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Extraction of Oil from Azadirachta indica and Moringa stenopetala Seeds and Evaluation of its Physicochemical Properties

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ABSTRACT

Azadirachta indica and *Moringa stenopetala* trees have been regarded as underutilized, tropical plants, fast-growing, drought-tolerant, robust, oleaginous, and evergreen perennial trees growing widely in various regions of Ethiopia. Almost every part of these plants (i.e., roots, stems, foliage, seeds, and barks) can be used as food additives and as raw materials for pharmaceuticals, cosmetics, soap, and biofuel processing industries. This study aimed at the extraction and characterization of oil from *A. indica* and *M. stenopetala* seeds using the solvent method. The Box-Behnken Design was employed in the experimental design and result analysis. The particle size (0.2, 0.5, 0.8 mm), solvent-to-solute ratio (3:1, 6:1, 9:1), and extraction time (2, 5, 8 hrs) were experimental variables with three levels of low, medium, and high, whereas, the extraction temperature was kept uniform. Seventeen experiments were conducted for each species thereby developing the quadratic models with a *P-value* < *0.0001*(significant). The quality and adequacy of the models were evaluated by analysis of variance (ANOVA) at 5% least significant difference. Results of the physicochemical determination of oils were triplicated and obtained as mean ± standard deviation. The determined physicochemical properties of *A. indica* and *M. stenopetala* seed oils were kinematic viscosity, specific gravity, pH value, refractive index, acid value, free fatty acid, saponification value, iodine value, and peroxide value. The obtained experimental results showed that the extracted oils from *A. indica* and *M. stenopetala* seeds exhibit good oil quality, and hence, they can be employed for commercial and industrial purposes, and the generation of renewable energy (biofuel).

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1. Introduction

Moringa stenopetala and *Azadirachta indica* are underutilized species growing in sub-tropical and tropical regions of Ethiopia. These tree species are considered multipurpose plants possessing several economic benefits, like industrial, medicinal, and nutritional values [1 -3]. Almost every part of these non-edible vegetables (i.e., the leaf, stem, bark, roots, and seed) is useful and has a broad range of applications. *Moringa stenopetala* tree offers the benefit of fuel wood, soil and water conservation, livestock forage, medicine, water purification, green manure, and dye and it generates income for farmers and *Moringa* growing enterprises [4 - 6]. On the other hand, *A. indica* (Neem) provides various industrial benefits (i.e., to produce cosmetics, biofuels and to synthesize medicines - Antiseptic, Anti-tuberculosis, Antiviral, Anti-tumour [2], environmental (i.e., it serves as fixation of dune, reclamation of soil in salinity areas, and organic manure or organic fertilizer from non-edible Oil cake); and socio-economic benefits such as employment generation, income generation [7].

Moringa stenopetala

Moringa is a tropical plant that originates and grows all around the tropics; and belongs to the *Moringaceae* family. The genus Moringa comprises fourteen species that cover the sub-tropics and tropical parts of the Earth's surface [3, 5, 8, 9], out of which five Moringa species including *Moringa stenopetala*, *Moringa rivae*, *Moringa oleifera*, *Moringa ruspoliana* and *Moringa longitude* exist in the Northeast tropic of Africa [5]. Among these species, *M. stenopetala* is predominantly found in the southern part of Ethiopia and the Northern part of Kenya; hence, considered an African Moringa tree. It has also been domesticated in the lowlands of East Africa, specifically, it is known as an indigenous species to Ethiopia among the

Moringaceae family [5, 9, 10]. It covers a wide range of the south-western part of Ethiopia, for instance, Konso, Gamo, Burji, and Gofa people cultivated it as a food crop and consume its leaves as a vegetable [1, 11]. Its English name is known as 'Cabbage tree', 'Ben oil tree', 'Africa Moringa tree', and 'Horse-radish tree'. In Ethiopia, *M. stenopetala* is known by various vernacular names, such as 'Haleko' in Wolaita and Gofa Zones, 'Shiferaw' in Amharic and 'Shelagda' in Konso areas [4, 10, 12].

M. stenopetala is a drought-tolerant, evergreen perennial plant, well-adapted to semi-arid, arid, and poor soil areas with a 500-1,400 mm annual rainfall. It grows naturally in riverine; and can also be cultivated in gardens and terraced fields due to its ease of propagation, multiple usages, and adaptability to degraded and harsh environments [1, 11]. Nonetheless, it does not grow in waterlogged or swampy soils; and it grows best in well-drained soils, with altitude varying from 400 - 2,100 m above sea level and annual temperature varying from 24-30 °C. Its seeds are triangular and covered with a thick, spongy, and yellowish seed coat. The seed kernel has an oval shape and a whitish-grey color (Figure 1). *M. stenopetala* tree can produce up to 4,500 - 10,000 seeds that weigh 2.3-5 kg from about 500-1,000 pods [13]. The *M. stenopetala* seed is a crucial source of oil that can be used for either cooking or various industrial applications, for instance, it is a potential feedstock for the production and utilization of biodiesel [14].

Figure 1. Moringa stenopetala Tree, its un-dehulled and dehulled seeds

Azadirachta indica Tree

The neem tree, *Azadirachta indica*, belongs to the family of *Meliaceae (Mahogany)*. It is native to regions of the Indian sub-continent and Burma [15]. It is an evergreen perennial tree species found in tropical and subtropical areas of South Asia, Africa, Australia, and America. *A. indica* tree has a broad range of adaptability potential to various topographic and climatic conditions. It is drought-tolerant and grows under humid and semi-arid conditions. The neem tree thrives well on poor soil fertility (i.e., calcareous, dry, and stony soils); and improves the soil conditions [16]. Currently, the tree is cultivated and grown in several countries across the globe (i.e., in Australia, Asia, Africa, and Central, Southern, and Northern parts of America). In Ethiopia, *A. indica* is planted and grown widely in the dry, moist 'Kolla' and 'Weyna Dega' agro-climatic regions, such as Gambella, Afar, Humara, Metema, Gonder, Kefa, Arsi, Hararge, Illubabor, Shoa, and other regions. It grows in altitudes ranging from 400 - 2,000 m above sea level. It is also a medium-sized and fast-growing tree that can reach over 20-25 m in height with an oval-shaped canopy and a dense leaf. The neem tree begins fruiting in 3-5 years and it becomes fully productive in about 10 years. Based on the climatic conditions and genotype of the plant, a single *A. indica* tree can produce about 30 - 100 kg of fruit annually (Figure 2). The neem seed comprises about 40-46% oil and the remaining part is the matrix of the seed. The neem oil is non-edible, brownish yellow with an unpleasant odor [17, 18]. Thus, neem oil, which is extracted from neem seeds, can be employed for the production of biodiesel, cosmetics, soap, and medicinal products in pharmaceutical industries.

