Original Article

In vitro Anticancer Activity and Antioxidant Properties of Essential Oils from *Populus alba* L. and *Rosmarinus officinalis* L. from South Eastern Anatolia of Turkey

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ABSTRACT

Background and Purpose: In recent years, essential oils (EOs) have been reported to possess interesting anti-tumor, anti-mutagenic and anti-carcinogenic activities against various cancer cells. Therefore, we aimed to investigate potential biological activities of EOs from white poplar (*Populus alba L.*, *Salicaceae*) and rosemary (*Rosmarinus officinalis* L., *Lamiaceae*). **Material and Methods:** EOs from *P. alba* L. and *R. officinalis* L. were extracted by hydrodistillation. MTT assay was carried out to determine the potential antiproliferative and cytotoxic properties of the essential oils as well as their corresponding IC_{50}, and the inhibition (%) calculated. Antioxidant activity was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay, and lipid peroxidation capacity was evaluated using thiobarbituric acid-reactive substances (TBARS) method, and the values were calculated using the standards. **Results:** The EOs were evaluated for their *in vitro* cytotoxic, antioxidant and lipid oxidation activities. Regarding cytotoxic activity rosemary essential oil possessed strong inhibition (IC_{50} = 3.06-7.38 μg/mL) of cell proliferation in comparison to that of *P. alba* L. (IC_{50} = 10.53-28.16 μg/mL). Additionally, EO from *R. officinalis* L. was found to have higher antioxidant and lipid peroxidation capacities with IC_{50} of 10.08 ± 0.15 and 1.76 ± 0.01, respectively. **Conclusion:** The results suggest that the EOs of both sources exhibited strong antiproliferative, cytotoxic and potent antioxidant properties and therefore they can have potential applications in the cancer treatment.

KEY WORDS: Cytotoxicity, *Populus alba* L., *Rosmarinus officinalis* L., DPPH, EOs, MTT, TBARS, IC_{50}.

INTRODUCTION

Medicinal and aromatic plants (MAPs) have been used in traditional medicine in all parts of the world since the ancient times, and nowadays interest in using both different MAPs and products derived from MAPs have continued almost in all sectors and industries.[1,2] In recent years, a large number of secondary metabolites, including essential oils (EOs) are considered as valuable sources for ingredients in food and pharmaceutical industries, cosmetics and medicinal applications for prevention and/or treatment of many diseases including neurodegenerative diseases, rheumatoid arthritis, diabetes, respiratory diseases, cardiovascular diseases, cancers and inflammatory and oxidative diseases.[2,3] EOs extracted from MAPs, contain 300 compounds such as limonene, α-pinene, β-myrcene, β-elemene, caryophyllene, humulene, α-farnesene, cembrene, kaurene, eucalyptol, camphorene, camphor, carveol, limonene oxide, caryophyllene oxide, farnesol, humulene epoxide, α-bisabolene oxide, carvacrol, catechol, eugenol, isopropyl

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alcohol, butyl alcohol, perillyl alcohol, geraniol, nerol, safrole, sesquiterpenes, rosmarinic acid, nerolidol, benzophenone, bornyl acetate, ethyl acetate, vernolide, cinnamaldehyde, acetophenone, helenin, fumarin, benzofuran etc., that exhibit many biological properties including antibacterial, antifungal, antioxidant, antiulcer, antispasmodic, antiproliferative, antitumor, antihepatotoxic, and others. EOs have thus become a focal point to improve and maintain human health needs and treatment of the diseases with less side effects.[3-5]

Previous researches both *in vivo* and *in vitro* showed that EOs possess major bioactive components and molecules, which have chemoprevention and cancer suppression activities. EOs have also been demonstrated to have potentials to fight against cancer cells by multiple pathways and mechanisms, including of apoptosis and cell cycle arrest, angiogenesis and cell proliferation, of DNA damage and to repair signaling and activation of detoxification enzymes such as glutathione reductase, catalase glutathione, superoxide dismutase, peroxidase, etc.[2-5]

