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All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer’s name and address. Subheadings should be used. Methods in general use need not be described in detail.
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The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


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ARTICLES

Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry
Mohammed Yahya Hadi, Ghaidaa Jihadi Mohammed and Imad Hadi Hameed

Determination of metabolites products by Cassia angustifolia and evaluate antimicrobial activity
Ali Hussein Al-Marzoqi, Mohammed Yahya Hadi and Imad Hadi Hameed
Full Length Research Paper

Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry

Mohammed Yahya Hadi¹, Ghaidaa Jihadi Mohammed² and Imad Hadi Hameed³*

¹College of Biotechnology, Al-Qasim Green University, Iraq.
²College of Science, Al-Qadisia University, Iraq.
³Department of Biology, Babylon University, Iraq.

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Phytochemicals are chemical compounds often referred to as secondary metabolites. Twenty eight bioactive phytochemical compounds were identified in the methanolic extract of *Nigella sativa*. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight, and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *Nigella sativa* revealed the existence of the β-Pinene, D-Glucose, 6-O-α-D-galactopyranosyl, O-Cymene, DL-Arabinose, Trans-4-methoxy thujane, 2-Propyl-tetrahydropyran-3-ol, Terpinen-4-ol, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1ɩ→3)-β-D-fruc, Thymoquinone, 2-Isopropylidene-5-methylhex-4-enal, Limonen-6-ol, pivalate, Longifolene, 2-(4-Nitrobutyryl)cyclooctanone, β-Bisabolene, 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol, Phenol, 4-methoxy-2,3,6-trimethyl, Pyrrolidin-2-one-3β-(propanoic acid, methyl ester), 5-methylene-4α, Cholestan-3-ol, 2-methylene-(3β,5α), l(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid (2,2), methyl ester, 1-Heptatriacotanol, 10,13-Eicosadienoic acid, methyl ester, E,E,Z-1,3,12-Nonadecatriene-5,14-diol, 9-Octadecenamide,(Z), 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[2-(dimethylar, Phthalic acid, decyl oct-3-yl ester, 1,2-Benzenedicarboxylic acid, bis(8-methyl) ester and Stigmasterol.

Key words: Gas chromatography-mass spectrometry, Fourier-transform infrared spectroscopy, *Nigella sativa*, phytochemicals.

INTRODUCTION

*Nigella sativa* L. (Ranunculaceae) is an annual herbaceous plant native to (and cultivated in) Southwest Asia, and cultivated and naturalized in Europe and North Africa (Al-Johar et al., 2008). The seeds and seed oil have been used as a diuretic, appetizer, hemorrhagic and anti-dandruff therapy in folk medicine (Al-Othman et al., 2006). *N. sativa* L. commonly known as Kalonji in Hindi, a member of Ranunculaceae family, also known as the black cumin seeds is one of the most revered medicinal seeds in history. It is an annual aromatic plant and its cultivation is traced back more than 3000 to the kingdom of the Assyrians and ancient Egyptians (Ashraf et al., 2006; Hameed et al., 2015a). *N. sativa* taxonomic classification (Ashraf, 2011), depicts it is a flowering...
dicotyledon plant belonging to family Ranunculaceae under kingdom plantae. Morphology **N. sativa** is an annual medicinal herb, about 30 to 60 cm high (Boskabady et al., 2007; Jasim et al., 2015), with finely divided, linear leaves. The flowers are usually pale blue and white, with 5 to 10 petals. The fruit is a large inflated capsule that composed of 3 to 7 united follicles, each containing numerous black trigonal seeds (Chaudhry and Tariq, 2008; Hameed et al., 2015b).

The black kalonji seeds possess the anthelmintic, insecticidal, antimalarial, antibacterial, antifungal, and antitumor effects. There are also reports that black seeds possess diuretic, carminative, digestive and antiseptic properties (Burits and Rouhou et al., 2007; Ali and Blunden, 2003; Saleh, 2006; Abdullah and Abidin, 2007; Ali et al., 2008). The seeds have also been used traditionally for centuries in the Middle East, Far East, and some Mediterranean and European countries for the treatment of different ailments, such as diabetes, hypertension, cardiac diseases, hemorrhoids, and sexual diseases and as an abortifacient (Kanter, 2008; Iqbal et al., 2010).

Seeds of **N. sativa** are reported to contain amino acids, carbohydrates, fixed and volatile oils. The yield of black seed fixed oil ranges from 22.0 to 40.35% (Ali and Blunden, 2003; Cheikh al., 2007). The extracts were also reported that black seeds have been used by patients to suppress coughs, disintegrate renal calculi (Hashem and El-Kiey, 1982), retard the carcinogenic process, treat abdominal pain, diarrhea, flatulence, and polio (Enomoto et al., 2001), exert cholereitic and uricosuric activities, anti-inflammatory and antioxidant effects (Mansour et al., 2002; Altameme et al., 2015b). Besides, the essential oil was shown to have antihelmintic, antischistosomal (Mahmoud et al., 2002), antimicrobial (Aboul-Ela et al., 1996), and antiviral.

**MATERIALS AND METHODS**

**Preparation of extract**

**N. sativa** were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the seeds were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Hameed et al., 2015c; Hamza et al., 2015). **N. sativa** seeds are washed with water, dried and ground into powder. **N. sativa** seeds powder was macerated using methanol for 1 x 24 h. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Hussein et al., 2015).

