QUALITATIVE, QUANTITATIVE ANALYSIS OF THE COMMERCIAL *LAURUS NOBILIS* ESSENTIAL OIL AND ITS BIOLOGICAL ACTIVITIES.

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Abstract

The chemical composition of the volatile oil of *Laurus nobilis* L. has been investigated. The essential oil was purchased as a commercial sample from Xherdo Co. Ltd, Albania and its analyses were performed by GC–MS. *L. nobilis* is composed mainly of oxygenated monoterpenes (65.9%) and monoterpene hydrocarbons (18.6%). The major constituents of *L. nobilis* were: eucalyptol (48.2%), sabinene (10.4%) and linalool (10.3%). Moreover, the essential oil was tested for its free radical scavenging activity using the following *in vitro* assays: i) interaction with the free stable radical of DPPH (1,1-diphenyl-2-picrylhydrazyl), ii) inhibition of linoleic acid peroxidation with the dihydrochlroic acid of 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH). Finally, their inhibitory activity toward soybean lipoxygenase was evaluated, using linoleic acid as substrate. The essential oil of *L. nobilis* is presenting the low interaction with the stable radical DPPH, the reducing activity is increased by the time e.g. it enhances after 20 min of interaction and it is higher after 60 min, we can conclude that it is time dependent. The tested sample *L. nobilis*, significant inhibit soybean lipoxygenase (69.76%), whereas it showed low anti-lipid peroxidation activity (23.11%) .

Key words: Essential oil; antioxidant activity; *Laurus nobilis*, GC-MS.

Përmbledhje

Në këtë studim paraqitet përbëja kimike e vajit volatile të *Laurus nobilis* L. Vaj esencial është marrë si kampion komercial nga kompania Xherdo Co. Ltd, Shqipëri dhe është analizuar me GC-MS. *L. nobilis* përbëhet kryesisht nga monoterpene të oksigjenuara (65.9%) dhe monoterpene hidrokarbone (18.6%). Përbërësit kryesor të *L. nobilis* rezultuan: eukaliptol (48.2%), sabinene (10.4%) dhe linalool (10.3%). Për më tëpër vaji esencial u testua për aktivitetin antioksidant duke përdorur testet e mëposhtëme *in vitro*: i) ndërveprimi me radikalin e qëndrueshëm të DPPH (1,1-diphenyl-2-picrylhydrazyl), ii) inhibimi i peroksidimit të acidit linoleic me acidin dihidro klorik 2,2'-azobis-2-metil-

propanimidamide, dihidro klorik (AAPH). Si përfundim u përcaktua aktiviteti inhibues kundrejt lipooksigjenazës së sojës, duke përdorur acidin linoleik si substrat. Vaj esencial i *L. nobilis* paraqet ndërveprim të ulët me radikalin e qëndrueshem DPPH, aktiviteti reduktues rritet me kohën p.sh rritet pas 20 min ndërveprimi dhe është më i lartë pas 60 min, ne arrijmë në përfundimin se ky aktivitet varet nga koha. Kampioni i testuar *L. nobilis*, inhibon në shkallë të konsiderueshme lipo oksigjenazën e sojës (69.76%), ndërkohë që paraqet aktivitet të ulët të peroksidimit anti lipidik (23.11%).

Fjalëkyçe: Vaj esencial; aktivitet antioksidant; *Laurus nobilis*; GC-MS.

Introduction

According to the World Health Organization (2008), more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs. Moreover, in many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (WHO, 2008). In the last decades, the consumption of herbal medicines has increased and there has been a growing interest in the investigation of natural products, in particular the essential oils extracted from plants, for the discovery of active compounds that can be applied to the food industry.

The fact that they can ally their aromatizing capacity with other functional uses, such as antimicrobial, antifungal, antioxidant and insecticide properties, have contributed to this (Ormancey *et al*., 2001; Figueiredo *et al.,* 2008). As consumers are avoiding the consumption of products with synthetic additives or preservatives, the natural products constitute an alternative, mainly because they are considered safe, natural and biodegradable, with low toxicity to mammals.

