*Egypt. J. Chem.* **Vol. 65** No 4 pp. 269 – 277, (2022)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Physiochemical Properties And Phytochemical Characteristics Of Bottle Gourd (*Lagenaria Siceraria*) Seed Oil

**Atef A. Abd El-Rahman [a]*, Akrum Z. Mahmoud[b], Ahmed A. Sayed[b] and Maha A. Abd El latif[b]**

[a]*Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minya 61519, Egypt*
[b]*Animal and Poultry Production Department, Faculty of Agriculture, Minia University, El-Minya 61519, Egypt*

*Corresponding author e-mail: atefnagi2000@yahoo.com; atef.ahmed@mu.edu.eg

**Abstract**

The physicochemical properties and phytochemical constituents of an extracted oil are important indicators for determining its compositional performance. In the current study, to assess the compositional quality of bottle gourd seed oil, physiochemical properties such as percentage yield, color, boiling point, specific gravity, acid value, peroxide value, saponification value, ester value and free fatty acid were estimated. In addition, GC-MS analysis was performed to identify the chemical components of bottle gourd seed oil. Physicochemical analysis revealed oil yield percentage of 47.22%, oil color is dark yellow, boiling point of $142^0$C, specific gravity of 0.77, acid value of 10.77 ± 0.06 (mg/g), peroxide value 0.55 ± 0.04 (mEq/kg), saponification value 71.52 ± 0.03 (mg/g), ester value 60.75 ± 0.03 (mg/g) and free fatty acid 2.23 ± 0.04 (mg/g). GC-MS fragment spectral analysis showed many fatty acids, including palmitic acid, stearolic acid, erucic acid (an omega-9 fatty acid), stearic acid and other active ingredients. According to these findings bottle gourd seed oil can be useful in nutritional approaches and has excellent purity, storage quality and a wide range of health benefits.

*Keywords:* bottle gourd; Lagenaria siceraria; physiochemical; phytochemical; seed oil

## 1. Introduction

Bottle gourd (*Lagenaria siceraria*) has been used as a source of medicinal agents since ancient times [1]. It belongs to the Cucubitaceae family [2], and can be found in tropical and sub-tropical climates [3]. It is used in pharmaceutical and dietary formulations. Bottle gourd is a tropical plant that is cultivated primarily for human consumption as a vegetable on the African continent [4]. Its seeds have high levels of oil, similar to sunflower seeds [5]. Bottle gourd seeds are high in phytochemicals, vitamins, minerals, amino acids, and fatty acids [6], making them a possible source of protein, lipid, micro and macronutrients [7]. Bottle gourd seed oil is used as a vegetable oil substitute in several African countries [3]. Bottle gourd is grown in Egypt for its seeds, which are used to make salad oil [8]. According to reports, bottle gourd seed oil is high in fatty acids and sterolic compounds [5]. It has many health benefits [2], which can be due to the high content of poly unsaturated fatty acids (PUFAs) including linoleic and linolenic acids, which lower the risk of cardiovascular and atherosclerosis diseases by their resistance to oxidation [9]. The compositional content of edible oils was monitored using a variety of physical and chemical parameters. The most important of these parameters are Iodine value (IV), saponification value (SV), viscosity, density, and peroxide value (PV) [10,11]. Gas chromatography (GC), high performance liquid chromatography (HPLC), ultra-visible spectrometry (UV), and fourier transform infrared (FT-IR) Spectroscopic techniques are several analytical methodologies used for quantitative evaluation of oils [12]. The active ingredients in bottle gourd seed oil haven't been extensively investigating. As a result, the aim of this study was to investigate the physicochemical

*Corresponding author e-mail: atefnagi2000@yahoo.com
Receive Date: 19 September 2021, Revise Date: 23 October 2021, Accept Date: 30 October 2021
DOI: 10.21608/EJCHEM.2021.96352.4529

characterization as well as the GC-MS analysis of bottle gourd seed oil extracted with hexane.

## 2. MATERIALS AND METHODS

*2.1. Sample preparation for the oil extraction*

The bottle gourd seeds used in this study were purchased from a local market in El-Minya governorate, Egypt. The seeds were manually shelled after being tested for bad seeds. They were then dried at room temperature and ground with a mechanical grinder. They were then refrigerated at about 4°C for further use.

*2.2. Procedure for extracting oil*

Using the Soxhlet extractor, complete extraction of oil with hexane was obtained. The powdered seed samples were placed in a porous thimble and placed in a Soxhlet extractor with 150 ml of n-hexane (boiling point 40-60°C) as the extracting solvent for 6 hours until the desired quantity was obtained. To remove the excess solvent from the extracted oil, the solvent was evaporated in a rotary evaporator at 55°C. [13].

*2.3. Oil yield as a percentage*

The extracted oil was placed in a beaker. Then put in a water bath for about 2 hours to allow full evaporation of the solvent, and the volume of the oil was measured and expressed as oil content (percent) using the following formula: [14].

$$\text{Oil content (\%)} = \frac{\text{Oil weight (g)}}{\text{Sample initial weight (g)}} \times 100$$

*2.4. Oil color determination*

The observation was used to decide the color of the oil, which was done by many qualified individuals. Color charts were used to correlate oil color [15].

*2.5. Measurement of the boiling point*

A thermometer was used to determine the boiling point of oil samples. The degree of unsaturation of fatty acids determines the boiling point [16].