**Gas chromatography-mass spectrum (GC-MS) analysis**

The GC-MS analysis of the plant extract was made in a Agilent 7890 A instrument under computer control at 70 eV. About 1 μl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected (Imad et al., 2014a; Hameed et al., 2015d). The greater the concentration in the sample, the bigger the signal obtained which was then processed by a computer. The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the gas chromatography column into the detector (Mohammed and Imad, 2013). The x-axis showed the RT and the y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called as the mass spectrum graph which is the fingerprint of a molecule (Imad et al., 2014b). Before analyzing the extract using GC-MS, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set at 1 ml/min. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries (Kareem et al., 2015; Imad et al., 2014c).

**RESULTS AND DISCUSSION**

Preparation of the extract was done by maceration method. Maceration was done using the appropriate solvent with several times shaking or stirring at room temperature (Rader et al., 2007). Maceration is a method that is suitable for compounds that do not withstand heating at high temperatures (Ramaa et al., 2006). The aim is to attract the chemical components based on the principle of mass transfer of substance into the solvent component, where the movement began to occur at the interface layer and then diffuses into the solvent (Sethi et al., 2008). Identification of the structure using a mass spectrometer conducted to determine the compounds contained in the samples analyzed can be seen from the relative abundance of mass fragments of molecules (m/e) of the molecular ion (M +). The more stable a molecular fragment that is formed is, then the fragment will be at a relative abundance of large and have a longer lifespan (Vuorela et al., 2004; Shama et al., 2009).

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract as shown in Table 1. The GC-MS chromatogram of the 28 peaks of the compounds detected are shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract...
<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>Exact mass</th>
<th>Chemical structure</th>
<th>MS Fragment- ions</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Pinene</td>
<td>3.173</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>136.1252</td>
<td><img src="1" alt="Chemical structure" /></td>
<td>53, 69, 93, 121</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>2</td>
<td>D-Glucose,6-O-α-Dgalactopyranosyl</td>
<td>3.613</td>
<td>C_{12}H_{22}O_{11}</td>
<td>342</td>
<td>342.11621</td>
<td><img src="2" alt="Chemical structure" /></td>
<td>60, 73, 85, 110, 126, 182, 212, 261</td>
<td>Anticoagulant, anti-inflammatory, psychotomimetic and anticancer activities</td>
</tr>
<tr>
<td>3</td>
<td>O-Cymene</td>
<td>3.939</td>
<td>C_{10}H_{14}</td>
<td>134</td>
<td>134.10955</td>
<td><img src="3" alt="Chemical structure" /></td>
<td>51, 58, 65, 77, 91, 103, 119, 134</td>
<td>Anti-oxidant activity</td>
</tr>
<tr>
<td>4</td>
<td>DL-Arabinose</td>
<td>4.815</td>
<td>C_{4}H_{9}O_{5}</td>
<td>150</td>
<td>150.052823</td>
<td><img src="4" alt="Chemical structure" /></td>
<td>55, 60, 73, 85, 96, 119, 132, 149</td>
<td>Antivirus activity</td>
</tr>
<tr>
<td>5</td>
<td>Trans-4-methoxythujane</td>
<td>4.952</td>
<td>C_{11}H_{20}O</td>
<td>168</td>
<td>168.151415</td>
<td><img src="5" alt="Chemical structure" /></td>
<td>55, 59, 72, 81, 85, 93, 107, 125, 136, 153, 168</td>
<td>Antibacterial and anti-Candida activities</td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>Properties</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>2-Propyl-tetrahydropyran-3-ol</td>
<td>C₄H₁₀O₂</td>
<td>144.115029</td>
<td>Anti-allergenic and anti-bacterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>Terpinen-4-ol</td>
<td>C₁₀H₁₆O</td>
<td>154.135765</td>
<td>Anti-tumoral activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1→3)-β-D-fruc</td>
<td>C₁₈H₃₂O₁₆</td>
<td>504.169035</td>
<td>Anticarcinogenic antimutagenic, antineoplastic and anti-thrombotic</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>Thymoquinone</td>
<td>C₁₀H₁₂O₂</td>
<td>164.08373</td>
<td>Anti-cancer activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>2-Isopropylidene-5-methylhex-4-enal</td>
<td>C₁₀H₁₆O</td>
<td>152.120115</td>
<td>Good antioxidant and anti-inflammatory properties</td>
<td></td>
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Table 1. Cont’d

