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# Phytochemical screening of plant extracts and GC-MS analysis of the n-hexane extracts of stems and roots of *Jatropha curcas* growing in Bangladesh

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**Abstract**
Phytochemical screening and gas chromatography-mass spectrometry (GC-MS) methods were used to determine the phytochemical components of various extracts of stems and roots of *Jatropha curcas*. Preliminary phytochemical screening of n-hexane, chloroform, ethyl acetate and methanol extracts of stems and roots of *Jatropha curcas* were carried out to identify the class of compounds present in each extract. The results showed that steroids, terpenoids, flavonoids and phenolic compounds were found in every extract of stems and roots. Alkaloids were also present in every extract except n-hexane. But carbohydrates were only present in methanol extract of stems and roots. The GC-MS analysis of the n-hexane extract of the stems and roots of *Jatropha curcas* were done which allowed the identification and quantification of 22 and 26 n-hexane soluble compounds present in the stems and roots respectively. In this experiment, Dotriacontane (17.22%) and Lycopene (12.46%) were identified as major components in the n-hexane extracts of stems and roots, respectively.

**Keywords:** *Jatropha curcas*, phytochemical screening, GC-MS analysis

## Introduction
Nature has been used as a major source of medicine for healing people all across the world for thousands of years, establishing the groundwork for the conventional medication system. As science and technology advanced, attention turned to identifying and characterizing the chemicals that were responsible for the therapeutic properties. The scientific and medicinal fields have benefited greatly from contributions made by nature [1]. *Jatropha curcas* is a woody shrub belonging to the Euphorbiaceae family and spreads all over the world. It is known as physics nut, goat nuts or just Jatropha in different regions all over the world [2, 3]. It is very much common plant grows in Bangladesh and locally known as Jamal Gota. According to earlier studies, the Jatropha plant is indigenous to Central and South America [4]. It is an important medicinal plant for novel pharmaceuticals. The plant has attracted the attention of chemists for its medicinal values. Different parts of the plants are used by the Ayuvedists and Yunans to cure various diseases [5]. It has long been used in different systems of medicine in the treatment of cancer [6], anti-inflammatory [7], Anti-coagulant [8] Anti-diarrheal activity [9] and also potent for anti-bacterial activities [10]. Previous phytochemical screenings also resulted in the isolation of Curcusones A, Eucurcusones B, Jatropholone A, Jatropholone B, 3,3′,4-trimethoxylellagic acid, Daucosterol, β-sitosterol, Sucrose, 5-hydroxy-6,7-dimethoxycoumarin, 6-methoxy-7-hydroxycoumarin, Caniojane, 3-hydroxy-4-methoxybenzaldehyde, Curcacycline A, Curcacycline B, Curcain [11-17]. Due to the presence of different class of compounds such as glycosides, flavonoids, coumarins, alkaloids, lignanes, phytosterols etc. in the plant, they contribute to the diverse biological functions such as anti-oxidant, hepatoprotective, anti-bacterial, anti-mutagenic, anti-tumor, anti-inflammatory and anti- helmintic activities. Phytochemical studies on this medicinal plant have already been conducted in several nations, including Bangladesh, India, Pakistan, Thailand, and other regions. However, the extracts from this plant's stems and roots have not yet been the subject of any comprehensive biological and phytochemical studies in Bangladesh. The current investigation aimed to determine whether the stems and roots of *Jatropha curcas* had any therapeutic value. GS-MS analysis provides the quantitative assessment of the phytochemicals of the plant extracts, whereas phytochemical screening gives the qualitative analysis.

## 2. Materials and Methods

### 2.1 Collection & Identification of the Plant Material

Stems and roots of *J. curcas* were collected from the roadsides of Mymensingh district in December 2021. The plant was recognized and identified by a taxonomist using a voucher specimen (No.=66331) that was placed at the Bangladesh National Herbarium.

