

Extraction and Physico-chemical Characterization of *Cordia africana* Lam Seed Oil

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ABSTRACT

C. africana used is used for skin disease, wound, diarrhea, and ascaris infection in human. The present study aimed to extract and characterize the physico-chemical characteristics of the *Cordia africana* Lam. seed oil. The solvent extraction was used to determining the percentage oil yield. The physical and chemical properties was determine of the extracted oil and showed that the oil was light yellow in colour, liquid with a characteristics smell, had a pH value of 4.10, and refractive index of 1.468. The oil had density of 0.883 g/cm³, saponification value of 182±0.325 mg/KOH/gram, acid value of 6.73, free fatty acid value of 3.365, and peroxide value of 8.16meq/kg. These results suggest that *Cordia africana* seeds may be a potentials viable source of oil going by its oil yield and also indicate that the *Cordia africana* seed oil can be used as a potential alternative to nutritional food and an important additive in soap making since its properties lies within the standard values of other oils used for that purposes.

Key words: *Cordia africana*, Solvent extraction, physico-chemical property.

INTRODUCTION

The worldwide seed oil production will face an increasing demand in the next thirty years due to the combination of factors, including a higher consumption for edible oil, the development of the biofuel industry and the needs for green chemistry. Nowadays, the annual worldwide oil production is close to 135 Mt with palm, soybean and rapeseed oils representing 31%, 24% and 15% of the total production respectively [1]. Oil seeds are mainly composed of oil and protein. For better oil extraction an in-depth understanding of the complex arrangement of polysaccharides in the oil seeds cell wall is a prerequisite [2].

Plant seeds have been used since antiquity as sources of vegetable oil [3]. Vegetable oil is an important and widely used lipid source for our everyday (diet products). It is also represents a particular importance as raw materials for industries like food (for their nutritional value), energetic (through their conversion in renewable biofuel), or chemical (detergents or materials industry, film-forming substances like varnishes, paints, and so on) [1]. Its application is increasing day by day for food purposes and for the manufacturing of a number of toiletry products. However, some vegetable oils are not up to standards to meet consumer satisfaction in terms of their physico-chemical properties or for the texture and stability of the food products [4]. The food value of the edible lipids also depends on chemical properties like iodine value, peroxide value, acid value, saponification value etc, as well as on some physical properties like solidification temperature, colour, appearance etc.

Cordia africana is a tree found wide spread in the Middle East, West, East, and Africa. It is known by the name Sudan

teak, East African *Cordia*, large-leaved *Cordia*, and Sebastian fruit [6]. In Ethiopia *Cordia africana* fruit is eaten by the local community during its fruiting season of March to May. Traditionally wood ash mixed with butter is used for skin disease locally referred to as "Spider disease" because they believe the spider causes the skin trouble by depositing its urine or other forms of excretion on the skin while they are asleep, wound, diarrhea, and ascaris infection in human [7, 8] The aqueous extract of *C. africana* promising anti-bacterial activity [7].



Figure 1: *Cordia africana* (Wanza) [Burie Woreda, Kebele 03, and February to April, 2015]

In continuation of the ongoing project to study the medicinal plants in Ethiopia, we here present extraction and Physico-chemical characterization of *Cordia africana* (Figure 1) (Wanza, in Amharic local language of Ethiopia) seed oil via soxhlet oil extraction methods and the levels of investigated crude oil were compared with recommended levels by edible oil from the data of FAO/WHO standards.

MATERIALS AND METHODS

Sampling and sample preparation

Healthy *C. africana* seeds were collected during three consecutive months directly plant harvesting seasons (February to April, 2015) from Amhara Region, West Gojjam Zone, Burie Woreda, Kebele 03 and further identified and authenticated by Dr. Birhanu a botanist, in the department of Biology, Bahir Dar University, Ethiopia. Soon after collection, the fruits were wrapped up with aluminum foil, taken to the laboratory and washed with tap water and then with distilled water in order to remove the dirty particle in the fruits. The seeds were then air dried for 15 days at room temperature away from the reach of sunlight before use. The fruit was decorticated using decortivating machine (disc-mill).

