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Chemical composition, antioxidant potential and cyto-protecting activity of essential oil of *Liriodendron tulipifera* L. leaves

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ABSTRACT

Objectives : The present study was under taken to characterize chemical composition, antioxidant and cyto-protecting capacity of essential oil obtained from leaves of *Liriodendron tulipifera* L.

Methods : Essential oil from the leafof *L. tulipifera* L. (EOLL) was extracted by hydro-distillation process and further its chemical composition was evaluated by GC-MS analysis. The *in vitro* antioxidant potential of the EOLL was determined by $DPPH^{\bullet}$, $ABTS^{\bullet+}$, superoxide and nitric oxide free radical scavenging activity using different concentrations in the range of 50-800 μg/mL. In addition, cyto-protecting property of the EOLLwas determined by MTT assay on Raw 264.7 macrophage cells challenged with hydrogen peroxide (H_2O_2).

Results : The result of GC-MS analysis showed presence of 34 volatile compounds, principally germacrene D, spathulenol, and α-cadinol in EOLL. The *in vitro* antioxidant assays of EOLL at the highest used concentration of 800 μg/mL showed 81.62, 84.29, 83.59 and 58.59% inhibition of $DPPH^{\bullet}$, $ABTS^{\bullet+}$, superoxide, and nitric oxide radicals, respectively. It also showed ferric reducing ability with 1310.04 mM Fe (II)/g of essential oil. The EOLL at three different concentrations (200, 400 and 800 μg/mL) protected the cells from H_2O_2-induced cell damage through scavenging intracellular ROS.

Conclusion : The findings from the study suggest that essential oil isolated from leaves of *L tulipifera* L. is a potent sources of natural antioxidants, which could be used to treat the diseases associated with oxidative stress condition.

Key words : *Liriodendron tulipifera* L., GC-MS, oxidative stress, MTT assay, antioxidant, cyto-protection.

Introduction

Oxidation is a key step for generation of energy in aerobic metabolism to fuel biological process. Free radicals such as superoxide radical ($O_2^{\bullet}$) hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) are constantly generated as byproduct of metabolism[1]. Besides endogenous metabolism process, exogenous sources such as ionizing radiations, UV light, and pesticides are also involved in production of highly reactive free radicals[2]. Fortunately, living beings possess an innate antioxidant enzymes defense mechanism including catalase, superoxide dismutase (SOD), glutathione (GSH) and peroxidase, to protect the functional and structural integrity of the biological molecules such as DNA, proteins, and lipids from detrimental effects of ROS[3,4]. However, during oxidative stress, which results from imbalance between formation and neutralization of pro-oxidants, antioxidant defence system become insufficient and this oxidative state is sole culprit for the physiological and pathological conditions such as cancer, aging, rheumatoid arthritis, atherosclerosis and neurodegenerative diseases[5-9].

In recent years research has been more focused towards to dugout non-toxic and more effective bioactive phyto-chemicals having antioxidant property, to substitute synthetic preservative antioxidants like butylated

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hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), whose applications are circumscribed because of their toxic and/or mutagenic concerns[10]. In this context, among different kinds of natural substances, essential oils and their components due to their relatively non-toxic nature, acceptance with consumers, and a wide spectrum of biological activity, have received a meticulous attention as a potential source of antioxidants[11]

Liriodendron tulipifera a L. commonly known as tulip tree is a rich source of various biologically active phyto-chemical including sesquiterpene, apophine, and alkaloids which makes it a suitable candidate for pharmacy[12,13]. The bark of *L. tulipifera* was used by the native Americans as a tonic, stimulant and febrifuge, and likely was used to treat the intermittent fevers associated with malaria[14,15]. However, there are no such reports about antioxidant activity and oxidative stress healing properties of the essential oil obtained from leaves of *L. tulipfera*. Therefore, the present study was under taken to assess the chemical composition, antioxidant and cyto-protective activity of essential oil obtained from the leaves of *L. tulipifera* (EOLL).