*2.6. Specific gravity determination*

Briefly, 10 ml of extracted oil was measured in a pre-weighed measuring cylinder. The cylinder and oil were both weighed, and the weight of the oil was calculated by subtracting the cylinder's weight from the total weight of the oil and cylinder. The specific gravity of oil was calculated using equations below [17].

$$\text{Density of water} = \frac{W_1 - W_0}{V_0}$$

Where:

$W_1$ = Weight of empty measuring cylinder + water
$W_0$ = Weight of empty measuring cylinder
$V_0$ = volume of water used

$$\text{Density of oil} = \frac{W_1 - W_0}{V_0}$$

Where:

$W_1$ = Weight of empty measuring cylinder + oil
$W_0$ = Weight of empty measuring cylinder
$V_0$ = volume of oil used

Therefore:

$$\text{Specific gravity} = \frac{\text{Density of water}}{\text{Density of oil}}$$

*2.7. Acid value determination*

In brief, 25 mL of 5% ethanol alcohol was boiled in a water bath to ensure that dissolved gases were removed. 2.5 g of extracted oil was combined with 25 ml of hot ethanol and brought to a boil. The acid value was calculated by adding a few drops of 1 % phenolphthalein indicator and titrating against 0.1 M KOH with continuous shaking until a permanent pink color was obtained. The acid value was calculated using the formula below [18].

$$\text{Acid value} = \frac{56.1 \times M \times V}{W}$$

Where: M = Concentration of KOH
V = Titre value
56.1 = Molecular weight of KOH
W = weight of oil sample (g)

*2.8. Peroxide value determination*

2 g of extracted oil was dissolved in 22 ml of a solution containing 12 ml chloroform and 10 ml acetic acid. 0.5 ml of saturated potassium iodide was added to the flask. The flask was corked and left for 1 minute with intermittent shaking. The mixture was then diluted with 30 ml distilled water and titrated against 0.1N $Na_2S_2O_3$ until the yellow color was almost gone. 0.5 mL starch indicator was quickly applied, and the titration was continued until the blue color was almost completely gone. At the same time, a blank titration was carried out. Using the formula below, the peroxide value was determined. [19].

$$\text{Peroxide value} = \frac{(S-B) \times N \times 1000}{W}$$

Where:

Peroxide value = Meq peroxide per 100g of sample
S = volume of titrant (ml) for sample
B = volume of titrant (ml) for blank
N = Normality of $Na_2S_2O_3$ solution (mEq/ml)

1000 = conversion of units (g/kg)
W = weight of oil sample (g)

*2.9. Saponification value determination*

Around 2 g of extracted oil was placed in a flask with 30 ml ethanolic KOH and condensed for 30 minutes to ensure that the sample was fully dissolved. Following the cooling of the samples, 1 ml of phenolphthalein was applied and titrated with 0.1 M HCl until a pink endpoint was reached. Saponification value was calculated using the following formula [20].

$$\text{Saponification value} = \frac{(S-B) \times M \times 56.1}{W}$$

Where: S = sample titre value
B = blank titre value
M = Molarity of the HCl
56.1 = Molecular weight of KOH
W = weight of oil sample (g)

*2.10. Ester value determination*

Ester value was calculated using the following formula [20].

Ester Value = Saponification Value – Acid Value

*2.11. Free fatty acid determination*

In a 250 ml Erlenmeyer flask, 2.0 g of oil was weighed, 100 ml of ethanol was added, and 2 ml of phenolphthalein indicator was added. The mixture was shaken and titrated against 0.1M NaOH with constant shaking until the endpoint was reached, which was marked by a faint pink color that lasted 30 seconds. The free fatty acid was expressed using the following formula [21].

$$\% \text{ FFA} = \frac{V \times M \times 282}{W} \times 100$$

Where: % FFA = Percent free fatty acid (g/100g)
V = Volume of NaOH (ml)
M = Molarity of NaOH
282 = Molecular weight of oleic acid
W = weight of oil sample (g)

*2.12. GC-MS analysis*

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) equipped with a split–splitless injector. The initial column temperature was kept at 50 °C for 3 min (isothermal) and programmed to 300 °C at a rate of 5 °C/min, and kept constant at300 °C for 10 min (isothermal). Injector temperature was 280 °C. Helium carrier gas flow rate was 1.41 ml/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 220°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15).

## 3. RESULTS AND DISCUSSION

*3.1. Physicochemical analysis*

Physicochemical properties such as percentage yield, color, boiling point, specific gravity, acid value, peroxide value, saponification value, ester value, and free fatty acids were used to assess the quality of extracted bottle gourd seed oil. The results of the physicochemical analysis are shown in Table 1 indicates the percentage yield of the oil was found to be 47.22%, which is so close with the oil percent (44.83%) found in a pervious study [22]. The color of the oil was dark yellow, the boiling point of the oil to be $142^0$C, the oil's specific gravity was 0.77, which is within the acceptable range of less than 1.0. [23] because the oils with lower values of specific gravity are highly appreciable to consumers.