### 2.2 Extraction of stems and roots of the *Jatropha curcas*

The stems and roots were cut into small pieces and dried thoroughly underneath the shed. After that, the dried stems and roots were turned into powder by using a grinder machine. The dried powder of the stems and roots was then extracted at room temperature with n-hexane, chloroform, ethyl acetate, and methanol successively. The dried crude extracts obtained by evaporation of the solvents using a rotary evaporator and denoted as JCSH, JCSC, JCSE, & JCSM for stems and JCRH, JCRC, JCRE, & JCRM for roots of *Jatropha curcas*.

Phytochemical analysis of the extracts of *Jatropha curcas* was done to identify various phytochemicals, including alkaloids, steroids, flavonoids, coumarins, glycosides, quinones, anthraquinones, tannins, carbohydrates, saponins, and others [18-20]. Phytochemical components were identified using the protocols outlined by Trease and Evans [21], Harborne [22] and Sofwara [23]. The following qualitative assays were used to perform a phytochemical screening on the various extracts of stems and roots of *Jatropha curcas* [24].

#### 2.3.1 Test for Steroids and Terpenoids Libermann - Burchard Test

Chloroform was added to 10 mg of the extract. After adding a few drops of $Ac_2O$, 1 ml of pure sulphuric acid was included. The presence of steroids is indicated by the blue chloroform layer that turned green existence of terpenoids by the emergence of the pink $CHCl_3$ layer.

#### 2.3.2 Test for Flavonoids Shin do's Test

Prepare extract solution (10 mg) in methanol. Then strong HCl was added, followed by magnesium turnings. Flavonoids could be seen as a pink colour.

#### 2.3.3 Test for Phenolic compounds

A few drops of a 2.5% $FeCl_3$ solution were included in a solution of 10 mg of extract dissolved in methanol. The red brown colour revealed the existence of the phenolic compound.

#### 2.3.4 Test for Coumarins

Alcoholic KOH was added after ten mg of the extract had been dissolved in methanol. The development of a yellow colour that turns gray when strong HCl is added indicated the existence of coumarins.

The extract, 10 mg, was dissolved in methanol and subjected to sulphuric acid treatment. Quinone was present because of the colour development.

#### 2.3.6 Test for Alkaloids Mayer's Test

Mayer's reagent was initially made to test for alkaloids. Mercuric chloride (1.36 g) and KI (5.0 g) were mixed in water to create the reagent (100 ml). Separately, ten mg of the extracts were dissolved in hydrochloric acid. A few solution drops were placed in the watch glass center. A glass rod was used to add Mayer's reagent along the watch glass's sides. The formation of a gelatinous white precipitate indicates a positive result.

#### 2.3.7 Tests for Saponins

A tiny amount of the extract was dissolved in distilled water and then vigorously shaken. Foams formed during the test exhibited the saponins present.

#### 2.3.8 Test for Carbohydrates Molisch's Test

After vigorously shaking with water, the extracts were filtered. The aqueous filtrate was then vigorously shaken before adding a few drops of Molisch's reagent (95% ethanol + 5% naphthol). To create a layer beneath the aqueous solution, concentrated $H_2SO_4$ (1 mL) was carefully added. The test was successful when there was a brown ring at the interface.

#### 2.3.9 Test for Tennis Lead Acetate Test

Aqueous extract (5 mL) were mixed with a few drips of a 1% solution of lead acetate (previously boiled in a water bath). Precipitation that was yellow or crimson denoted a positive test result.

The n-hexane extracts of *Jatropha curcas* stems and roots were investigated at the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories using the Electron Impact Ionization (EI) technique on a Shimadzu GC-17A gas chromatograph coupled to an MS 2010 plus mass spectrometer. In a capillary column conveying helium, a temperature of 40 °C was kept at continuous pressure of 90 kPa. A split ratio of 10 was used to administer the samples. Chloroform was used to dissolve the sample. Here are the working conditions: The column's name is RTS5MS, and its dimensions are 30 cm in diameter and 0.25 nm in length. 10% diethylene glycol succinate was used for column packing. He was used as carrier gas at the above pressure [25].