Materials and methods

1. chemicals and cell line

DPPH (1,1-diphenyl-2picrylhydrazyl), ABTS (2,2-azinobis -(3ethylthiazoline-6-sulphonic acid), ferric chloride, Griess reagent, nitro blue tetrazolium (NBT), xanthine, xanthine oxidase, 2,4,6-Tris (2-pyridyl)-*s*-triazine (TPTZ), quercetin, sodium nitroprusside (SNP), CM-H_2DCFDA stain, fetal bovine serum(FBS), Dulbeco's modified eagles medium (DMEM), trypsin-EDTA, antibiotics, phosphate buffer saline (PBS) and hydrogen peroxide, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used for isolation of essential oil were of highest analytical grade. Raw 264.7 macrophages, obtained from American Type Culture Collection (ATCC), were grown as monolayer in DMEM supplemented with 10% FBS, and 1% penicillin-streptomycin at 37 ℃ under 5% CO_2 in humidified chamber.

2. Plant material and isolation of essential oil

Leaves of *L. tulipifera* were collected from Kyoungsan city of Republic of Korea in August 2012. After air drying, leaves of *L. tulipifera* (500 g) were grinded and subjected to hydro distillation for 3 h using a Clevenger type apparatus. The essential oil from the aqueous distillate was extracted by liquid-liquid extraction method using dichloromethane. The essential oil was recovered from dichloromethane layer by drying it in anhydrous sodium sulfate. The obtained essential oil with a yield of 0.27% (w/w) was transfer into dark color vial and stored at 4 ℃ for further use.

3. Gas chromatography-mass spectrometry (GC-MS) analysis of EOLL

Quantitative and qualitative analysis of the EOLL was performed using a GC-MS (Model QP 2010, Shimadzu, Japan) equipped with a ZB-1 MS fused silica capillary column (30 cm × 0.25 cm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 220 and 290 ℃, respectively. The oven temperature was programmed from 50 to 150 ℃ at 3 ℃/min, then held isothermal for 10 min and finally raised to 250 ℃ at 10 ℃/min Diluted samples (1/100 (v/v), in methanol) of 1 mL were manually injected in the split less mode. The relative percentage of the oil constituents was expressed as percentage by peak area normalization.

Identification of components of the EOLL was based on their retention indices, relative to a homologous series of n-alkane (C8 - C20) on the ZB-1 capillary column under the same operating conditions and computer matching with the Wiley 6.0 libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature data[16].

4. *In vitro* antioxidant activity

1) Determination of DPPH• radical scavenging activity

DPPH• free radical scavenging activity of EOLLwas estimated using the method of Liyana-Pathirana and Shahid (2005)[17] with slight modifications. 100 μl of different concentrations (50, 100, 200, 400, 800 μg/mL) was added in different test tubes. A solution of 0.1 mM DPPH in methanol was prepared and 900 μl of this solution was added to these tubes, and then shaken vigorously. The tubes were allowed to stand for 20 min at 27 ℃ in dark. The control was prepared as above without any sample. The absorbance of the samples was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the extracts and was calculated by using the following formula :

% radical scavenging activity = [(absorbance of control

- absorbance of sample) / absorbance of control] × 100. IC_{50} was calculated from regression equastion. Quercetin was used as a standard antioxidant. All the concentrations were used in triplicate and the graph was plotted with the mean values.

2) Determination of $ABTS^{\bullet+}$ radical scavenging activity

The $ABTS^{\bullet+}$ radical scavenging activity of different concentration of EOLL was determined according to the procedure described by Re et al. (1999)[18] with slight modifications. $ABTS^{\bullet+}$ was dissolved in water to a 7 mM concentration. $ABTS^{\bullet+}$ radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted with ethanol [about 1 : 89 (v/v)] and equilibrated at 30 ℃ to give an absorbance of 0.7 ± 0.02 in a 1 cm cuvette at 734 nm 100 μl of different concentrations (50, 100, 200, 400, 800 μg/mL) of the EOLL was added to 900 μl $ABTS^{\bullet+}$ free radical solution and incubated for 30 min. OD was taken at exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the extract and was calculated using the following formula : % radical scavenging activity = [(absorbance of control-absorbance of sample)/absorbance of control] × 100. Quercetin was used as a standard antioxidant.