Table 1
Physicochemical properties of *Lagenaria siceraria* seed oil extracted with n-hexane.

| Properties | Obtained values ± SEM | Standard values Ref. [23] |
|---|---|---|
| Oil yield (%) | 47.22 ± 0.01 | |
| Color | dark yellow | |
| Boiling point ($^0$C) | 142 | |
| Specific gravity | 0.77 | Less than 1.0 |
| Acid value (mgKOH/g) | 10.77 ± 0.06 | 0.2 – 50mg/g |
| Peroxide value (mEq/kg) | 0.55 ± 0.04 | up to 15 mEq/kg |
| Saponification value (mgKOH/g) | 71.52 ± 0.03 | 170 – 260 mg/g |
| Ester value (mgKOH/g) | 60.75 ± 0.03 | |
| Free fatty acids | 2.23 ± 0.04 | 0.4 – 45 mg/g |

The acid value of a vegetable oil is a significant indicator of its quality. It is showing the free fatty acid present in the oil and expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil. The higher the acid value of an oil, the lower its storage quality [19], whereas, the lower acid value signifies a maximum purity [13]. Thus, the obtained acid value (10.77 ± 0.06 mg/g) (Table 1) which are within the range of vegetable oil's standard value of 0.2-50 (mg/g), shows that bottle gourd seed oil has an excellent purity and storage quality.

The peroxide value is an indicator of the degree to which rancidity reactions occurred during storage, and measured deterioration of oil from oxidation [21], it can be used to determine the consistency and

stability of fats and oils [24]. Therefore, the low obtained peroxide value 0.55 ± 0.04 (mEq/kg), which are within the range of the standard value of vegetable oil (up to 15 mEq/kg), indicates that the oil can be kept for a very long period of time [25].

The saponification value is a measure of the average molecular mass of fatty acids in a sample of oil [16]. A higher saponification value means that the oil is suitable for soap making [13]. The saponification value obtained for the oil sample in Table 1 showed 71.52 ± 0.03 (mg/g) which was less than the standard value for vegetable oil of 170 – 260 mg/g. As a consequence of this finding, this oil cannot be used to make soap. The amount of milligram of potassium hydroxide needed to saponify the esters in 1.0 g of the substance is known as the ester value. It can be calculated by subtracting the acid value from the saponification value. The obtained ester value (Table 1) was 60.75 ± 0.03 (mg/g). The amount of free fatty acids in an oil decides its suitability for human or industrial use. The hydrolysis of oils and fats produces free fatty acids (FFA). Since oils and fats are exposed to different conditions such as storage, refining, heating, or frying, the amount of FFA varies with time, temperature, and moisture content. FFAs are more vulnerable to oxidation and rancidity than neutral oils because they are less stable. As a result, FFA is an important factor in determining the consistency and commercial value of oils and fats [26]. The result of free fatty acids showed in Table 1 was found to be 2.23 ± 0.04 (mg/g) which is within the normal range of 0.4 – 45 mg/g, meaning that there is a small amount of free fatty acid in this oil. Thus, this oil is ideal for human and animal consumption due to its low free fatty acid content.

*3.2. GC-MS analysis*

GC-MS analysis was used to examine the main phytochemicals in bottle gourd seed oil. After separation depending on retention times, different groups of compounds were used to classify the chemical constituents obtained: alkanes, n-alkanes, cycloalkanes, aromatic hydrocarbons, bicyclic hydrocarbons, esters, fatty alcohols, saturated fatty acids, unsaturated fatty acids, triterpenes and vitamins. The phytochemicals found in bottle gourd seed oil, as well as their formula, are mentioned in Table 2. By using the percentage area as a guide, the analysis demonstrated the existence of Stearolic acid (9-Octadecynoic acid) (16.97 %), Decane (12.08%), Erucic acid (7.99%), Cycloeucalenol acetate (9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.) (7.17%), Toluene (6.82%) and Squalene (3.24%) as the major phytochemicals. Additionally, mass spectral analysis showed the existence of saturated fatty acids such as Palmitic acid (n-Hexadecanoic acid) (0.89%) and Stearic acid (Octadecanoic acid) (0.88%), as well as fat-soluble vitamins such as gamma tocopherol (1.34%). (the major form of vitamin E in many plant seeds) in bottle gourd oil (Figure 1 and Table 2). A recent investigation [27] identified in bottle gourd seed oil the following fatty acid composition: palmitic acid ($C_{16}H_{32}O_2$), stearic acid ($C_{18}H_{36}O_2$), eicosadenoic acid ($C_{21}H_{38}O_2$), linoleic acid ($C_{19}H_{34}O_2$), oleic acid ($C_{18}H_{34}O_2$), erucic acid ($C_{22}H_{42}O_2$), arachidic acid ($C_{20}H_{40}O_2$), and behhenic acid ($C_{23}H_{46}O_2$). These results are consistent with those found in the current investigation. The most abundant component found in the investigated oil is 9-Octadecynoic acid ($C_{18}H_{32}O_2$) (Figure 1 & Table 2). It's also known as Stearolic acid and it's an 18-carbon, unbranched acetylenic fatty acid (fatty acids which contain a triple bond). It has a triple bond at position 9 and is known to be cytotoxic and DNA binding agent. It has none of the structural characteristics of known DNA binding molecules and may bind to DNA via a novel mechanism [28]. To our knowledge, Stearolic acid was discovered in bottle gourd seed oil for the first time in the current investigation. In an *in vitro* antifungal examination, 9-octadecynoic acid and other acetylenic acids were found to have antifungal activity related to their chain lengths and positional triple bonds [29]. As a result of its Stearolic acid content, bottle gourd seed oil appears to have antimicrobial characteristics. Endogenous linear hydrocarbons, such as n-alkanes, are found in vegetable oils. Different vegetable oils, as well as oils of the same form but different varieties and provenience, have distinct n-alkane patterns that can be used to identify them [30]. In the current study Decane $CH_3(CH_2)_8CH_3$ as an acyclic saturated hydrocarbon and one of the n-alkanes was found in the investigated oil (Figure 1 and Table 2). Plants and other living organisms contain a large number of n-alkanes. Plants are thought to produce n-alkanes by elongating a preformed fatty acid and then losing the carboxyl carbon. The predominance of alkanes with an odd carbon number strongly indicates that fatty acid precursors only lose one carbon during conversion to alkanes [31, 32].