Oil Extraction

15 g of the powdered sample was placed into the thimble and inserted into the centre of soxhlet extractor. The extraction was carried out different solvent systems (100% n-hexane, mixture of solvents (9:1, 8: 2, 7: 3, 6: 4, 5:5, 4:6) Ethanol to hexane ratio and 95% ethanol) by using soxhlet apparatus. 300 mL of (n-hexane, ethanol) or the mixture of these two was poured into round bottom flask and connected to the extractor; the condenser was also connected to the extractor. Rubber hose attached to the inlet of the condenser was connected to a water tap where water could flow in and out through the outlet hole. Heater set at 70 °C supplied heat to the bottom of the flask placed on the heating mantle. When the solvent was boiled, the vapour started rising through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre, which contains the solid sample to be extracted. The extract then seeped through the pores of the thimble and filled the siphon tube, where it was start flowed back down into the round bottom flask. This was allowed to continue for 5 hours. The extracts were concentrated by using rotary evaporator, then cooled in the desiccators and all experiments were done in triplicate and the averages were calculated. Results were expressed in mean \pm S.D.

Determination physical parameters of *C. africana* seed oil

Determination of moisture Content

Moisture content of oils is the loss in mass of the sample on heating at 105 \pm 1°C under operating conditions specified. 2.1542 g, 2.2375g and 2.0562g of the cleaned sample was weighed in a crucible and dried in an oven at a temperature of 105 °C for 7hrs and weighed after every 2hrs. The procedure was repeated until a constant weight was obtained. The percentage moisture in the seed was calculated by using (Equation 1):

$$\text{Moisture(\%)} = \frac{W_1 - W_2}{W_1} \times 100 \dots \dots \dots (1)$$

Where, W_1 = Original weight of the sample before drying; W_2 = Weight of the sample after drying.

Determination of Ash contents

The remaining sample after determination of volatile matter was kept in furnace at a temperature of 550°C for 6 hours. The percentage of weight loss gave the ash content. The percentage of ash is computed by using (Equation 2):

$$\text{(\%)} \text{ Ash} = \frac{[\text{weight of ash left}]}{W_{to}} \times 100 \dots \dots \dots (2)$$

Where, W_{to} = Original weight of the sample before drying.

Determination of percentage yield of seed oil extracted

The extraction of oil using soxhlet extractor was repeated for the sample and the oil was recovered by solvent evaporation. The oil which was recovered by complete distilling of most of the solvent on a rotary vapor was then transferred to measuring cylinder. The volume of the oil was recorded and the yield expressed as oil content (%) by using (Equation 3):

$$\text{Yield\%} = \frac{W_1 - W_2}{W_1} \times 100 \dots \dots \dots (3)$$

Where: W_1 = sample weight initially placed in the thimble and W_2 = sample weight after dried in the oven

Determination of Specific Gravity and Density of the Oil

Specific gravity of the oil was determined by hydrometer in the range of 0.880-0.990. And the density of the oil was determined by using density bottle. A clean 25 mL specific gravity bottle was dried in an oven. Then it was allowed to cool in desiccators. The weight of the specific gravity bottle was obtained as W_1 , and then the specific gravity bottles were filled with clean water. The weight of the bottle plus water was obtained as W_2 . The water was poured out and allowed the bottle to dry. The specific gravity bottle was again filled with oil and the weight of the specific gravity bottle oil was obtained as W_3 . The specific gravity and relative density of the oil was calculated by using (Equation 4&5) [9, 10].

$$\text{Density} = \frac{\text{weight of oil}}{\text{volume of oil}} \quad \text{i.e.} = \frac{W_2 - W_1}{100} \dots \dots \dots (4)$$

$$\text{Specific gravity} = \frac{\text{weight of oil}}{\text{weight of equal volume of water}} \dots \dots \dots (5)$$

Determination of Viscosity of the oil

The viscosity of the oil was measured using viscometer at 100 rpm and the results shown (Table 1). 200 ml of oil was poured into a test tube and measured the viscosity at a temperature of 33 °C [9].

Determination of refractive index

The value of refractive index of *C. africana* seed oil measured using refractometer. Few drops of the sample were transferred into the glass slide of the refractometer. Water at 30 °C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value was noted and recorded as the refractive index [9].

Determination of pH value

The value of pH of *C. africana* oil was measured using pH meter. 2 g of the sample was poured into a clean dry 25 mL beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then put in cold-water bath to 25 °C. The pH electrode was standardized with buffer solution and then immersed into the sample and the pH value was read and recorded.

