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Proximate Composition and Physicochemical Characterization of Seed and Seed Oil of Solenostemma Argel

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ABSTRACT

The objective of this study is to determine the basic chemical components of the Hargal seeds (*Solenostemma Argel*) and physicochemical properties, minerals concentrations and fatty acids of its oil. The sample was collected from the local market of Abu Hammad city in the River Nile State, Sudan. The results showed that the seeds contained (43.3%) carbohydrate, (30.7%) crude protein, (13%) oil, (7%) moisture and (6%) ash. The results of the study also showed the physicochemical properties of oil as refractive index (at 25°C) 1.4, density 0.9104 g/cm^3, acid value 10.38 mg KOH/g oil, peroxide value 0.03 meq O_2/Kg oil, iodine value 121/100 g oil and saponification value 197 mg KOH/g oil. The minerals contents in the oil (mg/100 g oil) were found 133, 85.0482, 67.4500, 24.4533, and 3 for sodium, calcium, iron, magnesium and potassium respectively. The results of the study revealed that the major fatty acid in Hargel seed oil is linoleic acid (44.15%) and the lowest one is arachidic acids (2.70%).

Keywords: Herb, *Solenostemma Argel*, proximate composition, seed oil, fatty acid profile, mineral content

I. INTRODUCTION

A herb is a plant or plant part valued for it is medicinal, perfumed or flavorful qualities. Herb plants produce and contain a variety of chemical substances that act upon the body [1]. Therefore, herbs have been utilized, intensively, by the world population, as a natural, effective and low cost source for medicine, since centuries [2].

Sudanese people use plants, especially in rural areas, as a source for healthcare. Many Sudanese medicinal plants are considered potential sources of antioxidants compounds, Such as *Punica geranium* , *Acacia nilotica*, *Terminalia chebula* [3]. Hargel (*Solenostemma Argel*) was among some Sudanese plants that were reported for their use as antimicrobial agents. It is a member of the family *Asclepiadaceae.* This family includes many wild growing medicinal plants (e. g. *Calotropis procera* and S. *argel Leptadinea* s pp) [4]. It is a desert plant which widely distributed in Egypt, Libya, Chad, Algeria, Saudi Arabia, Palestine, central and Northern part of the Sudan. Among these above mentioned countries, Sudan is regarded as the richest source of the Hargel plant which found between Barbar and Abu Hamad. The part used of the plant is dried leaves and stems [5]. Many studies reports the medicinal benefits of Hargel leaves [3, 6, 7]

The present study aims to investigate the chemical composition of Hargel seeds. Specific purposes include:

- To determine the proximate composition (moisture, protein, carbohydrate, fat, crude fiber and ash) of Hargel seeds.
- To determine the physicochemical properties of the extracted oils such as density or specific gravity, viscosity, refractive index, acid value , peroxides, saponification value, iodine value, Fatty acids and some mineral contents such as Na, K ,Ca , Mg, and Fe.

II. MATERIAL AND METHODS

Seed Collection

Dried seed of Hargel were collected from the local market of Abo Hamad city in the River Nile State in the north of Sudan. The seeds were cleaned to remove damaged seeds, wood, leaves, stones, dust and any other foreign materials. Cleaned seeds were stored in black plastic bags and transported to the laboratory and characterized as received.

Proximate Composition of *Solenostemma Argel* Seeds

Moisture content, ash content, crude protein, crude fiber, fat content and carbohydrate were obtained based on the method described by AOAC [8]. Physicochemical properties and mineral content of the oil were determined according to Person [9].

Determination of Moisture Content in Seeds

2 grams of crushed sample were weighed accurately in clean preheated and cooled aluminum dish, heated at 100-105°C for one hour. The dish was covered while still in the oven, transferred to desiccators, and weighed after cooling. The moisture content was calculated from duplicate experiments as the loss of weight divided by the weight of the sample percent, as show in the following expression

$$Moisture\ content\ \% = (W - W1) \times 100 / W\ (g)$$

Where:

W= weight of the sample

W1= weight of the sample after drying.

Ash Content

A sample of 2 g ±1mg was weighed into a preheated, cooled, weight and tarred porcelain crucible and placed into a muffle furnace At 550 to 600 °C until a white gray ash was obtained. The crucible was transferred to desiccators, allowed to cool to room temperature and weighed. After that, the ash content was calculated as a percentage based on the initial weight of the sample.