Endogenous hydrocarbons have been shown to have discrimination potential in various studies. Indeed, the n-alkane profile can be used to decide if expensive edible oils are genuine or whether they've been blended with cheaper oils. Furthermore, the qualitative/quantitative n-alkane profile can be used to differentiate between crude and refined oils of different plant origins, as well as oils from different olive cultivars and/or proveniences [33, 34]. Erucic acid ($C_{22}H_{42}O_2$), was also detected in the spectra. (Figure 1 and Table 2). It is a monounsaturated omega-9 fatty acid and a major feedstock for the oleochemical industry It is a monounsaturated very long-chain fatty acid with a 22-carbon backbone and

a single double bond in the cis- structure that originates from the 9th position from the methyl end [35].

Table 2
Phytochemical constituents of bottle gourd *Lagenaria siceraria* seed oil

| Peak No | Retention Time (min) | Name of Compound | Molecular Formula | Nature of Compound | Peak Area (%) |
|---|---|---|---|---|---|
| **1** | 3.092 | Heptane, 2-methyl- | $C_8H_{18}$ | Alkanes | 1.63 |
| **2** | 3.185 | Toluene | $C_7H_8$ | Aromatic hydrocarbons | 6.82 |
| **3** | 3.337 | Cyclohexane, 1,3-dimethyl-, cis- | $C_8H_{16}$ | Cycloalkanes | 1.74 |
| **4** | 3.697 | Hexane, 2,4-dimethyl- | $C_8H_{18}$ | Alkanes | 1.69 |
| **5** | 7.774 | Cyclohexane, 1,1,2,3-tetramethyl- | $C_{10}H_{20}$ | Cycloalkanes | 0.55 |
| **6** | 8.057 | Nonane, 2-methyl- | $C_{10}H_{22}$ | Alkanes | 0.70 |
| **7** | 8.261 | Nonane, 3-methyl- | $C_{10}H_{22}$ | Alkanes | 0.35 |
| **8** | 8.634 | Cyclohexane,1-methyl-4-(1-methylethyl)-, trans- | $C_{10}H_{20}$ | Cycloalkanes | 0.65 |
| **9** | 8.723 | 1-Methyl-4-(1-methylethyl)-cyclohexane | $C_{10}H_{20}$ | Cycloalkanes | 0.55 |
| **10** | 9.182 | Decane | $C_{10}H_{22}$ | n-Alkanes | 7.96 |
| **11** | 9.887 | Decane, 4-methyl- | $C_{11}H_{24}$ | Alkanes | 1.18 |
| **12** | 10.180 | Cyclohexane, butyl- | $C_{10}H_{20}$ | Cycloalkanes | 1.02 |
| **13** | 10.908 | Naphthalene, decahydro-, trans- | $C_{10}H_{18}$ | Bicyclic hydrocarbons | 1.60 |
| **14** | 10.998 | Decane, 5-methyl- | $C_{11}H_{24}$ | Alkanes | 0.87 |
| **15** | 11.095 | Decane, 4-methyl- | $C_{11}H_{24}$ | Alkanes | 0.67 |
| **16** | 11.207 | Decane, 2-methyl- | $C_{11}H_{24}$ | Alkanes | 1.94 |
| **17** | 11.415 | Decane, 3-methyl- | $C_{11}H_{24}$ | Alkanes | 0.79 |
| **18** | 11.825 | Cyclohexane, 1-methyl-2-pentyl- | $C_{12}H_{24}$ | Cycloalkanes | 0.54 |
| **19** | 12.351 | Decane | $C_{10}H_{22}$ | n-Alkanes | 12.08 |
| **20** | 12.666 | Naphthalene, decahydro-2-methyl- | $C_{11}H_{20}$ | Bicyclic hydrocarbons | 0.54 |
| **21** | 12.888 | Oxalic acid, propyl undecyl ester | $C_{16}H_{30}O_4$ | Esters | 0.67 |
| **22** | 13.190 | 2-Tridecen-1-ol, (E)- | $C_{13}H_{26}O$ | Fatty alcohols | 1.00 |
| **23** | 13.435 | Cyclohexane, pentyl- | $C_{11}H_{22}$ | Cycloalkanes | 0.94 |
| **24** | 14.079 | Decane, 2-methyl- | $C_{11}H_{24}$ | Alkanes | 1.12 |
| **25** | 14.204 | Undecane, 4-methyl- | $C_{12}H_{26}$ | Alkanes | 0.74 |
| **26** | 14.336 | Undecane, 2-methyl- | $C_{12}H_{26}$ | Alkanes | 1.86 |
| **27** | 14.540 | Tridecane, 3-methyl- | $C_{14}H_{30}$ | Alkanes | 1.02 |
| **28** | 15.449 | Decane | $C_{10}H_{22}$ | n-Alkanes | 8.91 |
| **29** | 15.857 | Undecane, 2,6-dimethyl- | $C_{13}H_{28}$ | Alkanes | 0.87 |
| **30** | 17.592 | Octane, 2,3,3-trimethyl- | $C_{11}H_{24}$ | Alkanes | 0.53 |
| **31** | 34.398 | n-Hexadecanoic acid (Palmitic acid) | $C_{16}H_{32}O_2$ | Saturated fatty acids | 0.89 |
| **32** | 37.868 | 9-Octadecynoic acid (Stearolic acid) | $C_{18}H_{32}O_2$ | Unsaturated fatty acids | 16.97 |
| **33** | 37.928 | Erucic acid | $C_{22}H_{42}O_2$ | Unsaturated fatty acids | 7.99 |
| **34** | 38.261 | Octadecanoic acid (Stearic acid) | $C_{18}H_{36}O_2$ | Saturated fatty acids | 0.88 |
| **35** | 48.974 | Squalene | $C_{30}H_{50}$ | Triterpenes | 3.24 |
| **36** | 52.249 | gamma-Tocopherol | $C_{28}H_{48}O_2$ | Vitamins | 1.34 |
| **37** | 56.379 | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-Cycloeucalenol acetate | $C_{32}H_{52}O_2$ | Triterpenes | 7.17 |

