



(RESEARCH ARTICLE)



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

Divya Selvaraj <sup>1</sup>, Arivoli Subramanian <sup>1,\*</sup> and Tennyson Samuel <sup>2</sup><sup>1</sup> Department of Zoology, Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India.<sup>2</sup> Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India.

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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 subtropical 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

\* Corresponding author

E-mail address: [rmsarivoli@gmail.com](mailto:rmsarivoli@gmail.com)

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.

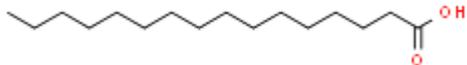
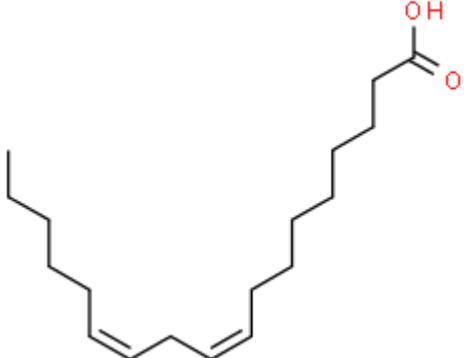
## 2.2. GC-MS Analysis

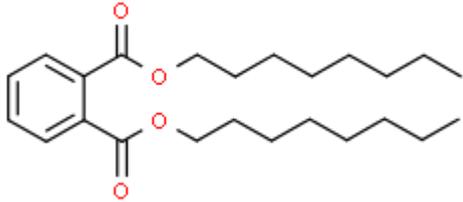
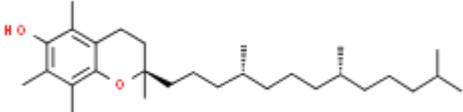
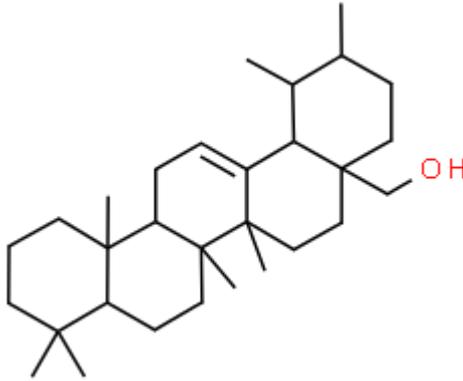
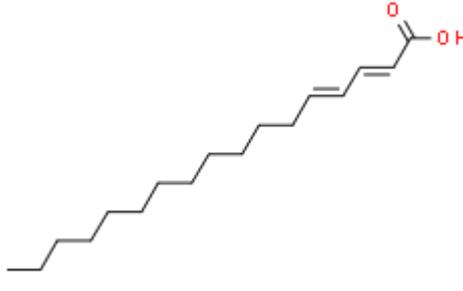
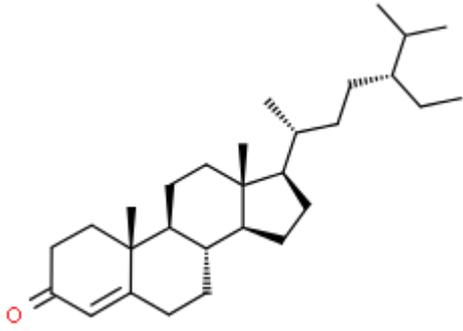
GC-MS analysis was carried out at the Sophisticated Instrumentation Facility (SIF), Chemistry division, School of Advanced Science, VIT University, Vellore, Tamil Nadu, India. The Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5 % biphenyl 95 % dimethylpolysiloxane, 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<sup>-1</sup>; 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.

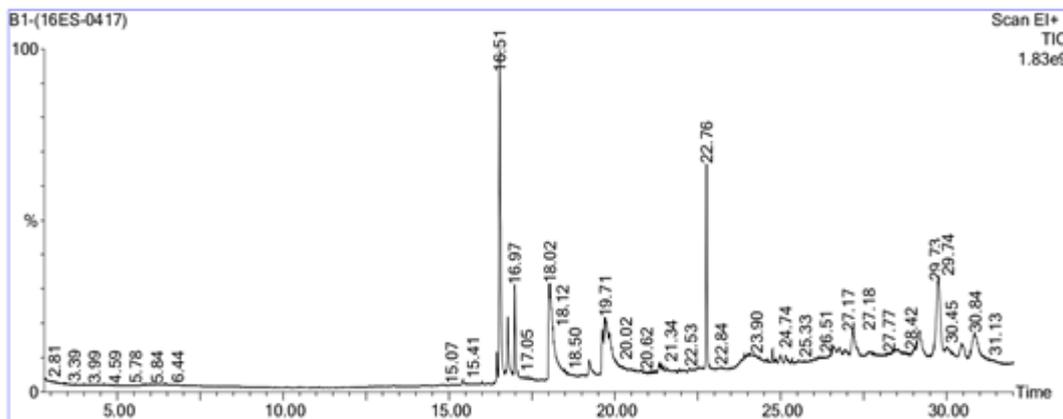
## 3. Results

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	C <sub>20</sub> H <sub>40</sub> O	
Palmitic acid	18.024	256.424	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	
Linoleic acid	19.620	280.445	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	

