed by EDXRF spectroscopy was illustrated in Figure (1). According to Table (3), it was found that meze seed kernel contains K, Ca, Fe and C.

Table (4) and Figure (2) show that different yield percentages of oil were extracted with respect to the different volumes of 95% ethanol, SBP and petroleum ether used. From these results, it is seen that increasing the amount of solvent did not increase the oil yield significantly. Although the increase amount of each of the solvent was used, each percentage of oil yield was not significantly increased. So, to recover the cost of solvent, 250 ml of 95%ethanol, 300 ml of SBP and 250 ml of petroleum ether were chosen as the suitable amount for each extraction. From these results, it can be considered that petroleum ether is the most suitable solvent to achieve the maximum oil yield. Effect of extraction time on yield of oil extracted with 95% ethanol, SBP and petroleum ether is shown in Table (5). Due to these results, it is seen that the most suitable extraction time is (4) hr for the respective solvent depending on the highest yield (Figure 3). This observation shows that the longer the extraction time beyond the most suitable time results in lower the oil yield. This result agrees to the finding reported by Mani et al.(2007) that any further increase in extraction time beyond suitable time did not increase the oil yield.

Interpretation data of FT-IR spectrum, shown in Figures (4) and (5), Tables (6) and (7) to identify that both the extracted meze seed oil and commercial oil contain the functional groups such as carboxylic acid and alkenes group significantly. Fatty acid composition of extracted oil investigated by Gas Chromatography is indicated in Figure (6) and Table (8). The results shown in Table (8) represent that extracted oil mainly contains palmitic acid (25.9%), oleic acid acid (43.7%)as predominant compounds while stearic acid (19.3%) and linoleic acid (9.8%)were low. The results are in agreement with Mishraet al. (2013).The results shown in Table (9) represent the comparative study on physico-chemical properties of extracted meze seed oil and commercial oil with literature value. Peroxide value, acid value and iodine value of extracted meze seed oil are much less than that of commercial oil and these lower values indicate the freshness and lower rancidity of extracted oil. This observation is in agreement with the findings of Chakrabarty (2003)that these detections give the initial evidence of rancidity in unsaturated fats and oils.

The results shown in Table (10) represents the comparative study of physico-chemical properties of meze seed oils extracted by different solvents. Because of higher boiling range of ethanol, oil extracted with 95% ethanol has greater peroxide value and acid value than that extracted with SBP and petroleum ether. In addition to simple distillation, vacuum distillation was also conducted to obtain meze seed oil. According to Table (11), it can be seen that the characteristics such as colour, acid value, peroxide value and saponification value of vacuum-distilled oil are less than that of simple-distilled oil indicating a better quality of meze seed oil. Besides these, the time taken for vacuum distillation is less than half of that taken for simple distillation.

Meze Tree and Fruits

Meze Seeds

Meze Seed kernels

Meze Seed Oilby expeller

Meze Seed Oilby petroleum ether

Table 1: Physico-Chemical Properties of Meze Seed Kernels(Myanmar)

Sr. No	Characteristics	Meze seed	Literaturevalues *
1.	Moisture content (w/w%)	4.18	7.8
2.	Ash content (w/w%)	1.64	3.4
3.	Protein (w/w%)	5.90	16.9
4.	Crude fiber (%)	8.86	3.2
5.	Crude fat (%)	53.71	46.7
6.	Carbohydrate (%)	25.71	22

Table 2: Phytochemical Characteristics of Meze Seed Kernel (Myanmar)

Sr. No.	Tests	Extract	Reagents	Observation	Inference
1	Alkaloids	1%HCl	Mayer's reagent Dragendorff's reagent	White ppt Orange ppt	+
2	Flavonoids	EtOH	H_2SO_4(conc:) +Mg turning	Pink colour	+
3	Glycosides	H_2O	10%$FeCl_3$	Purple colour	+
4	Phenolics	H_2O	10%$FeCl_3$	Blue-black colour	+
5	Reducing Sugar	H_2O	Fehling's solution	Brick red ppt	+
6	Tannins	H_2O	2% NaCl+ 1%$FeCl_3$	Deep blue ppt	+
7	Saponin	H_2O	-	Persistent foam	+
8	Carbohydrate	H_2O	10%α naphthol &H_2SO_4(conc:)	Red ring	+
9	α-amino acid	H_2O	Ninhydrin reagent	Purple colour	+
10.	Starch	H_2O	Iodine solution	-	-