<table>
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<tr>
<th></th>
<th>Compound Description</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>CAS Number</th>
<th>Main Activity</th>
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</thead>
<tbody>
<tr>
<td>11</td>
<td>Limonen-6-ol, pivalate</td>
<td>C_{15}H_{24}O_{2}</td>
<td>236</td>
<td>236.17763</td>
<td>57, 93, 107, 134, 185, 236, Antioxidant and anti-inflammatory</td>
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<tr>
<td>12</td>
<td>Longifolene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>204.1878</td>
<td>55, 67, 79, 94, 107, 119, 133, 147, 161, 175, 189, 204, Antifeedant, anti tumor, anti inflammatory, antioxidant and antibacterial</td>
</tr>
<tr>
<td>13</td>
<td>2-(4-Nitrobutyryl)cyclooctanone</td>
<td>C_{12}H_{19}NO_{4}</td>
<td>241</td>
<td>241.31408</td>
<td>55, 69, 97, 123, 135, 193, 213, 241, Anti-tumor activity</td>
</tr>
<tr>
<td>14</td>
<td>β-Bisabolene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>204.1878</td>
<td>55, 69, 93, 109, 135, 161, 189, 204, Anti-ulcer activity</td>
</tr>
<tr>
<td>No.</td>
<td>Name</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>Monoisotopic Mass</td>
<td>PubChem CID</td>
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<tr>
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<td>-------------------</td>
<td>------------------</td>
<td>-------------------</td>
<td>------------</td>
</tr>
<tr>
<td>15</td>
<td>1,1-Diphenyl-4-phenythiobut-3-en-1-ol</td>
<td>C$<em>{22}$H$</em>{20}$OS</td>
<td>332</td>
<td>332.123486</td>
<td>55, 81, 105, 121, 135, 150, 179, 205, 233, 314</td>
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<tr>
<td>16</td>
<td>Phenol, 4-methoxy-2,3,6-trimethyl</td>
<td>C$<em>{10}$H$</em>{14}$O$_2$</td>
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<td>166.09938</td>
<td>53, 67, 77, 83, 91, 107, 123, 135, 151, 166</td>
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<td>17</td>
<td>Pyrrolidin-2-one-3ß-(propanolic acid, methyl ester),5-methylene-4α</td>
<td>C$<em>{10}$H$</em>{14}$NO$_3$</td>
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<td>18</td>
<td>Cholestan-3-ol, 2-methylene-,(3ß,5α)</td>
<td>C$<em>{28}$H$</em>{48}$O</td>
<td>400</td>
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<td>69, 81, 95, 149, 175, 227, 260, 315, 400</td>
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<td>19</td>
<td>l-(+)-Ascorbic acid 2,6-dihexadecanoate</td>
<td>C$<em>{33}$H$</em>{60}$O$_8$</td>
<td>652</td>
<td>652.49142</td>
<td>57, 73, 85, 98, 115, 129, 143, 157, 185, 199, 213, 227, 256, 297, 322, 353</td>
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<th>No.</th>
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<th>Molecular Mass</th>
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<th>Activity</th>
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<tr>
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<tr>
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<td>1-Heptatriacotanol</td>
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<td>22</td>
<td>10,13-Eicosadienoic acid , methyl ester</td>
<td>C_{21}H_{38}O_2</td>
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<td>322.28718</td>
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<td>E,E,Z-1,3,12-Nonadecatriene-5,14-diol</td>
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<td>24</td>
<td>9-Octadecenamide , (Z)</td>
<td>C_{19}H_{35}NO</td>
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<tr>
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<td>26</td>
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<td></td>
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<td></td>
<td></td>
<td>Adamantane-di-carboxylic acid, bis(8-methylnonyl) ester</td>
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<td>28</td>
<td>Stigmasteryl glucoside</td>
<td>C_{29}H_{48}O_{4}</td>
<td>412</td>
<td>201.406</td>
<td>Biological activities such as anti-diabetic, anti-neoplastic, anti-hypertensive and anti-retroviral</td>
</tr>
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</table>

**Figure 1.** GC-MS chromatogram of methanolic seed extract of *Nigella sativa.*
of N. sativa showed the presence of 28 major peaks and the components corresponding to the peaks were determined as follows. The first setup peak were determined to be β-Pinene (Figure 2). The second peak showed D-Glucose, 6-O-α-Dgalactopyranosyl (Figure 3). The next peaks was considered to be O-Cymene, DL-Arabinose, Trans -4-methoxy thujane, 2-Propyltetrahydropyran-3-ol, Terpinen-4-ol, α- D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-

D-fruc, Thymoquinone, 2-Isopropylidene-5-methylhex-4-enal, Limonen-6-ol, pivalate, Longifolene, 2-(4- Nitrobutyryl) cyclooctanone, β-Bisabolene, 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol, Phenol, 4-methoxy-2,3,6-trimethyl, Pyrrolidin -2-one-3β-(propanoic acid, methyl ester),5-methylene-4α, Cholestan-3-ol, 2-methylene-,(3β,5α), l-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid (Z,Z), methyl ester, 1-Heptatriacotanol, 10,13-Eicosadienoic acid, methyl ester, E,E,Z-1,3,12-Nonadecatriene-5,14-diol, 9-Octadecenamide,(Z), 2H-Benzof[fg]xireno[2,3-E]benzofuran-8(9H)-one,9-[[2-(dimethylar, Phthalic acid, decyl oct-3-yl ester, 1,2-Benzenedicarboxylic acid, bis(8-methyl)) ester and Stigmasteryl (Figures 4 to 30). Plants are considered to be one of the natural bases for the production of bioactive compounds, many of which are used to support health and fight against pathological conditions and many of them are marketed as food or herbal medicines (Shama et al., 2009). The usage of herbal medicine has amplified dramatically for various diseases amongst general people over last few years not only because of their easy accessibility without prescription, low cost and appointment to the health care specialists and more with the belief that natural remedies have less lethal effects as compared to synthetic medicines (Ashraf et al., 2011). A qualitative investigation of N. sativa has revealed the presence of sterols, triterpenes, tannins, flavanoids, cardiac glycosides, alkaloids, saponins, volatile oils, volatile bases, glucosinolates and anthraquinones (A1-Yahya, 1986). Qualitative evaluation of the black seed oil via capillary GC-MS technique has enabled the identification of 67 compounds, when classified into various functional
Figure 5. Structure of DL-Arabinose present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 6. Structure of Trans-4-methoxy thujane present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 7. Structure of 2-Propyl-tetrahydropyran-3-ol present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 8. Structure of Terpinen-4-ol present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.
Figure 9. Structure of α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1→3)-β-D-fruc present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 10. Structure of Thymoquinone present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 11. Structure of 2-Isopropylidene-5-methylhex-4-enal present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 12. Structure of Limonen-6-ol, pivalate present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.
Figure 13. Structure of 2-Isopropylidene-5-methylhex-4-enal present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 14. Structure of Longifolene present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 15. Structure of 2-(4-Nitrobutyl) cyclooctanone present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 16. Structure of β-Bisabolene present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.
Figure 17. Structure of 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 18. Structure of Phenol, 4-methoxy-2,3,6-trimethyl present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 19. Structure of Pyrrolidin-2-one-3ß-propanoic acid, 5-methylene-4α present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 20. Structure of Cholesterol-3-ol, 2-methylene-(3ß,5α) present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.
Figure 21. Structure of l-(+)-Ascorbic acid 2,6-dihexadecanoate present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 22. Structure of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 23. Structure of 1-Heptatriacotanol present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.

