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Matiur Rahman
Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh

Raton Kumar Bishwas
Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh

Ali Ahsan Muzahid
Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh

Md Farid Uddin
Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh

Koushik Saha
Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh

Corresponding Author:
Koushik Saha
Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh

Phytochemical screening of plant extracts and GC-MS analysis of the n-hexane extracts of leaves, stems and roots of *Garcinia cowa* growing in Bangladesh

Matiur Rahman, Raton Kumar Bishwas, Ali Ahsan Muzahid, Md Farid Uddin and Koushik Saha

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Abstract
To identify the phytochemical constituents of different extracts of *Garcinia cowa* leaves, stems, and roots, phytochemical screening and gas chromatography-mass spectrometry (GC-MS) techniques were used. The leaves, stems, and roots of *Garcinia cowa* were extracted using n-hexane, chloroform, ethyl acetate, and methanol. A preliminary phytochemical screening was done to determine the class of compounds found in each extract. The findings demonstrated the presence of steroids, terpenoids and phenolic compounds in each extract of leaves, stems and roots. All extracts with the exception of n-hexane contained flavonoids as well. However, all extracts of the leaves, stems, and roots aside from methanol were free of carbohydrates. The identification and quantification of 19, 26 and 20 n-hexane soluble chemicals contained in the leaves, stems, and roots of *Garcinia cowa* respectively, were made possible by the GC-MS analysis of the n-hexane extract of those parts of the plant. In this experiments, Methyl hexadecanoate (32.79%), Oleanitrile (16.10%) and 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid (9.54%) detected in the n-hexane extract of the leaves, stems and roots, respectively as having the greatest concentration.

Keywords: Oleanitrile, hexane, extract

1. Introduction
The most abundant bioreservoirs of multiple phytochemicals are found in medicinal plants that are used to treat various illnesses and ails [1]. The phytochemical components of plants influence their therapeutic qualities [2]. Herbal remedies have been recognized for over half a century as being effective in the treatment of acute diseases such as cancer [3]. Numerous investigations have shown that the chemical compounds xanthones, coumarins, flavonoids, phenolics, alkaloids, tannins, terpenoids, polypeptides, essential oils, lectin and polyacetylenes are present in medicinal plants [4]. For the production of antibiotics to treat pathogenic infections, these bioactive substances are employed as starting materials [5]. Plants that have been shown to be useful as medicines and are regularly used in traditional medicine may include chemicals that are potential medication candidates [6]. Natural drug sources are often reported to have less adverse consequences. As a result, the local population of this subcontinent has a long history of employing herbal remedies [7]. Regarding its bioactive components, the genus *Garcinia*, which is a member of the family Clusiaceae and has roughly 300 species, has received much research. The plants, which are native to the regions of Asia, Africa, South America, and Polynesia, are small to medium-sized evergreen trees that may reach heights of 30 m and are found all over the globe in both tropical and temperate climates [8].

Garcinia cowa Roxb. a moderate-sized tree with edible fruit commonly referred to as asam kandis, grows to a height of around 30 m [9]. In addition to being used as a spice, the dried fruit of the *Garcinia cowa* plant is also used for the treatment of ulcers, constipation, and nausea [10]. Former phytochemical analyses of *Garcinia cowa*, xanthones, benzophenones, dyhydrobenzopyran, acylphloroglucinol, depsidone, and tetraprenyltoluquinone were isolated [11-14]. To perform biological functions, it absorbs nutrients from its parent organism. As a result, the host plant has a significant influence on the phytochemicals found [15]. The aim of the current research was to assess the therapeutic value of the whole *Garcinia cowa* plant. Gas chromatogram-mass spectrometric (GS-MS) analysis gives a numerical evaluation of the

phytochemical components that make up the plant sample in this experiment, whereas phytochemical screening offers a qualitative analysis.

2. Materials & Methods

2.1 Collection and Identification of the Plant Material

Garcinia cowa leaves, stems, and roots have been collected in April 2022 from a remote location of Cumilla, Bangladesh. The species was recognized by a taxonomist from the National Herbarium in Dhaka, who gave it the DACB Accession Number 78797.

2.2 Extraction of leaves, stems and roots of the *G. cowa*

The roots and stems were chopped into little pieces except leaves and dried completely beneath the shed. Then, a grinder machine was used to ground the dried leaves, stems, and roots into powder at room temperature. The dried powder of the leaves, stems, and roots were extracted with n-hexane, $CHCl_3$, ethyl acetate, and CH_3OH in that order. The dried crude extracts of *G. cowa*, labeled as MLH, MLC, MLE, MLM for leaves, MSH, MSC, MSE, & MSM for stems, and MRH, MRC, MRE, & MRM for roots and were prepared by evaporating the solvents that were used using a rotary evaporator [16].

