GC-MS analysis of *Abelmoschus manihot* (L.) Medik (Malvaceae) leaves

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Abstract

Plants are a rich source of bioactive phytochemicals which provide health benefits for humans further than those attributed to macronutrients and micronutrients. The phytochemical compounds isolated and purified are employed in a wide range of applications. In the present study, the bioactive compounds of *Abelmoschus manihot* benzene, chloroform, methanol and ethanol leaf extracts via GC-MS was analyzed and its biological properties being available in pure form, being nontoxic with a wide spectrum of biological functions, may find its application in the formation of various medicinal products.

Keywords: *Abelmoschus manihot*; Leaf extracts; GC-MS; Phytochemical compounds

1. Introduction

Plants of the genus *Abelmoschus* belong to the family of flowering plants called Malvaceae. This genus, also known as okra is composed of numerous species of flowering plants in the mallow family and are native to tropical and sub-tropical areas [1]. Interest in this genus is due principally to the high protein and mineral salt content of the pods, making okra a very good vegetable. Studies have shown that the daily consumption of 100g of okra provides 20, 15 and 50% calcium, iron, and Vitamin C of human dietary requirements respectively [2]. Onakpa [3] and Patil et al. [4] have documented the ethnomedicinal, phytochemical and pharmacological profile of the genus *Abelmoschus*. The species *Abelmoschus manihot* is cultivated mainly in the Far East, but also in the Indian sub-continent and northern Australia. It is less frequently found in America and tropical Africa. On the latter continent, Chevalier [5] described the variety zenkeri in Cameroon and the variety caillei in West Africa. The latter has also been observed in Zaire [6]. This species contains various chemical ingredients including flavonoids, organic acids, steroids, volatile constituents, coumarins, aliphatic hydrocarbons and nitrogenous compounds [7]. It has been used for treatment of chronic renal disease, mouth ulcers, and burns [8-10]. This plant species is also reported to possess analgesic [11], anti-inflammatory [12], antiviral [13], antibacterial [14], anticoagulant [15], larvicidal [16], wound healing [12] and osteoporosis [17, 18] properties. In the present study, the GC-MS analysis of this plant species has been analyzed as studies reported above have shown that there is scope to use this plant as a source of medicinal agent.

2. Material and methods

2.1. Plant collection and preparation of extracts

Mature and healthy leaves of *Abelmoschus manihot* were located and collected from in and around of Unaivaniyambadi village, Vellore district, Tamil Nadu, India (12.8730° N, 78.9714° E). Taxonomical identity of the plant was confirmed at the Department of Biotechnology, Thiruvalluvar University, Vellore, Tamil Nadu, India. Leaves were then washed under running tap water, air dried and shade dried for 10-15 days. The leaves were powdered using an electronic...
blender and sieved to get fine powder. The powdered leaves (500 g) were macerated with various solvents (1.5 L) each, viz., benzene, chloroform, methanol and ethanol using a Soxhlet apparatus [19] with their respective temperatures. The crude extract thus obtained was concentrated by evaporation and the yield was used for further phytochemical analysis.

2.2. GC-MS Analysis

GC-MS analysis was carried out at the Sophisticated Instrumentation Facility (SIF), Chemistry division, School of Advanced Science, VIT University, Vellore, Tamil Nadu, India. The Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5 % biphenyl 95 % dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/minute. The injector temperature was set at 260 °C during the chromatographic run. The extract sample (1 μL) injected into the instrument with the oven temperature was as follows: 60 °C (2 minutes); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for six minutes. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 seconds and a scan interval of 0.1 seconds. The fragments were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST library.

3. Results

The phytochemical compounds via GC-MS of the benzene leaf extract of *Abelmoschus manihot* indicated the presence of phytol, palmitic acid, linoleic acid, dioctyl phthalate, tocopherol, Urs-12-en-28-ol, (2E,4E)-2,4-heptadecadienoic acid and stigmast-4-en-3-one (Table 1; Figure 1). The GC-MS of chloroform extract revealed the presence of phytol, methyl isopalmitate, palmitic acid, linoleic acid, 25-hydroxycholesterol, DL-α-tocopherol acetate, 3,5-di-tert-butylbenzaldehyde, 12-hydroxy-8,10-heptadecadienoic acid, 22,23-dibromostigmasterol acetate and cholest-4-en-3-one (Table 2; Figure 2). The phytochemical compounds in the methanol leaf extract specified the presence of phytol, 2,3-dimethyl-8-oxo-non-2-enal, palmitic acid, 1,1'-bi(cyclohexyl), DL-α-tocopherol acetate, Urs-12-en-3-ol-acetate-(3β), 12-hydroxy-8,10-heptadecadienoic acid and fludrocortisone acetate (Table 3; Figure 3). The GC-MS study of ethanol extract showed presence of phytol, palmitic acid, linoleic acid, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, DL-α-tocopherol acetate, β-amyrone, 12-hydroxy-8,10-heptadecadienoic acid and stigmast-4-en-3-one (Table 4; Figure 4).