Figure 24. Structure of 10,13-Eicosadienoic acid, methyl ester present in the methanolic seeds extract of Nigella sativa by using GC-MS analysis.
Figure 25. Structure of E,E,Z-1,3,12-Nonadecatrien-5,14-diol present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 26. Structure of 9-Octadecenamide, (Z) present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 27. Structure of 2H-Benz[o]xireno[2,3-E]benzofuran-8(9H)-one, 9-[[2-(dimethylamino) present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.

Figure 28. Structure of Phthalic acid, decyl oct-3-yl ester present in the methanolic seeds extract of *Nigella sativa* by using GC-MS analysis.
groups corresponding with the following data: monoterpenes (~46%); carbonyl compounds (~25%); phenols (~1.7%); alcohols (~0.9%) and esters (~16%) (Abu-Jadayil et al., 1999).

**Conclusion**

*N. sativa* seed is a promising source for active ingredients that would be with potential therapeutic modalities in different clinical settings. The efficacy of the active ingredients, however, should be measured by the nature of the disease. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthmatic.

**ACKNOWLEDGEMENT**

The authors thank Dr. Abdul-Kareem Al-Bermani, Lecturer, Department of Biology, for valuable suggestions and encouragement.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**REFERENCES**


Phytochemicals are chemical compounds often referred to as secondary metabolites. Forty four bioactive phytochemical compounds were identified in the methanolic leaves extract of *Cassia angustifolia*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *C. angustifolia* revealed the existence of the 2,5-dimethyl-4-hydroxy-3(2H)-furanon, 2-propyltetrahydropyran-3-ol, estragole, benzene, 1-ethynyl-4-fluoro-, 5-hydroxymethylfurfural, anethole, 7-oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-, 2-methoxy-4-vinylphenol, 1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid, E-9-tetradecenoic acid, caryophyllene, cholesan-3-ol,2-methylene-, (3β,5α), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, β-curcumen, 7-epi-cis-sesquisabinene hydrate, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylenethyl-[(S)-[(R*,S*)]-m, octahydrobenzo[b]pyran, 4a-acetoxy-5,8a-trimethyl, dodecanoic acid, 3-hydroxy, tetraacetyl-d-xylonic nitrile, 1-ethyl-13, trans(1,1-dimethylhexyl)-4, cis-methylxyclohexan-1-ol, phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl, 5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, 5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, phytol, acetate, desulphosinquirin, oxiraneundecanoic acid, 3-pentyl-4-methyl ester, cis,Phytol, 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, butanoic acid, 1a,2,5,5a,6,9,10,10a-octahyd-ro-5,5adihydroxy-4-(h), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and Diisooctyl phthalate. *C. angustifolia* was highly active against *Aspergillus terreus* (6.01±0.27).

**Key words:** Antifungal, gas chromatography-mass spectrometry, fourier-transform infrared spectroscopy, phytochemicals, *Cassia angustifolia*.

**INTRODUCTION**

Medicinal plants are those plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs (Sofowora, 1982; Bako et al., 2005; Altamene et al., 2015a). The use of medicinal herbs to relieve and treat diseases is
increasing because of their mild features and few side effects (Basgel and Erdemoglu, 2006). These plants are unlicensed and freely available, however, and there is no requirement to demonstrate efficacy, safety or quality (Ernst, 1998). The genus Cassia comprises 580 species of shrubs and trees which are widely distributed throughout the world, of which only twenty species are indigenous to India which belongs to the family Caesalpiniaceae, which generally consist of trees, shrubs and a few woody herbs. Cassia angustifolia Vahl (Family: Caesalpiniaceae), popularly known as senna, is a valuable plant drug in Ayurvedic and modern system of medicine for the treatment of constipation. The pods and leaves of senna, as well as the pharmaceutical preparations containing sennosides A and B, are widely used in medicine because of their laxative properties. Senna is used in medicine as a cathartic; it is especially useful in habitual constipation. The laxative property of senna is based on two glycosides viz. sennoside A and sennoside B, whereas sennoside C and D have also been reported in the plant. Apart from sennoside, the pod and leaf also contain glycosides of anthraquinones rhein and chrysophenic acid, recently two naphthalene glycosides have also been isolated from leaves and pods (Gupta, 2010).