Figure 2. *Azadirachta indica Tree and Seeds*

Extraction of oil is a process of obtaining or separating triglycerides from oil-bearing vegetables or animal tissues. This process can be carried out via several extraction techniques or methods while diminishing the alteration of the desired product quality and maximizing the yield of the product. The extraction of oil from non-edible vegetable oils predominantly depends on the part of the plant, for instance, the seed kernel, the pulp, or the foliage [19]. In this study, the Soxhlet extraction method with the solvent n-Hexane is employed. The oil extraction involves a solubility-controlled phase which is a fast physical process that helps to separate oil from the outer surface of the considered particles; and the diffusion-controlled phase which is relatively slower process enables to obtain oil from the internal part of the subjected particle [20]. Moreover, this process of oil extraction from the oleaginous seed materials (i.e., *A. indica* and *M. stenopetala* seeds) is affected by a number several operational variables, such as moisture content of the oilseed, particle size, quality of the solvent used, the time allowed for the extraction, the solvent-to-solute ratio, and extraction temperature [21, 22] that in turn affects the quality and the yield of the desired oil [23]. Regardless of the method of extraction to be employed, pre-treatment of the non-edible oilseeds is an indispensable step before extraction. The oilseed pre-treatment step comprises, cleaning, removal of pod or seed coat (de-hulling), winnowing, sorting, and particle size reduction or milling [24, 25]. The removal of moisture content or drying of oilseeds by oven drying is necessitated before grinding proceeds [26]. Size reduction or grinding of the oilseeds (i.e., *A. indica* and *M. stenopetala* seeds) ruptures or breaks the cell embedded in the structure of fiber to increase the surface area of the oil-bearing minute cells for to ensure the release of the desired oil by leaching [27, 28].The present study aimed at the extraction of oil from *A. indica* and *M. stenopetala* seeds by employing the Soxhlet extraction technique and using n- Hexane as; and determination of physicochemical properties of extracted oils following the standard methods of American Society for Testing Materials (ASTM) and Association of Official Analytical Chemists (AOAC).

2. Materials and Methods

Sample collection and Preparation:

The sample of *A. indica* seed was collected from the Afar Region, district of *Amibara*, which is about 242 km from Addis Ababa, the capital city of Ethiopia, in the Northeastern part. The sample collection area has latitude of 09°15′ North, a longitude of 40°10′ East, and an altitude of 740m above sea level (a.s.l.). The sample of *M. stenopetala* seed was collected from Sidama Region Agricultural Center, 273 km from Addis Ababa, in the southern part of Ethiopia, with the geographical location of latitude 7° 3' north, longitude 38° 28' east, and altitude of 1,708 m a.s.l. The collected samples were transported to Addis Ababa Institute of Technology, School of Chemical and Bioengineering, laboratory to conduct the experiment. Then, the samples were cleaned and free from foreign materials, such as weed seeds, molds, stones, and other contaminants. Prior to oil extraction, the collected seeds were first de-hulled (i.e., decorticated). Following the decortication, the outer husk was separated from the kernels by winnowing. Then, samples of seed kernel were dried in an oven at 60 °C for 24 hours to remove the moisture content, and drying was continued until the moisture content was below 5% [29].

$$\textit{Moisture content [\%]} = \frac{W_0 - W_1}{W_0} * 100 \quad (1)$$

Where, W_0 - initial weight of the sample (before drying); W_1 - final weight of the sample (after drying)

The dried samples of *A. indica* and *M. stenopetala* seed kernels were ground into the paste at the particle sizes, 0.20 mm, 0.50 mm, and 0.80 mm turn-by-turn using grinder to provide a higher surface area of particles for the ease of extraction. The range of desired particle size was obtained through the vibrated sieve shaker. The milling operation ruptured the cell wall and released the solute for direct contact with the solvent during the oil extraction process (i.e., by Soxhlet Extraction Method). The ground samples were put into a Low-Density Polyethylene (LDPE), labeled, and stored until dispatch.

Experimental Design:

The response surface methodology (RSM), Box-Behnken Design (BBD), was employed in the design of the experiment. The operational variables in the study were particle sizes (A), solvent-to-solute ratio (B), and extraction time (C), with three experimental levels for each variable, low (-1), medium (0), and high (+1) and the yield of oil (%) was the response of the experiment (Table 1). The influence of operational variables on the response was examined along with their interaction effects. A total of 17 experimental runs were carried out with three levels and five central replication points following the BBD method [30]:

$$N = K^2 + K + Cp \quad (2)$$

Where, *N- t*otal experimental runs*; K*- number of variables; and C_p- central replication point

Table 1- Experimental levels for the considered factors of study following the BBD method

Symbols	Levels				
	Variables	Units	-1 (Low)	0 (Medium)	+1 (High)
A	Particle size	mm	0.20	0.50	0.80
B	Solvent-to-solute ratio	-	3	6	9
C	Extraction time	Hrs.	2	5	8

The powder of *A. indica* and *M. stenopetala* seeds was obtained turn-by-turn and subjected to an extraction process with various experimental variables including, particle size, solvent-to-solute ratio, and extraction time. The considered particle sizes were 0.20mm, 0.50mm, and 0.80mm; and the solvent-to-solute ratio of 3:1, 6:1, and 9:1, whereas, the extraction time was 2 hrs, 5 hrs, and 8 hrs. In addition, the extraction temperature (°C) and amount of powder sample (g) per run and were allowed to be constant during the experiment (Table 1).

The Procedure for Oil Extraction Process:

The Soxhlet extraction method, with *n-hexane* as a solvent, was employed for the extraction of oil from the considered species. A 120 g of ground sample was weighed and placed in thimble paper. Then, the thimble paper containing the sample was inserted into the Soxhlet apparatus. A measured solvent (*n- hexane*) at various proportions to solute (3:1, 6:1, and 9:1) was poured into a 1000 mL round bottom flask and adjusted in the set-up of the extraction vessel. The extraction temperature was kept constant at 72 °C (i.e., slightly higher than the boiling point of the solvent) and the extraction process was continued for 3 hrs, 5 hrs, and 8 hrs to obtain the desired crude oils. As the extraction temperature increased and the process of heating continued, the solvent commenced to evaporate and condensed back to the thimble containing the sample. The solvent was recycled and refluxed back to the flask receiving crude oil and this process was continued until the extraction hour was reached. Then, to recover the solvent from the crude oil-solvent mixture, a rotary evaporator was used at the temperature of about 72 °C. The extraction process was continued until a reasonable amount of crude oil was obtained [31].

Determination of Physicochemical Properties of Extracted Oil

The physicochemical properties of the extracted oil were determined following the American Society for Testing Materials (ASTM) and Association of Official Analytical Chemists (AOAC). The evaluated properties were acid value and free fatty acid content, saponification value, specific gravity, kinematic viscosity, pH value, iodine value, and refractive index.