Diocetyl phthalate	22.756	390.556	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	
Tocopherol	27.183	430.706	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	
Urs-12-en-28-ol	29.164	426.717	C <sub>30</sub> H <sub>50</sub> O	
(2E,4E)-2,4-heptadecadienoic acid	29.739	266.419	C <sub>17</sub> H <sub>30</sub> O <sub>2</sub>	
Stigmast-4-en-3-one	30.839	412.690	C <sub>29</sub> H <sub>48</sub> O	

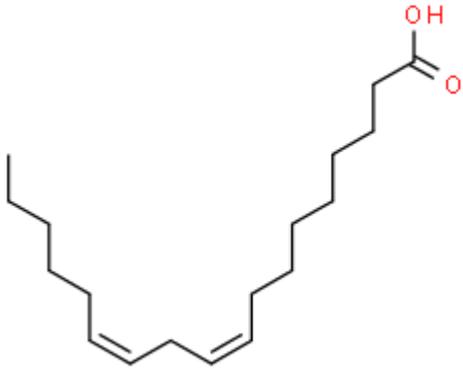
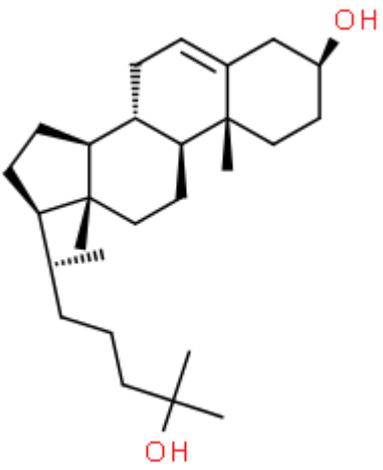
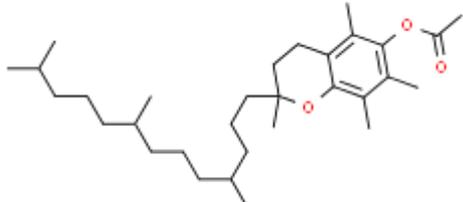
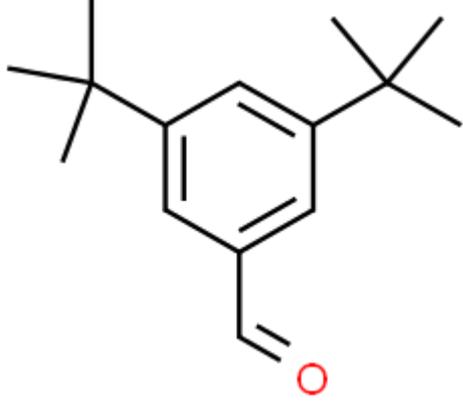
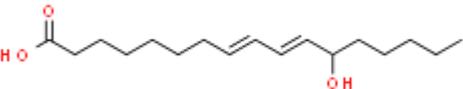


**Figure 1** GC-MS chromatogram of benzene leaf extract of *Abelmoschus manihot*

#	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	C <sub>20</sub> H <sub>40</sub> O	
Methyl isopalmitate	17.504	270.45	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	
Palmitic acid	18.024	256.424	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	

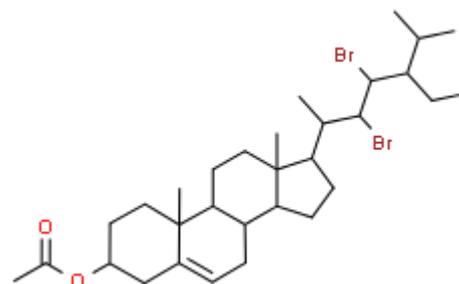
Linoleic acid	19.620	280.445	$C_{18}H_{32}O_2$	
25-Hydroxycholesterol	25.162	402.653	$C_{27}H_{46}O_2$	
DL- $\alpha$ -tocopherol acetate	27.193	472.743	$C_{31}H_{52}O_3$	
3,5-Di-tert-butylbenzaldehyde	29.184	218.335	$C_{15}H_{22}O$	
12-hydroxy-8,10-heptadecadienoic acid	29.759	282.418	$C_{17}H_{30}O_3$	