Antimicrobial activity has been reported in many plants by various workers (Sarin, 2005; Bansal et al., 2010; Chahal et al., 2010; Seth and Sarin, 2010; Malwal and Sarin, 2011; Hameed et al., 2015a). A new anthraquinone glycoside (emodin 8-0- sophorside) and seven known glycosides were isolated from the leaves of C. angustifolia and their structures were elucidated by spectral analysis (Kinjo et al., 1994). It has anti-inflammatory properties (Vanderperren et al., 2005), detoxification ability (Bournemouth, 1992) and also helps improve the function of the digestive system (Hoffmann, 1990). Cassia senna helps to reduce the nervous tension (Mills, 1993) and also helps in aiding the spleen and liver in production of blood and red blood cells (Spiller et al., 2003; Altameme et al., 2015b; Hamza et al., 2015). The present study was undertaken to investigate the antimicrobial activity and phytochemical analysis of C. angustifolia.

MATERIALS AND METHODS

Collection and preparation of plant material

C. angustifolia was purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the seeds were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Hameed et al., 2015b; Jasim et al., 2015).

Preparation of sample

About fifteen grams of methanolic leaves extract of C. angustifolia powdered was soaked in 30 ml methanol for ten hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Hussein et al., 2015; Hameed et al., 2015c).

Gas chromatography – mass spectrum analysis

The GC-MS analysis of the plant extract was made in a Agilent 7890 A instrument under computer control at 70 eV. About 1 μl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected (Imad et al., 2014a; Kareem et al., 2015). The greater the concentration in the sample, the bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the chromatography column into the detector (Mohammed and Imad, 2013; Imad et al., 2014b). The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectrocscopy) detector, where they were bombarded with a stream of electrons causing, them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass (Hameed et al., 2015d). The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called the mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures (Imad et al., 2014c). Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries.

Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 μl of the samples solutions (C. angustifolia) was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent (Hameed et al., 2015b). The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and differences among the means were determined for significance at P
RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *C. angustifolia*, as shown in Table 1. The GC-MS chromatogram of the forty four peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Althaea rosea* showed the presence of 44 major peaks and the components corresponding to the peaks were determined as follows. The first set up peaks were determined to be 2,5-dimethyl-4-hydroxy-3(2H)-furanon (Figure 2). The next peaks considered to be 2-Propyl-tetrahydropryan-3-ol, Estragole, Benzenec, 1-ethynyl-4-fluoro-, 5-Hydroxymethyl furfural, Anethole, 7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-, 2-Methoxy-4-vinylphenol, 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid, E-9-Tetradecenoic acid, Caryophyllene, Cholestan-3-ol,2-methylene-,(3β,5α)-, Benzene, 1-(1,5-dimethyl-4-}

hexenyl)-4-methyl-, β-curcumene, 7-epi-cis-sesquisabinene hydrate, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-m, Octahydrobenzo[b] pyran,4a-acetoxy-5,5,8a,-trimethyl, Dodecanoic acid, 3-hydroxy, Tetraacetyl-d-xylo nic nitrile, 1-Ethenyl 3,trans(1,1-dimethyllethyl)-4,cis-methoxycyclohexan-1-ol, Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl, 5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, 5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, Phytole, acetate, Desulphosiniqrin, Oxiraneundecanoic acid, 3-pentyl-,methyl ester,cis, Phytol, 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5adihydroxy-4-(h), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and Diisooctyl phthalate (Figures 3 to 45). Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, and *Aspergillus fumigatus* (Table 2). *C. angustifolia* was very highly active against *A. terreus* (6.01±0.27). *Aspergillus* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug amphotericin B and fluconazole to some extent.
Table 1. Major phytochemical compounds identified in *Cassia angustifolia*.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>Exact mass</th>
<th>Chemical structure</th>
<th>MS Fragment-ions</th>
<th>Pharmacological actions</th>
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</thead>
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<tr>
<td>1</td>
<td>2,5-dimethyl-4-hydroxy-3(2H)-furanone</td>
<td>4.883</td>
<td>C₆H₈O₃</td>
<td>128</td>
<td>128.047344</td>
<td><img src="image" alt="Structure" /></td>
<td>57, 72, 85, 94, 109, 128</td>
<td>Antimicrobial effect</td>
</tr>
<tr>
<td>2</td>
<td>2-Propyl-tetrahydropyran-3-ol</td>
<td>5.908</td>
<td>C₈H₁₆O₂</td>
<td>144</td>
<td>144.115029</td>
<td><img src="image" alt="Structure" /></td>
<td>55, 73, 87, 101, 116, 144</td>
<td>Anti-infective agent in human microbial infections.</td>
</tr>
<tr>
<td>3</td>
<td>Estragole,</td>
<td>6.303</td>
<td>C₁₀H₁₂O</td>
<td>148</td>
<td>148.088815</td>
<td><img src="image" alt="Structure" /></td>
<td>51, 55, 63, 77, 91, 105, 121, 133, 148</td>
<td>Anti-inflammatory activity</td>
</tr>
<tr>
<td>4</td>
<td>Benzene , 1-ethyl-4-fluoro</td>
<td>6.720</td>
<td>C₈H₇F</td>
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<td>50, 63, 74, 81, 94, 100, 120</td>
<td>Antibacterial activity / Antifungal activity</td>
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### Table 1. Cont’d