Determination of acid value and free fatty acid:

The amount of acid number or acid value and free fatty acid (FFA) are used to show the edibility and rancidity of vegetable oils. The acid number indicates the number of milligrams of potassium hydroxide (mg KOH) required to neutralize about 1g of FFA in the given vegetable oil; and the free fatty acid (i.e., usually half of the acid value) is the weight percent of the determined fatty acid like percentage of oleic acid ($C_{18}H_{34}O_2$) in the non-edible vegetable oils [32]. The acid value and free fatty acid determination for *M. stenopetala* and *A. indica* seed oil were conducted according to the standard test method of Association of Official Analytical Chemists (AOAC).

Determination of saponification value:

The saponification value of the non-edible vegetable oil is used to indicate the quality, size, and characteristics of chains of fatty acids that can be esterified into glycerol. It also provides the magnitude of the mean length of the fatty acid chain that comprises fat. In amalgamation with an acid number, the saponification value helps to offer information, such as the average weight, amount, and type of glycerides presented in the provided sample of the non-edible vegetable oils [33, 34]. The determination of the saponification value for both samples of crude oils (i.e., *M. stenopetala* and *A. indica* oil) was carried out following the AOAC standard test method [35].

Determination of iodine value:

The iodine number or iodine value indicates the mass of iodine (g) that is added to 100 g of the considered sample while measuring the unsaturation levels of the subjected organic compound. The iodine value also shows the number of double bonds that exist in the test samples; and the higher the iodine value presented in the sample, the higher the number of double bonds exists and vice-versa. The iodine value of samples of *M. stenopetala* and *A. indica* seed oil was determined according to the standard test method provided in AOAC [36].

Determination of peroxide value:

The peroxide value is a measure of the extent to which the vegetable oils undergo oxidation during the storage and it helps to predict the stability and nature of oils [37]. A high degree of oil unsaturation induces higher peroxide value; and the peroxide value increases with increasing duration of oil contact with atmospheric oxygen, storage temperature, and oil storage time [34] which in turn causes the oxidative rancidity of oil. The determination of peroxide value for each sample (i.e., *M. stenopetala* and *A. indica* oil) was conducted using the standard test method [38].

Determination of kinematic viscosity:

Viscosity is a measure of opposition or resistance of flowing fluid (i.e., liquid or gaseous) to a deformation at a provided rate. The viscosity of the non-edible vegetable oil can be expressed in two ways based on the dynamic viscosity (μ) and kinematic viscosity (υ). The dynamic viscosity of vegetable oil is its shear force or resistance to flow because of the internal friction force in the oil molecule. On the other hand, the kinematic viscosity (mm^2/s) of the oil is its shear force or opposition to flow due to gravity; and this can be determined by dividing the dynamic viscosity of the non-edible vegetable oil by its corresponding density (ρ). The dynamic viscosity of each oil sample (i.e., *M. stenopetala* and *A. indica* oil) was determined using the SV- 10 Vibro-viscometer following the standard test method [39]. The volume of the oil sample was kept uniform throughout the measurement and the corresponding dynamic viscosity of each oil sample was recorded at various temperatures (i.e., at 22 °C, 40 °C, and 60 °C) respectively. The required temperature was adjusted by a hot water bath. Then, the kinematic viscosity of the oil sample was computed as follows:

$$Kinematic\ viscosity\ (\upsilon) = \frac{dynamic\ viscosity\ of\ oil\ (\mu)}{density\ of\ oil\ (\rho)} \quad (3)$$

Where, υ (mm^2/s), μ (mPa.s), ρ (kg/m^3)

Determination of pH value:

The pH value of each sample of *M. stenopetala* and *A. indica* oils were determined using the standardized Digital pH meter. Three grams of each oil sample were weighed and added to the dry and clean beaker of 25 ml. Then, hot distilled water of 15 ml was added into a beaker containing the sample and gently stirred, and using the cold water bath, the sample was cooled. A mixture of NH_4OH and NH_4Cl buffer solution was used to standardize the pH electrode. Then, the electrode was immersed into the beaker containing the sample and the corresponding pH value of each oil sample was recorded [27].

Determination of specific gravity:

The specific gravity of the non-edible vegetable oils (i.e., *M. stenopetala* and *A. indica* oil) was determined by pycnometer according to the standard test method [40].

Determination of refractive index (RI)

The refractive index of oil, usually known as the index of refraction, is the ratio of the velocity of light in a vacuum to the velocity of light in a given non-edible vegetable oil [41]. The longer fatty acid chain in the oil results in a higher refractive index of oil and vice-versa. The refractive index of extracted *M. stenopetala* and *A. indica* seed oil were determined using a digital refracto-meter as per the AOAC standard test method [42].

3. Results and discussion

Determination of Moisture Content of the collected Seeds:
Determination of the moisture content was conducted for both *M. stenopetala* and *A. indica* seeds based on the dry biomass.

The initial moisture content was carried out using the moisture analyzer and the corresponding results were triplicated and the average percentages of moisture contents were provided below (Table 2).

The average mass per single seed was 0.48±0.02 g and 0.62±0.15 g for *M. stenopetala* and *A. indica* samples, respectively. The obtained initial average moisture content of *M. stenopetala* seed (6.48±0.32%) was comparable with the 5.70% value of moisture content obtained for *M. oleifera* [43].

To enhance the ease and effectiveness of oil extraction and to avoid challenges of oil water content in the downstream process, the collected samples were dried and pretreated properly. Thus, using the oven, the collected samples were dried continuously until the moisture content was below 5%. After drying, the average moisture contents of *M. stenopetala* and *A. indica* seeds were triplicated and obtained as 3.12±0.38% and 4.34±0.42%, respectively (Table 2).

Table 2 - *The moisture content of M. stenopetala and A. indica seeds based on dry biomass (DB)*

S/N	Experimental samples	Initial moisture (%) (mean ± SD)	Final moisture (%) (mean ± SD)	Average mass per seed (g) (mean ± SD)
(a)	*M. stenopetala* seed	6.48±0.32	3.12±0.38	0.48±0.02
(b)	*A. indica* seed	11.64±0.54	4.34±0.42	0.62±0.15

SD – standard deviation

The Statistical Evaluation of A. indica and M. stenopetala Oil Yield Using the RSM:

The experimental results were evaluated and interpreted by employing the RSM, Box-Behnken Design method thereby carrying out the analysis of variance (ANOVA), regression analysis, and coefficients of the determinations for the model equation. The adequacy of the model equation was evaluated by the results of ANOVA. The regression coefficients (R^2), variation coefficients (CV), adjusted coefficients (adj-R^2), predicted coefficients (Pred-R^2), and F-test were applied to evaluate the significance and quality of the model equation, where the main comparison was conducted at 5% of the least significant difference (LSD).