Essential oils (EOs) from aromatic and medicinal plants receive particular attention as potential natural agents with a wide spectrum of biological activities. EOs are proven to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic effects (Bowles, 2004; Lahlou, 2004).

Adding to this, there is also a trend interest oriented to the analysis of metabolites from food (vegetables and spices) with bioactivities, recently named *nutraceuticals* and *phytochemicals*. These metabolites have a great potential in the food industry because they can combine nutritional and medicinal benefits, to produce the so-called *Functional Foods*. Antioxidants minimize oxidation of the lipid components in foods. There is an increasing interest in the use of natural and/or synthetic antioxidants in food preservation, but it is important to evaluate such compounds fully for both antioxidant and pro-oxidant properties. In our screening project for the search of antioxidative agents from natural sources we evaluated the antioxidant and anti-inflammatory activity of *Laurus nobilis* essential oil.

L. nobilis L. (bay) is an evergreen tree or shrub that belongs to the Lauraceae family and is cultivated in many temperate and warm parts of

the world, particularly the Mediterranean countries of Turkey, Greece, Spain, Portugal and Morocco, and in Mexico. *L. nobilis* L. is used as an aroma in the food and cosmetics industries; dry fruits and dry leaves are used for adding fragrance to food and consumed as tea, respectively (Baytop, 2000). The antimicrobial, analgesic, anti-inflammatory, antitumoral, acetylcholine esterase inhibiting properties of the essential oil of *L. nobilis* L. have been reported (Sayyah *et al.* 2003, Ferreira *et al.* 2006, Soylu *et al.* 2006, Loizzo *et al.* 2007).

Materials and methods

Chemicals

L. nobilis L. essential oil was purchased as commercial sample from Xherdo Co. Ltd, Albania. 1,1-Diphenyl 2-picryl hydrazyl (DPPH), Lipoxygenase (1.13.11.12) type I-B (Soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis. MO, USA). Nordihydroguaiaretic acid (NDGA), Butyl hydroxytoluene (BHT) and caffeic acid were purchased from Merck. All other chemicals were of analytical grade. A Perkin Elmer Lambda 20 UV-Vis spectrophotometer has been used for the radical scavenging activity experiments.

Gas Chromatography-Mass Spectrometry

Essential oil analyses were performed on a Shimadzu GC-2010-GCMS-QP2010 system operating at 70eV. This was equipped with a split/splitless injector (230 ^{o}C) and a fused silica HP-5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The temperature program was from 50 ^{o}C to 290 ^{o}C, at a rate of 4 ^{o}C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of each sample was 1.0 μl. Arithmetic indices for all compounds were determined according to Van den Dool and Kratz (Van den Dool and Kratz, 1963), using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 (Massada, 1976), and by comparison of their retention indices with literature data (Adams, 2007). Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

Inhibition of linoleic acid lipid peroxidation

Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion is monitored at 234 nm in the presence of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) of 50 μl of 40 mM AAPH solution as a free radical initiator in 0.05 M phosphate buffer, pH 7.4. Oxidation was carried out in the presence of the tested samples (10 μl/10mg/ml stock solution). The rate of oxidation at room temperature was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides. Trolox was used as a reference drug. (Peperidou *et al.*, 2014)

Soybean lipoxygenase inhibition study *in vitro*

The tested samples dissolved in DMSO (10mg/ml stock solution) were incubated 10 μl at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution ($1/9x10^{-4}$ w/v in saline) in tris buffer pH 9. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm compared with the appropriate standard inhibitor (caffeic acid). (Peperidou *et al.*, 2014)

Interaction with DPPH

To a solution of DPPH (0.1mM in methanol) the tested samples dissolved in DMSO (10mg/ml stock solution) were added (10μl). After 20/60 min the antioxidant activity is recorded at 517nm and the percentage of reducing activity (RA) was calculated and compared to the reference compound NDGA (nordihydroguaiaretic acid). (Peperidou *et al.*, 2014)

Results and discussion

The results obtained by chemical analysis of *Laurus nobilus* L., essential oil is presented in Table 1.