Table 3: Elemental Composition of Meze Seed Kernel Analyzed by Energy Dispersive X-ray Fluorescence (EDXRF) Spectroscopy Method

Element	Composition (w/w%)
K	0.079
Ca	0.018
Fe	0.002
C	99.901

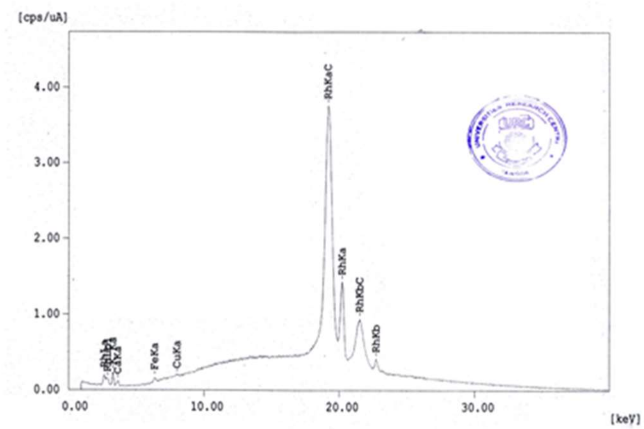

Figure 1: EDXRF Spectrum for Elemental Composition of Meze Seed Kernel

Table 4: Effect of the Volume of Solvent on Yield of Meze Seed Oil Extracted Using

Different Solvents

Weight of meze seed kernel = 5 g
Time of extraction = 4hr
Particle size of kernel powder = 10 mesh

Sr. No	Volume of Solvent (mL)	Ratio of meal to solvent (w/v)	95% Ethanol		SBP (62/82)		Petroleum Ether	
			Weight of oil (g)	Oil yield (%)	Weight of oil (g)	Oil yield (%)	Weight of oil (g)	Oil yield (%)
1.	200	1:40	2.52	50.4	2.61	52.2	2.830	56.6
2.	250 *	1:50	2.58	51.6	2.62	52.4	2.890	57.8
3.	300**	1:60	2.62	52.4	2.48	53.1	2.905	58.1
4.	350	1:70	2.64	52.8	2.65	54.4	2.915	58.3
5.	400	1:80	2.67	53.4	2.74	54.8	2.925	58.5

* Most suitable volumes of 95% ethanol and petroleum ether
** Most suitable volume of SBP

Table 5: Effect of Extraction Time on Yield of Meze Seed Oil, Extracted Using **Different Solvents (based on most suitable volume of each solvent)**

Weight of Meze seed kernel	= 5 g
Volume of 95% ethanol / Petroleum ether	= 250 mL
Volume of SBP(62/82)	= 300 mL
Particle size of kernel	= 10 mesh

Sr. No	Extraction Time (hr)	95% Ethanol		SBP (62/82)		Petroleum Ether	
		Weight of oil (g)	Oil yield (%)	Weight of oil (g)	Oil yield (%)	Weight of oil (g)	Oil yield (%)
1.	1	1.4	28	2.1	42	2.32	46.4
2.	2	1.52	30.4	2.53	50.6	2.61	52.2
3.	3	1.4	37.6	2.6	52	2.62	52.4
4.	4*	2.58	51.6	2.48	53.1	2.89	57.8
5.	5	1.76	45.2	2.42	48.4	2.87	57.4

* Most suitable extraction time using 95% ethanol, SBP(62/82) and petroleum ether

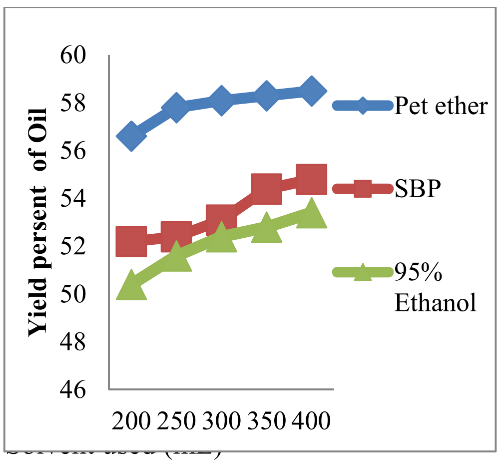

Figure 2: Effect of Extraction Timeon Yield of Meze Seed Oil ,Extracted Using Different Solvents