Calculation:

$$\boldsymbol{Ash}\ (\%) = \frac{[\,(\boldsymbol{Wt\ of\ \ crucible\ +\ Ash}) - (\boldsymbol{Wt\ of\ empty\ crucible})\,]\ \times \mathbf{100\%}}{\boldsymbol{Initial\ weight\ (Wt)}}$$

Crude Protein Content

A sample of 2g was accurately weighed and transferred together with kjeldahl catalysts (Na_2SO_4+ $CuSO_4.5H_2O$) and 8 ml of concentrated sulfuric acid into a Kjeldahl digestion flask. After that, the flask was placed into Kjeldahl digestion unit for about 3 hours until a colorless digest was obtained and the flask was left to cool to room temperature. The

distillation of ammonia was carried out with about 20 ml (40% sodium hydroxide solution into 50 ml boric acid (2%) and mixed indicator (Bromocreasol green + Methyl red) till the boric acid solution turned green. Finally, the distillate was titrated with standard solution of 0.1 N HCL until a brown reddish color was observed.

Calculation:

$$\textbf{\textit{Crude Protein}}\ (\%) = \frac{\textbf{\textit{voluom of HCL}} \times \boldsymbol{N} \times \mathbf{14}}{\textbf{\textit{Sample weight}}\ (\boldsymbol{g}) \times \mathbf{100}} \times \mathbf{100} \times \mathbf{6.25}$$

Where:

N= Normality of HCL

14 = Equivalent Weight of nitrogen.

6.25 = protein conversion factor.

Crude Fiber Content

About 3g ±1mg of a defatted sample (W1) was placed into a crucible and used for estimating the crude fiber by acid and alkaline digestion methods. 150 ml of 0.255N of preheated sulfuric acid were added to each crucible and heated for 30 minutes at the end, the acid was drained and washed with 30 ml distilled water three times. After the drainage of the last wash, the same operation was repeated using 150 ml of 0.313 N sodium hydroxide. After the drainage of the sodium hydroxide, crucibles were washed with 25 ml of acetone. Finally, the crucible was dried at 105°Cfor an hour, cooled in desiccators and weighed. This weight represents the crude fiber and ashes. The crucibles were placed into a muffle furnace at 550°C, started from room temperature, for 3 hours and weighed again after cooling in a desiccator. This weight W3 comprises the ashes only.

Calculations:

$$\%fiber = (W2 - W3/W1) \times 100$$

W1= sample weight (3g)

W2 = crucible weight with fiber and ashes

W3 = crucible weight with ashes

Fat Content

A sample of 5g± 1mg was weighed into an extraction thimble and covered with cotton that previously extracted with n- hexane. Then, the sample was placed into extraction unit and fitted with pre-weighed extraction flask containing about 100 ml n-hexane and the extraction process was conducted for 6 hrs. At the end of the extraction period, the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining hexane extract was put in an oven at 70ºC for 3 hours, to evaporate the remaining hexane, cooled to room temperature in a desiccators, reweighed and the dried extract was registered as fat content.

$$Fat\ content\% = weight\ of\ the\ crude\ fat / weight\ of\ the\ sample$$

Carbohydrate Determination

The carbohydrate content was calculated by subtracting the sum of the contents of moisture, protein lipid, fiber, and ash from 100. It was calculated using the following formula:

$$Carbohydrate\ (\%) = 100 - [Protein\ (\%) + Moisture\ (\%) + Ash\ (\%) + Fiber\ (\%) + Crude\ Fat\ (\%)\ ]$$

Physical and Chemical Properties of the Oil

Determination of Refractive Index

The Refractive index was determined using Abbe Refractometer. Few drops of the oil were placed between the two prisms. The instrument was left to stand for few minutes before reading to equilibrate the sample temperature with that of the instrument. The refractive index of oil was determined at 25°C.

Determination of Specific Gravity

The specific gravity of the oil was recorded using 25 ml density bottle.

Procedure

The density bottle was filled with the oil at room temperature (27°C) and weighed. The density bottle was emptied and thoroughly washed, dried, filled with water and reweighed. The specific gravity was calculated by the following formula:

$$S.G = (c - a/b - a) \times p$$

Where :

S.G =specific gravity.