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<tr>
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<td>C_10H_12O</td>
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<td><img src="image2" alt="Structure" /></td>
<td>Antihyperglycemic effect</td>
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<tr>
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<td>7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-</td>
<td>C_10H_16O_2</td>
<td>168.115029</td>
<td><img src="image3" alt="Structure" /></td>
<td>New chemical compound</td>
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<td>2-Methoxy-4-vinylphenol</td>
<td>C_9H_10O_2</td>
<td>150.068080</td>
<td><img src="image4" alt="Structure" /></td>
<td>Antioxidant, anti microbial and anti inflammatory</td>
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Table 1. Cont’d

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<tr>
<th>No.</th>
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<th>Property</th>
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<td>9</td>
<td>1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid</td>
<td>C₁₉H₁₆O₄</td>
<td>200</td>
<td>200.104859</td>
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<td>E-9-Tetradecoenoic acid</td>
<td>C₁₄H₂₆O₂</td>
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<td>226.19328</td>
<td>Analgesic and anti-inflammatory effect</td>
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<td>11</td>
<td>Caryophyline</td>
<td>C₁₅H₂₄</td>
<td>204</td>
<td>204.1878</td>
<td>Anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic</td>
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<tr>
<td>12</td>
<td>Cholestan-3-ol,2-methylene-(3β,5α)-</td>
<td>C₂₈H₄₈O</td>
<td>400</td>
<td>400.370516</td>
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<td>Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-</td>
<td>C₁₅H₂₂</td>
<td>202</td>
<td>202.172151</td>
<td>Antimicrobial and anti-inflammatory</td>
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<td>Compound Description</td>
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<tr>
<td>14</td>
<td>Β-curcumene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>204.1878</td>
<td>Anti-tumor, anti-cancer, anti-repellent, antitussive and anti-platelet</td>
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<td>15</td>
<td>7-epi-cis-sesquisabinene hydrate</td>
<td>C_{15}H_{24}O</td>
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<td>222.198365</td>
<td>nil-cancer</td>
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<td>16</td>
<td>Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- [S-(R*,S*)]-</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>204.1878</td>
<td>Anti-bacterial and antifungal</td>
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<tr>
<td>17</td>
<td>Octahydrobenzo[b]pyran,4-acetoxy-5,5,8a-trimethyl</td>
<td>C_{12}H_{24}O_{3}</td>
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<td>18</td>
<td>Dodecanoic acid, 3-hydroxy</td>
<td>C_{12}H_{24}O_{3}</td>
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<td>1-Ethynyl 3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol</td>
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<th>Molecular Weight</th>
<th>Antitumor, anti-inflammatory, antifungal, pesticidal and insecticidal</th>
<th>Anti-inflammatory, antileishmanial and antitypanosomal</th>
<th>New chemical compound</th>
<th>Anti-oxidant</th>
<th>Anti-cancer activities</th>
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<td>23 2H-Benzof[</td>
<td>loxireno[2,3-E]benzofuran-8(9H)-one,9-[[2-(dimethylamin]</td>
<td>12.877 C_{10}H_{11}N_{2}O_{3}</td>
<td>336</td>
<td>336.241293</td>
<td>58, 71, 81, 91, 109, 123, 149, 166, 185, 204, 219, 233, 248</td>
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<td>24 Phytol, acetate</td>
<td>13.953 C_{15}H_{12}O_{2}</td>
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<td>338.318481</td>
<td>57, 68, 81, 95, 109, 123, 137, 151, 179, 208, 249, 278</td>
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<td>25 Desulphosiniqrin</td>
<td>14.399 C_{10}H_{17}NO_{6}S</td>
<td>279</td>
<td>279.077658</td>
<td>60, 73, 85, 103, 127, 145, 163, 213, 262</td>
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<td>26 Oxiraneundecanoic acid, 3-pentyl-methyl ester, cis</td>
<td>16.482 C_{20}H_{40}O_{3}</td>
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<td>27 Phytol</td>
<td>16.665 C_{10}H_{16}O</td>
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<td>296.307917</td>
<td>57, 71, 81, 95, 111, 123, 137, 196, 221, 249, 278</td>
<td>Anti-cancer activities</td>
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<td>No.</td>
<td>Compound Description</td>
<td>Formula</td>
<td>Molecular Weight</td>
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<td>New chemical compound</td>
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<td>28</td>
<td>9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester</td>
<td>C_{28}H_{34}O_{6}</td>
<td>18.296</td>
<td>440</td>
<td>440.29266</td>
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<td>29</td>
<td>Butanoic acid , 1a,2,5,5a,6,8,9,10a-octahydro-5,5ahydroxy-4-(h)</td>
<td>C_{28}H_{34}O_{6}</td>
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<td>9-Octadecenoic acid , 1,2,3-propanetriyl ester , (E,E,E)-</td>
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<td>19.846</td>
<td>884</td>
<td>884.78329</td>
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<td>31</td>
<td>Diisooctyl phthalate</td>
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<td>32</td>
<td>8,14-Seco -3,19-epoxyandrostane-8,14-dione,17-acetoxy-35-methoxy</td>
<td>C_{28}H_{34}O_{6}</td>
<td>21.449</td>
<td>420</td>
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<th>Other References</th>
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<td>33</td>
<td>Squalene</td>
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<td>410</td>
<td>69, 81, 95, 121, 149, 175, 203, 231, 257, 265, 341, 367, 395</td>
<td>Widely used in the cosmetics industry as an anti-wrinkle agent</td>
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<td>34</td>
<td>Cyclopropanebutanoic acid 2-[2]-([2]-[2]-pentylcyclopropyl)methyl)cyclo</td>
<td>C_{20}H_{50}O_{2}</td>
<td>374</td>
<td>74, 121, 227, 270, 298, 334</td>
<td>Used as a poultice as an anti-inflammatory</td>
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<td>35</td>
<td>Cycloocta-1,7,16,22-tetraone</td>
<td>C_{20}H_{50}O_{2}</td>
<td>476</td>
<td>55, 81, 125, 183, 239, 279, 321, 337, 379, 419, 458</td>
<td>New chemical compound</td>
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<td>36</td>
<td>2-[[4-methyl-6-[(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclo</td>
<td>C_{20}H_{50}O_{2}</td>
<td>324</td>
<td>555, 91, 135, 173, 187, 239, 324</td>
<td>Antimicrobials and anti-virals</td>
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<td>37</td>
<td>Oxirane ,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-hen</td>
<td>C_{20}H_{50}O_{2}</td>
<td>426</td>
<td>69, 81, 95, 135, 203, 231, 271, 299, 357, 426</td>
<td>Anti-diarrhoeal activity</td>
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<td>38</td>
<td>9,19-Cyclolanol-24-en-3-ol,acetate , (30)-</td>
<td>C_{20}H_{50}O_{2}</td>
<td>468</td>
<td>55, 69, 81, 95, 109, 135, 203, 217, 266, 311, 365, 408, 424</td>
<td>Anti mosquito larvicidal activity</td>
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<td>9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol</td>
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<td>γ-Tocopherol</td>
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<td>C_{29}H_{48}O_{2}</td>
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<td>41</td>
<td>Olean-12-ene-3,15,16,21,22,28-hexol,(3β,15α,16α,21β,22α)-</td>
<td>25.683</td>
<td>C_{28}H_{40}O_{6}</td>
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<td>Carbonic acid , ( ethyl)(1,2,4-triazol-1-ylmethyl)diester</td>
<td>3.224</td>
<td>C_{6}H_{9}N_{3}O_{3}</td>
<td>171.064391</td>
<td>Anti-inflammatory</td>
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Table 2. Zone of inhibition (mm) of Aspergillus Spp. test to Cassia angustifolia bioactive compounds and standard antibiotics.

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<th>Plant/ antibiotics</th>
<th>Aspergillus niger</th>
<th>Aspergillus terreus</th>
<th>Aspergillus flavus</th>
<th>Aspergillus fumigatus</th>
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<td>Cassia angustifolia</td>
<td>3.08±0.10</td>
<td>6.01±0.27</td>
<td>5.00±0.16</td>
<td>4.03±0.20</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>2.01±0.20</td>
<td>2.99±0.16</td>
<td>4.05±0.10</td>
<td>4.90±0.30</td>
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<tr>
<td>Fluconazol</td>
<td>4.08±0.61</td>
<td>2.96±0.14</td>
<td>3.00±0.81</td>
<td>4.90±0.40</td>
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<tr>
<td>Control</td>
<td>0.00</td>
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</table>

Figure 2. Structure of 2,5-dimethyl-4-hydroxy-3(2H)-furanone present in Cassia angustifolia with RT= 4.883 using GC-MS analysis.

Figure 3. Structure of 2-Propyl-tetrahydropyran-3-ol present in Cassia angustifolia with RT= 5.908 using GC-MS analysis.

Figure 4. Structure of Estragole present in Cassia angustifolia with RT= 6.303 using GC-MS analysis.

Figure 5. Structure of Benzene, 1-ethyl-4- fluoro present in Cassia angustifolia with RT= 6.720 using GC-MS analysis.
Conclusion

From the results obtained in this study, it could be concluded that *C. angustifolia* possesses remarkable antimicrobial activity which is mainly due to 2-Propyltetrahydropyran-3-ol, 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid and Diisooctyl phthalate. According to these findings, it could be said that the methanol extract act as antifungal agent.

Conflict of Interests

The authors have not declared any conflict of interests.
Figure 10. Structure of 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid present in *Cassia angustifolia* with RT= 8.431 using GC-MS analysis.

Figure 11. Structure of E-9-Tetradecenoic acid present in *Cassia angustifolia* with RT= 8.746 using GC-MS analysis.

Figure 12. Structure of Caryophyllene present in *Cassia angustifolia* with RT= 9.301 using GC-MS analysis.

Figure 13. Structure of Cholestan-3-ol,2-methylene- (3β,5α) present in *Cassia angustifolia* with RT= 9.616 using GC-MS analysis.
Figure 14. Structure of Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl present in Cassia angustifolia with RT = 10.010 using GC-MS analysis.

