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# Research Article
# Effect of solvent on Chemical Composition and Antioxidant Activities of *Abelmoschus esculentus* (L.) Moench Pods' Oils Extracts

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## Abstract

**Background and Objective:** *Abelmoschus esculentus* (Okra) is an important vegetable crop because of the numerous uses of the fresh leaves, buds, flowers, pods, stems and seeds as food and medicine. Effect of polar and non-polar solvent on the chemical composition and phytochemical properties of *A. esculentus* pods' oils was determined since polarity of solvent has relative effect on the extractives which include chemical and nutritional constituents. **Materials and Methods:** The pods' oil was extracted using hexane and methanol and the chemical constituent was determined using Gas-Chromatography-Mass Spectroscopy (GC-MS) while the antioxidant activity was determined using the 2, 2-diphenylpicrylhydrazyl radical (DPPH) and total phenolic content measurement methods. **Results:** The result revealed that the oil obtained from hexane contained more phytochemicals, 19 constituents dominated with palmitic acid, 10, 13-octadecadienoic acid, methyl ester, methyl linoleate and methyl linolenate while palmitic acid, methyl ester, methyl linoleate and methyl linolenate were identified as the major compounds in the methanol extract containing 11 constituents. The methanol extract with 77.7% scavenging activity was significant in the antioxidant assay using the stable radical 2,2-diphenyl picrylhydrazyl (DPPH) when compared with ascorbic acid used as standard. **Conclusion:** It can be concluded that the hexane extract contain more fatty acid ester than the methanol extract but the methanol extract has free radical scavenging ability when compared with the hexane extract.

Key words: *Abelmoschus esculentus*, methyl linoleate, methyl linolenate, solvent, antioxidant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Abelmoschus esculentus* (L.) Moench also called Ladies' Finger is a popular vegetable of the north-eastern African origin. Okra being the local name in the Yoruba speaking region of Nigeria is a perennial flowering plant belonging to the Malvaceae family. The plant is cultivated throughout the tropical and warm temperature regions around the world for their fibrous fruits of 'pods'. The pods features small round, mucilaginous white coloured seeds arranged in vertical rows. The pods are gathered while still green, tender and at immature stage[1-3]. Okra is a popular health food due to its high fiber, vitamin C, folate and mineral contents such as calcium, potassium, sodium, iron and magnesium[3-5]. Okra is an affordable source of protein, carbohydrates, minerals and vitamins, dietary fibre and health promoting fatty acids[1,5-6]. Lipid components of Okra greatly contribute to the nutritional and sensory value of almost all types of foods and is high in unsaturated fats such as oleic acid and linoleic acid[6-8]. The pods contain healthy amounts of vitamin A and flavonoid anti-oxidants such as beta-carotene, xanthin and lutein[3,9]. Traditional medicine practitioners employ the use of Okra pods in the treatment of disease such as osteoporosis, common cold, headache, malaria, blood pressure, heart and kidney disorders, anxiety and stress, nausea and fatigue[1,10]. The *A. esculentus* gum has also been used as suspending and emulsifying agents and the mucilage obtained from it was reported to have a sustained release property in tablet formation[9-12]. Okra mucilage has medicinal applications when used as a plasma replacement or blood volume expander. High fiber content of Okra makes it useful as blood sugar stabilizer and body's cholesterol level controller. Studies on the chemical, nutritional composition and the anti-oxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus* Moench) flour, seeds and their kernels has been reported. Also, biological studies such as anti-nutrient, anti-diabetic and anti-hyperlipidemic potential of *A. esculentus* have be done[12-17]. More research however, is still on going to harness the medicinal values of Okra. Notably, side effects associated with the administration of synthetic drugs has necessitated the need to find alternative way of treating disease in recent times. The *A. esculentus* (L.) Moench pods is said to be a rich source of phytochemicals with nutritional and medicinal tendencies, thus this study aims to determine the phytochemical constituents (qualitative and quantitative) on the two oil extracts of *A. esculentus*, determine the chemical composition of the oils using Gas Chromatography-Mass Spectrometry (GC-MS) and carry out anti-oxidant assays on the two extracts using 2,2-diphenyl picryl hydrazyl (DPPH) radical and total phenolic acid (TPC) measurement methods.