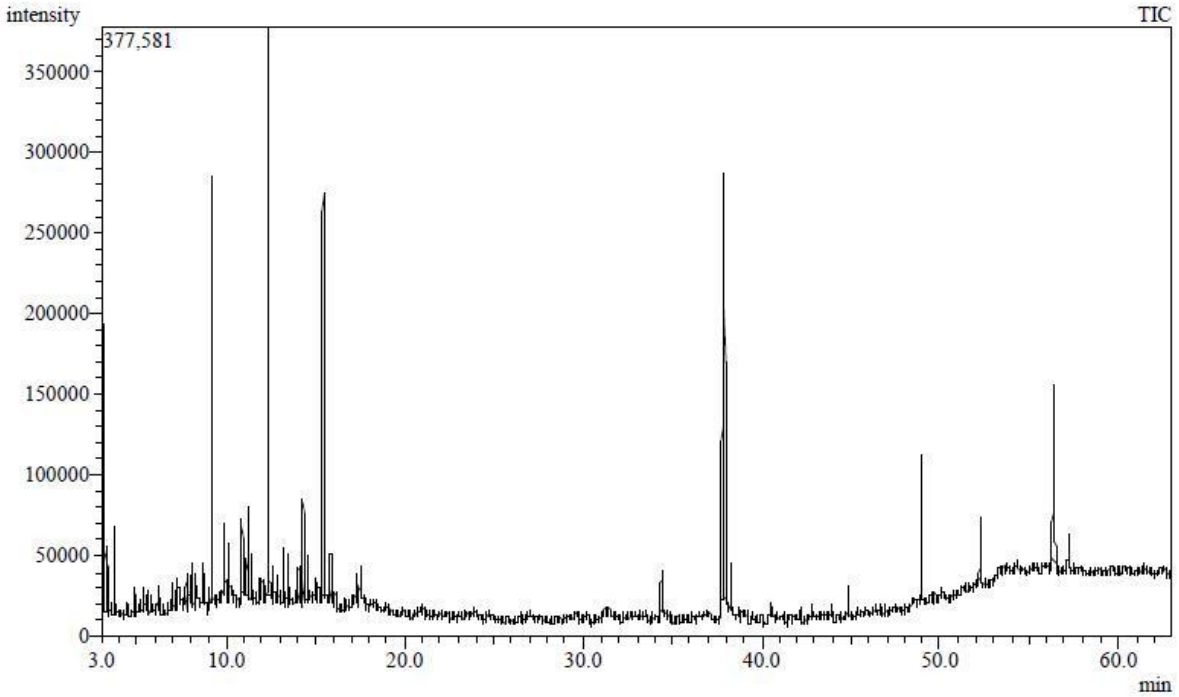


Fig. 1. GC-MS analysis of the of bottle gourd *Lagenaria siceraria* seed oil

Erucic acid levels in food can be harmful to one's health. Fatty degeneration of the heart (myocardial lipidosis), in which fats (lipids) accumulate in the heart tissue, is one of erucic acid's health-damaging impact. On the other hand, a recent study [36] found that erucic acid-rich plant oil reduced adipose tissue inflammation and insulin resistance. As a result of its erucic acid content, bottle gourd seed oil appears to have health benefits characteristics. Because of its erucic acid content, bottle gourd seed oil appears to have health-promoting benefits, according to the findings of this study (Table 2). One of the most common components found in the investigated oil is cycloeucalenol acetate. It is a triterpene with the molecular formula $C_{32}H_{52}O_2$. This compound was found to have potent antimicrobial properties against a wide range of bacterial and fungal strains [37], which can be due to its structural components, such as the acetoxyl groups and the oxygen role [38]. Its strong antimicrobial activity may also be due to the presence of methyl and carbonyl groups [39].

Toluene ($C_7H_8$), is also one of the most essential components contained in the investigated oil (Figure 1 and Table 2). It is also known as methyl benzene, is an aromatic hydrocarbon that can be used to melt a variety of materials and can also be used as a solvent due to its high dissolving strength [40].

Squalene ($C_{30}H_{50}$), one of the most common triterpenoids was found in the present investigated oil (Figure 1 and Table 2). It is a triterpenoid that occurs naturally and serves as a biochemical precursor to sterols. It was first discovered in shark liver oils [41], and it has since been shown to have important medical, therapeutic, and cosmetic uses [42, 43]. It was recently discovered to be a possible conjugate in anticancer drug development [44, 45]. Such interest has prompted efforts to classify squalene sources other than shark liver as a possible source, as well as the need for a rapid method for accurate squalene determination [46]. Squalene was detected as one of the major phytochemicals by GC analysis in the current study by 3.24 %, indicating that the oil under study has medicinal value.