Chemical Characterization of the *C. africana* seed oil

The quality of *C. africana* seed oil was determined as regards of the amount of free fatty acid present in the oil were determined through determination of acid value, saponification, Iodine value and peroxide value.

Determination of acid value

Acid value was determined by titrimetric method of Pearson [11]. 5g of the oil sample was weighed and 75ml of hot neutral alcohol with a few drops of phenolphthalein was added. The mixture was shook vigorously and titrated with 0.5N Potassium

hydroxide solution with constant shaking until the pink coloration remained permanent. Acid value was calculated using (Equation 6):

$$\text{Acid Value (AV)} = \frac{V \times C \times 56.11}{M} \dots\dots\dots (6)$$

Where V = Volume of standard potassium hydroxide (ml), C = Concentration of potassium hydroxide, 56.11 =Molecular weight of potassium hydroxide, M= sample weight [12].

The acidity frequently was expressed as free fatty acid according to standard formula by using (Equation 7) [13].

$$\text{Free fatty acids as oleic acid} = \frac{28.2 \times V \times N}{W} \times 100 \dots\dots (7)$$

Determination of saponification value

The oil sample was saponified by refluxing with 0.5N alcoholic potassium hydroxide solution. The alkali required for saponification was determined by titration of the excess potassium hydroxide with standard hydrochloric acid [12]. The saponification value was determined according to the titrometric method of Pearson [11]. 2g of oil sample was poured into a conical flask and 25ml of alcoholic Potassium hydroxide was added. The solution was then heated in boiling water bath for 1h and 1 mL of 1% Phenolphthalein was added followed by titration with 0.5N HCl Blank was prepared with alongside the oil samples. The saponification value was calculated by using (Equation 8) [13]:

$$\text{Saponification value} = \frac{56.1 N (A - B)}{W (g)} \dots\dots\dots (8)$$

Where: N = Normality of HCl, A = Volume (ml) of H₂SO₄, for blank, B = Volume (ml) of H₂SO₄, for sample, 56.1= Equivalent weight of potassium hydroxide, W= Weight of oil used (2g)

Determination of Iodine value

Iodine value was determined according to Pearson [11]. 2g of oil sample poured into a dry glass stoppered bottle of 250ml capacity and 10ml of carbon tetrachloride was added to the oil. About 20ml of Hanus solution was then added and allowed to stand in the dark for 30 min [13, 14]. 15ml of (10%) Potassium Iodide was added followed by 100ml of water and then titrated with 0.1M Sodium thiosulphate solution using starch as indicator just before the end point. Iodine value was calculated by using (Equation 9) [13]:

$$\text{Iodine value} = \frac{(V_2 - V_1) \times 1.269}{\text{weight of sample (g)}} \dots\dots\dots (9)$$

Where: V₂ = titer value for blank, V₁ = titer value for sample (s).

Determination of peroxide value

2 g oil sample was poured into a test tube and 1g of powdered Potassium iodide with 20ml of solvent mixture (glacial acetic acid and chloroform) was added. This mixture was then placed in boiling water bath for 30s. The content was poured into a conical flask containing 20ml of 5% iodide solution. The test tube was washed with 25ml of distilled water and then titrated with 0.002N Sodium thiosulphate solution using starch as indicator. Peroxide was obtained by using (Equation 10) [13]:

$$\text{Peroxide Value (PV)} = 2 \times \frac{[V_1 - V_2] mEq/Kg}{\text{Weight of sample (g)}} \dots\dots (10)$$

Where: V₂ = Blank titre value, V₁ = Sample (s) titre value.

RESULTS AND DISCUSSIONS

Physico-chemical properties of *C. africana* seed oil

Physical properties of *C. africana* seed oil

Moisture contents

The moisture content of seed samples at different time of drying was obtained as shown in (Table 1). The average moisture content of the three samples of the crude oil is 9.11±2.24 % indicating low moisture content might be as a result of effectiveness of the distillation apparatus used for oil recovery. The low moisture content of the oil is advantageous in terms of storage stability since the lower the moisture content, the better the storability and suitability to be preserved for a longer period.