3) Super oxide free radical scavenging activity

The super oxide anion radical protecting activity of EOLL was estimated according to the method Kuthan et al., (1986)[19] with slight modification. The reaction mixture contained 0.25 mL of 0.8 mM xanthine in 0.1 mM potassium phosphate (pH 7.8), 0.15 mL of 0.5 mM nitro-blue tetrazolium (NBT) in 0.1 mM potassium phosphate (pH 7.8) and 0.09 mL of different concentrations of EOLL. After incubation at 25 ℃ for 15 min, the reaction was started by adding 0.5 U/mL xanthine oxidase and reaction mixtures were kept at 25 ℃ for 30 min and after that, by adding 0.5 mL of 1 N HCl, reaction of samples was stopped. The absorbance was measured at 560 nm. Quercetin was used as a positive control.

4) Nitric oxide free radical scavenging activity

At physiological pH, SNP in aqueous solution spontaneously generates nitric oxide[20], which interacts with oxygen to produce nitric ions that can be determined by using Greiss reagent. Scavengers of nitric oxide compete with oxygen to minimize the production of nitric oxide. 10 mM of sulphanilamide (SNP) in 0.1 mM phosphate buffer saline (PBS) was mixed with different concentrations (50-800 μg/mL) of EOLL and incubated at 25 ℃ for 150 min. After the completion of incubation period, Greiss reagent A (2% SNP in 4% H_3PO_4) and Greiss reagent B (0.2% napthyl ethylenediamine dihydrochloride) were added to EOLL + SNP mixture and a further incubated for 10 min. The OD of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthyl ethylenediamine was measured at 540 nm. Quercetin was used as a standard antioxidant.

The percentage of inhibition was measured by the following formula :

% radical scavenging activity = [(absorbance of control - absorbance of sample) / absorbance of control] × 100. All the tests were performed in triplicate and the graph was plotted with the mean values.

5) Ferric- reducing (FRAP) assay

The antioxidant activity of EOLL was determined according to Pulido et al. (2000)[21]. Freshly prepared FRAP reagent (900 μl) was added in 30 μl of EOLL and incubated in water bath for 30 min the temperature of water bath was kept at 37 ℃ . The FRAP reagent contained 2.5 mL of 20 mM TPTZ in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6.

5. MTT assay

Cell viability in the presence of H_2O_2 was determined by MTT bioassay. Briefly, Raw 264.7 macrophage cells (1×10^6 cells/well) were cultured in 96-well plate. After incubation with different concentration of EOLL (200, 400, 800 μg/mL) for 24 h, cells were challenged with 0.33 mM H_2O_2 and further incubated for 45 min. After completion of incubation period, 10 μl/well of MTT solution (5 mg/mL in PBS) was added and plate was incubated for additional 4 h at 37 ℃ to produce the formazan crystals. Finally after the incubation, cells were dissolved in 100 μl of DMSO and absorbance was measured in ELISA plate reader (Bio-Tek instrument Co., WA, USA) at 540 nm.

6. Determination of intra-cellular ROS

H_2O_2 induced ROS (reactive oxygen species) inhibition activity of EOLL was evaluated by using CM-H2DCFDA assay. For this assay, Raw 264.7 macrophage cells (1×10^6 cells/well) were incubated with different concentrations (200, 400, 800 μg/mL) of EOLL for 24 h at 37 ℃ in CO_2 incubator. After that cells were assaulted with

0.33 mM H_2O_2 for 45 min. Positive and negative control Raw 264.7 macrophage (1×10^6 cells/well) were incubated only with PBS and 0.33 mM H_2O_2 respectively. After 45 min of incubation, cells were trypsinzed, and washed with PBS and cells were incubated for additional 10 min in presence of 5 mM CM-H_2DCFDA dye. The levels of fluorescence intensity were immediately detected by using an EPI fluorescent microscope (Nikon TS 100, JapaN) at 200 X magnification. Quercetin was used as a positive control.