## 3. Results and discussion

### 3.1 Study of Phytochemical Constituents

**Table 1:** The findings of the phytochemical components analysis of *Jatropha curcas* stem extracts

| Investigated Phytochemicals | JCSH (n-hexane extract) | JCSC (Chloroform extract) | JCSE (Ethyl acetate extract) | JCSM (Methanol extract) |
|---|---|---|---|---|
| Steroids | + | + | + | + |
| Terpenoids | + | + | + | + |
| Flavonoids | + | + | + | + |
| Phenolic Compounds | + | + | + | + |
| Coumarins | - | + | + | + |
| Quinones | - | - | + | + |
| Alkaloids | - | + | + | + |
| Saponins | - | - | - | + |
| Anthraquinones | - | - | + | - |

| | | | | |
|---|---|---|---|---|
| Carbohydrates | - | - | - | + |
| Tannins | - | - | + | + |

**Table 2:** The findings of the phytochemical components analysis *Jatropha curcas* root extracts

| Investigated Phytochemicals | JCRH (n-hexane extract) | JCRC (Chloroform extract) | JCRE (Ethyl acetate extract) | JCRM (Methanol extract) |
|---|---|---|---|---|
| Steroids | + | + | + | + |
| Terpenoids | + | + | + | + |
| Flavonoids | + | + | + | + |
| Phenolic Compounds | + | + | + | + |
| Coumarins | - | + | + | + |
| Quinones | - | - | + | + |
| Alkaloids | - | + | + | + |
| Saponins | - | - | - | + |
| Anthraquinones | - | - | + | + |
| Carbohydrates | - | - | - | + |
| Tannins | - | - | - | + |

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

Preliminary phytochemical screening of different extracts of stems and roots of *J. curcas* were carried out in order to identify the class of compounds present in each extract. The results of this experiment provide us a guideline to isolate pure compounds from these extracts. The results showed that steroids, terpenoids, flavonoids and phenolic compounds were present in every extract of stems and roots. Alkaloids were also present in every extract except n-hexane. But carbohydrates were only present in methanol extract of 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.

#### 3.2.1 GC-MS Analysis of n-Hexane Extract of *Jatropha curcas* Stems

The GC-MS analysis allowed for the identification and quantification of 22 compounds in the n hexane extract of *Jatropha curcas* stems. The National Institute of Standard and Technology (NIST) database, which contains more than 62000 patterns, was used to interpret the mass spectrum obtained by the GC-MS instrument. The spectrum of the unknown compound was compared to the spectrum of the known compound preserved in the NIST database. The retention time, molecular formula, molecular weight and composition percentage of the sample materials of *Jatropha curcas* were recorded in Table-3.