## MATERIALS AND METHODS

### Preparation of samples

**Sample collection and preparation:** Fresh sample of Okra pods (1000 g) were obtained from a farm in Lagelu Local Government Area of Oyo state, Ibadan, Nigeria in March, 2017 and identified by a Taxonomist at the Botany Department, University of Ibadan, Nigeria. They were sliced and air dried at room temperature for 14 days until a constant weight was achieved. Afterwards, it was blended to increase its surface area for maximum extraction.

**Reagents:** Hexane, methanol, ethyl acetate, ferric chloride, hydrogen peroxide, 2,2-diphenyl-1-picrylhydrazyl, potassium ferricyanide, ferric chloride, sulphuric acid, potassium permanganate, ammonium hydroxide, hydrochloric acid, ammonium thiocyanate, diethyl ether, ethanol, phenolphthalein indicator, sodium hydroxide, alcoholic solution of potassium hydroxide, garlic acid, methanol, Folin-ciocalteu reagent, sodium carbonate and trichloroacetic acid obtained from Sigma-Aldrich, Germany were used.

**Equipment/apparatus:** The following equipment and apparatus were used: OAUS vacuum oven and electronic weighing balance, heating mantle, desiccators, syringes, sample bottles and flasks. Rotary evaporator fitted with vacuum pump V-700 and B-490 heating bath (Heidolph Laborota 400 efficient, Germany, model 517-01002-002), Ultraviolet-visible (UV-visible) spectrophotometer (Unico1200 and Perkin Elmer lambda 25 model, UK) and Gas Chromatography-Mass (GC-MS) Spectrophotometer (Gas chromatograph/GC-MS (HP 6890), UK).

### Extraction process

**Extraction of the fat using hexane (non-polar solvent):** About 240 g of the ground dried sample was weighed, transferred into a glass container and 1 L of pure n-hexane was added, stirred at every 2 h with a glass rod and allowed to stay for 72 h. The solvent (now containing the fat) was collected using muslin bag and the filtrate was further

filtered using Whatman filter paper (1 mm). This process was repeated by adding 1 L of pure n-hexane to the shaft. The combined filtrate was then concentrated with the aid of rotary evaporator set at 35°C and transferred into a vacuum oven set at 40°C with a pressure of 700 mmHg.

**Extraction using methanol (Polar solvent):** About 240 g of the dried defatted sample was transferred into a glass container and 1 L of pure methanol was added, stirred at every 2 h with a glass rod and allowed to stay for 72 h. The solvent (now containing the extract) was collected using muslin bag and the filtrate was further filtered using Whatman filter paper (1 mm). This process was repeated twice on the shaft using 1 L of pure methanol. The combined filtrate was then concentrated with the aid of rotary evaporator set at 40°C and transferred into a vacuum oven set at 40°C with a pressure of 700 mmHg.

The extracts were weighed and their percentage yield calculated. Chemical composition was determined by use of GC-MS spectrometer. Chemical tests for the screening of the secondary plant metabolites present in the oils were carried out using standard procedures as described by Sofowora[14]. Table 1 shows the secondary plant metabolites obtained from the two solvent extracts. Antioxidant screening was carried out by use of DPPH and TPC methods[18-19].

### Analysis of the seed oil

**Gas chromatography:** The oils were analyzed using an HP 6890 Gas Chromatograph powered with ChemStation Rev. A09.01 [1206] Software using the following specifications: column type: HP 5MS, inlet temperature: 150°C, split ratio: (20:1) with hydrogen as carrier gas. Flow rate: 1.0 mL $min^{-1}$, column dimensions: 30 m×0.25 mm×0.25 µm, oven program: initial at 40°C, ramped at 5°C $min^{-1}$ to 200°C and run at 220°C for 5 min[18-20].