22,23-  
dibromostigmasterol  
acetate

30.494

614.536

C<sub>31</sub>H<sub>50</sub>Br<sub>2</sub>  
O<sub>2</sub>



Cholest-4-en-3-one

30.909

384.638

C<sub>27</sub>H<sub>44</sub>O

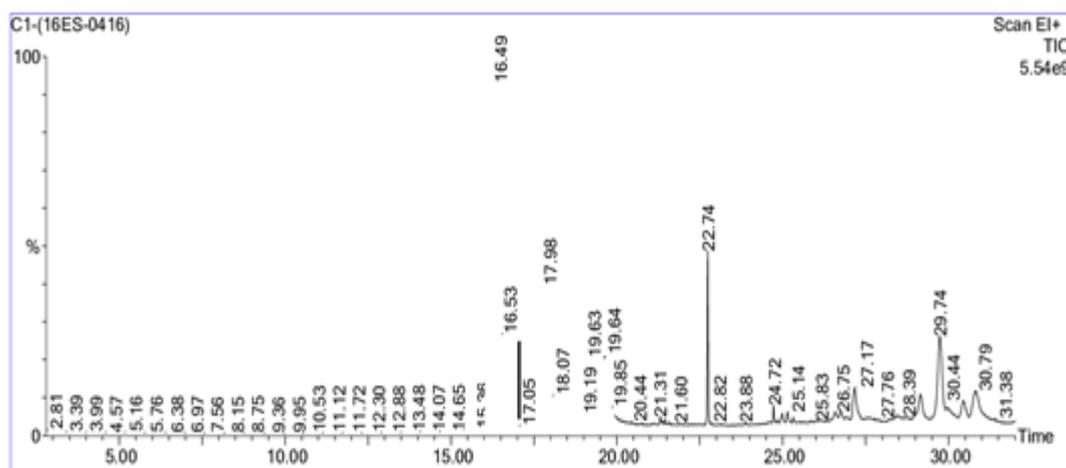
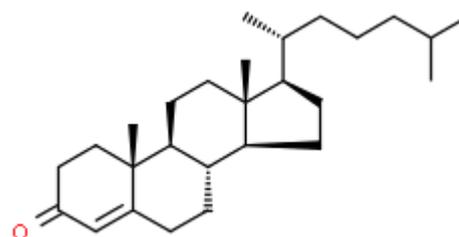
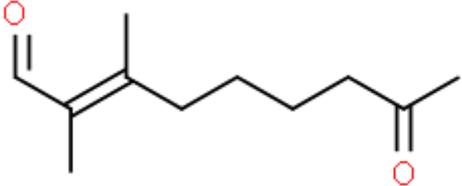
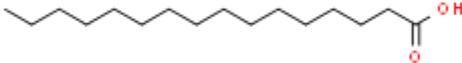
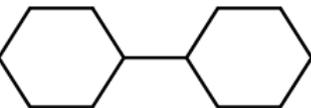
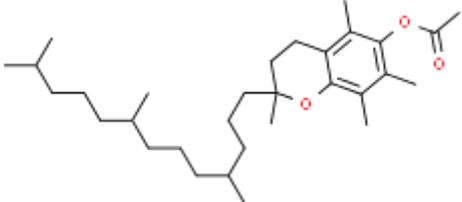
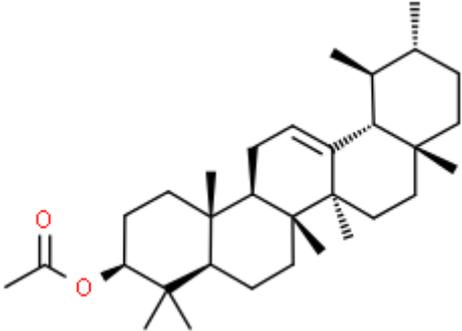
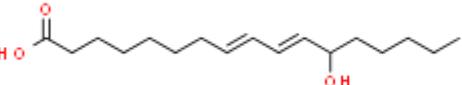