Ash contents

About 2.041 g, 2.045 g and 2.001 g of *C. africana* was taken; the ash content of the sample was evaluated by using (Equation 2). The average ash content of the three samples was 1.89±0.02 % w/w (Table 1). The result of ash content of seeds indicates the presence it could be minerals, abrasive solids, soluble metallic soaps, and silica residue in the seed and also indicates the food adulteration [14]. The value (1.89±0.02%) obtained for ash falls within acceptable limits for edible oils (1.5-2.5%) from literature [15, 16].

Specific gravity of the oil was determined by hydrometer which was measured in the range of 0.880-0.990. Specific gravity for the *C. africana* seed was 0.883 (Table 1) and this is very close to the values 0.89-0.92 reported for edible oils [15]. Then, using (equation 4), the density of the oil was obtained 0.883 g/cm³ indicated in (Table 1), and the crude oil of *C. africana* has a good value of specific gravity and relative density value 0.883, approaches within the range of 0.89 – 0.92 g/ml reported for edible oils [15].

Moreover, refractive index is used mainly to measure the change in unsaturation as the fat or oil is hydrogenated. It depends on their molecular weight, fatty acids chain length, degree of unsaturation and degree of conjugation [17, 18]. Refractive index for *C. africana* seed oil in this study was obtained 1.46 (Table 1) which closely agrees with values suggested for edible vegetable oils such as Neem and sesame oil [17]. This indicates that extracted *C. africana* oil was pure (no adulteration) and therefore minimizes purification procedures during processing such as filter pressing and/or centrifugation. The results suggest that *C. africana* seed oil is light making it desirable for many cooking purposes.

Viscosity of oil is a measure of the oil's resistance to shear. High viscosity implies a high resistance to flow while a low viscosity indicates a low resistance to flow [19]. As we have observed from the literature [20]. *C. africana* seed oil was compared with Cashaw seed oil it could be viscous liquids with viscosity values at 32 ±1.5 centripores. The *C. africana* seed oils have agreeable odour at room temperature (20-25°C), a liquids, with a light yellow to yellowish colouration.

The **pH** value of *C. africana* was 4.10 (Table 1). The pH value between 4.0-6.9 recommended value of acidifying fats and oils such as olive oil, sesame oil sunflower oil due to this reason *C. africana* seed oil probably grouped under edible oil [21].

Chemical analysis of the *C. africana* seed oil

In the present study chemical parameters such as, saponification value (SV), iodine value (IV), peroxide value (PV), acid value (AV), and ester value (EV) were determined (Table 2). The Acid Values (AV) was 6.73±0.082mg KOH g-1, Free Fatty Acids (FFA) was 3.37±0.082 evaluated as

(Equation 7); Saponification Value (SV) was 182 ± 0.32 mg KOH g⁻¹, Iodine Values (IV), was 71 ± 1.418 mg of I₂ g⁻¹ of oil and Peroxide Values (PV), 8.16 ± 0.2 mg O₂ kg⁻¹ for extract *C. africana* seed oils.

The low peroxide and acid values found for *C. africana* oil indicate that it could be used for human consumption, as the recommendation for edible oils is a peroxide value less than 10 meq O₂/kg [22]. The data also showed that the seed oils were edible inferring from their low AV and their corresponding low FFA contents [23]. Acid value indicates the amount of free fatty acids presents in an oil and it is good indicator of oil degradation caused by hydrolysis [24]. Industrially, the results revealed the seed oils to have great potentials in soap manufacturing industries because of their high SV [23].

Saponification value indicates the average molecular weight of triglycerides in the oil. The lower the saponification value, the larger the molecular weight of fatty acids in the glycerides and vice versa [24]. Low Iodine value was (less than 100) also shown to be non-drying due to their which also suggested that the oils contain few unsaturated bonds and therefore have low susceptibility to oxidative rancidity and deterioration as confirmed by their low PV which also serves as indicators of the presence of anti-oxidants in the oils [23]. Iodine value expresses the unsaturation level of the oil. Peroxide value is used as an indicator of oil rancidity. Rancidity is caused by aldehydes, ketones, and oxidation [24].