Table 3 - The effects of experimental variables on the yield of M. stenopetala and A. indica oil

Run	Factor A	Factor B	Factor C	Amount of sample per run (g)	Extraction temperature (°C)	Response: yield of oil (%)			
	Particle size (mm)	Solvent-to-solute ratio	Reaction time (hrs.)			*M. stenopetala* oil		*A. indica* oil	
						Actual value	Predicted value	Actual value	Predicted value
1	*0.50 (0)*	*6:1 (0)*	*5 (0)*	*120*	*72*	*45.96*	*45.35*	*44.23*	*42.99*
2	*0.20 (-1)*	*3:1 (-1)*	*5 (0)*	*120*	*72*	*39.24*	38.68	*36.18*	*35.43*
3	*0.80 (+1)*	*3:1 (-1)*	*5 (0)*	*120*	*72*	*37.84*	*37.54*	*35.32*	*34.91*
4	*0.50 (0)*	*6:1 (0)*	*5 (0)*	*120*	*72*	*45.52*	*45.35*	*42.64*	*42.99*
5	*0.80 (+1)*	*9:1 (+1)*	*5 (0)*	*120*	*72*	*40.72*	*41.28*	*37.17*	*37.92*
6	*0.20 (-1)*	*6:1 (0)*	*2 (-1)*	*120*	*72*	*30.92*	*31.51*	*28.12*	*28.83*
7	*0.50 (0)*	*3:1 (-1)*	*2 (-1)*	*120*	*72*	*30.84*	*30.81*	*27.51*	*27.55*
8	*0.80 (+1)*	*6:1 (0)*	*2 (-1)*	*120*	*72*	*30.62*	*30.94*	*27.86*	*28.22*
9	*0.50 (0)*	*9:1 (+1)*	*8 (+1)*	*120*	*72*	*49.48*	*49.51*	*46.22*	*46.18*
10	*0.50 (0)*	*9:1 (+1)*	*2 (-1)*	*120*	*72*	*30.78*	*32.90*	*31.25*	*30.13*
11	*0.20 (-1)*	*9:1 (+1)*	*5 ()*	*120*	*72*	*42.72*	*43.01*	*40.39*	*40.80*
12	*0.20 (-1)*	*6:1 (0)*	*8 (+1)*	*120*	*72*	*47.36*	*47.04*	*44.72*	*44.36*
13	*0.80 ()*	*6:1 (0)*	*8 (+1)*	*120*	*72*	*45.32*	*44.73*	*42.28*	*41.57*
14	*0.50 (0)*	*6:1 (0)*	*5 (0)*	*120*	*72*	*44.82*	*45.35*	*42.68*	*42.99*
15	*0.50 (0)*	*6:1(0)*	*5 (0)*	*120*	*72*	*45.28*	*45.35*	*43.28*	*42.99*
16	*0.50 (0)*	*6:1 (0)*	*5 (0)*	*120*	*72*	*45.15*	*45.35*	*42.12*	*42.99*
17	*0.50 (0)*	*3:1 (-1)*	*8 (+1)*	*120*	*72*	*41.64*	*43.52*	*39.26*	*40.38*

Table 4 - Results of ANOVA for the extracted oils at various experimental variables

Response	**Yield of *M. stenopetala* oil**					
ANOVA for Response surface quadratic model [partial sum of squares-type III]						
Source	**Sum of Squares**	**df**	**Mean square**	**F-value**	**P-value Prob > F**	
Model	*605.63*	*9*	*67.29*	*118.19*	*< 0.0001*	***significant***
A- Particle size	*4.12*	*1*	*4.12*	*7.23*	*0.0311*	
B- Solvent-to-solute ratio	*32.56*	*1*	*32.56*	*57.19*	*0.0001*	
C- Extraction time	*429.83*	*1*	*429.83*	*754.96*	*< 0.0001*	
AB	*0.090*	*1*	*0.090*	*0.16*	*0.7028*	
AC	*0.76*	*1*	*0.76*	*1.33*	*0.2868*	
BC	*3.80*	*1*	*3.80*	*6.68*	*0.0362*	
A^2	*35.97*	*1*	*35.97*	*63.19*	*< 0.0001*	
B^2	*22.14*	*1*	*22.14*	*38.88*	*0.0004*	
C^2	*63.00*	*1*	*63.00*	*110.65*	*< 0.0001*	
Residual	*3.99*	*7*	*0.57*			
Lack of Fit	*3.26*	*3*	*1.09*	*5.98*	*0.0584*	***not significant***
Pure Error	*0.73*	*4*	*0.18*			
Cor Total	*609.62*	*16*				
Response	**Yield of *A. indica* oil**					
Model	*623.22*	*9*	*69.25*	*61.85*	*< 0.0001*	***significant***
A- Particle size	*5.75*	*1*	*5.75*	*5.13*	*0.0579*	
B- Solvent-to-solute ratio	*35.11*	*1*	*35.11*	*31.36*	*0.0008*	
C- Extraction time	*416.74*	*1*	*416.74*	*372.20*	*< 0.0001*	
AB	*1.39*	*1*	*1.39*	*1.24*	*0.3016*	
AC	*1.19*	*1*	*1.19*	*1.06*	*0.3372*	
BC	*2.59*	*1*	*2.59*	*2.32*	*0.1719*	
A^2	*38.40*	*1*	*38.40*	*34.30*	*0.0006*	
B^2	*30.81*	*1*	*30.81*	*27.52*	*0.0012*	
C^2	*75.16*	*1*	*75.16*	*67.13*	*< 0.0001*	
Residual	*7.84*	*7*	*1.12*			
Lack of fit	*5.24*	*3*	*1.75*	*2.69*	*0.1815*	***not significant***
Pure error	*2.60*	*4*	*0.65*			
Cor total	*631.05*	*16*				

Table 5 - The model equation for the extracted oils at various factors of study

Response	**Yield of *M. stenopetala* oil**					
Sequential model sum of squares [Type I]						
Source	**Sum of Squares**	**Degree of freedom**	**Mean square**	**F-value**	**P-value Prob>F**	
Mean vs Total	28758.31	*1*	28676.31			
Linear vs Mean	466.51	*3*	155.50	*14.13*	*0.0002*	
2FI vs Linear	4.65	*3*	*1.55*	*0.11*	*0.9511*	
Quadratic vs 2FI	**134.47**	***3***	***44.82***	***78.73***	***< 0.0001***	***Suggested***
Cubic vs Quadratic	*3.26*	*3*	*1.09*	*5.98*	*0.0584*	*Aliased*
Residual	*0.73*	*4*	*0.18*			
Total	29285.92	*17*	1722.70			
Response	**Yield of *A. indica* oil**					
Sequential model sum of squares [Type I]						