### Table 1 Phytochemical compounds in the benzene leaf extract of *Abelmoschus manihot*

| Compound Name | Retention Time | Molecular Weight (g/mol) | Molecular Formula | Structure |
|---------------|----------------|--------------------------|------------------|-----------|
| Phytol        | 16.514         | 296.531                  | C₂₀H₄₀O          | ![Structure](phytol.png) |
| Palmitic acid | 18.024         | 256.424                  | C₁₆H₃₂O₂         | ![Structure](palmitic.png) |
| Linoleic acid | 19.620         | 280.445                  | C₁₈H₃₂O₂         | ![Structure](linoleic.png) |

The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 seconds and a scan interval of 0.1 seconds. The fragments were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST library.
| Compound                        | Molar Mass | Formula   | Molecular Structure |
|--------------------------------|------------|-----------|---------------------|
| Dioctyl phthalate              | 22.756     | C_{24}H_{38}O_{4} | ![Dioctyl Phthalate](image) |
| Tocopherol                     | 27.183     | C_{29}H_{50}O_{2} | ![Tocopherol](image) |
| Urs-12-en-28-ol                | 29.164     | C_{30}H_{50}O | ![Urs-12-en-28-ol](image) |
| (2E,4E)-2,4-heptadecadienoic   | 29.739     | C_{17}H_{30}O_{2} | ![2E,4E)-2,4-heptadecadienoic acid](image) |
| Stigmast-4-en-3-one            | 30.839     | C_{29}H_{48}O | ![Stigmast-4-en-3-one](image) |
Figure 1 GC-MS chromatogram of benzene leaf extract of *Abelmoschus manihot*

| #  | RT  | Scan | Height       | Area            | Area % | Norm % |
|----|-----|------|--------------|-----------------|--------|--------|
| 1  | 16.514 | 2742 | 1,716,226,304 | 76,951,480.0    | 18.433 | 100.00 |
| 2  | 16.769 | 2793 | 292,744,608  | 12,344,304.0    | 2.957  | 16.04  |
| 3  | 16.969 | 2833 | 475,076,000  | 17,730,502.0    | 4.247  | 23.04  |
| 4  | 18.024 | 3044 | 507,284,352  | 73,028,112.0    | 17.493 | 94.90  |
| 5  | 19.620 | 3363 | 236,198,192  | 13,875,433.0    | 3.324  | 18.03  |
| 6  | 19.705 | 3380 | 295,806,080  | 59,129,820.0    | 14.164 | 76.84  |
| 7  | 22.756 | 3990 | 1,090,984,832| 37,132,468.0    | 8.895  | 48.25  |
| 8  | 27.183 | 4875 | 124,673,560  | 16,795,720.0    | 4.023  | 21.83  |
| 9  | 29.164 | 5271 | 134,499,904  | 23,895,588.0    | 5.724  | 31.05  |
| 10 | 29.739 | 5386 | 411,425,792  | 57,607,016.0    | 13.799 | 74.86  |
| 11 | 30.839 | 5606 | 138,842,016  | 28,982,470.0    | 6.942  | 37.66  |

Table 2 Phytochemical compounds in the chloroform leaf extract of *Abelmoschus manihot*

| Compound Name            | Retention Time | Molecular Weight (g/mol) | Molecular Formula | Structure |
|--------------------------|----------------|--------------------------|-------------------|-----------|
| Phytol                   | 16.514         | 296.531                  | C₂₀H₄₀O          | ![Phytol Structure](image) |
| Methyl isopalmitate      | 17.504         | 270.45                   | C₁₇H₃₄O₂         | ![Methyl Isopalmitate Structure](image) |
| Palmitic acid            | 18.024         | 256.424                  | C₁₆H₃₂O₂         | ![Palmitic Acid Structure](image) |
Linoleic acid  
\[\text{C}_{18}\text{H}_{32}\text{O}_2\]  
\[
\begin{array}{c}
\text{OH} \\
\text{C} \\
\text{H} \\
\text{O} \\
\end{array}
\]