R. officinalis L., also known as rosemary, is native to the Mediterranean region and grown in many parts of the world. Possesses a wide range of biological activities and medicinal uses, particularly for the treatment of central nervous system complaints, liver, respiratory and genito-urinary disorders, cancer gastrointestinal and cardiovascular diseases.[6-8] In addition to its medicinal uses, it has been used for culinary, food flavoring and spice, cosmetics, and perfumery applications.[9,10] Recent studies revealed that rosemary EOs have greater biological effects than its extracts since the EOs have higher contents of major bioactive components, especially polyphenols and phenylpropanoids such as rosmarinic acid, carnosic acids, camphor, camphene, α-pinene, β-pinene, borneol, caryophyllene, limonene, terpineol and myrcene.[8-12] *P. alba* L., known as white poplar, growing in a wide range of environmental conditions, is belonging to the family *Salicaceae* and native to central and southern Europe.[13] Although there were some previous investigations on the EO of P. alba , to the best of our knowledge, this is the first report of its *in vitro* anticancer and antiproliferative activities.

Contrary to rosemary extracts, which have been widely investigated for their anticancer effects, the EOs of rosemary and white poplar have not yet been thoroughly investigated for their biological activities especially antioxidant, antiproliferative and cytotoxic properties. Therefore, we have evaluated potential antioxidant properties, lipid peroxidation capacity, *in vitro* cytotoxic and antiproliferative activities of EOs extracted from *P. alba* L. and *R. officinalis* L. against three human cancer cell lines: A549 cells (human lung adenocarcinoma), H1299 (human non-small lung cancer) and MCF-7 (human breast adenocarcinoma), obtained from the American Type Culture Collection (ATCC).

MATERIALS AND METHODS

Plant material: The aerial parts of *P. alba L.* and *R. officinalis* L. were collected from Gaziantep, south eastern Anatolia of Turkey, and kept at laboratory conditions at room temperature (25°C) with no direct light until the extraction.

Extraction of EOs: The leaves and flowers of *P. alba L.* and *R. officinalis* L. were used for extraction of EOs. All the plant parts (100 g for each plant species) were extracted separately by hydrodistillation using a Clevenger Apparatus for 4h at 100±5°C, and the extracted oils were dried over anhydrous Na_2SO_4, then the pure essential oils were obtained and stored at 4°C for further analysis.

***In vitro* anticancer activity assay by MTT method:** MTT assay was carried out to determine the potential antiproliferative and cytotoxic properties of the EOs from *P. alba* L. and *R. officinalis* L. against three human cancer cell lines: A549 cells (human lung adenocarcinoma), H1299 (human non-small cell lung cancer), MCF-7 (human breast adenocarcinoma), and non-tumor HUVEC cells, obtained from the American Type Culture Collection (ATCC). MTT assay was performed as previously reported by Al-Qubaisi *et al*.[14] with minor modifications. Cells were cultured on RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin in the flasks with 5% CO_2 at 37°C. The 5 x 10^3 cells per mL were treated with EOs with concentrations of 0, 1, 10, 100 µM, and incubated at 37°C for 48h. After incubation, 50 µL/well of MTT solution was added into each well, then the OD was measured at 570 nm and the inhibition (%) was calculated as;

[(mean OD without test agent (negative control) - mean OD with test agent / mean OD without test agent (negative control)] × 100

***In vitro* antioxidant activity assay by DPPH method:** Antioxidant activity was determined using microplate 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay described by Molyneux[15] with some modifications. Ascorbic acid was used as standard. The absorbance value was measured at 517 nm using a spectrophotometer in triplicate. The inhibition of the DPPH scavenging activity was calculated using the following formula:

% DPPH scavenging activity = $(1- A_{517\ sample} / A_{517\ control}) \times 100$

Where $A_{control}$ is the absorbance of all the reagents except the test sample, A_{sample} is the absorbance of the EOs. Ascorbic acid was used as standard antioxidant. In order to determine IC_{50} values (scavenge 50% of the DPPH radicals) of the EOs, a linear regression analysis was used.

Lipid peroxidation activity assay by TBARS method: Lipid peroxidation activity was evaluated using thiobarbituric acid-reactive substances (TBARS) method described by Raharjo *et al.*[16] and Ferreira *et al.*[17], 2-thiobarbituric acid (TBA) was used as indicator and 1,1,3,3-tetraethoxypropane (TEP) as standard. The absorbance of organic upper layer was read at 532 nm using a spectrophotometer in triplicate, and the TBARS values were expressed in terms of malondialdehyde (MDA) equivalents for quantifying lipid peroxides. The lipid peroxidation inhibition was calculated as;

$$\text{Inhibition ratio (\%)} = (1 - A_{532\ sample} / A_{532\ control}) \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample solution. IC_{50} values (50% of the lipid peroxidation inhibition) of the EOs were calculated and compared with the standard.