Source	Sum of Squares	Degree of freedom	Mean square	F-value	P-value Prob>F	
Mean vs Total	24947.09	*1*	24947.09			
Linear vs Mean	457.60	*3*	152.53	*11.43*	*0.0006*	
2FI vs Linear	4.65	*3*	*1.72*	*0.10*	*0.9568*	
Quadratic vs 2FI	**160.45**	***3***	***53.48***	***47.77***	***< 0.0001***	***Suggested***
Cubic vs Quadratic	*5.24*	*3*	*1.75*	*2.69*	*0.1815*	*Aliased*
Residual	*2.60*	*4*	*0.65*			
Total	29376.56	*17*	1728.03			

The amount of *A. indica and M. stenopetala* crude oils were recorded at the end of each step of the extraction process and the percentage of extracted oil was determined as follows:

$$\text{Oil yield [\%]} = \frac{mass\ of\ crude\ extracted\ oil}{total\ mass\ of\ seed\ kernel} * 100 \quad (4)$$

The results showed that the maximum oil yields, 49.48% and 46.22%, were recorded for *M. stenopetala* and *A. indica*, respectively, from the experimental run 9 at the particle size of 0.50mm, solvent-to-solute ratio of 9:1, and extraction time of 8 hrs (Table 3). The minimum oil yield (30.62%) was recorded for *M. stenopetala* from experimental run 8 and (27.51%) oil yield for *A. indica* from experimental run 7.

The model equation development and evaluation for the extracted oils

The BBD method and results of ANOVA were employed to develop the model equation and analyze its suitability and significance.

The ANOVA result indicated that the developed model equation for the conducted experiment is significant at the $P < 0.0001$ signifying a 0.01% probability that the model F-value is large because of noise. The model terms are regarded as significant at $P < 0.05$. Hence, for the oil extraction process from M. stenopetala seed, the model terms A^2, B^2, C^2, BC, A, B, and C are considered significant as their respective P – value < 0.05, whereas, the terms AB and AC are not significant terms of the model (Table 4). On the other hand, for the oil extraction process from A. indica seed, the model terms A^2, B^2, C^2, B, and C are significant at $P < 0.05$, whereas, A, AB, AC, and BC are insignificant terms of the model. To improve the quality of the model equation, the insignificant terms would not be counted and hence, removed from the model. Moreover, the model fit summary was analyzed for the conducted experiments and the quadratic equation was selected with the statistically significant terms of the model ($P < 0.0001$), where the model is not aliased (Table 5).

In addition, the adjusted determination coefficients of the developed model equations were in agreement with the prediction coefficients. The value of adequate precision is greater than 4 signifying the adequate signal-to-noise ratio and the desirability of the developed model (Table 6). Therefore, the developed model equations can be used to navigate the design space in the process of oil extraction via solvent method at various operational parameters.

Table 6 - The measures of model adequacy for the extracted oils

Adequacy measures	***M. stenopetala oil***	***A. indica oil***
Standard deviation	*0.75*	*1.06*
Mean	*41.07*	*38.31*
C.V. (%)	*1.84*	*2.76*
R^2	*0.9935*	*0.9876*
Adj-R^2	*0.9851*	*0.9716*
Pred-R^2	*0.9126*	*0.8607*
Adeq Precision	*32.204*	*22.95*
PRESS	*53.27*	*87.91*

C.V.- coefficient of variations, R^2- determination coefficient, Adj-R^2– adjusted determination coefficient, Pred-R^2– predicted determination coefficient

The determination coefficients (R^2) indicated that the estimated 99.35% (*M. stenopetala* oil) and 98.76% (*A. indica* oil) overall variations happened in the responses of the experiment were induced by the experimental variables. In addition, the minimum values of variation coefficients (i.e., the ratio of standard error to mean value of oil yield), fignified the experiment was reproducible, reliable, and precise. Hence, the developed model expressions were conceived as reproducible and they can be employed at various factors of study thereby carrying out the oil extraction process [30]:

The model equation with coded factors to extract oils according to the BBD method:

$$\text{Yield of } M.\ stenopetala \text{ oil} = -2.92*A^2 - 2.29*B^2 - 3.87*C^2 - 0.15*B - 0.44*A*C + 0.97*B*C - 0.72*A + 2.02*B + 7.33*C + 45.35 \quad (5)$$

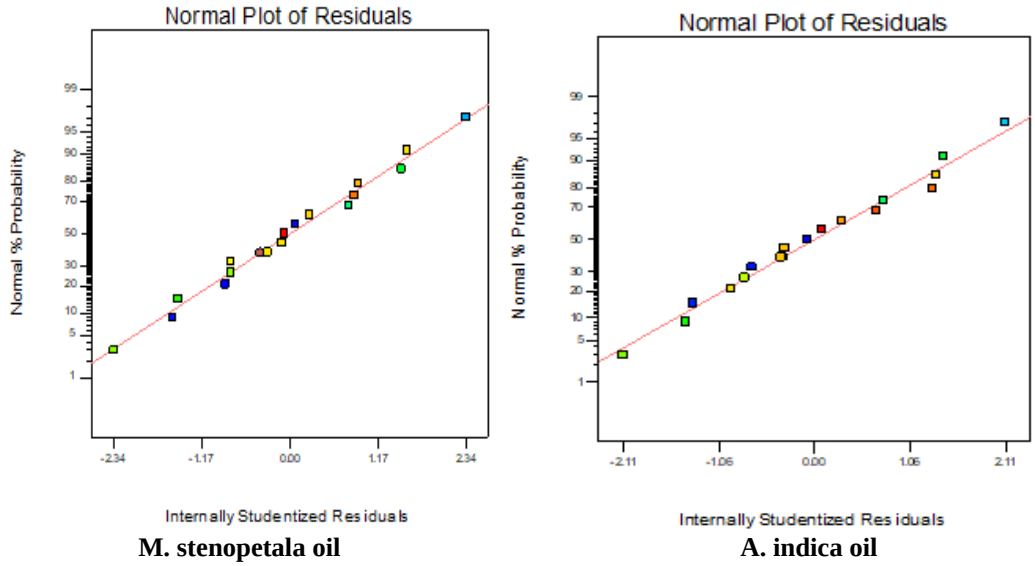
$$\text{Yield of } A.\ indica \text{ oil} = -3.02*A^2 - 2.70*B^2 - 4.23*C^2 - 0.59*A*B - 0.54*A*C + 0.81*B*C - 0.85*A + 2.09*B + 7.22*C + 42. \quad (6)$$

The diagnostics case statistics of the extracted oils:

Table 7 - The diagnostics case statistics of the extracted oils

	***M. stenopetala* seed oil**				***A. indica* seed oil**			
Run order	**Actual value**	**Predicted value**	**Internally Studentized residual**	**Externally Studentized residual**	**Actual value**	**Predicted value**	**Internally Studentized residual**	**Externally Studentized residual**
1	*45.96*	*45.55*	0.910	0.897	*44.23*	*42.99*	1.310	1.396
2	*39.24*	38.93	1.484	1.660	*36.18*	*35.43*	1.422	1.562
3	*37.84*	*37.80*	0.782	0.758	*35.32*	*34.91*	0.770	0.745
4	*45.52*	*45.55*	0.258	0.240	*42.64*	*42.99*	-0.370	-0.346
5	*40.72*	*41.03*	-1.484	-1.660	*37.17*	*37.92*	-1.422	-1.562
6	*30.92*	*30.81*	-1.557	-1.783	*28.12*	*28.83*	-1.342	-1.442
7	*30.84*	*30.81*	0.073	0.068	*27.51*	*27.55*	-0.080	-0.074
8	*30.62*	*30.69*	-0.855	-0.836	*27.86*	*28.22*	-0.662	-0.662
9	*49.48*	*49.51*	-0.073	-0.068	*46.22*	*46.18*	0.080	0.074
10	*30.78*	*30.40*	2.339	4.630	*31.25*	*30.13*	2.112	3.247
11	*42.72*	*42.77*	-0.782	-0.758	*40.39*	*40.80*	-0.770	-0.745
12	*47.36*	*47.29*	0.855	0.836	*44.72*	*44.36*	0.690	0.662
13	*45.32*	*44.98*	1.557	1.783	*42.28*	*41.57*	1.342	1.442
14	*45.82*	*45.55*	-0.779	-0.755	*42.68*	*42.99*	-0.328	-0.306
15	*45.28*	*45.55*	-0.098	-0.098	*43.28*	*42.99*	0.306	0.286
16	*45.15*	*45.55*	-0.290	-0.271	*42.12*	*42.99*	-0.919	-0.908
17	*41.64*	*42.02*	-2.339	-5.499	*39.26*	*40.38*	-2.112	-3.247

To evaluate the characteristics of the Model equations and the nature of their statistical distribution, the normal probability versus residual error was used in compliance with the Box-Behnken design and the plots showed that the normal distribution of residual errors in the linear patterns (Figure 3). Hence, in the experimental data distributions, a set of data points in the developed model equation were estimated to be in a straight line with no sign of abnormality. Moreover, a high correlation coefficients (i.e., close to unity) were obtained from the experimental data indicating that the actual experimental values of oil yield (i.e., *M. stenopetala* and *A. indica* oils) were in agreement with their respective predicted values (Table 7), and a data set fits the model equation properly thereby providing a precise prediction of the desired response or yield of the extracted oils (Figure 3).



M. stenopetala oil **A. indica oil**

Figure 3. *The normal probability versus residual errors plots for M. stenopetala and A. indica seed oils*

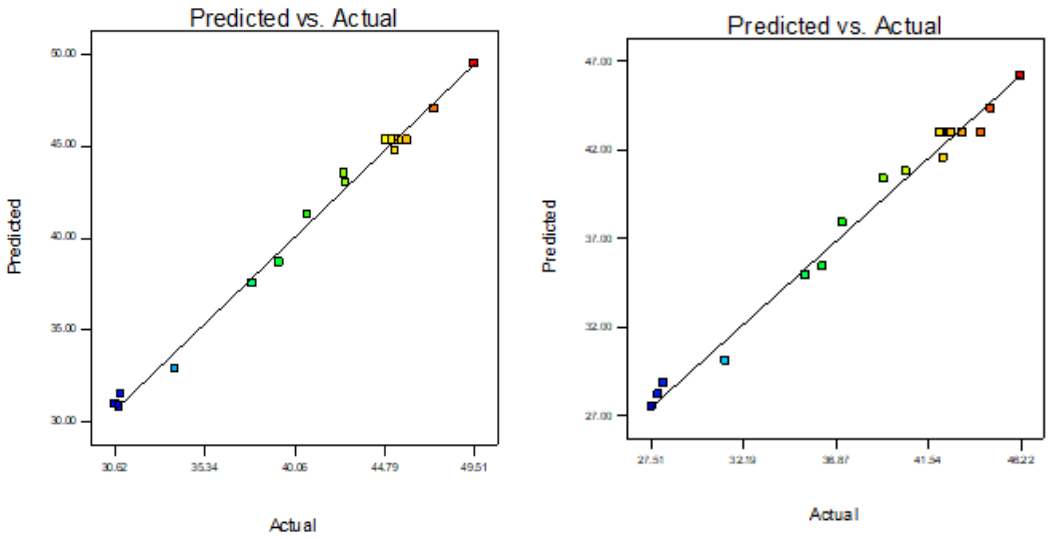


Figure 4. *The predicted versus actual value plots for M. stenopetala and A. indica seed oils*

The Effect of Experimental Variables on the Yield of Oils:

The effect of particle size and solvent-to-solute ratio on the yield of oils

The ANOVA results signified that the particle size and solvent-to-solute ratio significantly affect the yield of oils of *M. stenopetala* and *A. indica* with a P- P-value less than 0.05, respectively (Table 4). For instance, the maximum oil yield (49.48%) was obtained for *M. stenopetala* seed when the particle size was 0.5 mm and the solvent-to-solute ratio was 9:1, whereas, the minimum oil yield (30.62%) was recorded at the particle size (0.8 mm) and solvent-to-solute ratio (6:1) with extraction time (2 hrs). Similarly, the maximum *A. indica* oil yield (46.22%) was recorded for the particle size (0.5mm) and solvent-to-solute ratio (9:1) with 8 hrs extraction time, whereas, the minimum oil yield (27.5%) was obtained at the particle size (0.5 mm) and solvent-to-solute ratio (3:1) with an extraction time of 2 hrs (Table 3). For the considered species under experimentation, the maximum and minimum oil yields were obtained by varying the particle size and solvent-to-solute ratio while keeping the extraction time constant (Figure 5). The 3D surface plot of BBD indicated that at uniform extraction time, minimum particle size enhances the oil yield and increasing the solvent-to-solute ratio up to the optimum point enhances the yield of oil and vice-versa. However, using a more solvent-to-solute ratio beyond the optimum level no longer increases the yield of the oil (Figure 5).