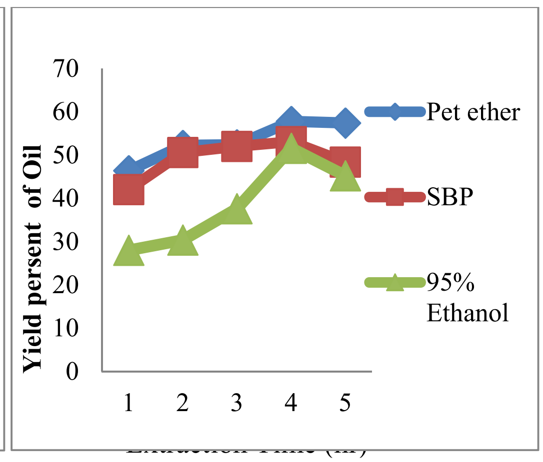

Figure 3: Effect of Extraction Timeon Yield of Meze Seed Oil, Extracted Using Different Solvents

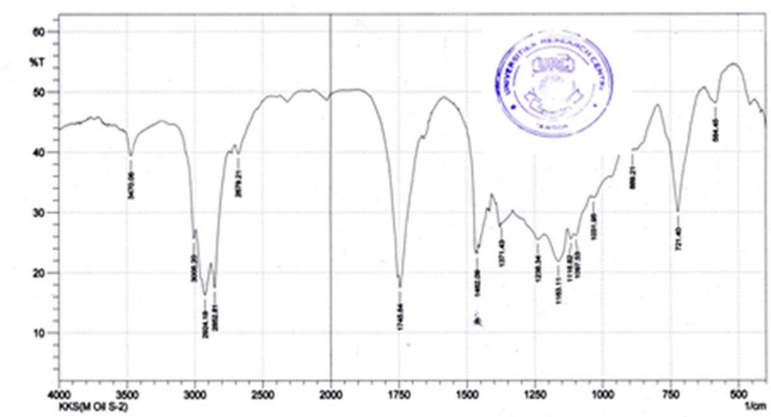

Figure 4: FT-IR Spectrum of Extracted Meze Seed Oil

Table 6: FT-IR Spectrum Data of Extracted Meze Seed Oil

Wave number, cm^{-1}			
Observed	**Literature ***		**Functional group**
3470	3500-3300	υ– NH	Stretching vibration of amines groups
3005	3560-3500	υ– OH	Stretching vibration of carboxylic acid
2924 2852	3000-2840	υ– CH	Stretching vibration of alkane group
2679	2700-2500	υ– OH	OH-in plane bending and C-O stretching
1745	1740-1720	υ – C=O	Stretching vibration of carboxylic acid
1462	1550-1220	υ – CO-H	Stretching vibration of alcohol and phenol group
1371	< 1400	υ– CH	CH symmetric bending vibration
1238 1163 1116	1260-1000	υ – C-O	Stretching vibration of alcohol and phenol group
889	~ 890	υ– CH	Stretching vibration of alkenes group (1,1-disubstituted)
721	~ 700-750	υ– CH	Stretching vibration of alkenes group (cis 1,2- disubstituted alkenes)