C = wt of density bottle plus oil .

a = wt of empty density bottle .

b = wt of density bottle plus water at 27°C.

p = density of water at 27°C.

Determination of Viscosity

The viscosity of the oil was measured by capillary tubes using an Ostwald device at room temperature (25°C). The viscosity was determined and calculated using the following equation:

$$\eta_s/\eta_w = d_s t_s/d_w t_w$$

Where; η_s is viscosity of the sample.

η_w = is viscosity of water.

d_s = is density of the sample.

t_s = is time flow of the sample.

d_w = is density of water .

t_w = is time flow of water.

Determination of Peroxide Value (PV)

Oil sample (2 g) were weighed into 250 ml conical flask and dissolved into 30 ml of a mixture (2:1) of glacial acetic acid and chloroform). Saturated KI solution (0.5 ml) was added to the contents of the flask and shaken well for 2 min. Distilled water (30ml) were added to the content and titrated against $Na_2S_2O_3$ (0.01N) with vigorous shaking until the blue color disappeared. The experiment was repeated two times and the mean and standard deviation were calculated and the peroxide value was determined using the following equation:

$$Peroxide\ value\ (Meq\ O_2/Kg) = (V \times N \times 100)/W$$

Where:

V= volume in ml of standard sodium thiosulphate required by the sample

N= normality of sodium thiosulphate solution used.

W= weight in gm of the sample.

Determination of Acid Value

2g of oil were weighed into a 250 ml conical flask and dissolved by addition of 50 ml of 95% ethanol and diethyl ether (1:1). The contents of flask were then shaken until the oil completely dissolved. Two drops of phenolphthalein indicator were added .Finally the content of the flask was titrated with 0.1M ethanolic potassium hydroxide under shaking until a pink color appeared. The volume of 0.1M ethanolic KOH used in titration was recorded .The acid value was calculated as follows:-

$$Acid\ value = 56.1 \times A \times M/W$$

Where:

A= volume of ethanolic potassium hydroxide solution.

M = Morality of ethanolic potassium hydroxide solution.

W = W=weight of the sample in (gram).

Determination of Saponification Value

2g of the oil were weighed into a 250ml saponification flask and then 25ml of 0.5 M ethanolic potassium hydroxide solution were added to the flask. The flask was connected to a condenser and the mixture was refluxed for one hour shaking from time to time. The solution, while still warm was titrated with 0.5M hydrochloric acid solution in presence of phenolphthalein as indicator. A blank experiment was carried out by refluxing without sample, under the sample conditions. The saponification value was calculated as follows

$$Saponification\ value = 56.1\ (b - a)\ \times M/W$$

Where:

b= volume of hydrochloric acid solution required for the titration of the blank.

a= volume of hydrochloric acid solution required for the titration of the sample.

M = morality of the hydrochloric acid solution

W=weight of the sample in (grams).

Iodine Value (IV)

0.25g of the sample was taken into an Erlenmeyer flask and weighed accurately to 0.01 mg digits. 20 ml of cyclohexane were added to dissolve the sample. 25 ml of Wijs solution was measured, exactly, with volumetric pipette and were added to the sample and the flask was closed with a stopper and left into dark room for 30 minutes. 20 ml of 1 mol/ L potassium iodide solution and 100 ml of pure water were added to the content of the flask. Then the contents of the flask were titrated against 0.1 N sodium thiosulfate standard solution. The blank sample was treated by

the same method. Iodine value was calculated by the following formula:

$$Iodine\ value = (B - S) \times N \times 12.69 / M$$

Where

B= titrant volume of the blank

S= volume of the sample

N= normality of the potassium thiosulfate

M= weight of the sample.

Determination of the Minerals

1 g sample was weighed and transferred quantitatively into a 250 ml conical flask and thereafter, digested with 10 ml of the digested acids mixture(ratio 1:2:2 of perchloric, nitric and sulphuric acids) with heating on a hot plate in a fume hood until evolution of white fumes . The digest was allowed to cool and 20 ml of distilled water was added to bring the metals into solution, and filtered using ashless Whatman filter paper into a 100 ml calibrated volumetric flask and made up to mark with distilled water . The digests were subsequently analyzed for Na, K, using flame spectrophotometer (manufacturer: GENWAY, UK, Model: PF7). Ca, Mg and Fe were determined using atomic absorption spectrophotometer (manufacturer: SHIMADZN, Japan AA6880).