Statistical analysis: All the assays were conducted in triplicate, three different microplate wells were used to evaluate for each concentration of the EOs. A linear regression analysis was performed to calculate IC_{50} values. P value of <0.01 was considered statistically very significant.

RESULTS AND DISCUSSION

The EOs from *P. alba* L. and *R. officinalis* L. were evaluated for their *in vitro* cytotoxic, antiproliferative, antioxidant and lipid peroxidation activities. Cytotoxicity (IC_{50} values after 48h treatment period) of the EOs from *P. alba* L. and *R. officinalis* L. to three cancer cell lines (A549, H1299, and MCF-7) and to the non-tumor HUVEC cells are given in the Table 1.

The EOs of *P. alba* L. and *R. officinalis* L. exhibited strong cytotoxic and antiproliferative properties against all the three tested human cancer cell lines, in a dose and time dependent manner. However, EO of *R. officinalis* was found to be more active (IC_{50} values ranging from 3.06 to 7.38 µg/mL) than that of *P. alba* (IC_{50} values ranging from 12.05 to 28.16 µg/mL). Interestingly, the EOs from both plants showed a selectivity toward the cancer cell lines, exhibiting strong activity against A459 and H1299 cells, moderate activity against MCF-7 and weak activity against the non-tumor HUVEC cells (IC_{50} > 30 µg/mL). The strong growth inhibition of EO from *R. officinalis* could be due to the presence of the cytotoxic components such as carnasol, methyl carnosate, carnosic and rosmarinic acids.[5,6-9] Although this is the first report of the cytotoxic and antiproliferative effects of the EO from *P. alba* , it is probable that saponins, which are present in this EO, could contribute to these effect.[13]

The results of *in vitro* antioxidant activity, expressed as IC_{50} values obtained from DPPH radical scavenging activity and lipid peroxidation activity by TBARS method, of the EOs from *P. alba* L. and *R. officinalis* L. are shown in Table 2. The EOs from both sources showed significant free radical scavenging activities against DPPH by when compared their values of IC_{50} with commercial with that of ascorbic acid, a commercial standard AA (IC_{50} = 12.64 ± 0.32 µg/mL). The EO from *R. officinalis* L. was found to have higher free radical scavenging activity, with IC_{50} at 10.08 ± 0.15 µg/mL ($p<0.01$), 18.05 ± 0.38 µg/mL ($p<0.05$) Polyphenols and phenylpropanoids, found in the rosemary oil are most likely responsible for a strong radical scavenging activity.[7,9,20]

The results of lipid peroxides radical scavenging capacity for both of the EOs, presented in Table 2, are consistent with the DPPH radical scavenging activity results. They were able to reduce the lipid peroxides radicals with IC_{50} of 1.76 ± 0.02 and 2.16 ± 0.14 respectively ($p<0.05$). The values of IC_{50} were compared with the commercial standard BHT. This indicates that the rosemary and white poplar EOs were able to inhibit the free lipid

Table 1: IC_{50} values, determined by MTT assay, of EOs from *R. officinalis* and *P. alba*.

Samples	IC_{50} values from MTT (µg/mL)			
	A549	H1299	MCF-7	HUVEC
R. officinalis L.	3.06 ± 0.37	4.34 ± 0.44	7.38 ± 0.52	30.68 ± 2.80
P. alba L.	12.05 ± 0.66	10.53 ± 0.38	28.16 ± 1.65	42.02 ± 2.04
Doxorubicin[a]	0.25 ± 0.01	0.36 ± 0.02	0.82 ± 0.05	1.02 ± 0.02
DMSO[b]	0	0	0	0

Data are shown as means ± SD from three independent experiments (n=3).
[a]Doxorubicin, positive control.
[b]DMSO: negative control

Table 2: The IC_{50} value of EOs of R. officinalis and *P. alba* , determined by DPPH and TBARS assays

Sample	IC_{50} values from DPPH (µg/mL)	IC_{50} values from TBARS (µg/mL)
R. officinalis L.	10.08 ± 0.15**	1.76 ± 0.02*
P. alba L.	18.05 ± 0.38*	2.16 ± 0.14*
AA[a]	12.64 ± 0.32	Not determined
BHT[b]	Not determined	0.78 ± 0.01