2.3 Phytochemical Screening

For the identification of multiple phytochemicals, comprising steroids, terpenoids, coumarins, flavonoids, alkaloids, quinones, carbohydrates, tannins, saponins and others, the extracts of *G. cowa* were subjected to phytochemical analysis [17]. Following the guidelines provided by Trease and Evans [18], Harborne [19] and Sofwara [20] phytochemical components were identified. The different extracts of *Garcinia cowa's* leaves, stems, and roots were screened for phytochemical content using the following qualitative tests.

2.3.1 Test for Steroids & Terpenoids

Libermann-Burchard-Test

10 mg of the extract were mixed with chloroform. A few drops of Ac_2O were added, and then 1 ml of pure sulfuric acid was added. The blue $CHCl_3$ layer that became green indicating the presence of steroids and the appearance of the pink $CHCl_3$ layer indicating the presence of terpenoids.

2.3.2 Test for Flavonoids

Shindo's Test

Prepare a 10 mg CH_3OH extract solution. Magnesium turnings were added next, followed by a strong HCl addition. appearance of a pink colour indicates flavonoids.

2.3.3 Test for Phenolic compounds

10 mg of extract were dissolved in CH_3OH, and a few drops of a 2.5% $FeCl_3$ solution were added. The presence of the phenolic component was identified by the reddish-brown color.

2.3.4 Test for Coumarins

After 10 mg of the extract had dissolved in CH_3OH, alc. KOH was added. Coumarins were detected by the appearance of a yellow color that becomes gray when strong HCl is applied.

2.3.5 Test for Quinones

10 mg of the extract were dissolved in CH_3OH and treated with sulfuric acid. The color development indicated that quinone was present.

2.3.6 Test for Alkaloids

Mayer's Test

Alkaloids were originally detected using Mayer's reagent. The reagent (100 ml) was made by combining KI (5.0 g) and mercuric chloride (1.36 g each) with water. Ten mg of the extracts were dissolved in HCl separately. In the middle of the watch glass, a few solution droplets were put. Mayer's reagent was added along the sides of the watch glass using a glass rod. A positive result is demonstrated by the production of a gelatinous white precipitate.

2.3.7 Tests for Saponins

The extract was dissolved in distilled water using a little quantity, followed by a vigorous shake. Formation of foam indicates the existence of saponins.

2.3.8 Test for Carbohydrates

Molisch's Test

The extracts were passed through filters after being vigorously shaken with water. After a thorough shaking, Molisch's reagent (95% ethanol + 5% naphthol) was added to the aqueous filtrate. 1 ml of Concentrated H_2SO_4 was gently added to form a layer below the aqueous solution. When there was a brown ring at the interface, the test was successful.

2.3.9 Test for Tennis

5 ml of aqueous extract were mixed with some drops of a lead acetate solution diluted to 1% that had previously been heated in water. Yellow or red precipitation revealed the presence of tanins.

2.4 Equipment and GC-MS analytical techniques

At BCSIR Laboratories, the n-hexane extracts of *Garcinia cowa* leaves, stems and roots were studied using the EI method on a Shimadzu GC-17A gas chromatograph connected to an MS 2010 plus mass spectrometer. A temperature of 40 °C was maintained at a constant pressure of 90 kPa in a capillary column that was transporting helium. The samples were distributed at a split ratio of 10. The sample was dissolved in $CHCl_3$. The working conditions are as follows: The column has a diameter of 30 cm and a length of 0.25 nm. Its name is RTS5MS. For packing the columns, 10% diethylene glycol succinate was employed. At the aforementioned pressure, it served as a carrier gas [21].

3. Results and Discussions

3.1 Study of Phytochemical Constituents

Table 1: The findings of the phytochemical component analysis of *G. cowa* leaves extracts

Examined phytochemicals	MLH	MLC	MLE	MLM
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	-	+	+	+
Phenolic Compounds	+	+	+	+
Coumarins	+	+	+	+
Quinones	-	+	+	+
Alkaloids	-	+	+	+
Saponins	-	-	+	+
Anthraquinones	-	-	+	+
Carbohydrates	-	-	-	+
Tannins	-	-	-	-

MLH: n-Hexane extract of leaves, MLC: Chloroform extract of leaves, MLE: Ethyl acetate extract of leaves, MLM: Methanol extract of leaves.