Fatty Acid Composition

Sample Preparation

2ml of the oil sample were mixed thoroughly with 7 ml of alcoholic sodium hydroxide (NaOH) 2% (W/W). 7 ml of 1% (W/W) alcoholic sulfuric acid were then added. The mixture was then shaken for 5 minutes. The content of the test tube was left to stand overnight. 1 ml of super saturated sodium chloride (NaCl) was then added and the content was shaken. 2 ml of normal hexane was added and the content was shaken thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe. 5 ml from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filter using 0.45 mm whatman filter paper and dried with 1 g of anhydrous sodium sulphate as drying agent and 1 ml of the diluted sample was injected into the GC/MS instrument [10].

GC/MS Conditions

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-ULtra) from japans shimadzu company, with serial number 02052510156SSAand capillary column(Rtx-5ms-30mx0.25pm).The sample was injected by using split model, helium as the carrier gas passed with flow rate 1.61ml/min ,the temperature program was started from 60°C with rate 10°C/min to 300°C as final temperature degree with 3 minutes hold time, the injection port temperature was 300°C as ,the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-300 charges to ratio and the total run time was 27 minutes, identification of components for the sample with achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National institute of standards and Technology (NIST), then results were recorded [10].

III. RESULTS AND DISCUSSION

Proximate Composition

Table 1 and **fig. 1** show the results of proximate analysis of *Solenostemma Argal* seeds. The highest value was observed for carbohydrate (43.3%) indicating the high energy content of *Solenostemma Argal* seeds. Whereas, the lowest value was obtained for the ash (6%). The moisture content of seeds was 7%. Ash content is important to determine the concentration of heavy metals and expresses the degree of purification. Whereas, high water content affects the shelf life of the seeds [10]. Crude protein content for the seeds of *Solenostemma Argal* was found 30.7% which is higher than the values (19.5 – 29%) reported for some Sudanese seeds by Elsayed *et al* [11].The oil content of the seeds under the study was found 13.33% which is in the range (3.2 – 21.3%)

reported for some Sudanese seed oils by Mariod and Matthäus [12].

Table 1. Proximate Compositions of *Solenostemma Argal* Seeds.

No	The Test	The Result (%)
1	Moisture Content	7
2	Ash Content	6
3	Crude Protein Content	30.7
5	Fat Content	13
6	Carbohydrate	43.3

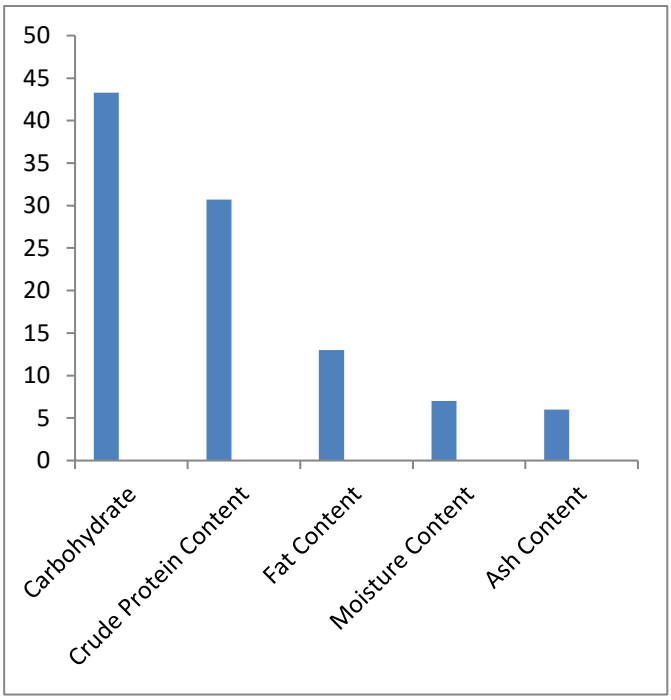

Fig. 1. Proximate Compositions of *Solenostemma Argal* Seeds

Physicochemical Properties of *Solenostemma Argal* seeds oil

Physical and chemical properties of *Solenostemma Argal* seed oil are listed in **Table 2.**

The Refractive Index

The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation [10].The refractive index of *Solenostemma Argal* seeds oil was found 1.4 which is similar to the common values that are observed to some Sudanese edible oil seeds [13].

