ABSTRACT

Objective: The aim of this research was to investigate the chemical composition of petroleum ether extract of *Tilia cordata* aerial parts as well as to evaluate its cytotoxic activity.

Methods: Gas chromatography and gas chromatography–mass spectrometry (GC-MS) were used to analyze the unsaponifiable matter and fatty acid methyl esters. Moreover, the cytotoxicity was examined against human hepatoma HepG2 cell line and breast adenocarcinoma MCF cell line.

Results: The result showed that thirteen compounds were identified in the fatty acid methyl esters fraction representing 93.71% of the total identified peak area. The major compounds were Octadecanoic acid methyl ester (36.26%) and eicosanoic acid methyl ester (29.42%), whereas nineteen compounds in the unsaponifiable fraction were identified representing 90.56% of the total peak area. The major compounds were 1-Nonene (30.44%), 1-Hexadecene (24.83%) and phytol (10.40%). Moreover, petroleum ether extract showed a potent cytotoxic effect against human hepatoma HepG2 cell line and a moderate cytotoxic effect on breast adenocarcinoma MCF7 human tumor cell line.

Conclusion: So the current research aims to be the first step toward the use of petroleum ether extract of *Tilia cordata* aerial parts as a potent cytotoxic drug.

Keywords: Aerial parts, chemical composition, cytotoxicity, petroleum ether extract, *Tilia cordata*.

INTRODUCTION

*Tilia cordata* belongs to family Tiliaceae, it is used in folk medicine for many purposes, and its flowers are widely used for the treatment of fever and anxiety. It contains flavonoids, volatile oils and tannins. The flower of *Tilia cordata* reported to have a potent antioxidant activity. The aerial parts of *Tilia cordata* showed antioxidant and anti-tyrosinase activities. Moreover, the aerial parts contain various phytoconstituents such as; coumarins, triterpenes, flavonoids, tannins, saponins and carbohydrates.

In addition, our recent research showed that aerial parts of *Tilia cordata* showed a powerful anti-inflammatory, antinociceptive and naphroprotective activities. Moreover, kaempferol 3-O-rutinoside, quercetin 3-O-β-galactoside, kaempferol 3-O-rutinoside, quercetin, vitexin and kaempferol were isolated and identified from aerial parts of *Tilia cordata*. The current research aims to find the correlation between the lipoidal matter of petroleum ether extract of *Tilia cordata* aerial parts and their effect on some human cell line carcinoma. So this research clarified the chemical composition of petroleum ether extract of *Tilia cordata* aerial parts as well as evaluated its cytotoxic activity.

So current study aims to be the first step toward the use of petroleum ether extract of *Tilia cordata* aerial parts as a potent cytotoxic drug with the aim of producing a natural drug.

MATERIALS AND METHODS

Plant material

*Tilia cordata* aerial parts were collected from the Agricultural Research Centre, Giza, Egypt, in March 2017. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National research centre (NRC).

Preparation of the lipoidal matter

The powder of the air-dried aerial parts of *Tilia cordata* (800g) was exhaustively extracted with light petroleum (60–80°C) in a continuous extraction apparatus (Soxhlet). The extract was evaporated under vacuum to

Raoof et al. Universal Journal of Pharmaceutical Research

Available online on 15.9.2019 at http://ujpr.org

Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal

Open access to Pharmaceutical research
This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial Share Alike 4.0 License which permits unrestricted non commercial use, provided the original work is properly cited

Volume 4, Issue 4, 2019

RESEARCH ARTICLE

CYTOTOXIC EFFECT AND PHYTOCHEMICAL STUDY OF PETROLEUM ETHER EXTRACT OF *TILIA CORDATA* MILL

Gehan F. Abdel Raoof1*, Hala M. Mohammed2†

1Pharmacognosy Department, National Research Centre, Dokki, 12622, Giza, Egypt.
2Home Economics department (Nutrition & Food Science), Faculty of Agriculture, Cairo University, Egypt.

