**Reviewer’s Comments**

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**Cytotoxic Effect and Phytochemical Study of Petroleum Ether Extract of *TiliaCordata***

**ABSTRACT**

The aim of this research was to investigate the chemical composition of petroleum ether extractof *Tilia Cordata*aerial parts as well as to evaluate its cytotoxic activity.Gas chromatography and gas chromatography–massspectrometry(GC-MS)were used to analyzethe unsaponifiable matter and fatty acid methyl esters. Moreover, the cytotoxicity was examined againsthuman hepatoma HepG2 cell line and breast adenocarcinoma MCFcell line. 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 beak 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 moderatecytotoxic effect on breast adenocarcinoma MCF7human tumor cell line.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.

***Key words:***Aerial parts, chemical composition ,cytotoxicity, petroleum ether extract***,*** *Tilia Cordata* ,

**INTRODUCTION**

*Tilia cordata* belongs to Tiliaceae, it is used in folk medicine for many purposes,itsflowers are widely used for the treatment of fever and anxiety. It contains flavonoids, volatile oils and tannins 1. The flower of *Tilia cordata* reported to have a potent antioxidant activity2.The aerial parts of *Tilia*cordata showed antioxidant and anti-tyrosinase activities3. Moreover, the aerial parts contain various phytoconstituents such as; coumarins, triterpenes , flavonoids, tannins ,saponins and carbohydrates3.In addition, our recent research showed thataerial parts of *Tilia* cordatashowed a powerful anti-inflammatory, antinociceptive and nephroprotective activities4. Moreover, kaempferol 3-Orutinoside, quercetin 3-O-β-galactoside, kaempferol 3-O-α-rhamnoside , quercetin, vitexin and kaempferol were isolated and identified from aerial parts of *Tilia* cordata4. The current research aims to find thecorrelation between the lipoidal matter of petroleum ether extract of *Tilia cordata* aerial parts and their effect on some human cell line carcinoma.So this researchclarified the chemical composition of petroleum ether extract of *Tilia Cordata*aerial parts as well as evaluated its cytotoxic activity.So our study aims to be the first step toward the use of petroleum ether extract of*Tilia Cordata*aerial parts as a potent cytotoxic drugwith 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* (800 g) was exhaustively extracted with light petroleum (60°–80°C) in a continuous extraction apparatus. The extract was evaporated under vacuum to yield 28 g 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) (1 g) was subjected tosaponification according to the method reported by Tsuda *et al*.5. Percentages of the unsaponifiable matter and thetotal fatty acid were found to be 38 and 60%, respectively.

***Preparation of fatty acid methyl esters***

Free fatty acids obtained by saponification were methylatedaccording to the method reported by Finar 6.

***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 massspectral fragmentation patterns with those of the availabledatabase libraries, Wiley (Wiley International, Colorado,USA) and NIST (Nat. Inst. St Technol., Colorado, USA),and/or published data7,8. Quantitative determinationwas carried out on the basis of the peak area integration.

**Cytotoxicty 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 (-180ºC) 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 ALORICH) supplemented with 10% fetal calf serum (SIGMA, USA) in presence 1% antibiotic 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).

***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-dimethylthiazol2-yl)- 2,5-diphenyltetrazolium bromide) method, Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug.The method was described in9.

**RESULTS AND DISCUSSION**

The results showed that nineteen compounds in the unsaponifiable fraction were identified representing 90.56 % of the total beak area.The major compounds were 1- Nonene (30.44%), 1-Hexadecene (24.83%) and phytol (10.40%) (table 1).Moreover,

thirteencompounds 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 thecorrelation between the lipoidal 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 Doxorubicinshowed activity with IC50 (µg/ml ) =3.62 and 3.34, respectively.So this result showed thatpetroleum ether extract of *Tilia cordata* aerial partshad 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 partsas well as to evaluate its cytotoxicity againsthuman hepatoma HepG2 cell line and breast adenocarcinoma MCFcell line. The result revealed that petroleum ether extract showed a potent cytotoxic effect against human hepatoma HepG2 cell line and a moderatecytotoxic effect on breast adenocarcinoma MCFhuman tumor cell line.So this study aims to be the first step toward the use of petroleum ether extract of*Tilia cordata* aerial partsas anticancer agent upon further clinical studies.