25-Hydroxycholesterol  
\[\text{C}_{27}\text{H}_{46}\text{O}_2\]  
\[
\begin{array}{c}
\text{OH} \\
\text{C} \\
\text{H} \\
\text{O} \\
\end{array}
\]

DL-\(\alpha\)-tocopherol acetate  
\[\text{C}_{31}\text{H}_{52}\text{O}_3\]  
\[
\begin{array}{c}
\text{OH} \\
\text{C} \\
\text{H} \\
\text{O} \\
\end{array}
\]

3,5-Di-tert-butylbenzaldehyde  
\[\text{C}_{15}\text{H}_{22}\text{O}\]  
\[
\begin{array}{c}
\text{OH} \\
\text{C} \\
\text{H} \\
\text{O} \\
\end{array}
\]

12-hydroxy-8,10-heptadecadienoic acid  
\[\text{C}_{17}\text{H}_{30}\text{O}_3\]  
\[
\begin{array}{c}
\text{OH} \\
\text{C} \\
\text{H} \\
\text{O} \\
\end{array}
\]
22,23-dibromostigmasterol acetate

|     | RT  | Scan | Height    | Area          | Area %  | Norm % |
|-----|-----|------|-----------|---------------|---------|--------|
| 1   | 16.494 | 2738 | 5,332,435,968 | 199,765,216.0 | 18.764 | 100.00 |
| 2   | 16.749 | 2789 | 760,535,616   | 25,432,190.0  | 2.389  | 12.73  |
| 3   | 16.949 | 2829 | 1,245,597,696 | 43,947,284.0  | 4.128  | 22.00  |
| 4   | 17.979 | 3035 | 2,091,796,352 | 151,928,192.0 | 14.271 | 76.05  |
| 5   | 19.555 | 3350 | 676,800,768   | 38,003,944.0  | 3.570  | 19.02  |
| 6   | 19.635 | 3366 | 989,754,816   | 160,485,808.0 | 15.075 | 80.34  |
| 7   | 22.736 | 3986 | 2,544,867,840 | 80,274,888.0  | 7.540  | 40.18  |
| 8   | 27.168 | 4872 | 446,307,072   | 56,145,404.0  | 5.274  | 28.11  |
| 9   | 29.154 | 5269 | 365,878,112   | 59,010,672.0  | 5.543  | 29.54  |
| 10  | 29.739 | 5386 | 1,129,086,336 | 164,345,792.0 | 15.437 | 82.27  |
| 11  | 30.819 | 5602 | 383,930,848   | 85,270,976.0  | 8.010  | 42.69  |

Figure 2 GC-MS chromatogram of chloroform leaf extract of *Abelmoschus manihot*
| Compound Name                          | Retention Time | Molecular Weight (g/mol) | Molecular Formula | Structure |
|---------------------------------------|----------------|--------------------------|-------------------|-----------|
| Phytol                                | 16.514         | 296.531                  | C_{20}H_{40}O     | ![Phytol structure](image) |
| 2,3-Dimethyl-8-oxo-non-2-enal         | 16.694         | 182.259                  | C_{11}H_{18}O_{2} | ![2,3-Dimethyl-8-oxo-non-2-enal](image) |
| Palmitic acid                         | 18.024         | 256.424                  | C_{16}H_{32}O_{2} | ![Palmitic acid structure](image) |
| 1,1′-bi(cyclohexyl)                   | 19.635         | 166.303                  | C_{12}H_{22}      | ![1,1′-bi(cyclohexyl) structure](image) |
| DL-α-tocopherol acetate               | 27.193         | 472.743                  | C_{31}H_{52}O_{3} | ![DL-α-tocopherol acetate structure](image) |
| Urs-12-en-3-ol, acetate, (3,β)-       | 29.194         | 468.754                  | C_{32}H_{52}O_{2} | ![Urs-12-en-3-ol, acetate, (3,β)- structure](image) |
| 12-hydroxy-8,10-heptadecadienoic acid | 29.759         | 282.418                  | C_{17}H_{30}O_{3} | ![12-hydroxy-8,10-heptadecadienoic acid structure](image) |
Fludrocortisone acetate  30.910  422.487  

\[ \text{C}_{23}\text{H}_{31}\text{FO}_6 \]