ABSTRACT

Objective: The aim of this research was to investigate the chemical composition of petroleum ether extract of *Tilia cordata* aerial parts as well as to evaluate its cytotoxic activity.

Methods: Gas chromatography and gas chromatography–mass spectrometry (GC-MS) were used to analyze the unsaponifiable matter and fatty acid methyl esters. Moreover, the cytotoxicity was examined against human hepatoma HepG2 cell line and breast adenocarcinoma MCF cell line.

Results: The result showed that thirteen compounds were identified in the fatty acid methyl esters fraction representing 93.71% of the total identified peak area. The major compounds were Octadecanoic acid methyl ester (36.26%) and eicosanoic acid methyl ester (29.42%), whereas nineteen compounds in the unsaponifiable fraction were identified representing 90.56% of the total peak area. The major compounds were 1-Nonene (30.44%), 1-Hexadecene (24.83%) and phytol (10.40%). Moreover, petroleum ether extract showed a potent cytotoxic effect against human hepatoma HepG2 cell line and a moderate cytotoxic effect on breast adenocarcinoma MCF7 human tumor cell line.

Conclusion: So the current research aims to be the first step toward the use of petroleum ether extract of *Tilia cordata* aerial parts as a potent cytotoxic drug.

Keywords: Aerial parts, chemical composition, cytotoxicity, petroleum ether extract, *Tilia cordata*.

INTRODUCTION

* Tilia cordata* belongs to family Tiliaceae, it is used in folk medicine for many purposes, and its flowers are widely used for the treatment of fever and anxiety. It contains flavonoids, volatile oils and tannins. The flower of *Tilia cordata* reported to have a potent antioxidant activity. The aerial parts of *Tilia cordata* showed antioxidant and anti-tyrosinase activities. Moreover, the aerial parts contain various phytoconstituents such as; coumarins, triterpenes, flavonoids, tannins, saponins and carbohydrates. In addition, our recent research showed that aerial parts of *Tilia cordata* showed a powerful anti-inflammatory, antinociceptive and naphroprotective activities. Moreover, kaempferol 3-O-rutinoside, quercetin 3-O-β-galactoside, kaempferol 3-O-rutinoside, quercetin, vitexin and kaempferol were isolated and identified from aerial parts of *Tilia cordata*. The current research aims to find the correlation between the lipoidal matter of petroleum ether extract of *Tilia cordata* aerial parts and their effect on some human cell line carcinoma. So this research clarified the chemical composition of petroleum ether extract of *Tilia cordata* aerial parts as well as evaluated its cytotoxic activity.

So current study aims to be the first step toward the use of petroleum ether extract of *Tilia cordata* aerial parts as a potent cytotoxic drug.

Keywords: Aerial parts, chemical composition, cytotoxicity, petroleum ether extract, *Tilia cordata*.

MATERIALS AND METHODS

Plant material

*Tilia cordata* aerial parts were collected from the Agricultural Research Centre, Giza, Egypt, in March 2017. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National research centre (NRC).

Preparation of the lipoidal matter

The powder of the air-dried aerial parts of *Tilia cordata* (800g) was exhaustively extracted with light petroleum (60–80°C) in a continuous extraction apparatus (Soxhlet). The extract was evaporated under vacuum to

Raoof GFA, Mohammed HM

Address for Correspondence

Dr. Gehan F. Abdel Raoof, Pharmacognosy Department, National Research Centre, Dokki, 12622, Giza, Egypt. E-mail: gehankandeel9@yahoo.com.

Cite this article-


DOI: https://doi.org/10.22270/ujpr.v4i4.292

Article Info: Received 4 August 2019; Revised 22 August; Accepted 8 September, Available online 15 September 2019

ISSN: 2456-8058 CODEN (USA): UJPRA3
yield 28g of dry residue, representing 3.5% of the air-dried aerial parts.

**Investigation of the lipoidal matter**

**Saponification of the petroleum ether extract**

The petroleum ether extract (PtE) (1g) was subjected to saponification according to the method reported by Tsuda et al.\(^5\) Percentages of the unsaponifiable matter and the total fatty acid were found to be 38 and 60%, respectively.