7. Statistical analysis

The data were presented as mean ± SEM (n = 3) and analysed using the analysis of variance (ANOVA) procedure of Statistical Analysis System (SPSS, Version 11).

Results

1. Chemical composition of essential oil (EOLL)

Exactly thirty four compounds were identified representing 98.38 % of the total oil (Table 1). EOLL was found to be rich in sesquiterpenes with germacrene D (13.51 %), spathulenol (13.09 %), α-cadinol (12.98 %), methoxy-eugenol (9.76 %), trans-Nerolidol (9.08 %), 10,12-pentacosadiynoicacid (6.28 %), as main representatives.

Table 1

Essential oil composition of leaves of *L. tulipifera*.

RI	Compound	% RA	Indentification
10.88	α- Hydroxytoluene	0.98	RI,MS
11.24	Eucalyptol	2.97	RI,MS
11.40	Santolina triene	0.36	RI,MS
11.65	Cyclopropane	0.40	RI,MS
12.27	β-Linalool	2.77	RI,MS
13.90	α -terpineol	1.04	RI,MS
13.99	Benzofuran	0.65	RI,MS
14.84	Cis -4-Decen-1-ol	0.94	RI,MS
15.01	Trans -4-Decen-1-ol	0.59	RI,MS
15.23	Benzenepropanoic acid	0.87	RI,MS
16.88	Eugenol methyl ether	3.20	RI,MS
17.30	Cyclohexane	2.02	RI,MS
18.35	α-Caryophyllene	0.57	RI,MS
18.75	**Germacrene**	13.51	RI,MS
19.56	Trans- Nerolidol	9.08	RI,MS
19.89	Methoxy-eugenol	9.76	RI,MS
20.15	**Spathulenol**	13.09	RI,MS
20.27	Caryophyllene oxide	2.29	RI,MS
20.95	10,12-Pentacosadiynoic acid	6.28	RI,MS
21.20	α-Cadinol	12.98	RI,MS
21.85	Trans-Farnesol	3.72	RI,MS
23.22	Isothujol	1.39	RI,MS
23.88	Patchoulane	2.65	RI,MS
24.34	Tans-Z-alphaBisabolene epoxide	0.58	RI,MS
25.59	Myristic acid	2.73	RI,MS
28.45	9,12,15-octadecatrien-1-ol	0.30	RI,MS
34.33	Hexadecanoic acid	0.78	RI,MS
37.60	Penta deconoic acid	1.71	RI,MS
	TOTAL	**98.38**	

RI, comparison of retention index with bibliography.

a Retention indices relative to n-alkanes C8–C20 on ZB-1 capillar column.

b Relative area (peak area relative to the total peak area).

c Identification: MS comparison of mass spectra with NIST MS libraries.

2. *In vitro* antioxidant activity

1) DPPH$^\bullet$ radical scavenging activity

The DPPH$^\bullet$ radical is a stable radical with a maximum absorbance at 517 nm that can readily undergo reduction by the antioxidant molecule. The radical scavenging capacity of EOLL is showed in Fig. 1, expressed as a percentage reduction of the initial DPPH radical absorption by the tested compounds. In this assay, the EOLL at the concentration of 800 μg/mL showed high scavenging of DPPH radical with a percentage inhibition of 81.62% (IC_{50} 348.18 μg/mL). A significant difference ($p<0.05$) in the DPPH$^\bullet$ radical scavenging activity was observed at different concentration.