L. nobilis is composed mainly of oxygenated monoterpenes (65.9%) and monoterpene hydrocarbons (18.6%). The major constituents of *L. nobilis* were: eucalyptol (48.2%), sabinene (10.4%), linalool (10.3%) and sabinene (10.4%). These results were partly similar to those reported before from *L. nobilis* composition of essential oil mainly in the absence of high amounts of β-ocimene (Kilic *et al.*2004).

The antioxidant activities of *Laurus nobilis* essential oil have been evaluated (Table 2). The essential oil of *L. nobilis* is presenting the low interaction with the stable radical DPPH, the reducing activity is increased by the time e.g. it enhances after 20 min of interaction and it is higher after 60 min, we can conclude that it is time dependent. The tested sample *L. nobilis*, significant inhibit soybean lipoxygenase (69.76%), whereas it showed low anti-lipid peroxidation activity (23.11%) (Table 2)

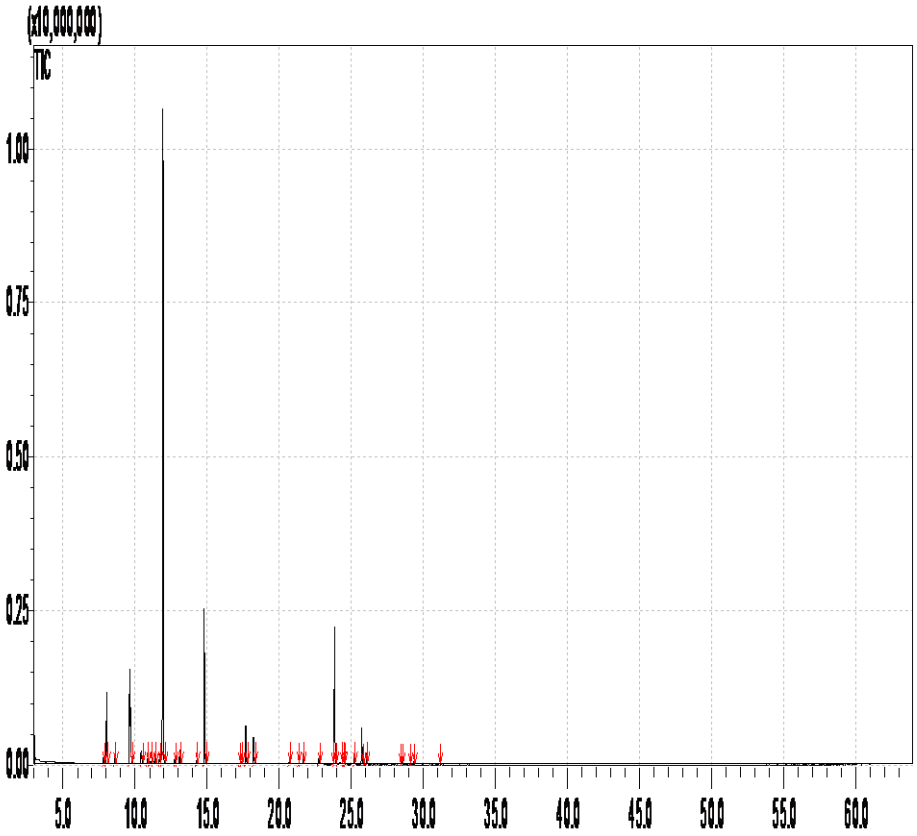

Figure 1. GC-MS chromatogram of L. nobilis commercial essential oil

Table 1: Composition of the commercial essential oils of *L. nobilis* (LN)