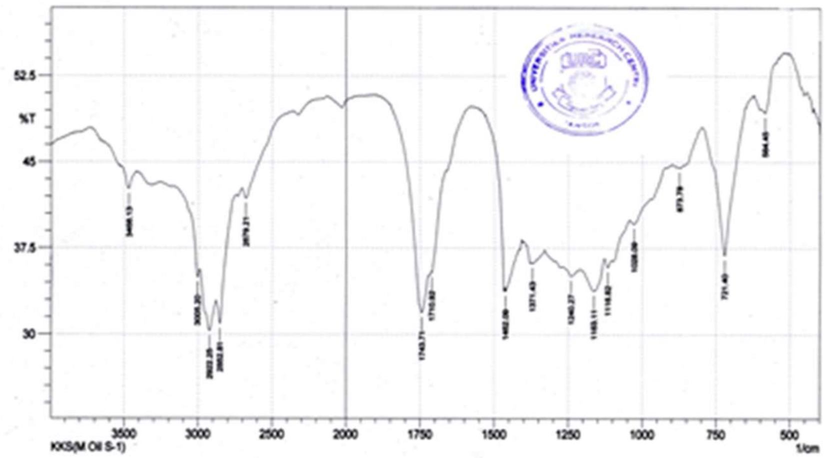

Figure 5: FT-IR Spectrum of Commercial Oil

Table 7: FT-IR Spectrum Data of Commercial Meze Seed Oil

Wave number, cm^{-1}			**Functional group**
Observed	**Literature ***		
3468	*3500-3300*	***υ**– NH*	*Stretching vibration of amines groups*
3005	3560-3500	υ– OH	Stretching vibration of carboxylic acid
2922 *2852*	*3000-2840*	***υ**– CH*	*Stretching vibration of alkane group*
2679	~2700-2500	υ– OH	OH-in plane bending and C-O stretching
1743 *1710*	*1740-1720*	***υ** – C=O*	*Stretching vibration of carboxylic acid*
1462	1550-1220	υ– OH	Stretching vibration of alcohol and phenol group
1371	< 1400	δs- CH	CH symmetric bending vibration
1240 1163 1117	1260-1000	υ – C-O	Stretching vibration of alcohol and phenol group
874	~ 890	υ– CH	Stretching vibration of alkenes group (1,1-disubstituted)
721	~ 700-750	υ– CH	Stretching vibration of alkenes group (cis 1,2- disubstituted alkenes)

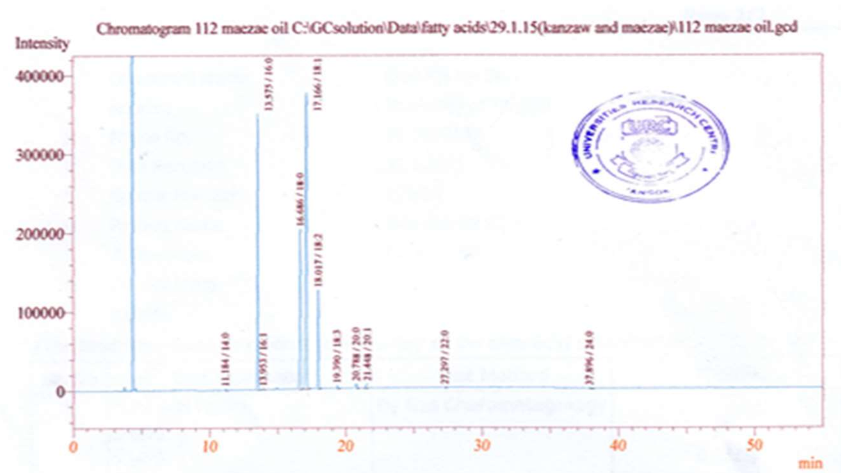

Figure 6 : Fatty Acid Gas chromatogramme of Extracted Meze Seed Oil

Table 8: Fatty Acid Composition of Extracted Meze Seed Oil by GC Analysis

Fatty acids composition by GC	Values (%w/w)	Literature values (%w/w) *
C 12:0 (Lauric)	Not detected	-
C 14:0 (Myristic)	0.073	-
C 14:1	Not detected	-
C 16:0 (Palmitic)	25.913	24.5
C 16:1 (Oleo-Palmitic)	0.059	-
C 18:0 (Stearic)	19.330	22.7
C 18:1 (Oleic)	43.716	37
C 18:2 (Linoleic)	9.824	14.3
C 18:3 (Linolenic)	0.283	-
C 20:0 (Arachidic)	0.526	-
C 20:1	0.125	-
C 22:0 (Behenic)	0.052	-
C 22:1	Not detected	-
C 24:0 (Lignoceric)	0.1	-

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Table 9: Comparison of Physico-Chemical Properties of Extracted Meze Seed Oil with Commercial Oil

Sr. No	Properties	Extracted meze seed oil	Commercial oil *	Literature values **
1.	Refractive index	1.465± 0.02	1.466± 0.01	1.452-1.462
2.	Specific gravity	0.908± 0.01	0.193± 0.53	0.862-0.875
3.	Colour	2.3R, 1.5B, 20Y	2.5 R, 0.2 B, 40.5Y	Pale Yellow
4.	Saponification value (mg KOH per g)	195.49± 0.56	197.223± 0.53	187-196
5.	Unsaponifiable matter (%)	0.984± 0.09	2.95± 0.06	1-3
6.	Iodine value (mgI_2/g)	53.50± 0.32	55.352± 0.372	58-70
7.	Peroxide value (milliequi peroxide oxygen per kg)	9.369± 0.62	26.422± 0.67	-
8.	Acid value (mg KOH per g)	1.752± 0.22	5.044± 0.045	0.5-20
9.	Moisture (loss on drying) %	0.046± 0.01	1.27 ± 0.11	-
10.	Relative density (at 20°C)	0.913± 0.01	0.182± 0.006	-