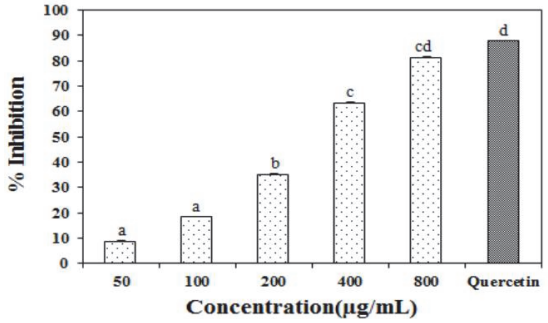


Fig. 1. DPPH$^\bullet$ radical scavenging activity of EOLL. Quercetine (200 μg/mL) used as standard compound. (Values are mean of ± SD, n=3). Values with different superscripts differ significantly from each other ($p<0.05$).

2) ABTS$^{\bullet+}$ radical scavenging activity

Furthermore, the antioxidant capacity of EOLL confirmed by ABTS$^{\bullet+}$ radical scavenging activity at different concentrations. As demonstrated in Fig. 2, EOLL showed 84.28 % (IC_{50} 330.11 μg/mL) inhibition at 800 μg/mL. EOLL showed a significant ($p<0.05$) scavenging activity toward ABTS radical cations in a concentration dependent manner, showing a direct role in quenching of free radicals. Standard compound quercetin at 1.25 μg/mL showed 95.07% inhibition

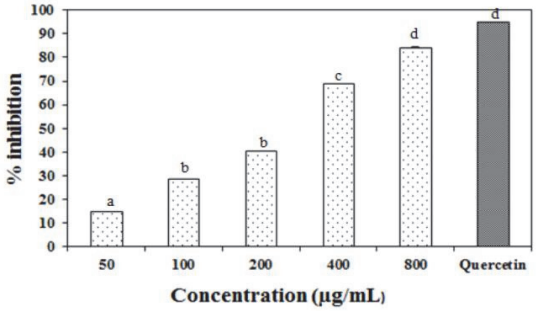


Fig. 2. Determination of ABTS$^{\bullet+}$ free radical scavenging activity of EOLL. Quercetine (1.25 μg/mL) used as standard compound (Values are mean of ± SD, n=3). Values with different superscripts differ significantly from each other ($p<0.05$).

3) Super oxide free radical scavenging activity

Superoxide radical protecting activity EOLL measured by the NBT which generates superoxide radicals. As shown in Fig. 3, EOLL showed a significant ($p < 0.05$) inhibition of superoxide free radicals in a concentration dependent manner with IC_{50} of 243 μg/mL, maximum activity of 83.59% inhibition reported at 800 μg/mL EOLL concentration. Standard compound quercetin showed 96.63% of inhibition at 20 μg/mL.

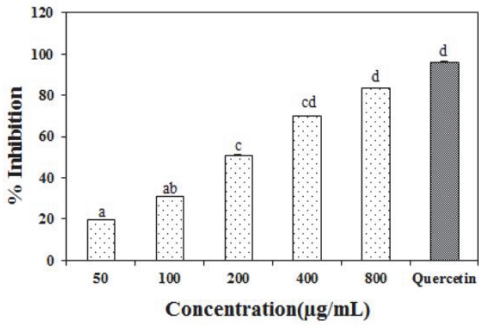


Fig. 3. Effect of EOLL against super oxide free radical scavenging activity. Quercetine (20 μg/mL) used as standard compound (Values are mean of ± SD, n=3). Values with different superscripts differ significantly from each other ($p < 0.05$).

4) Nitric oxide free radical scavenging activity

In this study, EOLL reduced the levels of nitrite by competing with oxygen to react with nitric oxide radical, a possible protective effect against oxidative damage. As shown in Fig. 4, EOLL showed most efficient inhibition of nitric oxide radicals with 58.50% at 800 μg/mL in concentration-dependent manner (IC_{50} 448 μg/mL). Standard compound, quercetin showed 67.2 % of inhibition at 500 μg/mL.