Compounds [a]	**AI** [b]	**LN**	**ID** [c]
Tricyclene	919	nd	AI, MS
α-Thujene	926	0.4	AI, MS
α-Pinene	931	**5.0**	AI, MS, Co-GC
Camphene	945	0.4	AI, MS
Thuja-2,4(10)-diene	952	nd	AI, MS
Sabinene	972	**10.4**	AI, MS
β-Pinene	973	nd	AI, MS, Co-GC
Octen-3-ol	983	nd	AI, MS
β-Myrcene	992	0.9	AI, MS, Co-GC

α-Phellandrene	1003	0.4	AI, MS
δ-3-Carene	1015	0.3	AI, MS, Co-GC
α-Terpinene	1016	0.5	AI, MS
p-Cymene	1024	0.7	AI, MS, Co-GC
Eucalyptol	1029	**48.2**	AI, MS
cis-Ocimene	1050	0.3	AI, MS
γ-Terpinene	1059	1.0	AI, MS, Co-GC
Terpinolene	1087	0.3	AI, MS
trans-Sabinenehydrate	1098	nd	AI, MS
Linalool	1101	**10.3**	AI, MS, Co-GC
Borneol	1164	tr	AI, MS, Co-GC
δ-Terpineol	1169	0.2	AI, MS
Terpinene-4-ol	1176	2.6	AI, MS, Co-GC
α-Terpineol	1191	1.9	AI, MS
Carvacrol methyl ether	1244	nd	AI, MS
Linalool acetate	1258	0.1	AI, MS
Bornyl acetate	1286	0.4	AI, MS, Co-GC
δ-Terpinyl acetate	1318	0.5	AI, MS
α-Terpinyl acetate	1350	**9.8**	AI, MS
Eugenol	1362	0.4	AI, MS
Neryl acetate	1368	0.1	AI, MS
α-Ylangene	1371	tr	AI, MS
β-Elemene	1392	0.5	AI, MS
Methyl eugenol	1407	2.5	AI, MS
β-Caryophyllene	1419	0.8	AI, MS, Co-GC
β-Selinene	1486	tr	AI, MS
γ-Cadinene	1514	tr	AI, MS
δ-Cadinene	1524	0.2	AI, MS
Carryophyllene oxide	1583	0.1	AI, MS, Co-GC
Total		**99.3**	
Monoterpene Hydrocarbons		18.6	

Oxygenated Monoterpenes	65.9
Sesquiterpene Hydrocarbons	1.3
Oxygenated Sesquiterpenes	0.1

[a] Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c] Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound. Concentrations below 0.05% are marked as tr (traces).

Table 2. Percentage (%) interaction of essential oil with DPPH, its % soybean LOX inhibitory activity and its % Inhibition of lipid peroxidation

Essential Oil	**% Interaction with the stable free radical of DPPH**		**% Inhibition of LOX**	**% Inhibition of lipid peroxidation**
Time	**20 min**	**60 min**		
Concentration	**10 µl**	**10 µl**	**10 µl**	**10µl**
L. nobilis	24.73	57.19	69.76	23.11
NDGA	81			
TROLOX			73	
CA				IC50 = 600 µM

Conclusions

The present study showed that due to the diversity and complexicity of *Laurus nobilis* essential oil, it is rather difficult to conclude about their antioxidant activities. There are also some antioxidant activities in herbs that may be attributable to synergistic interactions.

It is well known that free radicals play an important role in the inflammatory process. Consequently, herbs with antioxidant/scavenging properties could be expected to offer protection in rheumatoid arthritis and inflammation. Due to their excellent protective features exhibited in antioxidant activity tests, as well as interesting anti-inflammatory properties (LOX inhibition) the essential oil of *Laurus nobilis* could be

used as a natural source and find applications as "nutraceutical" and culinary herbs.

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