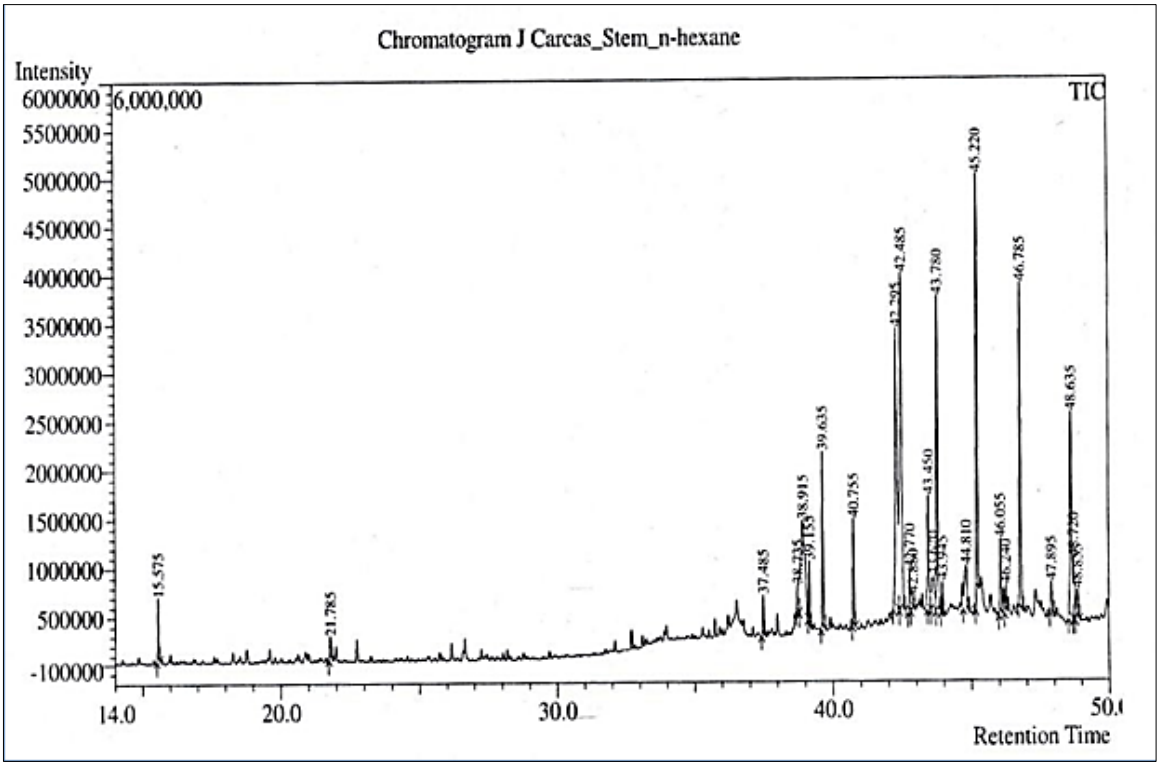


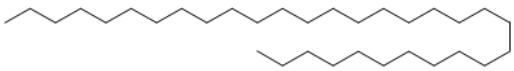
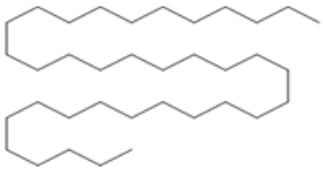
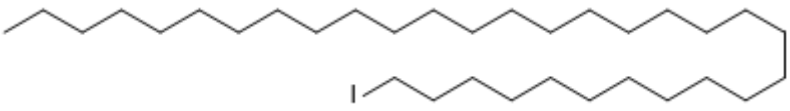