Palmitic acid $CH_3(CH_2)_{14}COOH$ also known as n-Hexadecanoic acid, is one of the most commonly found saturated fatty acids in the analyzed oil (Figure 1 and Table 2). It is the main component of palm oil. In recent decades, due to the high palmitic acid content of palm oil, controversial studies have identified possible harmful effects of palm oil [47,48]. It has long been vilified for its alleged negative health effects, obscuring its many important physiological functions. It is the most common saturated fatty acid in the human body, accounting for 20–30% of total fatty acids. It can be obtained from food or synthesized endogenously by de novo lipogenesis (DNL) [49]. Several reports, on the other hand, support the positive effects of palmitic acid. A study of postmenopausal women discovered a protective link between palmitic acid intake and breast cancer risk [50]. Furthermore, the connection between palmitic acid consumption and cancer has shown controversial results. Eventually, unique fatty acids such as palmitic acid can play a key role in tumor growth regulation [51].

Stearic acid $CH_3(CH_2)_{16}COOH$, also known as octadecanoic acid, is another of the most common saturated fatty acids present in the studied oil.

Spectral results showed stearic acid (Figure 1 and Table 2), It is found in many animal and plant fats as a glycerol ester. [52]. It is found in higher concentrations in animal fat (up to 30%) than in vegetable fat (typically 5%). Cocoa butter and shea butter are notable exceptions, with stearic acid content (as a triglyceride) ranging from 28 to 45 %. Stearic acid, unlike the other long-chain saturated fatty acids, has no effect on the levels of lipoprotein cholesterol in men or women [53]. A study found that dietary stearic acid reduces thrombogenic and atherogenic risk factors, and proposed that the food industry consider adding stearic acid to foods [54].

The ratio of unsaturated to saturated fatty acids ratio (UFA:SFA ratio) is an essential factor in fat and oil nutritional properties. The UFA/SFA index is typically used to evaluate the effect of diet on cardiovascular health. It is hypothesized that all unsaturated fatty acids in the diet will lower low-density lipoprotein cholesterol (LDL-C) and serum cholesterol levels, while all saturated fatty acids can boost serum cholesterol levels. As a result, the higher the ratio, the more beneficial the impact [55]. Thus, the UFA:SFA ratio of the examined oils in the current study was calculated, and the oil had a high UFA:SFA ratio of 14.1:1. Furthermore, a recent animal study found that increasing the unsaturated fatty acid to saturated fatty acid ratio (UFA:SFA ratio) leads to a curvilinear increase in digestible energy (DE) values. [56].

In human plasma, vitamin E is the strongest lipid-soluble antioxidant. Alpha, beta, gamma, and delta tocopherols, as well as alpha, beta, gamma, and delta tocotrienols, are dietary components with antioxidant activity of vitamin E. Tocopherols and tocotrienols are compounds with a chromanol ring bound to a saturated phytyl (tocopherols) or unsaturated phytyl (tocotrienols), and the number of methyl groups on the chromanol ring varies [57]**.** Gamma tocopherol ($C_{28}H_{48}O_2$) is the major form of vitamin E (fat-soluble vitamin) in many plant seeds was detected in the analyzed oil (Figure 1 and Table 2). Among the isomers of vitamin E, gamma-tocopherol is thought to be the most effective free radical fighter. Furthermore, studies show that gamma-tocopherol has potent anti-inflammatory properties and is linked to carcinogenesis inhibition [58].

## 4. Conclusion

The physico-chemical and GC-MS analyses revealed that the bottle gourd seed oil has good quality. Peroxide value, acid value, and free fatty acid levels in physiochemical analyses were all within the appropriate range for excellent storage properties. Bottle gourd seed oil, according to GC-MS analysis, contains a variety of active compounds, including Cycloeucalenol acetate, Stearolic acid, Vitamin E, Erucic acid, and Squalene, which respectively have antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer properties. As a result of these findings, bottle gourd seed oil suggests to be useful in nutritional and pharmacological approaches and has excellent purity, storage stability, and a wide range of health benefits.

## 5. Conflict of interests

The author declare that they have no competing interests.

## 6. References


1. Chimonyo V.G.P., Modi A.T. Seed performance of selected bottle gourd (*Lagenaria siceraria* (Molina) Standl.). *American J. Exp. Agric*., 3(4), 740- 766 (2013).
2. Milind P., Satbir K. Is bottle gourd a natural guard?? Int. Res. J. Pharm., 2, 3–17 (2011).
3. Grubben G.J.H., Denton O.A. (eds) Plant resources of Tropical Africa 2. Vegetables. - PROTA Foundation, Wageningen, Netherlands I Bakhuys hblishers, Leiden, Netherlands/ CTA, Wageningen, Netherlands. 668 pp. ISBN 90-5782-147-148 (2004).
4. Decker-Walters D.S., Wilkins-Ellert M., Chung S.S., Staub J.E. Discovery and genetic assessment of wild bottle gourd [*Lagenaria siceraria* (MOL.) standley; cucurbitaceae] from Zimbabwe Economic Botany 58(4), 501-508 (2004).
5. Axtell B.L., Fairman R.M. Minor oil crops. *FAO* Agricultural Service Bulletin N°94, FAO, Rome (1992).
6. Hemeda H.M., Ibrahim H.S., Khattab H.A.H. Bakery Fortification and Biological Evaluation of Bottle Gourd Seeds (*Lagenaria siceraria*). Egyptian J. Food Sci., 36(1), 93–113 (2008).
7. Hassan L.G. Sani N.A., Dangoggo S.M. Nutritional Value of Bottle gourd (*Lagenaria siceraria*) Seeds. Global J. Pure and Applied Sci., 14(3), 301– 306 (2008).
8. Hegazy E.M., El Kinawy O.S. Characteristics, of pumpkin and bottle gourd in Egypt and potentially their contamination by mycotoxins and mycotoxigenic Fungi. Journal of American Science, 7, 615-622 (2011).
9. Abramovic H., Butinar B. Nikolic V. Changes occurring in phenolic content, tocopherol composition and oxidative stability of Camelina sativa oil during storage. Food Chem. 104, 903–909 (2007).
10. Ceriani R., Paiva F.R., Alves C.B.G., Batista E.A.C., Meirelles A.J.A. Densities and viscosities of vegetable oils of nutritional value. J. Chem. Eng. Data 53 (8), 1846–1853 (2008).