The Density

Solenostemma Argal seeds oil exhibited, relatively, low value of density (0.9104 g/cm^3) and high viscosity (0.5183). Viscosity is directly proportional with the content of saturated fatty acids [11].

Acid Value

The concentration of free fatty acids in *Solenostemma Argal* seeds oil was found 10 mg KOH/g. Acid value is used to quantify the amount of acids present in the oil and indicates its level of freshness. The high acid value, obtained in this study, indicates the hydrolysis of the triglycerides and the fresh less of the oil [12].

Peroxide Value

The peroxide value (PV) measures the extent of primary oxidation of oils (rancidification). It is considered as initial parameter of oxidative rancidity **[13]**. High values of PV are indicative of high levels of oxidative rancidity of the oils and also suggest absence or low levels of antioxidant. The WHO/FAO (1994) stipulated a permitted maximum peroxide level of not more than 10 equivalents of oxygen/kg of the oils [14]. The obtained value of PV in this study (0.03mequivalent of oxygen/kg of the oils) is very low and hence indicates the high oxidative stability and antioxidant contents of *Solenostemma Argal* seeds oil [15].

Iodine Value

The iodine value is directly proportional to the number of unsaturation bonds in the oil and hence, reflects the susceptibility of the oil to oxidation. Iodine value less than 100 gI_2/100g of oil are non-drying oils [16]. This study revealed that *Solenostemma Argal* seed oil is classified among drying oils, since it had high iodine value (121 gI_2/100g oil). Also, the high iodine value indicates that *Solenostemma Argal* seed oil has a high content of unsaturated fatty acids and this was also shown in the present study by determination of fatty acid composition of *Solenostemma Argal* seeds.

Saponification Value

Saponification value is, inversely, proportional to the length of the carbon chains of the fatty acids. High saponification value enhances the quality of the oil because it shows the presence of lower molecular weight components in 1 g of the oil which will yield more energy on combustion [13].The saponification value obtained for *Solenostemma Argal* seed oil (197 g KOH/g oil) indicates that the majority of the fatty acids in the oil are of medium chin (C16 and C18) which was confirmed by the GC/Ms analysis explained before.

Table 2. Physical and chemical properties of *Solenostemma Argal* seeds oil

The Test	Refractive Index	Density (g/cm^3)	Acid value Mg KOH/l g	Peroxide Value Ml equiv O_2/kg	Iodine Value g/100g	Saponification Value Mg KOH/g
Result	1.4	0.9104	10.38	0.03	121	197

Minerals of *Solenostemma Argal* Seeds Oil

Solenostemma Argal oil seeds contained important amounts of minerals as presented in the **Table 3** and exhibited at **fig. 2**. The most abundant mineral was Sodium followed by, Calcium, Iron, Magnesium and Potassium. The *Solenostemma Argal* seeds oil is considered as a good source of sodium.

Table 3. Minerals of *Solenostemma Argal* seeds oil

No	1	2	3	4	5
The Test	Sodium	Calcium	Iron	Magnesium	Potassium
Observed Value mg/100 g oil	133	85.0482	67.4500	24.4533	3.00