*Purchased from Daw San Oil Milll, Amarapura Township, Mandalay Region.
** :en.m.wikipedia. org/-----/ *Madhucalongifolia*

Table 10: Comparison of Physico-Chemical Properties of Meze Seed Oils Extracted by Different Solvents

Sr. No.	Properties	Extracted meze seed oil			Literature values *
		95% Ethanol	SBP(62/82)	Petroleum Ether	
1.	Refractive index	1.466±0.01	1.469±0.02	1.465±0.1	1.452-1.462
2.	Specific gravity	0.918±0.002	0.945±0.29	0.907±0.02	0.862-0.875
3.	Colour	2.5R, 0.9B, 25.5Y	2.3R, 1.3B, 20Y	2.1R, 1.2B, 25.2Y	Pale Yellow
4.	Saponification value (mg KOH per g)	197.892±0.06	196.167±0.81	192.659±0.75	187-196
5.	Unsaponifiable matter (%)	2.372±0.14	1.617±0.01	1.612±0.005	1-3
6.	Iodine value (mg I_2/g)	54.126±0.66	54.486±0.23	54.124±0.88	58-70
7.	Peroxide value (milliequi peroxide oxygen per kg)	28.753±0.52	7.226±0.5	15.66±0.27	-
8.	Acid value (mg KOH per g)	22.598±0.22	6.689±0.19	8.569±0.37	0.5-20
9.	Moisture (loss on drying) %	0.045±0.008	0.039±0.03	0.099±0.01	-
10.	Relative density (at 20°C)	0.921±0.03	0.928±0.01	0.918±0.008	-

* :en.m.wikipedia. org/-----/ *Madhucalongifolia*

Table 11: Comparison of Physico-Chemical Properties of Extracted Meze Seed Oil using Simple and Vacuum Distillation

Sr. No	Properties	Extracted with 95% Ethanol		Extracted with SBP(62/82)		Extracted with Petroleum Ether		Literature values*
		Simple-distillation	Vacuum-distillatio	Simple-distillati	Vacuum-distillatio	Simple-distillati	Vacuum-distillatio	
1.	Colour	2.5 R, 0.9 B, 17.5 Y	4.8 R, 0 B, 17 Y	2.3R, 1.3B, 17 Y	6.2 R, 0 B, 17 Y	2.1R, 1.2B, 17 Y	5.8 R, 0 B, 17 Y	Pale Yellow
2.	Saponification value (mg KOH per g)	197.892 ±0.06	189.912 ±0.29	196.167 ±0.81	189.636 ±0.21	192.659± 0.75	198.665 ±0.23	187-196
3.	Peroxide value (milliequivalent peroxide oxygen per kg)	28.753 ±0.52	14.510 ±0.19	7.226 ±0.5	2.491 ±0.21	15.66 ±0.27	12.560 ±0.2	-
4.	Acid value (mg KOH per g)	22.598 ±0.22	7.32 ±0.32	6.689 ±0.19	5.921 ±0.1	8.569 ±0.37	6.176 ±0.05	0.5-20

Conclusion

The seed kernels having average moisture content between 4 and 6 % gave the maximum oil yield. Extraction of oil from meze seeds were conducted by using expeller and also by using the organic solvents like 95% ethanol, SBP and petroleum ether. 4 hours of extraction time gave a maximum oil yield percentage of 51.6% by 95% ethanol, 53.1% by SBP and 57.8% by petroleum ether. Petroleum ether is the most suitable solvent to achieve the maximum oil yield. But the defect of using petroleum ether and SBP is that the odour of these solvents remained in the extracted oil although the oil colour is better than using ethanol. The properties like acid value, peroxide value, saponification value and colour of meze seed oil extracted by solvents are also better than that extracted by expeller. But to extract the oil by solvent extraction is more expensive than using the expeller. Using vacuum distillation in oil extraction can give meze oil of better quality. Meze seed oil can be used to make soaps, candles and biodiesel production.

Acknowledgements

The author would like to acknowledge Professor Dr Cho Cho Oo, Head of the Department of Industrial Chemistry, University of Yangon, for giving permission to perform this research and advice on this research work.

I would like to express my gratitude to Dr Pansy Kyaw Hla, Professor (Retd.) of the Department of Industrial Chemistry, University of Yangon and Dr Yi Yi Myint, Professor and Head (Retd.) of the Department of Industrial Chemistry, Yadanabon University for their valuable suggestions and advice throughout this research work.

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