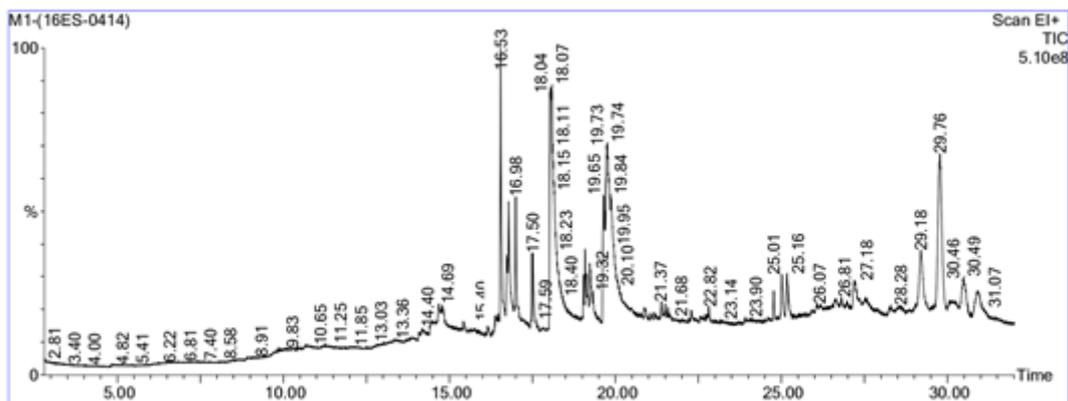
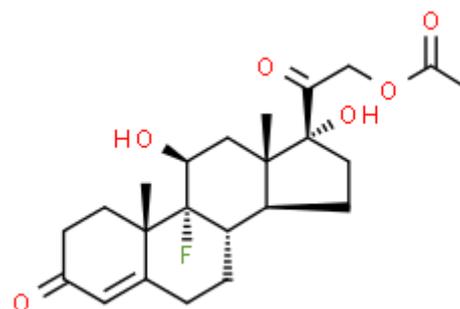
Figure 2 GC-MS chromatogram of chloroform leaf extract of *Abelmoschus manihot*

#	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

**Table 3** Phytochemical compounds in the methanol leaf extract of *Abelmoschus manihot*

Compound Name	Retention Time	Molecular Weight (g/mol)	Molecular Formula	Structure
Phytol	16.514	296.531	C <sub>20</sub> H <sub>40</sub> O	
2,3-Dimethyl-8-oxo-non-2-enal	16.694	182.259	C <sub>11</sub> H <sub>18</sub> O <sub>2</sub>	
Palmitic acid	18.024	256.424	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	
1,1'-bi(cyclohexyl)	19.635	166.303	C <sub>12</sub> H <sub>22</sub>	
DL-α-tocopherol acetate	27.193	472.743	C <sub>31</sub> H <sub>52</sub> O <sub>3</sub>	
Urs-12-en-3-ol, acetate, (3.β.)-	29.194	468.754	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	
12-hydroxy-8,10-heptadecadienoic acid	29.759	282.418	C <sub>17</sub> H <sub>30</sub> O <sub>3</sub>	

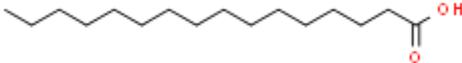
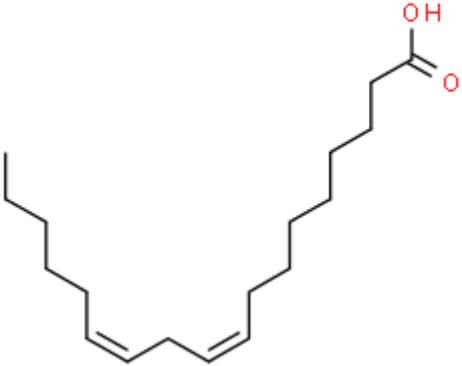
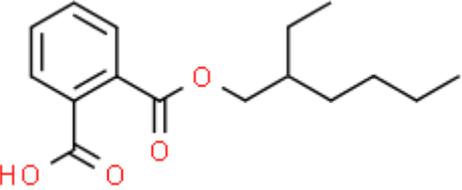
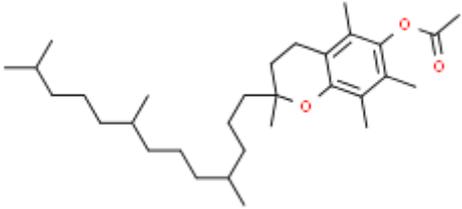
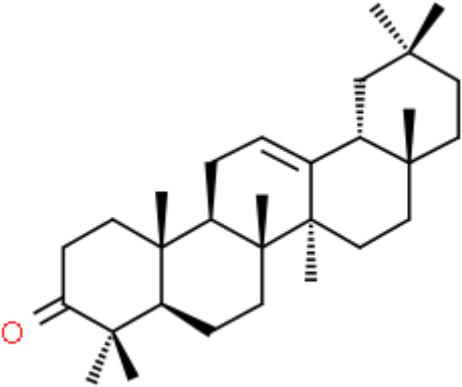
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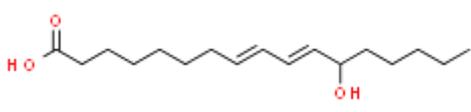
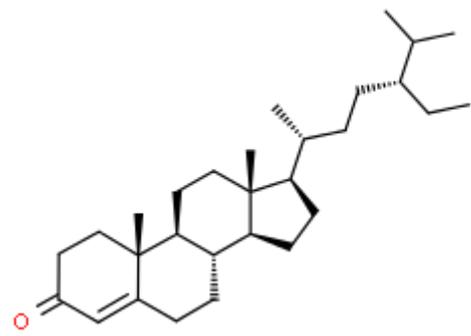