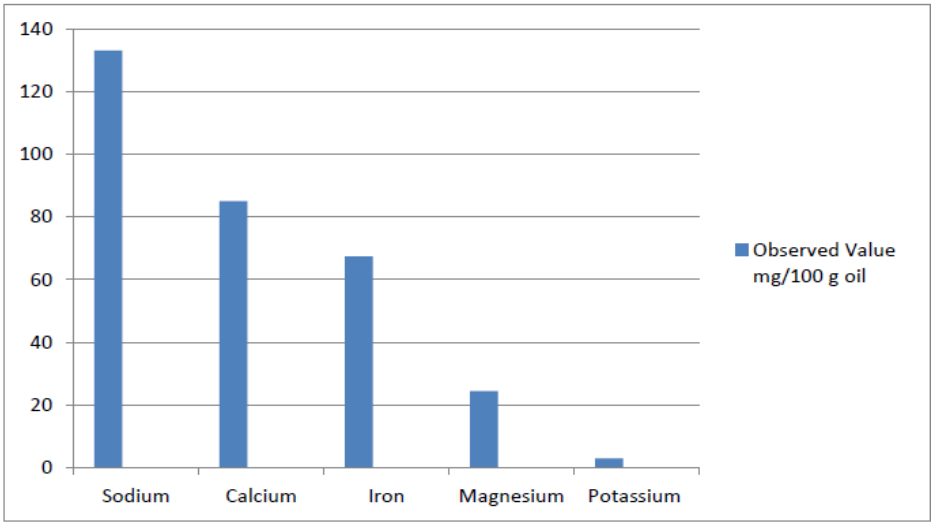

Fig. 2. Minerals of *Solenostemma Argal* seeds oil

Fatty Acid Composition of *Solenostemma Argal* Seeds Oil

The fatty acids in the *Solenostemma Argel* seeds oil were determined using GC/MS analysis. **Table 4** shows the names of fatty acids, their retention times and peak area percentages. This study shows that *Solenostemma Argel* seeds are an excellent source of essential fatty acids. The analysis revealed that *Solenostemma Argel* oil seed contains mixture of saturated and unsaturated fatty acids. The fatty acid profile plays an important role to the chemical properties; therefore, this study provides useful knowledge for further researches.

The results showed that the major component of *Solenostemma Argal* seeds oil, as presented on **fig. 3,** are linoleic acids (44.15%), followed by palmitic acids (15.45%), followed by stearic acids (12.52%), palmitoleic acid (6.76%), Oxiraneoctanoic acid, 3-octyl-,methyl ester (5.35%), oleic acid (4.07%), lignoceric acid (2.70%) and arachidic acids (2.08%). **Fig. 4** illustrates a comparison between the saturated and unsaturated fractions of fatty acids. The total unsaturated fatty acids comprised (54.98%) higher than the saturated fatty acids (38.1%). Other constituents with concentration below 1% appeared in the GC-Ms chromatogram was not considered in this discussion.

Table 4. Fatty Acids of *Solenostemma Argal* seeds oil

Fatty acids	Chemical formula	Acronym	Retention Time	Area %
Linoleic acids	$C_{18}H_{32}O_2$	C18:2	18.368	44.15 %
Palmitic acids	$C_{16}H_{30}O_2$	C16:0	16.474	15.45 %
Stearic acids	$C_{18}H_{36}O_2$	C18:0	18.524	12.52 %
Palmitoleic acid	$C_{16}H_{30}O_2$	C16:1	16.221	6.76%
9,10-Ethoxysteric acid	$C_{19}H_{36}O_3$	C19:0 (9,10 –O-)	20.045	5.35 %
Oleic acid	$C_{18}H_{34}O_2$	C18:1	18.397	4.07%
Lignoceric acid	$C_{24}H_{48}O_2$	C24:0	23.539	2.70 %
Arachidic acids	$C_{20}H_{40}O_2$	C20:0	20.262	2.08%
Total unsaturated fatty acids	-	-	-	54.98%
Total saturated fatty acids	-	-	-	38.1%