11. Mousavi K., Shoeibi S., Ameri M. Effects of storage conditions and PET packaging on quality of edible oils in Iran. Adv. Environ. Biol. 6 (2), 694–701 (2012).
12. Che Man Y.B., Syahariza Z.A., Rohman A. Chapter 1. Fourier transform infrared (FTIR) spectroscopy; development techniques and application in the analyses of fats and oils In Ress OJ.(Ed.) Fourier transform infrared (FTIR) spectroscopy, Nova science publishers, New York: USA ,1-26 (2010).
13. Warra A.A., Ukpanukpong R.U., Wawata I.G. Physico-chemical and GC-MS Analysis of Calabash (*Lagenaria siceraria*) seed oil. International Journal of Biochemistry Research & Review, 14(1), 1-7 (2016).
14. Pant K.S., Koshla V., Kumar D., Gairola, G. Seed oil content variation in Jatropha curcas L indifferent altitudinal ranges and site conditions in H.P. India. Lyonia., 11(2):31-34 (2006).
15. Okolie P., Uaboi-Egbenni P.O., Ajekwene A.E. Extraction and quality evaluation of sandbox tree seed huracrepitan oil. World J. Agric. Sci., 8(4),359-365 (2012).
16. Zahir E., Saeed R., Abdul Hameed M., Yousuf A. Study of physicochemical properties of edible oil and evaluation of frying oil quality by Fourier Transform-Infrared (FT-IR) Spectroscopy. Arabian Journal of Chemistry, 10, 3870–3876 (2017).
17. John K. Analytical chemistry for technicians 3rd Edition, Lewis Publisher, Kalyani Publisher, New Delhi, India. Pp 347-349 (2003).
18. AOAC. Official methods of analysis of the association of official analytical chemists, 16th Edition, Gaithersburg, Pp1-3 (1998).
19. Nielsen, S. Food analysis, laboratory manual 3rd Edition Kluwer academic premium. Publishers, London. Pp 88-89 (2003).
20. Akpan U.G., Jimoh D., Mohammed A.D. Extraction, characterization and modification of castor seed oil. Leonardo Journal of science, 8, 43-52 (2006).
21. Chopra S.L., & Kanwar J.S. Analytical agricultural chemistry 4th Edition, New Delhi, Kalyami publishers, Pp 12-15 (1991).
22. Abdulhamid A., Fakai I.M., Sani I., Warra A.A., Bello F. Extraction, physicochemical characterization and phytochemical screening of bottle gourd (*Lagenaria siceraria*) seed oil. Continental J. Food Science and technology, 7:29-34 (2013).
23. Danjuma M.N., Dandago M. Extraction and characterization of calabash (*Lageneria siceratia*) seed oil. Techno Science Africana Journal, 3, 67-69 (2009).
24. Ekwu F.C., Nwagu, A. Effect of processing on the quality of cashew nut oils. J. Sci. Agric. Food Tech. Environ. 4, 105–110 (2004).
25. Ogbuagu M.N. Inhibitory effect of onion and garlic extracts on the rancidity of palm and palm kernel oils. Journal chemistry society of Nigeria, 33, 43-44 (2008).
26. Mahesar S.A., Sherazi S.T.H. Khaskheli A.R., Kandhroc A.A., Uddina S. Analytical approaches for the assessment of free fatty acids in oils and fats. Anal. Methods, 6, 4956–4963 (2014).
27. Warra A.A., Ukpanukpong R.U., Wawata, I.G. Physico-chemical and GC-MS Analysis of Calabash (Lagenariasiceraria) Seed Oil. IJBCRR, 14(1): 1-7 (2016).
28. Berry D.E., Chan J.A., MacKenzie L., Hecht S.M. 9-Octadecynoic acid: a novel DNA binding agent. Chem. Res. Toxicol.4:195-198 (1991).
29. Li X.C., Melissa R., Khan J.S., Khalid A.M., Babu K.S., Agarwal A.K., El-Sohly H.N., Manly S.P., Clark A.M. Potent *In Vitro* antifungal activities of naturally occurring acetylenic acids. Antimicrobial agents and chemotherapy. 52, 2442–2448 (2008).
30. Srbinovska A., Conchione C., Ursol L.M., Lucci P., Moret S. Occurrence of n-Alkanes in vegetable oils and their analytical determination. Foods, 9,1-19 (2020).
31. Bognar A.L., Paliyath G., Rogers L., Kolattukudy P.E. Biosynthesis of alkanes by particulate and solubilized enzyme preparations from pea leaves (*Pisum sativum*). Arch. Biochem. Biophys., 235, 8–17 (1984).
32. Samuels L., Kunst L., Jetter R. Sealing plant surface: Cultivar wax formation by epidermal cells. Annu. Rev. Plant. Biol., 59, 683–707 (2008).
33. Koprivnjak O., Moret S., Populin T., Lagazio C., Conte L.S. Variety decentration of virgin olive oil based on n-alkane profile. Food Chem., 90, 603–608 (2005).
34. Mihailova A., Abbado D., Kelly S.D., Pedentchouk N. The impact of environmental factors on molecular and stable isotope compositions of n-alkanes in Mediterranean extra virgin olive oils. Food Chem., 173, 114–121 (2015).
35. Xueyuan Li , Eibertus N, Loo Y., Gruber J., Fan J., Guan R., Frentzen M., Stymne S., Zhu L.H. Development of ultra-high erucic acid oil in the industrial oil crop *Crambe abyssinica* Plant Biotechnol J., 10(7):862-70 (2012).
36. Takahashi A., Ishizaki M., Kimira Y., Egashira Y., Hirai S. Erucic acid-rich yellow mustard oil improves insulin resistance in KK-Ay Mice. Molecules, 26: 546-556 (2021).
37. Kandasamy S., Ramub S., Aradhyac S.M. *In vitro* functional properties of crude extracts and isolated compounds frombanana pseudostem and rhizome. J Sci Food Agric, 96, 1347–1355 (2016).