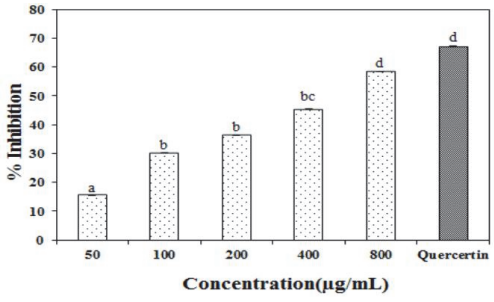


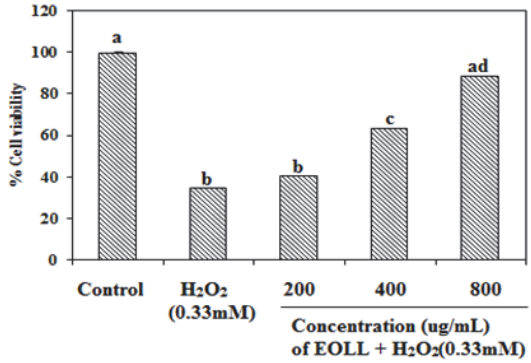
Fig. 4. Nitric oxide free radical scavenging activity of EOLL. Quercetine (500 μg/mL) used as standard compound (Values are mean of ± SD, n=3). Values with different superscripts differ significantly from each other ($p < 0.05$).

5) Ferric- reducing (FRAP) assay

The antioxidant potential of EOLL was examined for its capacity to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). EOLL showed 1310.484 mM Fe (II)/g of essential oil ferric reducing capacity. Essential oils containing terpene hydrocarbons and oxygenated terpenes showed antioxidant potential through their reductive capacity TPTZ-Fe (III) complex to TPTZ-Fe (II)(57).

3. Cyto-protective effect against H_2O_2-induced ROS

Cytotoxicity modulating activity of EOLL was evaluated on Raw 264.7 macrophage cell survival challenged with H_2O_2 which is a strong inducer of oxidative stress with its cytotoxic property in biological system. From Fig. 5, in the absence of EOLL, H_2O_2-treated cell survival was reduced to 34.47% as we supposed 100% viability in control cells, but cytotoxic effect of H_2O_2 was undermined by the pre-incubation with the different concentrations of EOLL (200, 400, 800 μg/mL) as cell survival was significantly ($P < 0.05$) increased to 40.51, 63.38, 88.39% respectively. It showed that EOLL has the potential to reduce H_2O_2 induced cytotoxicity due to having antioxidant activity at cellular level.



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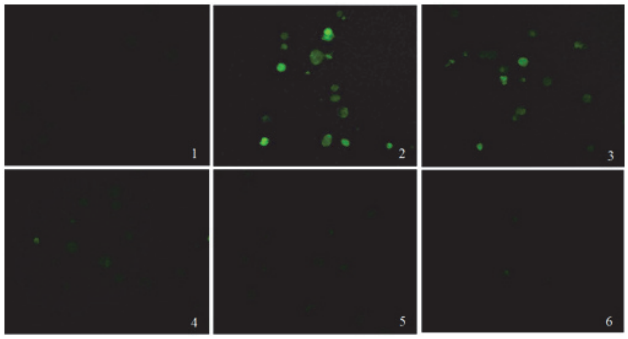
4. Inhibition of intracellular ROS

To further explore the mechanism of the protective effect of EOLL on H_2O_2-induced oxidative damage in Raw 264.7 macrophage cells, we examined the effect of EOLL on inhibition of intracellular ROS induced by H_2O_2 by using CM-H_2DCFDA stain, which freely can penetrate to cell membranes. After penetrating inside the cells, CM-H_2DCFDA is hydrolyzed to DCF due to esterase activity and it interacts with free radicals and forms the fluorescent 2', 7'- dichlorofluorescein, which produces green color and is detected by using EPI fluorescence microscope (Nikon TS 100, Japan). As shown in Fig. 6 (A and B), the intracellular elevation of ROS resulting from H_2O_2 exposure was significantly ($p < 0.05$) suppressed by pre-incubation of EOLL at 200, 400, 800 μg/mL in a dose-dependent manner. This showed antioxidant mechanism of EOLL that scavenged from intracellular ROS.