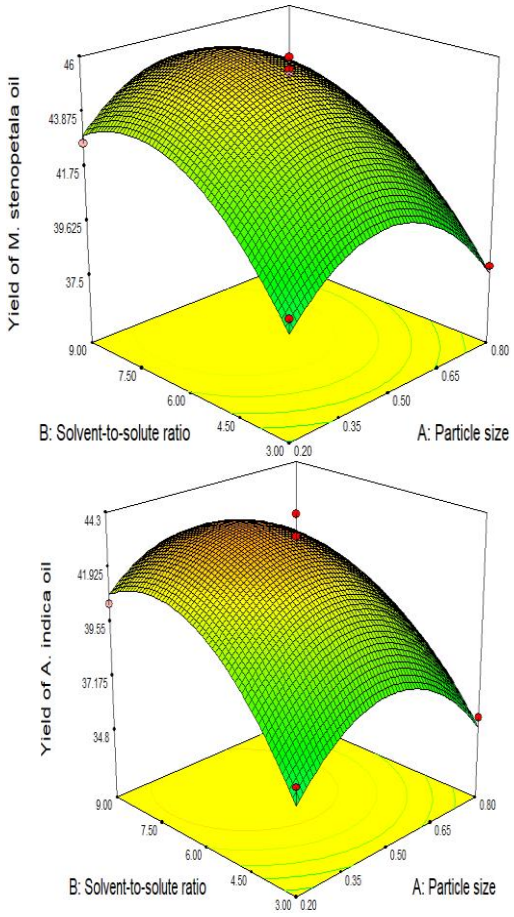


Figure 5. The effect of particle size and solvent-to-solute ratio on the oil yield

The effect of particle size and extraction time on the oil yield

Results of ANOVA showed that the particle size and extraction time have significant effects on the yield of oil for both species with $P < 0.05$ (Table 4). The respective maximum and minimum percentages of oil yields were obtained for both species by keeping the solvent-to-solute ratio uniform. Larger particle size and shorter extraction time diminish the oil yield and vice-versa while keeping the solvent-to-solute ratio constant. Increasing the extraction time from 2 - 8 hrs with smaller particle sizes increases the oil yield as shown in the 3D surface plot of the BBD (Figure 6).

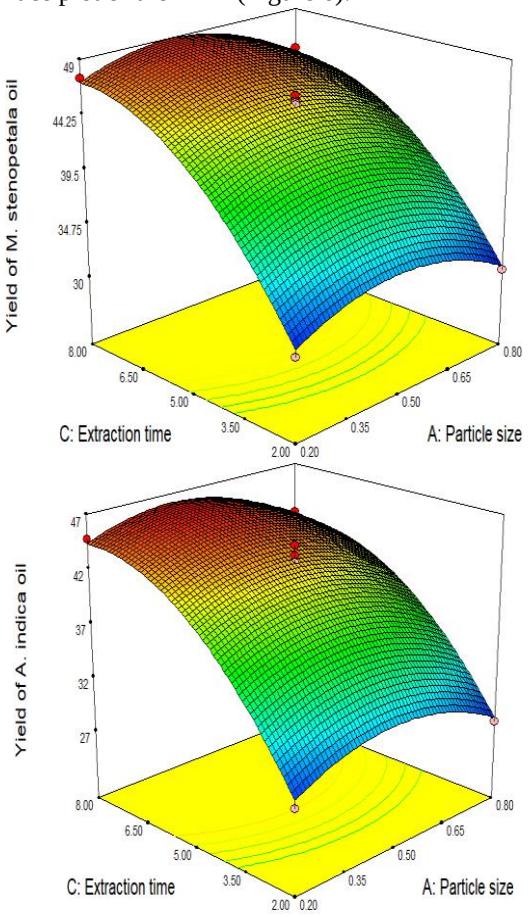


Figure 6. The effect of particle size and extraction time on the oil yield

The effect of solvent-to-solute ratio on the oil yield

As presented in the results of ANOVA, the solvent-to-solute ratio and extraction time significantly affect the yield of oil for both species with $P < 0.05$ (Table 4). The respective maximum and minimum percentages of oil yields were obtained for both species by keeping the particle size constant. It has been indicated that a more solvent-to-solute ratio and longer extraction time increase the oil yield and vice-versa while keeping the particle size constant. Increasing the extraction time from 2 - 8 hrs with increasing the solvent-to-solute ratio (3:1 - 9:1), increases the oil yield for both *M. stenopetala* and *A. indica* as shown in the 3D surface plot of BBD (Figure 7).

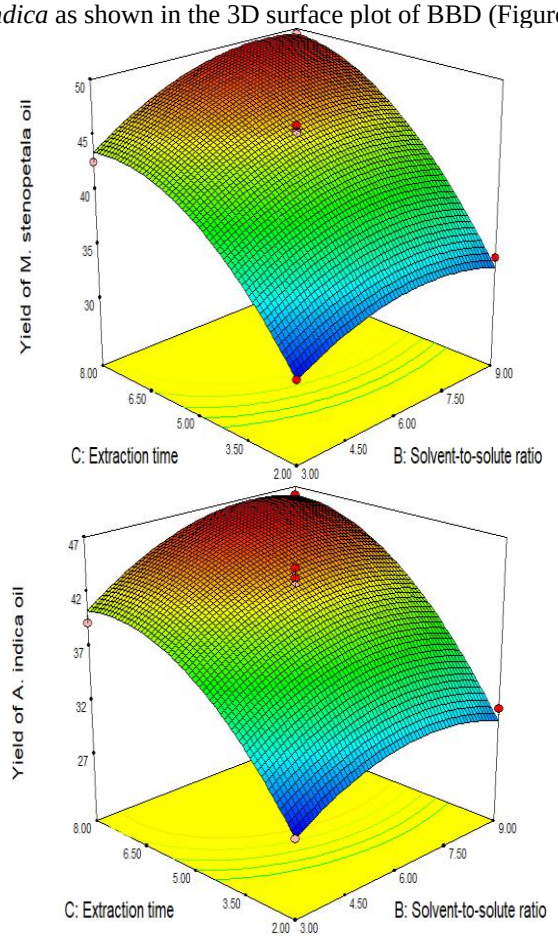


Figure 7. *The effect of solvent-to-solute ratio and extraction time on the oil yield*

The Physicochemical Properties of Extracted Oil from M. stenopetala and A. indica Seed:

The physicochemical properties of *M. stenopetala* and *A. indica* seed oil were evaluated according to the standard test methods. The results were triplicated and their corresponding mean values with standard deviation were provided in Table 8. The main physicochemical characteristics of *M. stenopetala* and *A. indica* seed oil were acid value, free fatty acid, saponification value, iodine value, peroxide value, kinematic viscosity, specific gravity, pH value, and refractive index.