**Fig 3.26:** TIC of n-hexane extract of stem of *J. curcas*

**Table 3:** GC-MS analysis of the n-hexane extract of the stems of *J. curcas*

| Symbol | Retention time | Name of the Compound | Molecular Weight (g/mol) | Molecular Formula | Conc.% |
|---|---|---|---|---|---|
| J-1 | 15.573 | 1, 6-Cyclodecadiene | 136 | C10H16 | 1.30 |
| J-2 | 39.156 | Heneicosane | 296 | C21H44 | 2.58 |
| J-3 | 38.730 | 1,2,3,4,4a,9,10,10a- octahydro-7-methoxy-1,1,4a-trimethyl 2-Phenanthrenol | 274 | C18H26O2 | 0.60 |
| J-4 | 38.915 | Stigmasterol | 412 | C29H48O | 3.02 |
| J-5 | 39.630 | 1,2-Benzenedicarboxylic acid | 166 | C8H6O4 | 4.86 |
| J-6 | 40.754 | Eicosane | 282 | C20H42 | 4.41 |
| J-7 | 42.295 | 1-Iododotriacontane | 576 | C32H65I | 8.55 |
| J-8 | 42.483 | β-Sitosterol | 414 | C29H50O | 3.36 |
| J-9 | 42.770 | 22-Tricosenoic acid | 352 | C23H44O2 | 0.68 |
| J-10 | 42.868 | Betulinaldehyde | 440 | C30H48O2 | 0.28 |
| J-11 | 43.449 | (Z)-13-Docosenamide | 337 | C22H43NO | 3.43 |
| J-12 | 43.620 | 24-Noroleana-3,12-diene | 394 | C29H46 | 1.81 |
| J-13 | 43.783 | Tetracontane | 563 | C40H82 | 12.23 |
| J-14 | 43.944 | Squalene | 410 | C30H50 | 1.31 |
| J-15 | 44.822 | 9,19-Cyclolanost-24-en-3-ol, acetate, (3β)- | 468 | C32H52O2 | 0.72 |
| J-16 | 45.222 | Dotriacontane | 450 | C32H66 | 17.22 |
| J-17 | 46.054 | Ergosta-5,7,9 (11), 22-tetraen-3-ol, (3β,22E) | 394 | C28H42O | 1.69 |
| J-18 | 46.785 | Hexatriacontane | 506 | C36H74 | 14.85 |
| J-19 | 47.885 | Cholest-22-ene-21-ol, 3,5-dehydro-6- methoxy-, pivalate | 498 | C33H54O3 | 0.93 |
| J-20 | 48.634 | Pentacosane | 352 | C25H52 | 11.70 |
| J-21 | 48.721 | 1-Heptacosanol | 396 | C27H56O | 1.34 |
| J-22 | 48.869 | 26-Hydroxycholesterol | 402 | C27H46O2 | 0.60 |

From Table-3 the total number of identified components can be observed. Total amount of identified compound was approximately 96% and the composition of nearly 4% remained unidentified. Here the major compounds were identified as Dotriacontane (J-16, 17.22%), Hexatriacontane (J-18, 14.85%), Tetracontane (J-13, 12.23%), Pentacosane (J-20, 11.70%), 1- Iododotriacontane (J-7, 8.55%), 1,2-Benzenedicarboxylic acid (J-5, 4.86%).

**Table 4:** Structures of key compounds discovered from stems of *J. curcas* n-hexane extracts

| Name of the Compound | Chemical structure |
|---|---|
| Dotriacontane (J-16) |  |
| Hexatriacontane (J-18) |  |
| Tetracontane (**J-13)** |  |
| Pentacosane (**J-20)** |  |
| 1-Iododotriacontane (J-7) |  |

**3.2.2 GC-MS Analysis of n-Hexane Extract of *J. curcas* Roots:** The GC-MS analysis allowed for the identification and quantification of 26 compounds in the n- hexane extract of *Jatropha curcas* roots. The National Institute of Standard and Technology (NIST) database, which contains more than 62000 patterns, was used to interpret the mass spectrum obtained by the GC-MS instrument. The spectrum of the unknown compound was compared to the spectrum of the known compound preserved in the NIST database. The retention time, molecular formula, molecular weight and composition percentage of the sample materials of *Jatropha curcas* were recorded in in Table-5