Table 2: The findings of the phytochemical component analysis of *G. cowa* stem extracts

Examined phytochemicals	MSH	MSC	MSE	MSM
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	-	+	+	+
Phenolic Compounds	+	+	+	+
Coumarins	+	+	+	+
Quinones	-	-	+	+
Alkaloids	-	-	+	+
Saponins	-_	-_	+	+
Anthraquinones	-	+	+	+
Carbohydrates	-	-	-	+
Tannins	-	-	-	-

MSH: n-Hexane extract of stems, MSC: Chloroform extract of stems, MSE: Ethyl acetate extract of stems, MSM: Methanol extract of stems.

Table 3: The findings of the phytochemical component analysis of *G. cowa* root extracts

Examined phytochemicals	MRH	MRC	MRE	MRM
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	-	+	+	+
Phenolic Compounds	+	+	+	+
Coumarins	+	+	+	+
Quinones	-	+	+	+
Alkaloids	-	+	+	+
Saponins	-	-_	-	+
Anthraquinones	-	+	+	+
Carbohydrates	-	-	-	+
Tannins	-	-	-	-

MRH: n-Hexane extract of roots, MRC: Chloroform extract of roots, MRE: Ethyl acetate extract of roots, MRM: Methanol extract of roots.

Note: symbol (+) indicates the presence and (-) indicating the absence

Preliminary phytochemical studies on leaves, stems and roots of *G. cowa* extracts were utilized to determine the class of the compounds in each extract. The findings showed that every extract of leaves, stems, and roots contained steroids, terpenoids, and phenolic compounds. Flavonoids were also identified in all extracts except n-hexane. But carbohydrates were found in methanol extracts of leaves, stems and roots only. Tannins were absent in every extracts of leaves, stems and roots.

3.2 GC-MS study of the plant extracts

The mass spectrum of the GC-MS instrument was decoded using a library of more than 62000 designs from the NIST. The spectrum of the identified component kept in the NIST collection was contrasted with the spectrum of the unidentified molecule. To identify the components, the spectrum of the extracts was compared to the spectrum of the known compound from the NIST library.

33.2.1 GC-MS Analysis of n-Hexane Extract of *Garcinia cowa* Leaves

By using GC-MS analysis, 19 components from the n-hexane extract of *G. cowa* leaves could be identified and measured. The outcomes of the GC-MS analysis of the n-hexane extract from *G. cowa* leaves were displayed in Fig. 1 and Table 4.