Figure 15. Structure of 7-epi-cis-sesquisabinene hydrate present in Cassia angustifolia with RT = 10.274 using GC-MS analysis.

Figure 16. Structure of 7-epi-cis-sesquisabinene hydrate present in Cassia angustifolia with RT = 10.274 using GC-MS analysis.

Figure 17. Structure of 7-epi-cis-sesquisabinene hydrate present in Cassia angustifolia with RT = 10.508 using GC-MS analysis.
Figure 18. Structure of Octahydrobenzo[b]pyran,4a-acetoxy-5,5,8a-trimethyl present in *Cassia angustifolia* with RT= 10.771 using GC-MS analysis.

Figure 19. Structure of Dodecanoic acid, 3-hydroxy present in *Cassia angustifolia* with RT= 11.218 using GC-MS analysis.

Figure 20. Structure of Tetraacetyl-d-xylonic nitrile present in *Cassia angustifolia* with RT= 11.012 using GC-MS analysis.

Figure 21. Structure of 1-Ethenyl 3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol present in *Cassia angustifolia* with RT= 11.246 using GC-MS analysis.
Figure 22. Structure of Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl present in Cassia angustifolia with RT= 11.378 using GC-MS analysis.

Figure 23. Structure of 5-Benzofuranacetic acid, 6-ethenyl -2,4,5,6,7,7a-hexahydro-3,6-dime present in Cassia angustifolia with RT= 12.036 using GC-MS analysis.

Figure 24. Structure of 2H-Benzofuroxireno[2,3-E]benzofuran-8(9H)-one, 9-[[2-(dimethylamin present in Cassia angustifolia with RT= 12.877 using GC-MS analysis.

Figure 25. Structure of Phytol, acetate present in Cassia angustifolia with RT= 13.953 using GC-MS analysis.
Figure 26. Structure of Desulphosinigrin present in Cassia angustifolia with RT= 14.399 using GC-MS analysis.

Figure 27. Structure of Oxiraneundecanoic acid, 3-pentyl- methyl ester, cis present in Cassia angustifolia with RT= 16.482 using GC-MS analysis.

Figure 28. Structure of Phytol present in Cassia angustifolia with RT= 16.665 using GC-MS analysis.

Figure 29. Structure of 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester present in Cassia angustifolia with RT= 18.296 using GC-MS analysis.
Figure 30. Structure of Butanoic acid, 1α,2,5,5a,6,9,10,10a-octahydro-5,5adihdroxy-4-(h) present in Cassia angustifolia with RT = 18.874 using GC-MS analysis.

Figure 31. Structure of 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) present in Cassia angustifolia with RT = 19.846 using GC-MS analysis.

Figure 32. Structure of Diisooctyl phthalate present in Cassia angustifolia with RT = 20.373 using GC-MS analysis.

Figure 33. Structure of 8,14-Seco-3,19-epoxyandrostan-8,14-dione, 17-acetoxy-3β-methoxy present in Cassia angustifolia with RT = 21.449 using GC-MS analysis.
Figure 34. Structure of Squalene present in *Cassia angustifolia* with RT= 22.604 using GC-MS analysis.

Figure 35. Structure of Cyclopropanebutanoic acid, 2-[[2-[[2-[[2-pentylcyclopropyl)methyl]cyclo present in *Cassia angustifolia* with RT= 22.845 using GC-MS analysis.

Figure 36. Structure of Cyclotriaconta-1,7,16,22-tetraone present in *Cassia angustifolia* with RT= 23.159 using GC-MS analysis.

Figure 37. Structure of 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclo present in *Cassia angustifolia* with RT= 23.451 using GC-MS analysis.
Figure 38. Structure of Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-hen) present in Cassia angustifolia with RT= 23.657 using GC-MS analysis.

Figure 39. Structure of 9,19-Cyclolanost-24-en-3-ol,acetate , (38) present in Cassia angustifolia with RT= 23.686 using GC-MS analysis.

Figure 40. Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol present in Cassia angustifolia with RT= 25.025 using GC-MS analysis.

Figure 41. Structure of γ-Tocopherol present in Cassia angustifolia with RT= 25.236 using GC-MS analysis.
Figure 42. Structure of Olean-12-ene-3,15,16,21,22,28-hexol, (3ß,15α,16α,21ß,22α) present in *Cassia angustifolia* with RT= 25.683 using GC-MS analysis.

Figure 43. Structure of Vitamin E present in *Cassia angustifolia* with RT= 26.581 using GC-MS analysis.

Figure 44. Structure of Campesterol present in *Cassia angustifolia* with RT= 28.315 using GC-MS analysis.

Figure 45. Structure of Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl)diester present in *Cassia angustifolia* with RT= 3.224 using GC-MS analysis.
ACKNOWLEDGEMENT

The authors thank Dr. Abdul-Kareem Al-Bermani, Lecturer, Department of Biology, for valuable suggestions and encouragement.

REFERENCES


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- Research in Pharmaceutical Biotechnology
- Medical Practice and Reviews
- Journal of Clinical Pathology and Forensic Medicine
- Journal of Medicinal Plant Research
- Journal of Drug Discovery and Development
- Journal of Clinical Virology Research