### Table 1: GC/MS analysis of USM from petroleum ether extract of *Tilia cordata* aerial parts

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Compound</th>
<th>Mol. Formula</th>
<th>M.Wt</th>
<th>B.P.</th>
<th>RRT</th>
<th>Relative area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-Nonene</td>
<td>C(_6)H(_8)</td>
<td>126</td>
<td>55</td>
<td>3.7</td>
<td>30.44</td>
</tr>
<tr>
<td>2</td>
<td>1-Tetradecene</td>
<td>C(<em>{14})H(</em>{28})</td>
<td>196</td>
<td>55</td>
<td>4.9</td>
<td>2.58</td>
</tr>
<tr>
<td>3</td>
<td>1-Hexadecane</td>
<td>C(<em>{16})H(</em>{32})</td>
<td>224</td>
<td>55</td>
<td>7.2</td>
<td>24.83</td>
</tr>
<tr>
<td>4</td>
<td>Hexadecane</td>
<td>C(<em>{16})H(</em>{34})</td>
<td>226</td>
<td>57</td>
<td>7.5</td>
<td>3.45</td>
</tr>
<tr>
<td>5</td>
<td>Atlantic - β</td>
<td>C(<em>{15})H(</em>{26})O</td>
<td>220</td>
<td>91</td>
<td>7.4</td>
<td>4.16</td>
</tr>
<tr>
<td>6</td>
<td>Tetradecanol</td>
<td>C(<em>{14})H(</em>{26})O</td>
<td>214</td>
<td>55</td>
<td>8.2</td>
<td>1.66</td>
</tr>
<tr>
<td>7</td>
<td>Pentadecanol</td>
<td>C(<em>{15})H(</em>{26})O</td>
<td>228</td>
<td>55</td>
<td>8.6</td>
<td>3.24</td>
</tr>
<tr>
<td>8</td>
<td>Phytol</td>
<td>C(<em>{20})H(</em>{34})O</td>
<td>296</td>
<td>71</td>
<td>8.8</td>
<td>10.40</td>
</tr>
<tr>
<td>9</td>
<td>Docosene</td>
<td>C(<em>{22})H(</em>{44})</td>
<td>308</td>
<td>55</td>
<td>9.0</td>
<td>3.42</td>
</tr>
<tr>
<td>10</td>
<td>Docosane</td>
<td>C(<em>{22})H(</em>{46})</td>
<td>310</td>
<td>57</td>
<td>9.1</td>
<td>1.09</td>
</tr>
<tr>
<td>11</td>
<td>Tetracosene</td>
<td>C(<em>{24})H(</em>{48})</td>
<td>336</td>
<td>55</td>
<td>9.8</td>
<td>0.25</td>
</tr>
<tr>
<td>12</td>
<td>Tetracosane</td>
<td>C(<em>{24})H(</em>{50})</td>
<td>338</td>
<td>57</td>
<td>9.9</td>
<td>1.20</td>
</tr>
<tr>
<td>13</td>
<td>Pentacosane</td>
<td>C(<em>{25})H(</em>{52})</td>
<td>352</td>
<td>57</td>
<td>10.6</td>
<td>0.12</td>
</tr>
<tr>
<td>14</td>
<td>Heptacosane</td>
<td>C(<em>{27})H(</em>{56})</td>
<td>380</td>
<td>57</td>
<td>11.1</td>
<td>0.21</td>
</tr>
<tr>
<td>15</td>
<td>Octacosane</td>
<td>C(<em>{28})H(</em>{58})</td>
<td>392</td>
<td>55</td>
<td>11.3</td>
<td>0.08</td>
</tr>
<tr>
<td>16</td>
<td>Squalene</td>
<td>C(<em>{30})H(</em>{60})</td>
<td>410</td>
<td>69</td>
<td>11.4</td>
<td>0.24</td>
</tr>
<tr>
<td>17</td>
<td>Cholesterol</td>
<td>C(<em>{27})H(</em>{46})O</td>
<td>386</td>
<td>43</td>
<td>11.9</td>
<td>0.17</td>
</tr>
<tr>
<td>18</td>
<td>β-Sitosterol</td>
<td>C(<em>{27})H(</em>{48})O</td>
<td>414</td>
<td>43</td>
<td>13.15</td>
<td>1.01</td>
</tr>
<tr>
<td>19</td>
<td>γ-Sitosterol</td>
<td>C(<em>{27})H(</em>{48})O</td>
<td>426</td>
<td>218</td>
<td>13.9</td>
<td>2.01</td>
</tr>
</tbody>
</table>