**Figure 3** GC-MS chromatogram of methanol leaf extract of *Abelmoschus manihot*

#	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 <sub>20</sub> H <sub>40</sub> O	
Palmitic acid	18.024	256.424	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	
Linoleic acid	19.620	280.445	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	
1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.736	278.344	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	
DL-α-tocopherol acetate	27.193	472.743	C <sub>31</sub> H <sub>52</sub> O <sub>3</sub>	
β-amyrone	29.154	424.702	C <sub>30</sub> H <sub>48</sub> O	

12-hydroxy-8,10-heptadecadienoic acid	29.759	282.418	C <sub>17</sub> H <sub>30</sub> O <sub>3</sub>	
Stigmast-4-en-3-one	30.839	412.691	C <sub>29</sub> H <sub>48</sub> O	

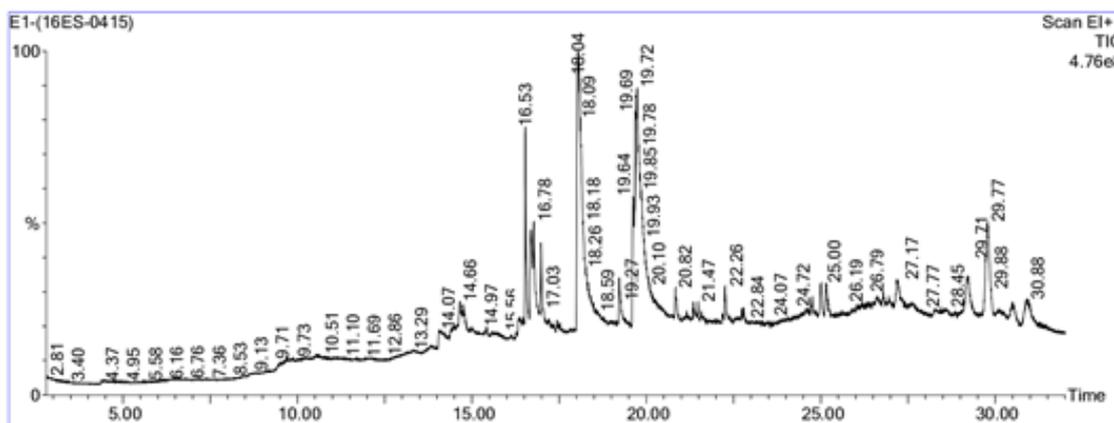


Figure 4 GC-MS chromatogram of ethanol leaf extract of *Abelmoschus manihot*

#	RT	Scan	Height	Area	Area %	Norm %
1	16.529	2745	274,019,040	10,499,938.0	4.710	14.91
2	16.694	2778	129,632,176	10,122,956.0	4.541	14.38
3	16.779	2795	139,496,160	9,295,630.0	4.170	13.20
4	16.969	2833	109,948,152	4,705,987.0	2.111	6.68
5	18.040	3047	385,880,288	65,543,028.0	29.400	93.08
6	19.215	3282	64,294,592	4,270,705.5	1.916	6.07
7	19.635	3366	174,992,736	8,233,253.0	3.693	11.69
8	19.715	3382	322,436,800	70,413,712.0	31.585	100.00
9	27.193	4877	37,345,408	4,941,854.0	2.217	7.02
10	29.194	5277	53,843,832	8,319,476.0	3.732	11.82
11	29.769	5392	128,888,256	18,541,098.0	8.317	26.33
12	30.910	5620	37,380,732	8,044,664.5	3.609	11.42

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#### 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, antieczemic, antiacne, antiarthritic, anticoronary, antiseptic, antidermatic, antispasmodic, antibronchitic, antidiabetic, antiandrogenic, antitumour, hypocholesterolemic, hepatoprotective, hypoglycemic, lubricant, nematocide 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].

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#### 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.

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#### 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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