**Gas chromatography-mass spectrometry:** The GC oven temperature and conditions were as described above. HP 6890 powered with ChemStation Rev. A09.01 [1206] Software was used and Mass spectra were recorded[18,21] at 70 eV.

**Identification of components:** Electronic integration measurement obtained from Flame Ionization Detector (FID) set at a temperature of 300°C was used to determine the relative percentage compositions of constituents while individual components of the oil were identified on the basis of their retention indices determined with reference to a homologous series of n-alkanes and by comparison of their mass spectra fragmentation pattern (NIST 0.8 L database/chem. Station system) with data previously reported in literature[22]. Table 2 and 3 show the relative percentages of the characterized components and their refractive index values.

**Determination of antioxidant activity using DPPH method:** Test samples were evaluated for anti-oxidant potential by free radical scavenging method. This measures and monitors change in optical density of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. DPPH radical (3.94 mg) was dissolved in 100 mL of methanol to give a 100 µM solution. Samples were prepared with methanol using concentrations ranging from 100-1000 µg $mL^{-1}$. About 1 mL of the different concentrations was transferred into test tube and 1 mL of DPPH solution (0.5 mM) was added. After 30 min, the decrease in absorbance was measured at 517 nm in UV spectrophotometer. Analysis was carried out in triplicate and the average result was recorded. The percentage of the DPPH radical scavenging was calculated using the equation:

$$\text{Inhibition (\%) of DPPH radical} = \frac{\text{Abr} - \text{Aar}}{\text{Abr}} \times 100$$

where, Abr is the absorbance before reaction and Aar is the absorbance after reaction has taken place[18,23].

**Determination of antioxidant activity by measuring Total Phenolic Compound (TPC):** Plants with phenolic constituents have been reported to have antioxidant ability[19]. Total phenolic content was analyzed using the Folin-Ciocalteu method. An aliquot of 0.1 mL of different concentrations (100-1000 µg $mL^{-1}$) of the extracts were mixed with 2 mL of sodium carbonate (0.2%), allowed to incubate at room temperature for 2 min followed by the addition of Folin-Ciocalteu phenol reagent (0.1 mL) and allowed to incubate at room temperature for 30 min. The absorbance of the mixture was measured at 720 nm. Standard calibration curve for garlic acid in the range of 100-1000 µg $mL^{-1}$ was also prepared and analysed[23].

## RESULTS AND DISCUSSION

The hexane and methanol extracts obtained from *A. esculentus* pods gave yield of 0.52 and 5.78%,

Table 1: Phytochemical screening result (Qualitative and Quantitative) of *A. esculentus*

| | Hexane extract | | Methanol extract | |
|---|---|---|---|---|
| Secondary metabolite | Qualitative | Quantitative | Qualitative | Quantitative |
| Saponins | -ve | Nil | ++ve | 9.0% |
| Tannins | -ve | Nil | +ve | ND |
| Flavonoids | +ve | 49% | +ve | 3.0% |
| Cardiac glycosides | -ve | Nil | -ve | Nil |
| Terpenoids | +ve | ND | ++ve | ND |
| Steroids | +++ve | ND | ++ve | ND |
| Anthraquinones | ++ve | ND | ++ve | ND |
| Alkaloids | +++ve | 8.5% | ++ve | 1.0% |