(A)



(B)

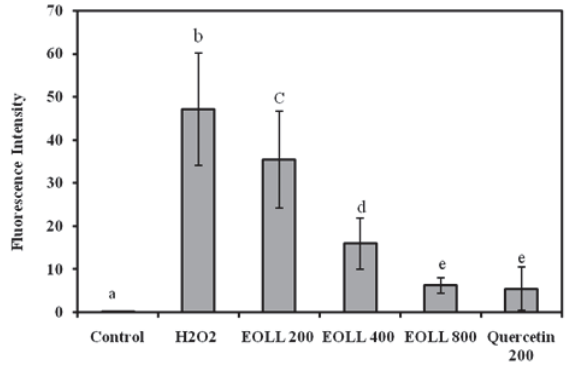


Fig. 6. Effect of essential oil isolated from leaves of *Liriodendron tulipifera* (EOLL) on intracellular ROS induced by treatment of H2O2 0.33 mM) in Raw 264.7 macrophage. A. (1) Control; without treatment (2) Only H_2O_2 treatment; (3) EOLL 200 μg/mL + H_2O_2; (4) EOLL 400 μg/mL + H_2O_2; (5) EOLL 800 μg/mL + H_2O_2; (6) Standard compound, Quercetin 200 μg/mL + H_2O_2. All images taken by EPI Florescence microscope (Nikon TS 100, Japan) at 200X.

Discussion

Plant based secondary metabolites such as essential oils are used in the many food industries and generally regarded as safe[22]. These secondary metabolites posses many biological activities such as antimicrobial, antioxidant, anti-inflammatorty and anticancer[23,24]. Antioxidants are the substance that protects the body from damage caused by harmful molecules called. synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) are now suspected due to their potentially harmful and variety of side effect to human health[25] as a region an antioxidant substance of natural origins like essential oils are gaining more interest. For this reason the present study was carried out to determine the antioxidant potential of essential oil isolated from *L. tulipifera* L. leaves. In order to determine the chemical composition of *L. tulipifera leaves* essential oil it was subjected to detailed GC/MS analysis. Results showed that it is composed of mainly 34 compounds with abundance of sesquiterpenes with germacrene D (13.51%), spathulenol (13.09%) and α-cadinol (12.98%). In recent years, phenolic compounds, terpenes and their oxygenated phyto-constituents have been reported to have enormous antioxidant and free radical scavenging capacity[26]. In general, the antioxidant compounds of essential oils are terpenes, which are phenolic in nature, and it would seem rational that their antioxidant mode of action might be related to that of other compounds. Interestingly, the EOLL was also found to rich in sesquiterpenes, which acts as direct free radical scavengers[27]. Moreover, it stimulates the synthesis of cellular antioxidant enzymes and alleviates cellular damage under elevated oxidative stress conditions[28,29].

The antioxidant potential of a substance is primarily evaluated by the DPPH• assay[22]. The DPPH• radical is a stable radical with a maximum absorbance at 517 nm that can readily undergo reduction by the antioxidant molecule. Consequently, the more rapidly the absorbance decreases, the more potent antioxidant capacity of EOLL observed in terms of hydrogen atom or electron donating ability. In this study we used Quercetin as a reference compounds at the concentration of 200 μg/mL which showed high inhibitory effect on scavenging DPPH• radical with about 88.03% and fairly comparable with 800 μg/mL EOLL. In a similar kind of the study antioxidant potential of *Metasequoia glyptostroboides* and *Iris planifolia* (Mill) plant-based essential oils have been proved by using a DPPH assay model for confirmation of antioxidant activity where they found high DPPH scavenging capacity, which warrants the significance of our study and validates the results obtained by us as well[30-32].