Table 1: Physical properties of *C. africana* seed oil

Characteristics (Unit)	Obtained value
Refractive index (at 25°C)(nD)	1.468 ± 0.0057
Color	Light yellow
Specific gravity (at 27°C)	0.883 ± 0.391
Viscosity (Cp) at 33 °C	32 ± 1.5
Odor	Agreeable
PH	4.10 ± 0.0056
Physical state at 28°C	Liquid
Oil yield (%)	7.2 ± 0.245
Moisture (%)	9.4357 ± 2.2492
Ash (%)	1.89 ± 0.02
Density (g/cm ³) 25°C	0.883 ± 0.034

Table 3: Comparison of extraction efficiency of oil from *Cordia africana* seed with different solvent ratio

Run	Hexane :Ethanol							
	100:0	90:10	80:20	70:30	60:40	50:50	40:60	0:100
1	7.5	7.0	6.8	6.6	6.3	5.9	5.5	4.75
2	7.2	6.8	6.7	5.3	4.9	4.2	4.5	4.5
3	6.9	6.9	6.4	6.2	5.5	4.85	4.3	4.8
	7.2 ± 0.24	6.9 ± 0.08	6.63 ± 0.02	6.03 ± 0.29	5.57 ± 0.32	4.98 ± 0.49	4.77 ± 0.27	4.68 ± 0.01

Values are mean and standard deviation (n=3).

Table 4: Comparison of some edible oil seeds and *C. africana* seed with respect to oil contents and some oil quality properties.

Parametrs	Cotton seed	Soybean seed	Peanut	Sesame seed	Niger seed	<i>C. africana</i> seed
pH	4.82	4.5	4.10	4.33	5.2-7.3	4.10 ± 0.005
%yield	15.05	17.0-21.0	24.0-40.0	35-63	30-50	7.2 ± 0.245

Table 2: Chemical properties of the *C. africana* seed oil seed with the standard of FAO value

Characteristics	Obtained value	FAO/ WHO standard
Acid value (mg/KOH/g)	6.73 ± 0.082	5.78-7.28
Free fatty acid (mg/KOH/g)	3.37 ± 0.082	4
Saponification value (mg/g)	182 ± 0.325	181.4 ± 2.60
Iodine value (g/100g)	71 ± 1.418	80-106
Peroxide value	8.16 ± 0.232	Below 10

Values are mean and standard deviation (n=3).

I. Effect of solvent type on Oil yield quantity of oil

The effect of five main factors which are type of solvent, temperature, reaction time, solvent to solid ratio and agitation speed were investigated to optimize the extraction operating conditions for achieving maximum oil yield. Solvent type is the most important parameter for solvent extraction specifically in case of oil extraction from *C. africana* seed as it affects the quantity of oil [24]. The yield can be calculated using (Equation 3). The effect of extraction solvent nature with different sample to solvent ratio on the determination of *C. africana* seed oil was shown (Table 3).

The results obtained from this work as shown (Table 3), using only n-hexane for extracting oil from the seeds has the best results (7.2%) at 1:15 w/v ratio of seed powder to solvent volume at 6h extraction time which is by far better than that of pure Ethanol (4.68%) at the same conditions. Polarity of the solvent increases from hexane to ethanol, the color of the oil that was extracted also changed from yellow to dark blue. This may most probably be due to the extraction of other polar compounds from the seeds when the solvent polarity is increasing [24]. The extraction rate is fast at the beginning of the extraction but gets slow gradually. The reason is that when the kernel powder is exposed to the fresh solvent, the free oil on the surface of seeds is solubilized and oil gets extracted quickly inducing a fast increase in the extraction rate [9].

CONCLUSION

In the present study was discussed extraction of oil using solvent extraction method. The results revealed that 100% n-hexane gives better oil yield compared mixture of n-hexane to ethanol in different ratio and 100% ethanol. The pH value, refractive index, specific gravity, colour of oil and viscosity and Ash content of extracted oils are reasonable in good agreement with the standard values for edible oil purpose from the literature. All these are important properties to determine the physical state of the oil. Also the results obtained presented saponification value, acid value, free fatty acid, peroxide and iodine value were determined are important properties to determine the chemical state and quality of the oil. From this analysis, that fell within the range of those acceptable as having good potential for edible oil, since its properties lie within the standard values of other oils used for this purpose. It may however be useful for other purposes such as soap making by their high saponification values in the range of 123-261. The results in this analysis indicated good quality for the *C. africana* oil that have high value but low volume.

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