**Figure 3** GC-MS chromatogram of methanol leaf extract of *Abelmoschus manihot*

|   | RT  | Scan | Height       | Area       | Area % | Norm % |
|---|-----|------|--------------|------------|--------|--------|
| 1 | 16.534 | 2746 | 420,916,928  | 16,048,945.0 | 6.381  | 23.95  |
| 2 | 16.789 | 2797 | 121,285,480  | 4,035,635.5  | 1.605  | 6.02   |
| 3 | 16.984 | 2836 | 176,739,520  | 5,988,394.5  | 2.381  | 8.94   |
| 4 | 17.504 | 2940 | 116,851,536  | 5,817,687.5  | 2.313  | 8.68   |
| 5 | 18.069 | 3053 | 377,771,200  | 66,083,348.0 | 26.275 | 98.61  |
| 6 | 19.650 | 3369 | 196,293,456  | 10,834,127.0 | 4.308  | 16.17  |
| 7 | 19.740 | 3387 | 276,188,416  | 67,011,896.0 | 26.644 | 100.00 |
| 8 | 25.162 | 4471 | 65,625,952   | 4,790,231.0  | 1.905  | 7.15   |
| 9 | 27.193 | 4877 | 42,989,608   | 5,609,520.5  | 2.230  | 8.37   |
| 10| 29.184 | 5275 | 94,510,936   | 14,102,153.0 | 5.607  | 21.04  |
| 11| 29.759 | 5390 | 243,846,832  | 34,815,536.0 | 13.843 | 51.95  |
| 12| 30.494 | 5537 | 52,523,988   | 7,831,631.5  | 3.114  | 11.69  |
| 13| 30.909 | 5620 | 39,656,244   | 8,534,596.0  | 3.393  | 12.74  |
Table 4 Phytochemical compounds in the ethanol leaf extract of *Abelmoschus manihot*

| Compound Name                                         | Retention Time | Molecular Weight (g/mol) | Molecular Formula | Structure |
|--------------------------------------------------------|----------------|--------------------------|-------------------|-----------|
| Phytol                                                 | 16.514         | 296.531                  | C_{20}H_{40}O     | ![Phytol](image) |
| Palmitic acid                                          | 18.024         | 256.424                  | C_{16}H_{32}O_{2} | ![Palmitic acid](image) |
| Linoleic acid                                          | 19.620         | 280.445                  | C_{18}H_{32}O_{2} | ![Linoleic acid](image) |
| 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester | 22.736         | 278.344                  | C_{16}H_{22}O_{4} | ![Benzenedicarboxylic acid](image) |
| DL-α-tocopherol acetate                                | 27.193         | 472.743                  | C_{31}H_{52}O_{3} | ![Tocopherol acetate](image) |
| β-amyrone                                              | 29.154         | 424.702                  | C_{30}H_{40}O     | ![Amyrone](image) |
12-hydroxy-8,10-heptadecadienoic acid  
29.759  282.418  C_{17}H_{30}O_{3}  

Stigmast-4-en-3-one  
30.839  412.691  C_{29}H_{48}O

**Figure 4** GC-MS chromatogram of ethanol leaf extract of *Abelmoschus manihot*
4. Discussion

Plants are a rich source of bioactive phytochemicals which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Todarwal et al. [20] have reviewed the ethnobotany, phytochemistry and pharmacological properties of *Abelmoschus manihot*. This plant is known for secondary metabolites like flavonoids [21] and steroids [12]. The phytochemical constituents obtained via GC-MS from different solvent leaf extracts of *Abelmoschus manihot* from the present study were found to have various biological properties reported elsewhere, viz., antimicrobial, anti-inflammatory, antioxidant, antidiuretic, antifungal, anticezemic, antiacne, antiarthritic, anticonorinary, antiseptic, antidermic, antispasmodic, antbranchitic, antidiabetic, antiandrogenic, antitumour, hypcholeterolemic, hepatoprotective, hypoglycemic, lubricant, nematicide and pesticide. Besides these, this plant is popular for its young, tender, juicy pods which can be consumed in different forms like boiled, fried or cooked [22, 23]. High protein source due to high lysine level in seeds make this plant as an alternative to soybean and therefore could be used as a supplement to cereal based diets [24, 25]. In medical application, it has been found as a good component for plasma replacement or blood volume expander [26-29]. It also has been reported as medicine for the control of fertility, childbirth and also to act as a stimulator in milk production for lactating mothers [30-33].

5. Conclusion

Studies on phytoprinciples from *Abelmoschus manihot* need to be evaluated in a scientific manner so as to identify potential lead compounds for further development, as ethnobotanical and traditional uses of natural compounds, especially those of plant origin, are often very effective and generally believed to be safe for human use.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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