-ve: Absent, +ve: Present, ++ve, +++ve: Abundantly present, ND: Not determined

Table 2: GC-MS analysis result of hexane oil extract of *A. esculentus*

| RT (mins) | Compounds | Molecular formula | AI | Area (%) |
|---|---|---|---|---|
| 18.050 | Methylpentadecanoate | $C_{16}H_{32}O_2$ | 1807 | 0.57 |
| 19.509 | Palmitic acid, methyl ester | $C_{17}H_{34}O_2$ | 1915 | 15.56 |
| 20.579 | Palmitic acid | $C_{16}H_{32}O_2$ | 1968 | 15.69 |
| 21.060 | Methylheptadecanoate | $C_{18}H_{36}O_2$ | 2008 | 1.23 |
| 22.101 | Palmitic acid, propyl ester | $C_{19}H_{38}O_2$ | 2072 | 1.65 |
| 22.319 | 10, 13-octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 2123 | 19.50 |
| 22.713 | Methyl stearate | $C_{19}H_{38}O_2$ | 2130 | 2.41 |
| 23.566 | Methyl linoleate | $C_{19}H_{34}O_2$ | 2097 | 18.35 |
| 23.657 | Methyl linolenate | $C_{19}H_{32}O_2$ | 2078 | 12.19 |
| 24.853 | n-propyl 9,12-octadecadienoate | $C_{12}H_{38}O_2$ | 2096 | 1.61 |
| 24.991 | n-propyl 9,12,15-octadecatrienoate | $C_{12}H_{36}O_2$ | 2109 | 0.71 |
| 25.786 | Eicosanoic acid, methyl ester | $C_{21}H_{42}O_2$ | 2312 | 1.27 |
| 27.045 | Heneicosanoic acid, methyl ester | $C_{22}H_{44}O_2$ | 2418 | 0.40 |
| 27.291 | Malonic acid, 3,3-dimethylbut-2-yl heptadecyl ester | $C_8H_{16}O_2$ | 1387 | 0.38 |
| 28.149 | Docosanoic acid, methyl ester | $C_{23}H_{46}O_2$ | 2531 | 2.55 |
| 29.145 | Tricosanoic acid methyl ester | $C_{24}H_{48}O_2$ | 2574 | 1.38 |
| 30.060 | Tetracosanoic acid, methyl ester | $C_{25}H_{50}O_2$ | 2715 | 1.14 |
| 30.913 | Pentacosanoic acid, methyl ester | $C_{26}H_{52}O_2$ | 2935 | 0.38 |
| 33.608 | Fumaric acid, 3-chlorophenyl hexyl ester | $C_8H_{12}O_2$ | 1256 | 0.96 |
| Total | | | | 97.93% |

Percentages calculated from the flame ionization detection data, RT: Retention Time, AI: Arithmetic Retention Index on HP-5MS column

Table 3: GC-MS result of methanol oil extract of *A. esculentus*

| RT (mins) | Compounds | Molecular formula | AI | Area (%) |
|---|---|---|---|---|
| 19.446 | Palmitic acid, methyl ester | $C_{17}H_{34}O_2$ | 1915 | 28.14 |
| 20.430 | Palmitic acid | $C_{16}H_{32}O_2$ | 1968 | 7.20 |
| 22.067 | Tritetracontane | $C_{43}H_{88}$ | 4300 | 2.79 |
| 22.227 | Methyl linoleate | $C_{19}H_{34}O_2$ | 2097 | 28.85 |
| 22.410 | Methyl linolenate | $C_{19}H_{32}O_2$ | 2078 | 11.23 |
| 22.7.8 | Methyl stearate | $C_{19}H_{38}O_2$ | 2130 | 3.91 |
| 23.348 | Linoleic acid | $C_{18}H_{32}O_2$ | 2173 | 7.61 |
| 24.848 | Citral | $C_{10}H_{16}O$ | 1240 | 1.31 |
| 25.815 | Tricosanoic acid, methyl ester | $C_{21}H_{42}O_2$ | 2312 | 1.73 |
| 28.166 | Docosanoic acid, methyl ester | $C_{23}H_{46}O_2$ | 2531 | 4.08 |
| 29.173 | Tricosanoic acid, methyl ester | $C_{24}H_{48}O_2$ | 2574 | 1.08 |
| Total | | | | 97.93 |

Percentages calculated from the flame ionization detection data, RT: Retention time, AI: Arithmetic retention index on HP-5MS column

respectively. Both were oily substances. The result of phytochemical analysis is presented in Table 1 while the chemical compositions of the oils are presented in Table 2 and 3. Figure 1 also showed the gas chromatography spectra of *A. esculentus* oils.