Table 8 - *The physicochemical properties of extracted M. stenopetala and A. indica seed oil*

Physicochemical properties oil		Units	Experimental results (*mean±SD*)		Standard Methods
			***M. stenopetala* oil**	***A. indica* oil**	
Kinematic Viscosity	at 22 °C	mm^2/s	47.73±0.42	49.84±0.33	[39]
	at 40 °C	mm^2/s	26.50±0.30	28.32±0.42	
	at 60 °C	mm^2/s	12.30±0.20	1 4.12±0.12	
Specific gravity		-	0.89±0.01	0.92±0.02	[40]
pH value		-	7.35± 0.05	8.26±0.35	-
Refractive index		-	1.4132±0.03	1.4628±0.02	[42]
Odor		-	Odorless	Unpleasant	-
Color		-	Pale yellow	Brownish-yellow	-
Acid value		mg KOH/g	5.68±0.08	18.60±0.64	[44]
Free fatty acid (FFA)		mg KOH/g	2.84±0.04	9.30±0.32	[44]
Saponification value		mg KOH/g	188.42±0.46	194.36±0.54	[35]
Iodine value		g I_2/100 g	68.20±0.35	72.78±0.36	[36]
Peroxide value		meq/kg	17.12±0.15	16.24±0.74	[38]

SD – standard deviation

It has been found that the acid number of *M. stenopetala* seed oil (5.68±0.08 mg KOH/g oil) was relatively lower than that of *A. indica* seed oil (18.60±0.64 mg KOH/g oil) (Table 8). These values showed that the extracted oil from the two species (i.e., *M. stenopetala* and *A. indica* seeds) could not be subjected to utilization directly, for instance, in the biodiesel production via transesterification process. Thus, they necessitated acid pretreatment steps (i.e., esterification reaction) to convert the free fatty acid in the considered crude oils until their respective FFA values were less than 0.5%.

Moreover, the obtained saponification values of *M. stenopetala* seed oil (188.42±0.46 mg KOH/g oil) and *A. indica* seed oil (194.36±0.54 mg KOH/g oil) were of high value signifying that high free fatty acid (FFA) was presented in each extracted crude oils (Table 8). The result indicated that the obtained saponification value of *M. stenopetala* seed oil was in agreement with the reported values in the previous work, 186 mg KOH/g saponification value of oil was reported by *Anwar et al*., [45] and 178.23 mg KOH/g by *Andinet et al.,* [14], whereas, the obtained saponification value of *A. indica* seed oil (194.36±0.54 mg KOH/g oil) was in comparable with the previous value, 199.810±1.584 mg KOH/g oil, reported by Bakari *et al.,* [46], for the sample of *A. indica* seed oil collected from *Zidim*, Cameroon.

The iodine number indicates the degree of unsaturation of oils. The lower iodine value of the oil signifies the lower degree of unsaturation and vice-versa. The experimental result showed that the iodine number of *M. stenopetala* seed oil was 68.20±0.35 g I_2/100 g oil (Table 8). The obtained value of iodine number was relatively lower and hence, it showed that the *M. stenopetala* seed oil was less likely susceptible to oil rancidity. The result was also in agreement with the report of the previous study, 69 g I_2/100 g oil by Andinet *et al.,* ([14], 2010) and 65.8 g I_2/100 g oil by Lalas *et al.,* [47]. Besides, the obtained value of iodine number for the *A. indica* seed oil was 72.78±0.36 g I_2/100 g oil. This value was in line with the previously reported result, 74.448±0.564 g I_2/100 g oil and 73.814±0.366 g I_2/100 g oil in *A. indica* seed oil collected from the districts of *Maroua* and *Zidim*, Cameroon, respectively [46]. On the other hand, the peroxide number determines the amount of hydro-peroxides presented in the crude extracted vegetable oil [48]. The crude oil with lower number of peroxide is highly resistant to oxidation reaction. The peroxide value of *M. stenopetala* seed oil obtained in this study was 17.12±0.15 meq/kg, and this value was in comparable with a 17.60 meq/kg peroxide value of *M. stenopetala* seed oil obtained from *Konso*, Ethiopia according to the report by *Meseret et al.,* [49]. The peroxide value of *A. indica* seed oil (16.24±0.74 meq/kg) was less than that of the *M. stenopetala* seed oil signified that the *A. indica* seed oil was more resistant to oxidation than that of *M. stenopetala* seed oil (Table 8).

The kinematic viscosities obtained in experiment were recorded at various temperature (i.e., at 22 °C, 40 °C, and 60 °C) and the corresponding values at each specified temperature were recorded as 47.73±0.42 mm^2/s, 26.50±0.30 mm^2/s, and 12.30±0.20 mm^2/s, respectively, for *M. stenopetala* seed oil, whereas, for the *A. indica* seed oil, the kinematic viscosities were recorded as 49.84±0.33 mm^2/s, 28.32± 0.42 mm^2/s, and 14.12±0.12 mm^2/s at 22 °C, 40 °C, and 60 °C, respectively (Table 8). For both crude oil samples, the result showed that the kinematic viscosities were decreased with increasing temperature of oil and vice-versa. The evaluated physicochemical properties of the *M. stenopetala* and *A. indica* seed oils (i.e., kinematic viscosity, specific gravity, and refractive index) were in agreement with the reported values in the previous work. The specific gravity of *M. stenopetala* and *A. indica* seed oil were 0.89 and 0.92, respectively, and these values were in comparable with the previously reported value [50], whereas, the values of refractive index, 1.4132±0.03 and 1.4628±0.02, for *M.*

stenopetala and *A. indica* seed oil, respectively (Table 8) were in line with the result shown in the previous study [45].

4. Conclusion

In this study, samples of *A. indica* and *M. stenopetala* seeds were collected from different regions of Ethiopia. The collected seeds were first de-hulled (i.e., decorticated). Following the decortication, the outer husk was separated from the kernels by winnowing. The dried samples of *M. stenopetala* and *A. indica* seed kernels were ground into the paste at particle sizes of 0.20 mm, 0.50 mm, and 0.80 mm turn-by-turn using a grinder thereby providing a higher surface area of particles for the ease of oil extraction and to obtain better oil yield. The Soxhlet extraction method, with *n-hexane*, was employed for the extraction of oil from the considered species. In the extraction process, the maximum oil yield, 49.48% *M. stenopetala* oil, and 46.22% *A. indica* oil were obtained from experimental run 9, with particle size 0.5mm, solvent-to-solute ratio of 9:1, and 8 hrs extraction time. On the other hand, the minimum values of oil yield, 30.62% *M. stenopetala* oil was recorded at the particle size of 0.8 mm, solvent-to-solute ratio of 6:1, and 2 hrs extraction time, whereas, the minimum oil yield for *A. indica* (27.51%) was obtained at the particle size 0.5 mm, 3:1 solvent-to-solute ratio, and extraction time of 2 hrs. The physicochemical properties of the extracted oils were determined following the methods of ASTM and AOAC. The results were triplicated and obtained as mean ± standard deviation (Table 8). The determined physicochemical properties of *A. indica* and *M. stenopetala* seed oil were kinematic viscosity, specific gravity, pH value, refractive index, acid value, free fatty acid, saponification value, iodine value, and peroxide value. The results of this study indicated that the extracted oils from the considered species had good oil quality, and therefore, they can be utilized for several applications (i.e., commercial and industrial purposes, and production of biofuels).

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