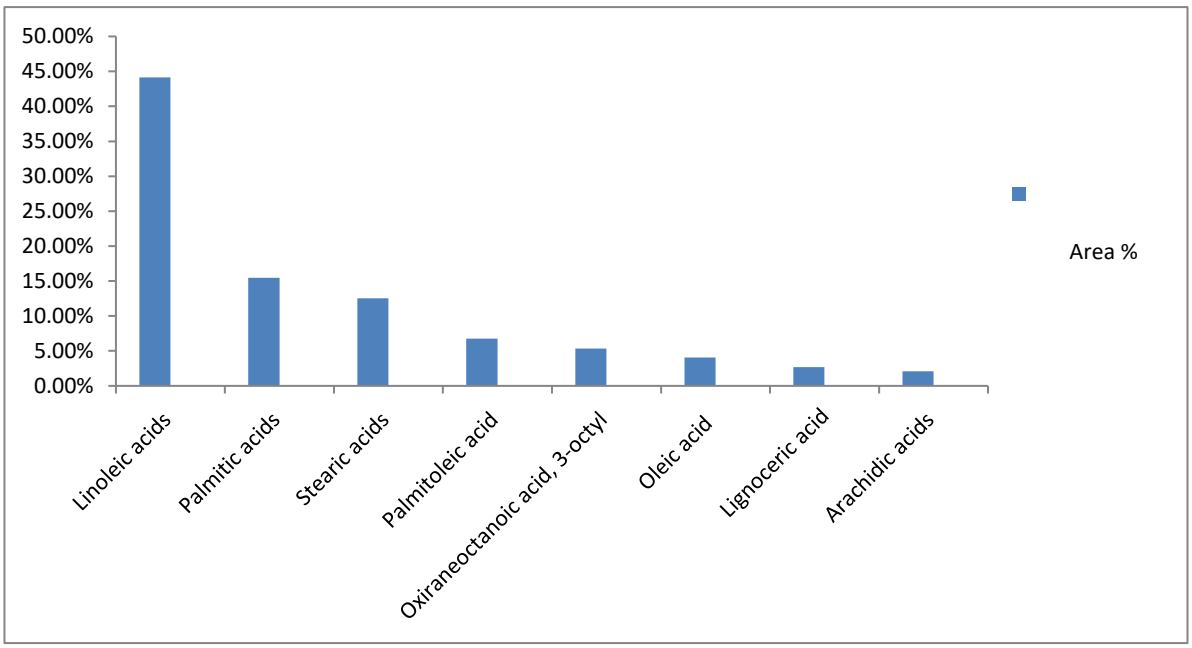

Fig. 3. Fatty Acids of *Solenostemma Argal* seeds oil

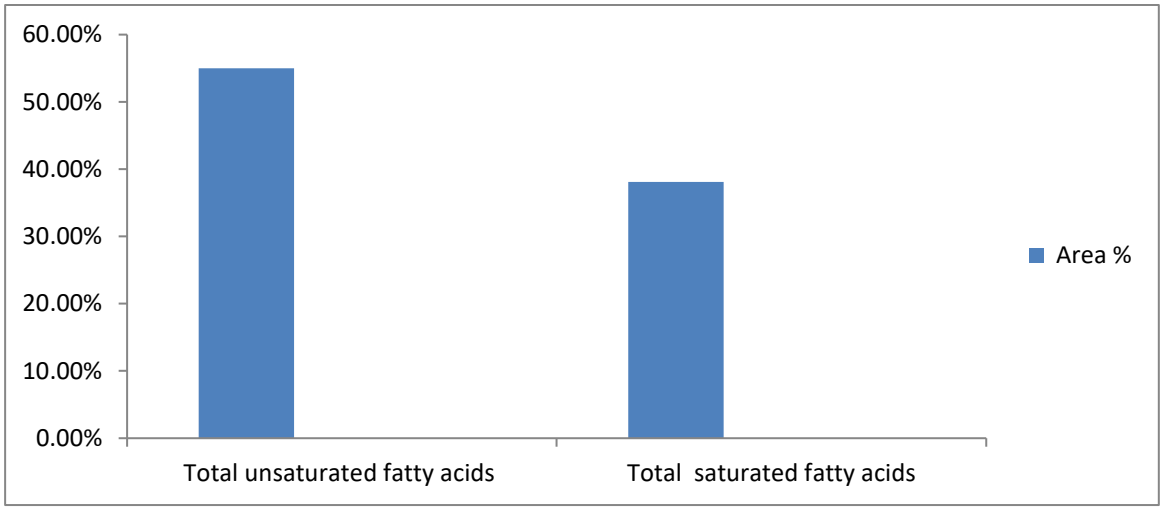

Fig 4. Total unsaturated and saturated fatty acids of *Solenostemma Argal* seeds oil

IV. CONCLUSION

The present study on the chemical composition, physicochemical properties and nutritional value of *Solenostemma Argal* seeds suggests that these seeds could be considered as an alternative source of oil, protein, and carbohydrate. *Solenostemma Argal* seeds oil contains a healthy mixture of fatty acids saturated and unsaturated, Linoleic acid is the highest value (44.15%).

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