The hexane extract is rich in flavonoids (49%) and alkaloids (8.5%) unlike the methanol extract having flavonoids (3%) and alkaloids (1%), respectively. Saponin was obtained only from the methanol extract (9.0%) (Table 1).

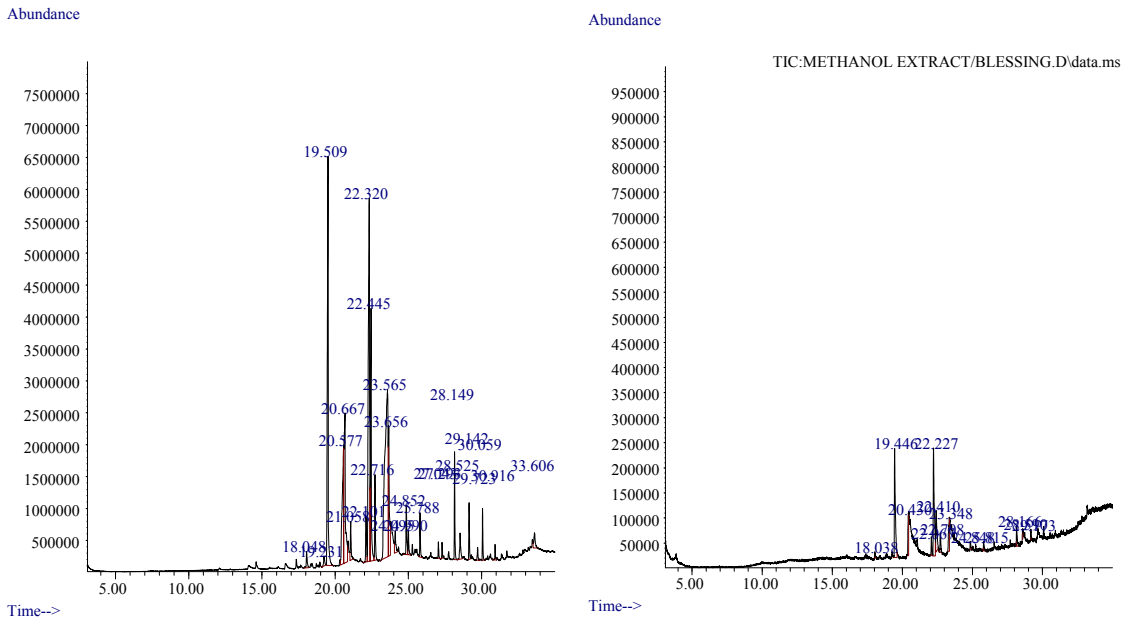


Fig. 1(a-b): Gas chromatography spectra of (a) Hexane and (b) Methanol oil extracts of *A. esculentus*

The GC-MS result of *A. esculentus* showed that hexane and methanol extracts contain 19 and 11 constituents mainly dominated with esters of fatty acids. The compounds were identified from the total ion chromatogram of the GC (Table 2, 3). Also, the GC spectrum of the pods' oils shown in Fig. 1 confirmed that the hexane extract has more peaks (constituents) than the methanol extract. Palmitic acid (15.69%), 10, 13-octadecadienoic acid, methyl ester (19.50%), methyl linoleate (18.35%) and methyl linolenate (12.19%) were the major compounds in the hexane extract while palmitic acid, methyl ester (28.14%), methyl linoleate (28.85%) and methyl linolenate (11.23%) were identified as the major compounds in the methanol extract. Palmitic acid, palmitic acid, methyl ester, methyl stearate, methyl linoleate, methyl linolenate, docosanoic acid, methyl ester and Tricosanoic acid, methyl esters are common to both solvent extracts. The pods of *A. esculentus* contain majorly esters of fatty acids with simpler alcohols (such as methyl-, ethyl-, n-propyl-, isopropyl-) which are applicable in the production of soap, emollients and lubricants for cosmetic purposes and metallic soaps, respectively. They are also used as emulsifiers, texturizing, anti-foam and stabilizing agents fatty acids are also converted via their methyl esters, to fatty alcohols and amines, which are precursors to lubricants, surfactants and detergents[24-25].

The oils obtained from both hexane and methanol extracts showed moderate antioxidant activity. At

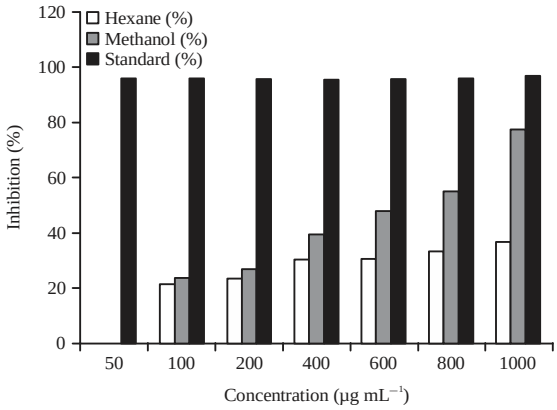


Fig. 2: Scavenging (%) activity of hexane and methanol extracts of *A. esculentus* against standard (Ascorbic acid)

1000 µg $mL^{-1}$, the hexane extract gave 36.8% percentage inhibition while the methanol extract gave 77.7% scavenging activity which was the highest at that concentration. This compared favourably well with ascorbic acid used as standard with scavenging activity (%) of 96.9% at the same concentration (Fig. 2). Therefore, the methanol extract has the ability to scavenge free radicals in the DPPH method.

The total phenolic content of the different extracts was determined against garlic acid used as standard by measuring the reduction in absorbance at 720 nm. The hexane extract had absorbance of 0.237 at 1000 µg $mL^{-1}$ which was better

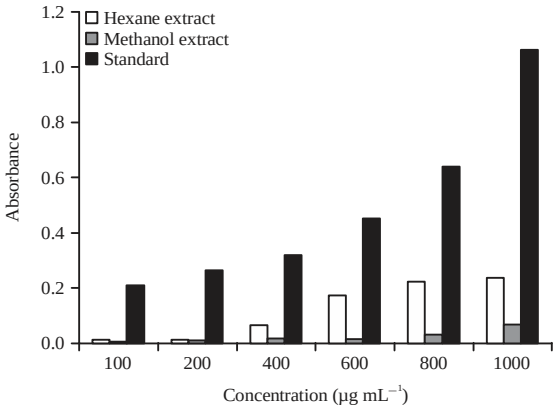


Fig. 3: Absorbance measurement of hexane and methanol extracts in the total phenolic content determination of *A. esculentus* against standard (garlic acid)

than that of the methanol extract indicating the presence of more phenolic substances. This was confirmed by the presence of flavonoids (49%) obtained in the phytochemical screening of the hexane extract. The standard (garlic acid) however contains more phenolic compounds (1.062) at this concentration (Fig. 3). Compounds with more phenolic compounds have been reported to have antioxidant activity[14-23].

## CONCLUSION

*Abelmoschus esculentus* (L.) Moench (Okra) pods was extracted with non polar (hexane) and polar (methanol) solvents to give oily substances which were analyzed using Gas Chromatography-Mass spectrometry. These revealed that fatty acid and their esters such as palmitic acid, methyl stearate, methyl linoleate and methyl linolenate were common to both oils. The methanol extract was more significant as an anti-oxidant being able to scavenge free radicals in the 2,2-diphenyl picryl hydrazyl radical method when compared with standard ascorbic whereas the hexane extract was better antioxidant with more phenolic content when activity was compared with garlic acid in the total phenolic acid content measurement method.

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