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RESEARCH ARTICLE

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

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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 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 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[1]. The flower of *T. cordata* reported to have a potent antioxidant activity[2]. The aerial parts of *T. cordata* showed antioxidant and anti-tyrosinase activities[3]. Moreover, the aerial parts contain various phytoconstituents such as; coumarins, triterpenes, flavonoids, tannins, saponins and carbohydrates[3].
In addition, our recent research showed that aerial parts of *T. cordata* showed a powerful anti-inflammatory, antinociceptive and nephroprotective activities[4]. 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 *T. cordata*[4]. The current research aims to find the correlation between the lipoidal matter of petroleum ether extract of *T. cordata* aerial parts and their effect on some human cell line carcinoma. So this research clarified the chemical composition of petroleum ether extract of *T. 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 *T. cordata* aerial parts as a potent cytotoxic drug with the aim of producing a natural drug.

MATERIALS AND METHODS

Plant material

T. 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 *T. cordata* (800 g) was exhaustively extracted with light petroleum (60–80°C) in a continuous extraction apparatus (Soxhlet). 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 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. **Preparation of fatty acid methyl esters**

Free fatty acids obtained by saponification were methylated according to the method reported by Finar 1967[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 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.

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 Aldrich) 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).

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

Compound	Mol. Formula	M.Wt	B.P	RRt	Relative area %
1- Nonene	C_9H_{18}	126	55	3.7	30.44
1-Tetradecene	$C_{14}H_{28}$	196	55	4.9	2.58
1-Hexadecene	$C_{16}H_{32}$	224	55	7.2	24.83
Hexadecane	$C_{16}H_{34}$	226	57	7.3	3.45
Atlantol – β	$C_{15}H_{24}O$	220	91	7.4	4.16
Tetradecanol	$C_{14}H_{30}O$	214	55	8.2	1.66
Pentadecanol	$C_{15}H_{32}O$	228	55	8.6	3.24
phytol	$C_{20}H_{40}O$	296	71	8.8	10.40
Docosene	$C_{22}H_{44}$	308	55	9.0	3.42
Docosane	$C_{22}H_{46}$	310	57	9.1	1.09
Tetracosene	$C_{24}H_{48}$	336	55	9.8	0.25
Tetracosane	$C_{24}H_{50}$	338	57	9.9	1.20
Pentacosane	$C_{25}H_{52}$	352	57	10.6	0.12
Heptacosane	$C_{27}H_{56}$	380	57	11.1	0.21
Octacosene	$C_{28}H_{58}$	392	55	11.3	0.08
Squalene	$C_{30}H_{50}$	410	69	11.4	0.24
Cholesterol	$C_{27}H_{46}O$	386	43	11.9	0.17
β-Sitosterol	$C_{29}H_{50}O$	414	43	13.15	1.01
α-amyrin	$C_{30}H_{50}O$	426	218	13.9	2.01

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

Compound	Mol. Formula	M.Wt	B.P.	RRt	Relative area %
Methyl decanoate	$C_{11}H_{22}O_2$	186	74	0.70	0.23
Methyl dodecanoate	$C_{13}H_{26}O_2$	214	74	0.72	0.15
Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	74	0.75	0.13
14-methyl-Pentadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	74	0.78	2.27
9-Hexadecenoic (Palmitoleic) acid, methyl ester	$C_{17}H_{32}O_2$	268	55	0.80	0.25
Hexadecanoic acid methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$	270	74	0.81	7.75
11-Hexadecenoic (Palmitoleic) acid methyl ester	$C_{17}H_{32}O_2$	268	55	0.84	7.37
Octadecanoic acid methyl ester (Methyl stearate)	$C_{19}H_{38}O_2$	298	74	0.94	36.26
Eicosanoic acid methyl ester (Methyl arachidate)	$C_{21}H_{42}O_2$	326	74	1.05	29.42
13-Eicosenoic acid methyl ester	$C_{21}H_{40}O_2$	324	55	1.07	9.35
Methyl docosanoate methyl	$C_{23}H_{46}O_2$	354	74	1.09	0.32
Methyl tetracosanoate	$C_{25}H_{50}O_2$	382	74	1.20	0.26
Methyl hexacosanoate	$C_{27}H_{54}O_2$	410	410	1.22	0.40

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[9].

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%) (Table 1). 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).

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

	IC_{50} (µg/ml)	
Human Cell line	HepG2	MCF7
petroleum ether extract of *T. cordata* aerial parts	5.42	9.67
Doxorubicin	3.62	3.34

IC_{50}: the concentration that produces 50% inhibition

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 lipoidal matter of petroleum ether extract of *T. cordata* aerial parts and their effect on some human cell line carcinoma. The cytotoxicity of petroleum ether extract of *T. 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 (IC_{50} (µg/ml)=5.42 and 9.67), respectively, while Doxorubicin showed activity with IC_{50} (µg/ml)=3.62 and 3.34, respectively. So this result showed that petroleum ether extract of *T. cordata* aerial parts had a potent cytotoxic effect against HepG2 and moderate activity against MCF7 (Table 3).

CONCLUSIONS

This work was carried out to investigate the chemical composition of petroleum ether extract of *T. 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. *In conclusion*, this study aims to be the first step toward the use of petroleum ether extract of *T. cordata* aerial parts as anticancer agent upon further clinical studies.

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AUTHOR'S CONTRIBUTION

Raoof GFA: writing original draft, methodology, investigation. **Mohammed HM:** formal analysis, data curation, conceptualization. Final version of manuscript is approved by all authors.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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