### Table 2: GC/MS analysis of fatty acids of petroleum ether extract of *Tilia cordata* aerial parts identified as the methyl esters

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Compound</th>
<th>Mol. Formula</th>
<th>M.Wt</th>
<th>B.P.</th>
<th>RRT</th>
<th>Relative area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl decanoate</td>
<td>C(<em>{10})H(</em>{20})O</td>
<td>186</td>
<td>74</td>
<td>0.70</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>Methyl dodecanoate</td>
<td>C(<em>{12})H(</em>{24})O</td>
<td>214</td>
<td>74</td>
<td>0.72</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>Methyl tetradecanoate</td>
<td>C(<em>{14})H(</em>{28})O</td>
<td>242</td>
<td>74</td>
<td>0.75</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>14-methyl-pentadecanoic acid methyl ester</td>
<td>C(<em>{17})H(</em>{30})O</td>
<td>270</td>
<td>74</td>
<td>0.78</td>
<td>2.27</td>
</tr>
<tr>
<td>5</td>
<td>9-Hexadecenoic (Palmitoleic) acid, methyl ester</td>
<td>C(<em>{18})H(</em>{32})O</td>
<td>268</td>
<td>55</td>
<td>0.80</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>Hexadecanoic acid methyl ester (methyl palmitate)</td>
<td>C(<em>{16})H(</em>{32})O</td>
<td>270</td>
<td>74</td>
<td>0.81</td>
<td>7.75</td>
</tr>
<tr>
<td>7</td>
<td>11-Hexadecenoic (Palmitoleic) acid methyl ester</td>
<td>C(<em>{17})H(</em>{30})O</td>
<td>268</td>
<td>55</td>
<td>0.84</td>
<td>7.37</td>
</tr>
<tr>
<td>8</td>
<td>Octadecanoic acid methyl ester (Methyl stearate)</td>
<td>C(<em>{18})H(</em>{36})O</td>
<td>298</td>
<td>74</td>
<td>0.94</td>
<td>36.26</td>
</tr>
<tr>
<td>9</td>
<td>Eicosanoic acid methyl ester (Methyl arachidate)</td>
<td>C(<em>{20})H(</em>{40})O</td>
<td>326</td>
<td>74</td>
<td>1.05</td>
<td>29.42</td>
</tr>
<tr>
<td>10</td>
<td>13-Eicosanoic acid methyl ester</td>
<td>C(<em>{22})H(</em>{44})O</td>
<td>324</td>
<td>55</td>
<td>1.07</td>
<td>9.35</td>
</tr>
<tr>
<td>11</td>
<td>Methyl docosanoate methyl</td>
<td>C(<em>{24})H(</em>{48})O</td>
<td>354</td>
<td>74</td>
<td>1.09</td>
<td>0.32</td>
</tr>
<tr>
<td>12</td>
<td>Methyl tetracosanoate</td>
<td>C(<em>{32})H(</em>{64})O</td>
<td>382</td>
<td>74</td>
<td>1.20</td>
<td>0.26</td>
</tr>
<tr>
<td>13</td>
<td>Methyl hexacosanoate</td>
<td>C(<em>{32})H(</em>{64})O</td>
<td>410</td>
<td>410</td>
<td>1.22</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**Preparation of fatty acid methyl esters**

Free fatty acids obtained by saponification were methylated according to the method reported by Fimar 1967\(^7\).