38. Naigre R., Kalck P., Rouques C., Roux I., Michel G. Comparison of antimicrobial properties of monoterpenes and their carbonylated products. Planta Med, 62,275–277 (1996).
39. Policegoudra R.S., Divakar S., Aradhya S.M. Identification of Difurocumenonol, a new antimicrobial compound isolated frommango ginger (Curcuma amada Roxb.) rhizome. J Appl. Microb. 102,1594–1602 (2007).
40. Cruz S.L., Rivera-García1 M.T., Woodward J.J. Review of toluene action: clinical evidence, animal studies and molecular targets. J. Drug Alcohol Res., 3,1-15 (2014).
41. Heller J.H., Heller S.M., Springer S., Clark E. Squalene content of various shark livers, Nature 179, 919–920. (1957).
42. Huang Z.R., Lin Y.K., Fang J.Y. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules 14, 540–554 (2009).
43. Reddy L.H., Couvreur P. Squalene: a natural triterpene for use in disease management and therapy. Adv. Drug Deliv. Rev. 61, 1412–1426 (2009).
44. Irby D., Du C., Li F. Lipid_drug conjugate for enhancing drug delivery. Mol. Pharm., 14, 1325–1338 (2017).
45. Kotelevets L., Chastre E., Caron J., Mougin J., Bastian G., Pineau A., Walker F., Lehy T., Desmaële D., Couvreur P. A squalene- based nanomedicine for oral treatment of colon cancer. Cancer Res. 77, 2964-2975 (2017).
46. Budge S.M., Barry C. Determination of squalene in edible oils by transmethylation and GC analysis. Methods X, 6,15–21 (2019).
47. Fattore E., Fanelli RPalm oil and palmitic acid: A review on cardiovascular effects and carcinogenicity. Int. J. Food Sci. Nutr., 64, 648–659 (2013).
48. Fattore E., Bosetti C., Brighenti F., Agostoni, C. Fattore G. Palm oil and blood lipid-related markers of cardiovascular disease: A systematic review and meta-analysis of dietary intervention trials. Am. J. Clin. Nutr., 99, 1331–1350 (2014).
49. Carta G., Murru E., Banni S., Manca C. Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. Front. Physiol. 8:902 (2017).
50. Sczaniecka A.K., Brasky T.M., Lampe J.W., Patterson R.E., White E. Dietary intake of specific fatty acids and breast cancer risk among postmenopausal women in the VITAL cohort. Nutr. Cancer, 64, 1131–1142 (2012).
51. Mancini A., Imperlini E., Nigro E., Montagnese C., Daniele A., Orrù S., Buono P. Biological and nutritional properties of palm oil and palmitic Acid: Effects on Health. Molecules, 20, 17339-17361 (2015).
52. Beare-Rogers J., Dieffenbacher A., Holm J. Lexicon of lipid nutrition (IUPAC technical report). Pure and Applied Chemistry, 73, 685-744 (2001).
53. Yu S., Derr J., Etherton T.D., Kris-Etherton P. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. The American Journal of Clinical Nutrition, 61, 1129-1139 (1995).
54. Kelly, F., Sinclair, A., Mann, N., Turner, A., Abedin L., Li D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. European Journal of Clinical Nutrition, 55, 88-91 (2001).
55. Chen J., Liu H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review Int. J. Mol. Sci., 21, 1-24 (2020).
56. Tartrakoon W., Tartrakoon T., Kitsupee N. Effects of the ratio of unsaturated fatty acid to saturated fatty acid on the growth performance, carcass and meat quality of finishing pigs. Animal Nutrition, 2,79-85 (2016).
57. Traber M.G., Vitamin E. In J. W. Erdman, I. A. MacDonald & S. H. Zeisel (Eds.), Present Knowledge in Nutrition (cap. 14, pp. 383- 397). Oxford: Wiley-Blackwell. (2012).
58. Ju J., Picinich S.C., Yang Z., Zhao Y., Suh N., Kong A.-N., Yang C.S. Cancer-preventive activities of tocopherols and tocotrienols. Carcinogenesis, 31(4), 533-542 (2010).