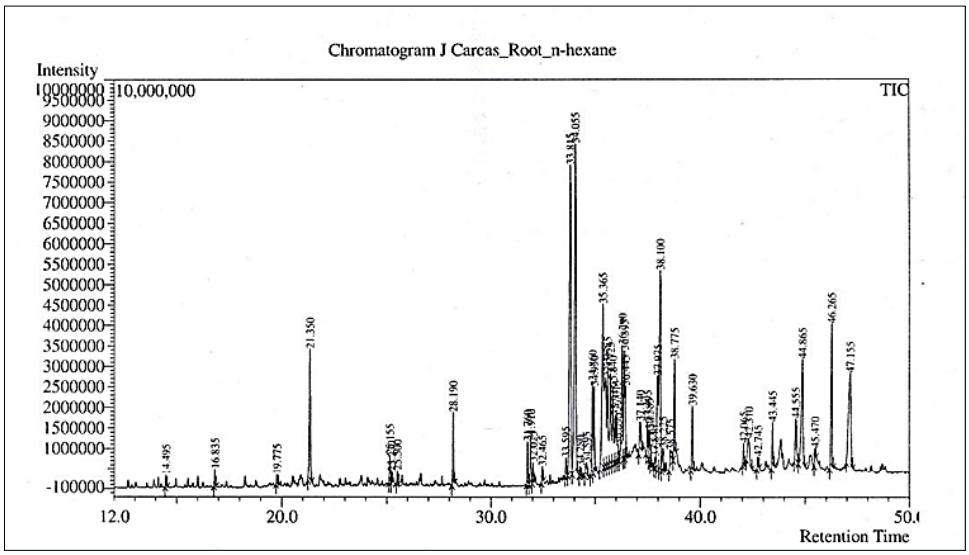


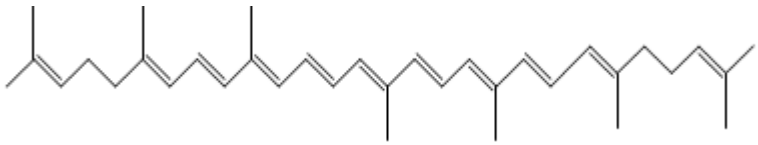
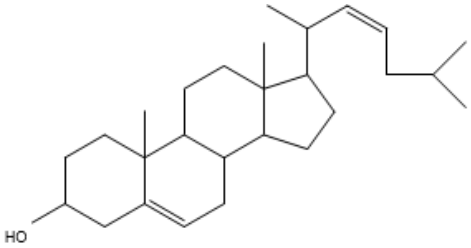
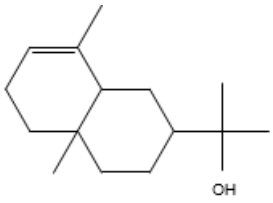
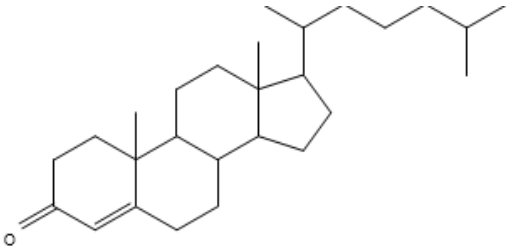
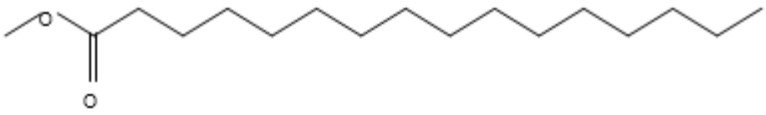