In the living beings superoxide radicals, a highly toxic species, are continuously produced in metabolic and physiological processes. Superoxide free radicals can generate more toxic and reactive free radicals i.e. hydroxyl radicals or singlet oxygen. Biological molecules such as DNA, lipids and proteins are prominent targets of these oxygen species and cause metabolic and cellular disturbances[6]. The above mentioned reason provoked us to determine the superoxide radicals scavenging activity of EOLL. On the basis of this assay, we have evidenced that EOLL can quench superoxide free radicals and can terminate radical chain reaction. A research carried out by Chikhi et al., 2012 on essential of *Iris planifolia* (Mill) also reported the similar kind of result which states that their essential oil potentially sacavenged the superoxide radicals to prove a potent antioxidant activity[32]. Also, an extensive review by Miguel MG, 2010 explored that those essential oils that efficiently can scavenge the superoxide radical possess a potent

antioxidant activity is in supports of our finding[33].

Nitric oxide radical is a key molecule that involved in several physiological processes such as neural signal transmission, vasodilatation and regulation of blood pressure[34]. Due to emergence in level of nitric oxide radical during oxidative stress, it becomes the major culprit for numerous carcinomas and inflammatory conditions including arthritis, ulcerative colitis juvenile diabetes, and multiple sclerosis[35]. During aerobic metabolism, nitric oxide molecule reacts with oxygen molecule and generates intermediates such as NO_2, N_2O_4 and N_3O_4. These products are highly genotoxic; causing deamination of purines, pyrimidines and denaturation of enzymes such as DNA ligase and DNA alkyl transferase[36]. Our results of nitric oxide radical scavenging activity showed that EOLL at 800 μg/mL inhibit 58.5% of nitric oxide radicals as compare to 67.2% of standard quercetin at 200 μg/mL. The nitric oxide radical scavenging capacities of various plant-based essential oils have been reported previously[32,33] and our data designate that EOLL could be an important source of natural nitric oxide radical scavenger.

Antioxidants can be explained as reductants, and neutralizer of pro-oxidants[37]. Therefore, the antioxidant potential of EOLL was evaluated for its capacity to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) by donating electron. We observed a positive ferric reducing activity of EOLL and our results are in accordance with previous findings where essential oils extracted from *Eucalyptus* and *Curcuma* species showed a positive ferric reducing activity[38].

ROS plays a vital role equally in health and disease. The maintenance of low ROS levels is critical to normal cell functions[39]. Hence a substance that can inhibit intracellular ROS can be very useful in combating many diseases. For this reason we evaluated the role of EOLL in inhibition of intracellular ROS induced by H_2O_2. Our findings suggested the dose dependent inhibition of ROS level in the Raw 264.7 macrophages suggesting EOLL a potential candidate as a natural antioxidant.

Conclusion

In this study, we evaluate the antioxidant and cyto-protective potential of the essential oil isolated from the leaves of *L. tulipifera* L. (EOLL) and further its GC-MS analysis to characterized it.

1. The GC-MS analysis showed the presence of several important phyto-constituents majorly germacrene D, spathulenol, α-cadinol, methoxy-eugenol, trans-nerolidol, 10,12-Pentacosadiynoicacid. Also, the EOLL was found to exhibit good antioxidant and free radical scavenging capacities.

2. The EOLL was found to exhibit good antioxidant and free radical scavenging capacities in an *in vitro* examination.

3. Moreover, EOLL showed potent cyto-protecting activity against the H_2O_2 by reducing the generation of intracellular ROS.

These experimental evidences proved that EOLL contains bioactive compounds, as sources of natural antioxidants, which might be beneficial to prevent various oxidative stress induced diseases. This study provided the evidence required for the utilization of newly characterized EOLL for its possible use as a source of cost effective natural antioxidant.

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