**GC/MS analysis**

Both the unsaponifiable and the saponifiable fractions were studied to identify their contents using GC/MS analysis. The constituents were identified by comparison of their mass spectral fragmentation patterns with those of the available database libraries, Wiley (Wiley International, Colorado, USA) and NIST (Nat. Inst. St' Technol., Colorado, USA), and/or published data\(^7,8\). Quantitative determination was carried out on the basis of the peak area integration.

**Cytotoxicity assay procedures**

**Tumor cell lines**

Human hepatocellular liver carcinoma (HepG2) and human breast carcinoma (MCF-7) cell lines were obtained in frozen state under liquid nitrogen (-180°C) from the American Type Culture Collection. The tumor cell lines were maintained by serial sub-culturing in the National Cancer Institute, Cairo, Egypt.

**Culture media**

The cells were suspended in RPMI 1640 medium (Sigma Aldrich) supplemented with 10% fetal calf
serum (SIGMA, USA) in presence 1% antibiotic and antimycotic mixture (10.000 U/ml K-penicillin, 10.000 µg/ml streptomycin sulphate and 25 µg/ml amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

Table 3: Cytotoxic activity of petroleum ether extract of Tilia cordata aerial parts

<table>
<thead>
<tr>
<th>Human Cell line</th>
<th>HepG2</th>
<th>IC₅₀ (µg/ml)</th>
<th>MCF7</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>petroleum ether extract of Tilia cordata aerial parts</td>
<td>5.42</td>
<td>9.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3.62</td>
<td>3.34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IC₅₀ is the concentration that produces 50% inhibition

Assay method for cytotoxic activity

The cytotoxicity against Hep-G2 and MCF-7 cells were tested in the National Cancer Institute, according to the SRB (Sulforhodamine B) assay by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) method, Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug. The method was described in

RESULTS AND DISCUSSION

The results showed that nineteen compounds in the unsaponifiable fraction were identified representing 90.56 % of the total peak area. The major compounds were 1- Nonene (30.44%), 1-Hexadecene (24.83%) and phytol (10.40%) (Table1). Moreover, thirteen compounds were identified in the fatty acid methyl esters fraction representing 93.71% of the total identified peak area. The major compounds were Octadecanoic acid methyl ester (36.26%) and Eicosanoic acid methyl ester (29.42%) (Table 2).

The cytotoxic activity

There are many researches that showed the cytotoxic effect of hydrocarbons and triterpenoids against many human tumor cell lines.10,11 The current research aims to find the correlation between the lipidal matter of petroleum ether extract of Tilia cordata aerial parts and their effect on some human cell line carcinoma. The cytotoxicity of petroleum ether extract of Tilia cordata aerial parts was evaluated against, HepG2 and MCF7 cell lines using Doxorubicin as reference drug. The results showed that the extract had cytotoxic activity against the tested cell lines (IC₅₀ (µg/ml)=5.42 and 9.67), respectively, while Doxorubicin showed activity with IC₅₀ (µg/ml)=3.62 and 3.34, respectively. So this result showed that petroleum ether extract of Tilia cordata aerial parts had a potent cytotoxic effect against HepG2 and moderate activity against MCF7 (Table 3).

CONCLUSION

This work was carried out to investigate the chemical composition of petroleum ether extract of Tilia cordata aerial parts as well as to evaluate its cytotoxicity against human hepatoma HepG2 cell line and breast adenocarcinoma MCF7 cell line. The result revealed that petroleum ether extract showed a potent cytotoxic effect against human hepatoma HepG2 cell line and a moderate cytotoxic effect on breast adenocarcinoma MCF7 human tumor cell line. This study aims to be the first step toward the use of petroleum ether extract of Tilia cordata aerial parts as anticancer agent upon further clinical studies.

ACKNOWLEDGEMENT

We acknowledge to National Research Centre and Faculty of Agriculture, Cairo University for using laboratory instruments in doing research.

AUTHOR’S CONTRIBUTION

All authors have worked equally for this work.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES