Industrial Crops and Products 53 (2014) 60–64

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of *Liquidambar orientalis* Mill. var. *orientalis*

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ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 6 December 2013
Accepted 9 December 2013

Keywords:
AMES
Biological activity
HPLC
Radical scavenging
Salmonella typhimurium

ABSTRACT

The ethanolic extract of the leaves of *Liquidambar orientalis* var. *orientalis*, an endemic tree species in Turkey, has medicinal and cosmetic properties; this extract was isolated, and its antioxidant, mutagenic, and antimutagenic activities were investigated. The antioxidant activity was determined by the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant activity, and phenolic compounds. The mutagenic and antimutagenic activities were investigated by Ames *Salmonella*/microsome mutagenicity test. The IC_{50} value for DPPH radicals was 3.11 ± 0.024 mg/ml. The total antioxidant activity increased with an increase in the concentration of the extracts (0.5, 2.5, 5, 10, and 20 mg/ml), which contained linoleic acid emulsion. The total phenolic content was 333.14 ± 7.96 mg gallic acid equivalent/g extract. HPLC analysis of the phenolic compounds showed the presence of protocatechuic acid (12.232 ± 0.118 mg/g extract), (−)-epicatechin (7.954 ± 0.493 mg/g extract) and gallic acid (3.258 ± 0.035 mg/g extract) as a major phenolic acids in *L. orientalis* var. *orientalis*.

The results showed that the ethanolic extract can be considered genotoxically safe, because it does not have any mutagenic effect at the tested concentrations. As a result, the ethanolic extract of the leaves exhibited antimutagenic effects at 2.5, 0.25, and 0.025 mg/plate concentrations. To our knowledge, this is the first study of the antioxidant, mutagenic, and antimutagenic activity of the ethanolic extract of the leaves of *L. orientalis* var. *orientalis*. Also, phenolic compounds of this plant are unknown. These activities are an important topic in the medical field as well as in the food industry.

1. Introduction

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potential (Khalaf et al., 2008). In recent years, much attention has been devoted to natural antioxidants and their association with health benefits (Arnous et al., 2001). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer (Devasagayam et al., 2004).

Mutations are the cause of inborn errors of metabolism, resulting in morbidity and mortality in living organisms. Besides inherited metabolic disorders, a spectrum of age-related human diseases, including cancer, are caused by mutations (Shon et al., 2004). A mutagen is considered an agent that is capable of destroying the integrity of the hereditary mechanism of a cell or an organism. Any substance causing increased mutations can also increase the probability of cancer (Zaveri et al., 2011).

In recent years, there has been greater interest in investigating compounds originating from plants and their effects on DNA (Horn and Vargas, 2003). However, research has shown that a lot of plants which are used as food ingredients or in traditional medicine have in vitro mutagenic (Mohd-Fuat et al., 2007) and antimutagenic properties (Bouhlel et al., 2007). Natural antimutagens from edible and medicinal plants are of particular importance, because they may be useful for human cancer prevention and have no undesirable xenobiotic effects on living organisms (Zahin et al., 2010). Herbal remedies and phytotherapy drugs containing active principles are currently being developed to protect against an electrophile (e.g. free radical) attack on DNA and its widespread outcomes such as aging and cancer (Ghazali et al., 2011).

Liquidambar is the only genus in the family of Hamamelidaceae (Li and Bogle, 1997). *L. orientalis* Mill. tree is commonly known as 'Sığla ağacı' or 'Günlük ağacı' in Turkey, and it has a local distribution in the south-western coastal district of Turkey (Duru et al., 2002). This species is a herbaceous plant known to have medicinal and cosmetic properties and is widely used in phytotherapy in the Mediterranean region (Sağdıç et al., 2005).

The antibacterial (Sağdıç et al., 2005), genotoxic, and cytotoxic (Karadeniz et al., 2011) activities of *L. orientalis* storax; the antioxidant activity of the essential oil (Topal et al., 2008); and the

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http://dx.doi.org/10.1016/j.indcrop.2013.12.015

antimicrobial activity of the ethanolic extract of the leaves (Oskay and Sarı, 2007; Oskay et al., 2009) were previously studied.

This is the first study using the ethanolic extract of the leaves of *L. orientalis* var. *orientalis* to evaluate antioxidant, mutagenic, antimutagenic activity and phenolic compounds in order to enable their use in phytomedicine.

2. Materials and methods

2.1. Plant material

The young leaf samples of *L. orientalis* var. *orientalis* naturally growing plants belonging to the Hamamelidaceae family were collected in April–May, 2012 from Köyceğiz, Muğla, Turkey. A voucher specimen (Herbarium No: K.A. 270586) has been taxonomically identified by Kenan Akbas and deposited in the Herbarium of the Faculty of Science, University of Mugla Sitki Kocman, Turkey. The leaves were air-dried at room temperature for later analysis.

2.2. Preparation of the ethanolic extract

The air-dried and powdered leaf samples (25 g) were extracted with ethanol (Merck) (300 ml) using the Soxhlet apparatus. The extract was evaporated and then extracted in ethanol/water (1:1, v/v), and then kept in small sterile opac bottles under refrigerated conditions until used.

2.3. Bacterial strains

S. typhimurium TA98 and *S. typhimurium* TA100 were used for the mutagenity and antimutagenity tests. The strains were analyzed for their histidine requirement, biotin requirement, the combination of both, rfa mutation, excision repair capability, the presence of the plasmid pKM101, and spontaneous mutation rate according to Mortelmans and Zeiger (2000). Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37 °C with gentle agitation (Oh et al., 2008).

2.4. Antioxidant activity

2.4.1. Determination of DPPH radical scavenging activity

Antioxidant activity of the extract was determined based on its ability to react with the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Yamasaki et al., 1994). Fifty μl of the extract (1.25, 2.5, 5 and 10 mg/ml in ethanol/water (1:1, v/v)) was added to 5 ml DPPH solution (0.004%) in ethanol. After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (IC_{50}) were determined. BHT and ascorbic acid were used as a positive control.

2.4.2. Total antioxidant activity by the β-carotene-linoleic acid method

The total antioxidant activity of the extract was evaluated by the β-carotene-linoleic acid model (Jayaprakasha and Jaganmohan Rao, 2000). 0.5 mg of the β-carotene in 1 ml of chloroform, 25 μl of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed together. The chloroform was completely evaporated using a vacuum evaporator and the resulting solution was diluted with 100 ml of oxygenated water. 2.5 ml aliquots of this mixture were transferred into different tubes containing 0.5 ml of samples at 0.5, 2.5, 5, 10 and 20 mg/ml concentrations in ethanol/water (1:1, v/v). The same procedure was repeated with the positive control BHT, ascorbic acid, and a blank. The emulsion system was incubated for up to 2 h at 50 °C. Measurement of absorbance was continued until the color of β-carotene disappeared in the control. After this incubation period, absorbance of the mixtures was measured at 490 nm. All determinations were performed in triplicate.

The bleaching rate (R) of β-carotene was calculated using the following formula. $R = \ln(a/b)/t$ where ln is the natural log, a is the absorbance at time 0, b is the absorbance at time t (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the formula $AA = [(R_{Control} - R_{Sample})/R_{Control}] \times 100$. Antioxidative activity of the extract was compared with those of BHT and ascorbic acid at 0.5 mg/ml.

2.4.3. Determination of total phenolic compounds

The phenolic constituent of the extract was determined by the method involving the Folin–Ciocalteu reagent and gallic acid as a standard (Slinkard and Singleton, 1977; Chandler and Dodds, 1983). Two hundred microliter of extract solution containing 0.1 mg extract was added to a test tube. Then, 100 μl Folin–Ciocalteu reagent was added and tube was shaken vigorously. After 3 min, a 2 ml solution of Na_2CO_3 (5%) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm.

Content of phenolic compound was determined as mg gallic acid equivalents per gram of extract (mg/g GAE extract) using the following linear equation based on the calibration curve: $A = 0.0265\,C$, $R^2 = 0.9935$ where A is the absorbance and C gallic acid equivalents.

2.5. Quantification of phenolic compounds by RP-HPLC

Phenolic compounds were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size). The eluates were detected at 278 nm. The mobile phases were A: 3.0% acetic acid in distilled water and B: methanol. For analysis, the samples were dissolved in ethanol: bidistilled water (1:1, v/v), and 20 μl of this solution was injected into the column. The elution gradient applied at a flow rate of 0.8 ml min^{-1} was: 93% A/7% B for 0.1 min, 72%A/28%B in 20 min, 75%A/25%B in 8 min, 70%A/30%B in 7 min and same gradient for 15 min, 67%A/33%B in 10 min, 58%A/42%B in 2 min, 50%A/50%B in 8 min, 30%A/70%B in 3 min, 20%A/80%B in 2 min 100%B in 5 min until the end of the run. This method has a good repeatability, since several analyses carried out on the same sample produce a repeatability coefficient of percent variation (CV%) of 7%. Phenolic compositions of the extracts were determined by a modified method of Caponio et al. (1999). Gallic acid, protocatechuic acid, (+)-catechin, chlorogenic acid, caffeic acid, (−)-epicatechin, p-coumaric acid, ferulic acid, trans-cinnamic acid, quercetin, kaempferol and apigenin were used as standard. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as mg per gram of extract.

2.6. Mutagenic and antimutagenic activity

2.6.1. Viability assays and determination of test concentrations

Cytotoxic dose of the extract was determined by the method of Mortelmans and Zeiger (2000). The toxicity of the extract toward *S. typhimurium* TA98 and TA100 was determined as described in