Data are shown as means IC_{50} (µg/mL) value from three independent experiments (n=3).
**P value of <0.01, *P value of <0.05.
[a]AA: Ascorbic acid, standard commercial antioxidant as positive control for DPPH assay.
[b]BHT: Butylated hydroxyl toluene, standard commercial antioxidant as positive control for TBARS assay.

peroxides radicals, as well as the activities of free radicals, this might be due to the fact that rich chemical compositions of the oils.[5,13,20]

CONCLUSION

However, further studies using cell culture and animal models to understand the mechanisms underlying the cytotoxicity and anticancer activity of these EOs are needed to better understand the biological events and molecular targets of EOs to safeguard their usage as therapeutic agents. In the near future, it would be possible to develop therapeutic drugs containing rosemary and white poplar EOs.

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CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

ABBREVIATIONS

ATCC: American Type Culture Collection**; BHT:** Butylated hydroxyl toluene**; DMSO:** dimethyl sulphoxide**; DPPH:** 2,2-diphenyl-2-picrylhydrazyl**; EOs:** essential oils**; IC_{50}:** inhibitory concentration 50%**; MAPs:** medicinal and aromatic plants**; MDA:** malondialdehyde**; MTT:** [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]**; OD:** optical density**; RPMI-1640 medium:** roswell park memorial institute medium**; TBARS:** thiobarbituric acid-reactive substances**; TEP:** 1,1,3,3-tetraethoxypropane.

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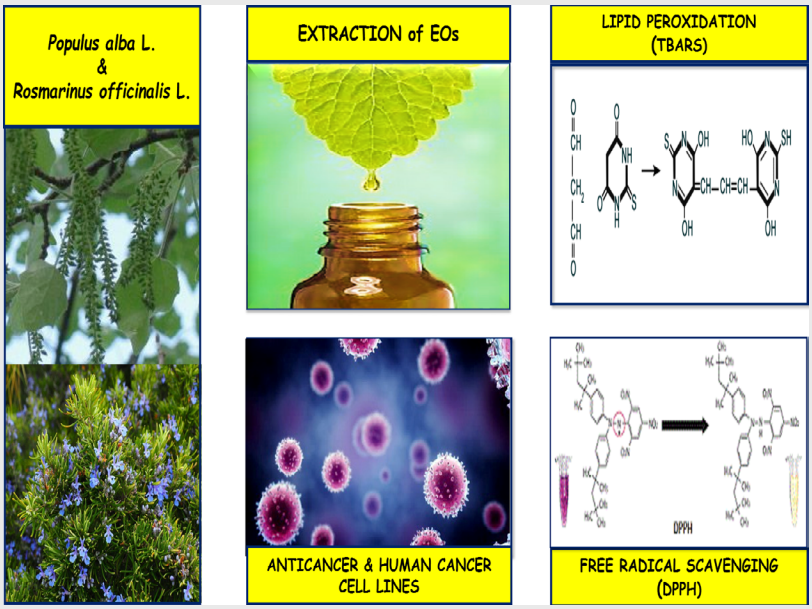
PICTORIAL ABSTRACT



SUMMARY

- EOs extracted from MAPs, are valuable sources due to their interesting anti-tumor, anti-mutagenic and anti-carcinogenic activities against various cancer cells.
- Potential antioxidant properties, lipid peroxidation capacity, *in vitro* cytotoxic and antiproliferative activities of EOs extracted from *P. alba* L. and *R. officinalis* L. were evaluated in this research.
- To the best of our knowledge, this is the first report of EO of *P. alba in vitro* anticancer and antiproliferative activities.
- As a result, both of the EOs exhibited strong cytotoxic and antiproliferative properties against tested cancer cell lines.
- Regarding antioxidant and lipid peroxidation activity, EO from *R. officinalis* L. was found to have higher antioxidant and lipid peroxidation capacities.
- This research clearly showed these oils have significant potential for developing therapeutic drugs in pharmaceutical industry.

ABOUT AUTHORS

Dr. Sevgi Gezici: After completing her Ms in Molecular Biology and Genetic at Department of Biology, Gaziantep University, Turkey, she earned her Ph.D degree in the Molecular Biology and Genetics form the same University. She has received scholarships for her MSc and Ph.D from TUBITAK, which is the best research center in Turkey. She is currently doctor at Gaziantep University, Department of Molecular Biology.

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