**Fig 3.27:** TIC of n-hexane extract of root of *J. curcas*

**Table 5:** GC-MS analysis of the n-Hexane extract of the roots of *J. curcas*

| Symbol | Retention time | Name of the Compound | Molecular formula | Molecular Weight (g/mol) | Conc.% |
|---|---|---|---|---|---|
| J-1 | 16.832 | Kessane | C15H26O | 222 | 0.64 |
| J-2 | 21.353 | 2-(4a, 8-Dimethyl-2,3,4,5,6,8a-hexahydro- 1H-naphthalen-2-yl)propan-2-ol | C15H26O | 222 | 8.69 |
| J-3 | 25.503 | Cryptomeridiol | C15H22O2 | 234 | 0.46 |
| J-4 | 28. 190 | Methyl hexadecanoate | C17H34O2 | 270 | 5.41 |
| J-5 | 31.761 | Methyl 9,12-octadecadienoate | C19H34O2 | 294 | 1.36 |
| J-6 | 31.911 | Methyl 11-octadecenoate | C19H36O2 | 296 | 1.36 |
| J-7 | 32.026 | 9-Octadecenoic acid | C18H34O2 | 282 | 0.51 |
| J-8 | 32.465 | Methyl stearate | C19H38O2 | 298 | 1.24 |
| J-9 | 33.595 | Khusimyl methyl ether | C16H26O | 234 | 0.30 |
| J-10 | 33.816 | 17-(1,5-Dimethyl-hex-2-enyl)-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H- cyclopenta[a]phenanthren-3-ol | C27H32O | 382 | 10.47 |
| J-11 | 34.057 | Lycopene | C40H56 | 536 | 12.46 |
| J-12 | 34.862 | 2,5-cyclohexadiene-1,4-dione | C6H4O2 | 108 | 1.81 |
| J-13 | 35.359 | 1,8,15,22-Tricosatetrayne | C23H32 | 308 | 2.07 |
| J-14 | 35.588 | Thunbergol | C20H34O | 290 | 1.51 |
| J-15 | 35.728 | Resibufogenin | C24H32O4 | 384 | 1.02 |
| J-16 | 36.289 | Retinol | C20H30O | 286 | 1.55 |
| J-17 | 37.975 | 1,3,5-triethenyl-2,4,6-triethyl-Benzene | C18H24 | 240 | 2.77 |
| J-18 | 38.577 | Dehydroabietylamine | C20H31N | 285 | 0.56 |
| J-19 | 38.776 | 2-phenanthrenol | C14H10O | 194 | 3.93 |
| J-20 | 39.629 | Diisooctyl phthalate | C24H38O4 | 390 | 4.77 |
| J-21 | 42.285 | β- Sitosterol | C29H50O | 414 | 0.83 |
| J-22 | 42.740 | Methyl triacontanoate | C31H62O2 | 466 | 0.49 |
| J-23 | 43.447 | (Z)-13-Docosenamide | C22H43NO | 337 | 2.15 |
| J-24 | 44.868 | 4,22-Cholestadien-3-one | C27H42O | 382 | 4.45 |
| J-25 | 46.267 | Lanosterol | C30H50O | 426 | 2.72 |
| J-26 | 47.155 | Cholest-4-en-3-one | C27H44O | 384 | 8.48 |

From Table-5 the total number of identified components can be observed. Total amount of identified compound was approximately 82% and the composition of nearly 18% remained unidentified. Here the major compounds were identified as Lycopene (J-11, 12.46%), 17-(1,5- Dimethyl-hex-2-enyl)-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H- cyclopenta[a]phenanthren-3-ol (J-10, 10.47%), 2-(4a, 8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H naphthalen-2-yl)propan-2-ol (J-2, 8.69%), Cholest-4-en-3-one (J-26,8.48%), Methyl hexadecanoate (J-4, 5.41%), Diisooctyl phthalate (J-20, 4.77%).

**Table 6:** Structures of key compounds discovered from stems of *J. curcas* n-hexane extracts

| Name of the Compound | Chemical structure |
|---|---|
| Lycopene (J-11) |  |
| 17-(1,5-Dimethyl-hex 2-enyl)- 10,13-dimethyl- 2,3,4,9,10,11,12,13,14,15,16,17 -dodecahydro-1H- cyclopenta[a]phenanthren-3-ol (J-10) |  |
| 2-(4a, 8-Dimethyl-2,3,4,5,6,8a- hexahydro-1H naphthalen-2- yl)propan-2-ol (J-2) |  |
| Cholest-4-en-3-one (J-26) |  |
| Methyl hexadecanoate (J-4) |  |

## 4. Conclusion

A broad overview of the presence of secondary metabolites across the whole *J. curcas* 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.

## 5. Acknowledgement

The laboratory space used for the research was provided by the Department of Chemistry at Jahangirnagar University in Savar, Dhaka, and the BCSIR handled the GC-MS analysis.

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