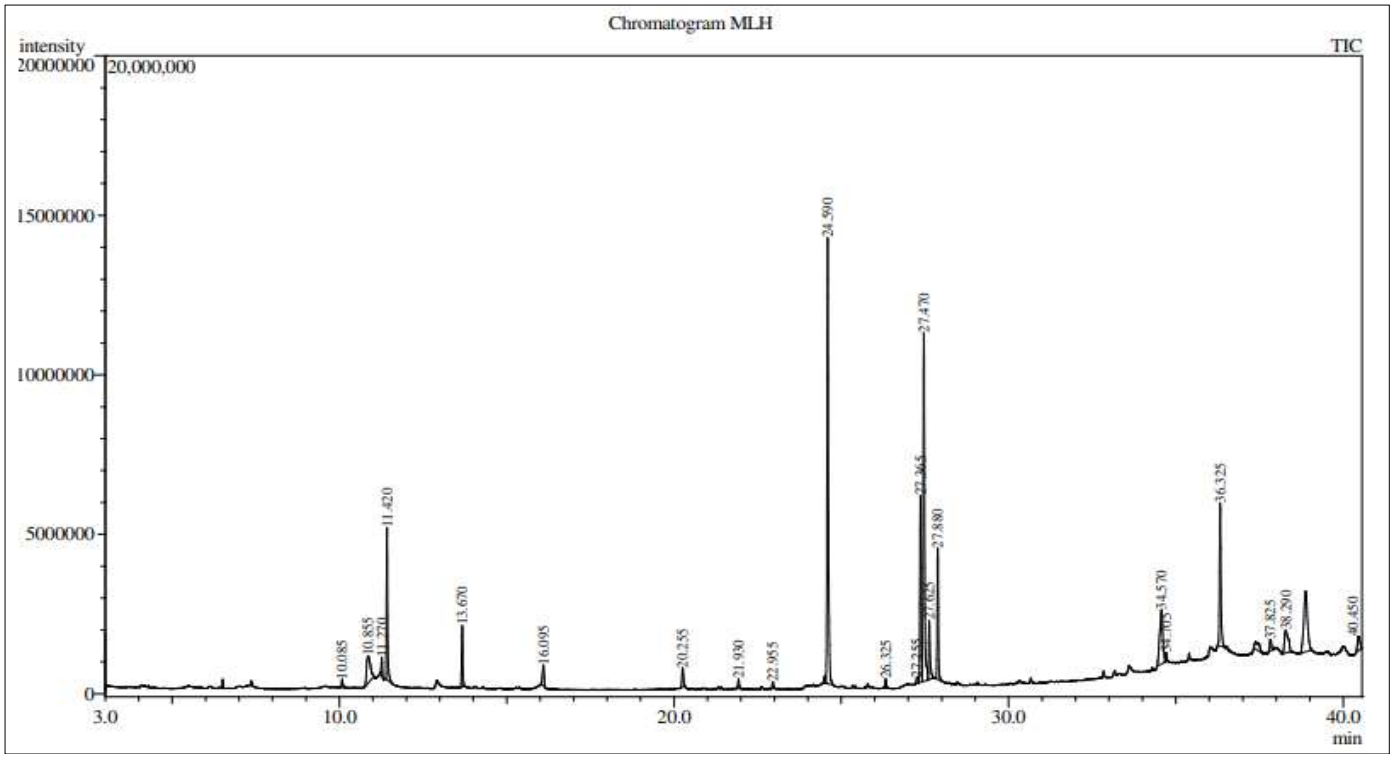


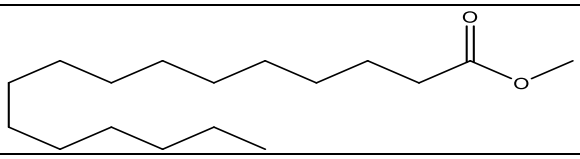
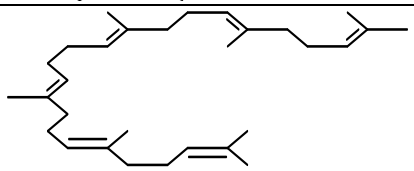
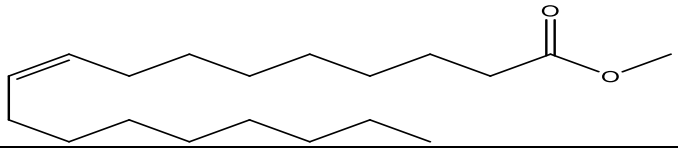
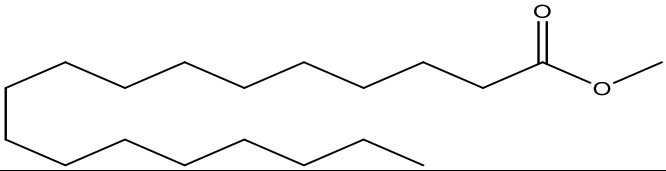
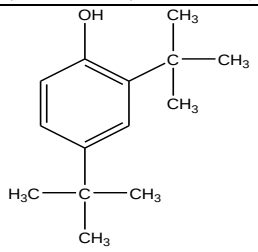
Fig 1: TIC of *G. cowa* leaves n-hexane extract

Table 4: GC-MS studies of the n-hexane extract of the leaves of *G. cowa*

Symbol	Retention Time	Name of the Compound	Molecular Weight	Molecular Formula	Conc.%
M-1	10.851	1-methyl-1-propyl-benzenemethanol	164.244	$C_{11}H_{16}O$	3.14
M-2	11.269	(E)-3-Tetradecene	196.372	$C_{14}H_{28}$	0.86
M-3	11.420	Estragole	148.201	$C_{10}H_{12}O$	5.56
M-4	13.669	2,4-bis(1,1-dimethylethyl) phenol	206.323	$C_{14}H_{22}O$	6.36
M-5	16.091	(E)-9-Octadecene	252.478	$C_{18}H_{36}$	1.60
M-6	20.257	Methyl tetradecanoate	242.397	$C_{15}H_{30}O_2$	2.67
M-7	21.931	E-14-Hexadecenal	238.408	$C_{16}H_{30}O$	0.46
M-8	22.958	6,10,14-trimethyl-2-Pentadecanone	268.477	$C_{18}H_{36}O$	0.37
M-9	24.592	Methyl hexadecanoate	270.450	$C_{17}H_{34}O_2$	32.79
M-10	26.326	Methyl heptadecanoate	284.477	$C_{18}H_{36}O_2$	0.66
M-11	27.259	1-Hexadecanol	242.440	$C_{16}H_{34}O$	0.21
M-12	27.365	Methyl 9,12-octadecadienoate	294.472	$C_{19}H_{34}O_2$	5.76
M-13	27.471	Methyl 9-octadecenoate	296.487	$C_{19}H_{36}O_2$	9.96
M-14	27.625	Phytol	296.531	$C_{20}H_{40}O$	2.12
M-15	27.882	Methyl stearate	298.503	$C_{19}H_{38}O_2$	9.00
M-16	34.575	Stigmasterol	412.690	$C_{29}H_{48}O$	3.29
M-17	36.324	Squalene	410.718	$C_{30}H_{50}$	12.01
M-18	38.282	4,22-Stigmastadiene-3-one	410.674	$C_{29}H_{46}O$	1.30
M-19	40.447	Cholest-4-en-3-one	384.637	$C_{27}H_{44}O$	0.41

The total quantity of identified compounds can be seen in Table 4. About 98.16% of the total compounds were identified, and nearly 1.83% were still unidentified. The key chemicals were identified here as Methyl hexadecanoate (M-9, 32.79%), Squalene (M-17, 12.01%), Methyl 9-octadecenoate (M-13, 9.96%), Methyl stearate (M-15, 9.00%), 2,4-bis(1,1-dimethylethyl) phenol (M-4, 6.36%). Majority of the compounds identified from n-hexane extract of leaves are fatty acid esters and hydrocarbons.

Table 5: Structures of key compounds discovered from Leaves of *G. cowa* n-hexane extracts

Name of the Compound	Chemical structure
Methyl hexadecanoate (M-9)	
Squalene (M-17)	
Methyl 9-octadecenoate (M-13)	
Methyl stearate(M-15)	
2,4-bis(1,1-dimethylethyl) phenol (M-4)	

3.2.2 GC-MS Analysis of n-Hexane Extract of *Garcinia cowa* Stems: 26 components from the n-hexane extract of *Garcinia cowa* stems were able to be identified and measured using GC-MS. The results of the GC-MS analysis of the *G. cowa* stem n-hexane extract are shown in Fig. 2 and Table 6.

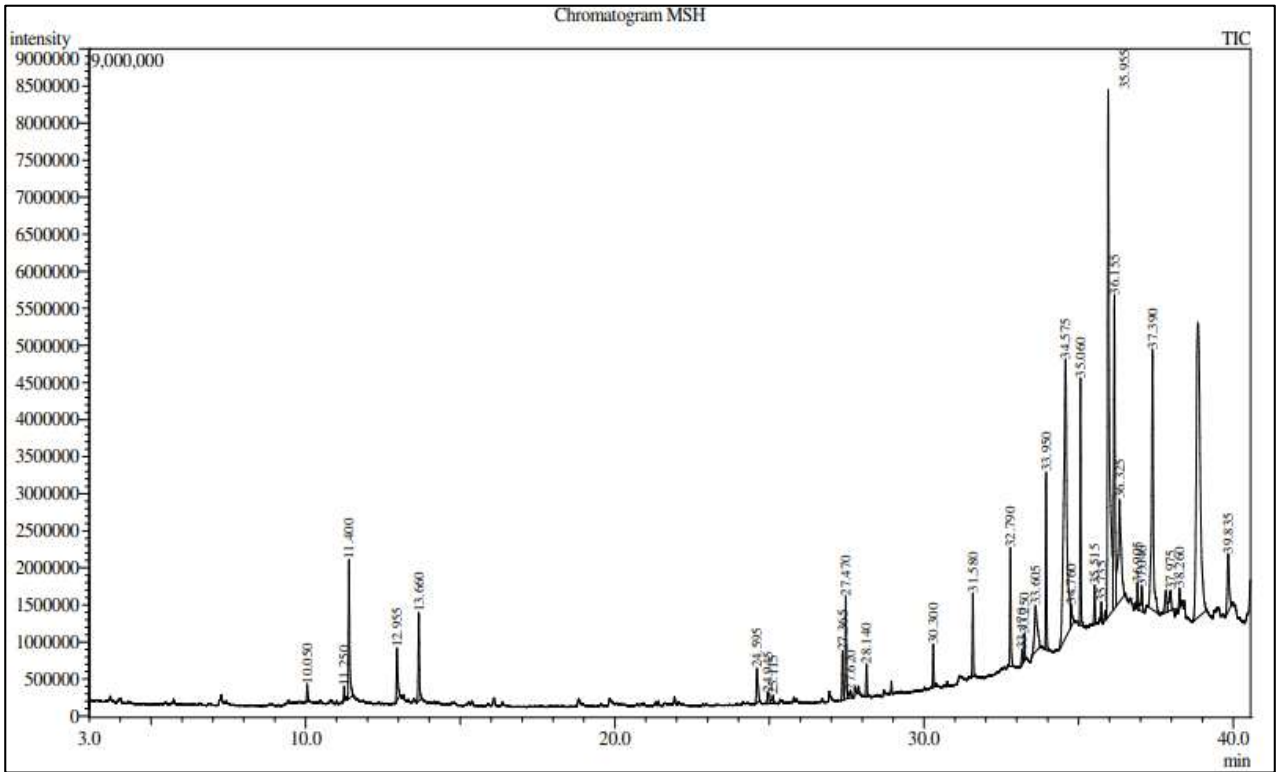


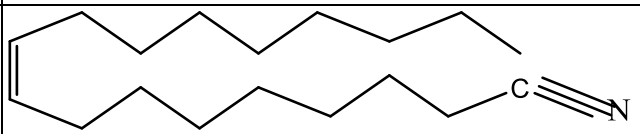
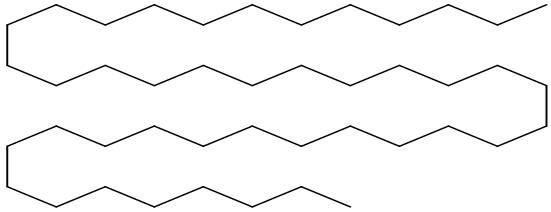
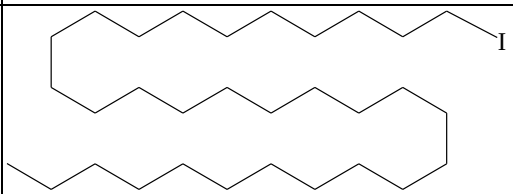
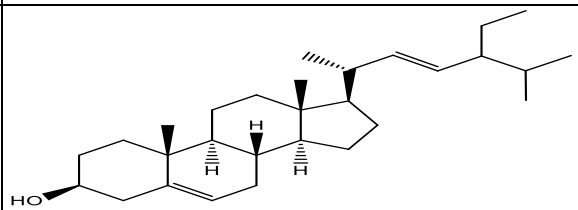
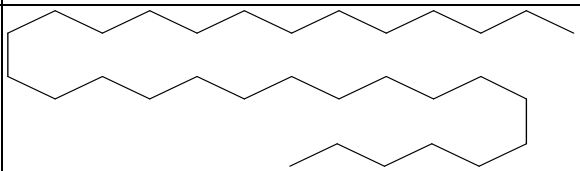
Fig 2: TIC of *G. cowa* stem n-hexane extract

Table 6: GC-MS studies of the *G. cowa* stem n-hexane extract

Symbol	Retention Time	Name of the Compound	Molecular Weight	Molecular Formula	Conc.%
F-1	10.050	5-Methoxyindane	148.201	$C_{10}H_{12}O$	0.54
F-2	11.249	1-Tridecene	182.345	$C_{13}H_{26}$	0.36
F-3	13.659	3,5-Bis(1,1-dimethylethyl)-phenol	206.323	$C_{14}H_{22}O$	5.93
F-4	24.593	Methyl hexadecanoate	270.450	$C_{17}H_{34}O_2$	1.70
F-5	27.361	Methyl 9,12-octadecadienoate	294.472	$C_{19}H_{34}O_2$	0.90
F-6	27.470	Methyl 9-octadecenoate	296.487	$C_{19}H_{36}O_2$	1.74
F-7	27.618	Phytol	296.531	$C_{20}H_{40}O$	0.21
F-8	28.140	Oxacycloheptadec-8-en-2-one	252.392	$C_{16}H_{28}O_2$	0.50
F-9	30.299	Heneicosane	296.574	$C_{21}H_{44}$	1.54
F-10	31.579	Tetracosane	338.653	$C_{24}H_{50}$	3.21
F-11	32.792	Hexacosane	366.707	$C_{26}H_{54}$	4.37
F-12	33.166	Di-n-octyl phthalate	390.556	$C_{24}H_{38}O_4$	0.65
F-13	33.249	(Z)-9-Eicosenoic acid	310.514	$C_{20}H_{38}O_2$	0.57
F-14	33.615	Campesterol	400.680	$C_{28}H_{48}O$	0.46
F-15	33.950	Octacosane	394.760	$C_{28}H_{58}$	6.04
F-16	34.575	Stigmasterol	412.690	$C_{29}H_{48}O$	8.87
F-17	35.059	Hentriacontane	436.839	$C_{31}H_{64}$	8.13
F-18	35.514	cis-13-Eicosenoic acid	310.514	$C_{20}H_{38}O_2$	0.68
F-19	35.735	Tetracontane	563.079	$C_{40}H_{82}$	0.62
F-20	35.954	Oleanitrile	263.502	$C_{18}H_{33}N$	16.10
F-21	36.155	Tetratetracontane	619.185	$C_{44}H_{90}$	10.80
F-22	36.324	(2E,6E,10E)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-tetraen-1-yl formate	318.493	$C_{21}H_{34}O_2$	3.54
F-23	36.902	1-Iodo-octacosane	520.656	$C_{28}H_{57}I$	1.04
F-24	37.391	1-Iodo-dotriacontane	576.763	$C_{32}H_{65}I$	9.61
F-25	38.261	2-methyloctacosane	408.786	$C_{29}H_{60}$	0.76
F-26	39.836	(R)-2,8-Dimethyl-2-((3E,7E)-4,8,12-trimethyltrideca-3,7,11-trien-1-yl)chroman-6-ol	396.605	$C_{27}H_{40}O_2$	2.32

The total amount of identified constituents can be seen in Table 6. The total quantity of reported components was around 91.19% and almost 8.81% remained unidentified. The key chemicals in this instance were identified as Oleanitrile (F-20, 16.10%), Tetratetracontane (F-21, 10.80%), 1-Iodo-dotriacontane (F-24, 9.61%), Stigmasterol (F-16, 8.87%), Hentriacontane (F-17, 8.13%). Different types of compounds were identified from the n-hexane extract of stems of *G. cowa* like fatty acid esters, steroids, hydrocarbons, substituted hydrocarbons, etc.

Table 7: Structures of key compounds revealed from the stem of *G. cowa* n-hexane extracts

Name of the compounds	Chemical structures
Oleanitrile (F-20)	
Tetratetracontane (F-21)	
1-Iodo-dotriacontane (F-24)	
Stigmasterol (F-16)	
Hentriacontane (F-17)	

3.2.3 GC-MS Analysis of n-Hexane Extract of *Garcinia cowa* Roots

Utilizing GC-MS, 20 compounds from the n-hexane extract of *Garcinia cowa* roots could be detected and measured. The GC-MS analysis of the *G. cowa* root n-hexane extract produced the results, which were shown in Fig. 3 and Table 8.

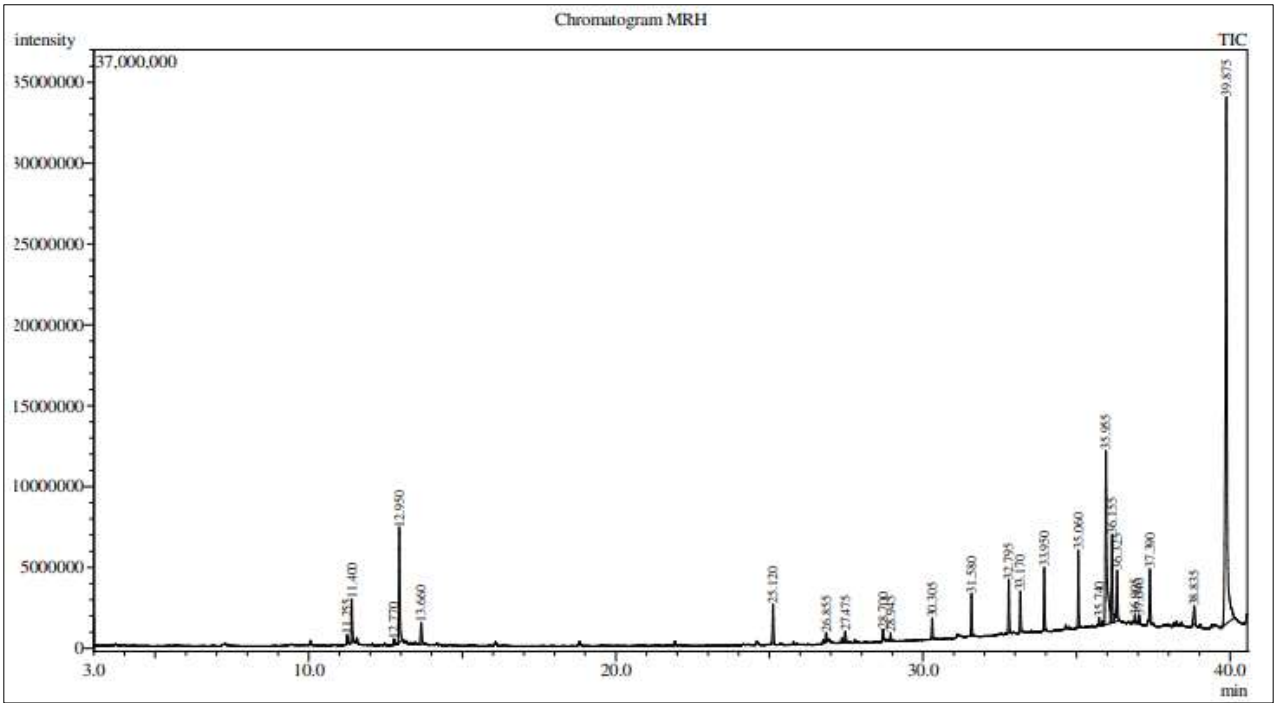


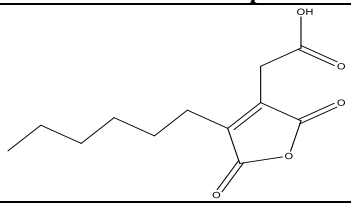
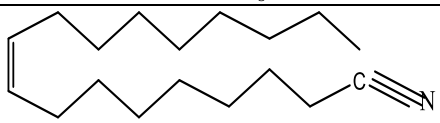
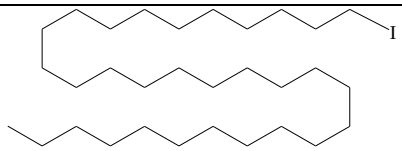
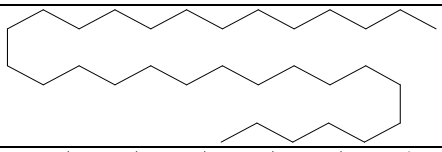
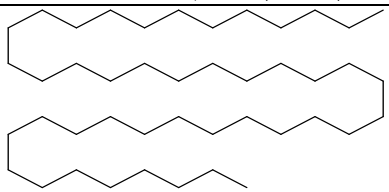
Fig 3: TIC of *G. cowa* root n-hexane extract

Table 8: GC-MS studies of the *G. cowa* roots n-hexane extract

Symbol	Retention Time	Name of the Compound	Molecular Weight	Molecular Formula	Conc.%
U-1	11.401	Estragole	148.201	$C_{10}H_{12}O$	1.77
U-2	12.950	4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid	240.250	$C_{12}H_{16}O_5$	9.54
U-3	13.658	3,5-bis(1,1-dimethylethyl)-phenol	206.323	$C_{14}H_{22}O$	3.06
U-4	25.120	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	272.468	$C_{20}H_{32}$	1.20
U-5	26.856	(E)-9-Octadecen-1-ol	268.477	$C_{18}H_{36}O$	0.50
U-6	27.473	Methyl 10-octadecenoate	296.487	$C_{19}H_{36}O_2$	0.37
U-7	28.702	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	272.468	$C_{20}H_{32}$	1.07
U-8	28.945	Heneicosane	296.574	$C_{21}H_{44}$	0.57
U-9	30.303	Tetracosane	338.653	$C_{24}H_{50}$	1.50
U-10	31.581	Pentacosane	352.680	$C_{25}H_{52}$	2.84
U-11	32.796	Hexacosane	366.707	$C_{26}H_{54}$	3.47
U-12	33.169	Di-n-octyl phthalate	390.556	$C_{24}H_{38}O_4$	3.06
U-13	33.951	Octacosane	394.760	$C_{28}H_{58}$	4.33
U-14	35.061	Hentriacontane	436.839	$C_{31}H_{64}$	5.01
U-15	35.738	2-methyloctacosane	408.786	$C_{29}H_{60}$	0.50
U-16	35.958	Oleanitrile	263.502	$C_{18}H_{33}N$	8.44
U-17	36.158	1-iodo-dotriacontane	576.763	$C_{32}H_{65}I$	5.59
U-18	36.325	Squalene	410.718	$C_{30}H_{50}$	4.49
U-19	37.043	2-methyl- eicosane	296.574	$C_{21}H_{44}$	0.64
U-20	37.389	Tetratetracontane	619.185	$C_{44}H_{90}$	4.56

From Table 8, the total amount of identified compounds was approximately 62.51%, and nearly 37.49% remained unidentified. Here the major compounds were identified as 4-hexyl-2, 5-dihydro-2, 5-dioxo-3-furanacetic acid (U-2, 9.54%), Oleanitrile (U-16, 8.44%), 1-iodo-dotriacontane (U-17, 5.59%), Hentriacontane (U-14, 5.01%), Tetratetracontane (U-20, 4.56%). Majority of the compounds identified from n-hexane extract of roots are fatty acid esters and hydrocarbons. Oleanitrile and 1-iodo-dotriacontane were also identified as major components from the n-hexane extract of stems of the plant.

Table 9: Structures of key compounds revealed from the root of *G. cowa* n-hexane extracts

Name of compounds	Structures of the compounds
4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid (U-2)	
Oleanitrile (U-16)	
1-iodo-dotriacontane (U-17)	
Hentriacontane (U-14)	
Tetratetracontane (U-20)	

4. Conclusion

A broad overview of the presence of secondary metabolites across the whole Garcinia cowa plant is provided in the present research. It indicates that various kinds of compounds may be present with potential therapeutic usefulness. The structures of the bioactive chemicals must therefore be identified and clarified through further study of this plant material.

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6. Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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