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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No.  | Compound  | Mol. Formula  | M.Wt  | B.P  | \*RRt  | Relativearea % |
| 1 | 1- Nonene  | C9H18 | 126 | 55 | 3.7 | 30.44 |
| 2 | 1-Tetradecene | C14H28 | 196 | 55 | 4.9 | 2.58 |
| 3 | 1-Hexadecene | C16H32 | 224 | 55 | 7.2 | 24.83 |
| 4 | Hexadecane | C16H34 | 226 | 57 | 7.3 | 3.45 |
| 5 | Atlantol – β | C15H24O | 220 | 91 | 7.4 | 4.16 |
| 6 | Tetradecanol | C14H30O | 214 | 55 | 8.2 | 1.66 |
| 7 | Pentadecanol | C15H32O | 228 | 55 | 8.6 | 3.24 |
| 8 | phytol | C20H40O | 296 | 71 | 8.8 | 10.40 |
| 9 | Docosene | C22H44 | 308 | 55 | 9.0 | 3.42 |
| 10 | Docosane | C22H46 | 310 | 57 | 9.1 | 1.09 |
| 11 | Tetracosene | C24H48 | 336 | 55 | 9.8 | 0.25 |
| 12 | Tetracosane | C24H50 | 338 | 57 | 9.9 | 1.20  |
| 13 | Pentacosane | C25H52 | 352 | 57 | 10.6 | 0.12 |
| 14 | Heptacosane | C27H56 | 380 | 57 | 11.1 | 0.21 |
| 15 | Octacosene | C28H58 | 392 | 55 | 11.3 | 0.08 |
| 16 | Squalene | C30H50 | 410 | 69 | 11.4 | 0.24 |
| 17 | Cholesterol | C27H46O | 386 | 43 | 11.9 | 0.17 |
| 18 | *β-*Sitosterol | C29H50O | 414 | 43 | 13.15 | 1.01 |
| 19 | α-amyrin | C30H50O | 426 | 218 | 13.9 | 2.01 |

**Table 2. GC/MS analysis of fatty acids of petroleum ether extract of*Tilia cordata* aerial partsidentified as the methyl esters**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Compound** | **Mol.****Formula** | **M.Wt** | **B.P** | **\*RRt** | **Relative****area %** |
| **1** | Methyl decanoate  | C11H22O2  | 186  | 74  | 0.70  | 0.23  |
| **2** | Methyl dodecanoate  | C13H26O2 | 214  | 74  | 0.72  | 0.15  |
| **3** | Methyl tetradecanoate  | C15H30O2 | 242  | 74  | 0.75  | 0.13  |
| **4** | 14-methyl-Pentadecanoic acid methyl ester  | C17H34O2 | 270  | 74  | 0.78  | 2.27  |
| **5** | 9-Hexadecenoic (Palmitoleic) acid, methyl ester | C17H32O2 | 268  | 55  | 0.80  | 0.25  |
| **6** | Hexadecanoic acid methyl ester (methyl palmitate)  |  [C17H34O2](http://www.chemspider.com/Molecular-Formula/C17H34O2) | 270  | 74  | 0.81  | 7.75  |
| **7** | 11-Hexadecenoic (Palmitoleic) acid methyl ester  | C17H32O2 | 268  | 55  | 0.84  | 7.37  |
| **8** | Octadecanoic acid methyl ester (Methyl stearate)  | [C19H38O2](http://www.chemspider.com/Molecular-Formula/C19H38O2) | 298  | 74  | 0.94  | 36.26 |
| **9** | Eicosanoic acid methyl ester (Methyl arachidate)  | C21H42O2 | 326  | 74  | 1.05  | 29.42 |
| **10** | 13-Eicosenoic acid methyl ester  | C21H40O2 | 324  | 55  | 1.07  | 9.35  |
| **11** | Methyl docosanoate methyl  | C23H46O2 | 354  | 74  | 1.09  | 0.32  |
| **12** | Methyl tetracosanoate  | C25H50O2 | 382  | 74  | 1.20  | 0.26  |
| **13** | Methyl hexacosanoate  | C27H54O2  | 410  | 410  | 1.22  | 0.40  |

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

|  |
| --- |
| (µg/ml)IC50 |
| MCF7 | HepG2 | Human Cell line  |
| 9.67 | 5.42 | petroleum ether extract of *Tilia cordata* aerial parts |
| 3.34 | 3.62 | Doxorubicin |

IC50: the concentration that produces 50% inhibition