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Assessment of Volatile Oil Composition, Phenolics and Antioxidant Activity of Bay (*Laurus nobilis*) Leaf and Usage in Cosmetic Applications

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Abstract: In this study, the components of the volatile oil obtained from *Laurus nobilis* leaves by steam distillation were determined using Agilent 6890 Gas Chromatography (GC) - 5975 Mass Spectrometry (MS). The antioxidant activities of different extracts of *L. nobilis* leaves were determined by using DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, β-carotene-linoleic acid bleaching assay and ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical decolorization assay. Determination of the total phenolic contents of *L. nobilis* leaf extracts were performed using the Folin-Ciocalteau procedure and total flavonoid contents were measured using a spectrophotometric assay. According to the GC/MS results, 1,8-cineole (46.16%), *alpha*-terpinyl acetate (10.62%), *alpha*-pinene (6.27%), terpinen-4-ol (5.07%) and sabinene (4.99%) were found to be the major compounds in volatile oil. The obtained volatile oil was used to make skin care lotion. Stability tests and organoleptic analyses of final product were performed after 1, 5, 30 and 90 days of production. The highest amounts of total flavonoid content were found to be 5.48 ± 0.65 and 8.60 ± 0.12 µg QEs/mg in ethyl acetate and ethanol extracts, respectively. The highest amounts of total phenolic compounds were found to be 54.42 ± 0.14 and 25.32 ± 0.10 µg PEs/mg in ethyl acetate and ethanol extracts, respectively. According to the results of ABTS$^{\bullet+}$, DPPH$^{\bullet}$ and β-carotene linoleic acid assays, ethyl acetate extract was found to be the most active extract (24.98±0.87 µg mL^{-1}, 75.65±0.77 µg mL^{-1} and 19.32±1.04 µg mL^{-1}).

Keywords: *Laurus nobilis*, Volatile oil, Antioxidant, Cosmetic, GC/MS

1. Introduction

Volatile oils are secondary plant metabolites, which are found in different parts of plants including flowers, roots, bark, leaves, seeds, peel, fruit and wood produced in cytoplasm and plastids of plant cells [1]. These oils, also known as essential oil, etheric oil by people, can contain terpenic hydrocarbons and their oxygenated derivatives as well as organic acids, alcohols, phenols and ketones [2]. The main components of volatile oils are usually mono and sesquiterpenes. In some cases their main derivatives are hydrocarbons (e.g. turpentine, formed

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by *alpha* and *beta*-pinene), while in others the main constituents are oxygenated (e.g. cloves formed by eugenol) [3, 4]. Some of the aromatic plants have a volatile oil formed mainly of aromatic compounds derived from allyl or isoallyl phenol. The plants containing those compounds, although they are less frequent than plants containing terpenes, only allow such compounds to be selective.

There has always been a great deal of interest in volatile oils throughout history. Although many of the intended uses of volatile oils have disappeared over time, it has generally been accepted that since the beginning of mankind, humans have obtained these oils from aromatic plants. Volatile oils have various applications for different purposes. Volatile oils are not only used for cooking in order to improve the taste and health of the food, but also for the manufacture of perfumes and cosmetic products [5].

The ancient Egyptians have used volatile oils in perfumery, medicine and even in the preparation of bodies and organs for mummification. In ancient Asia, Vedas coded intended uses of perfume and aromatics for therapeutic purposes. Indeed, throughout history, many civilizations have used volatile oils and fragrances for a variety of purposes, including religious rituals, perfumes and therapeutic against infectious diseases. During the Renaissance period, the use of volatile oils in perfumery and cosmetic products has been spread to the world [6].

Volatile oils can be obtained by water distillation, water and steam distillation, or steam distillation alone, which are the most commonly used methods. The part of the plant where the volatile oil is to be obtained may be fresh, partially or completely dried, but if the volatile oil is to be obtained from the flower part, the flowers should be fresh [7].

Different methods have been used to control and analyze essential oils [8]. Currently, however, the identification of essential oil components is usually carried out with the aid of gas chromatography–mass spectrometry (GC/MS) equipped with flame ionization detector (FID) and mass spectrometer (MS) detectors, a capillary column and a split. Test conditions may vary depending on the column and the sample [9].

The main sources of volatile oils are medicinal and aromatic plants, which are widely used since ancient times in medicine, cosmetics and preserving and improving the flavor of foods. Especially in recent years, there are numerous artificial chemical-free productions, mainly cosmetics, due to the increased interest in natural products [10]. As a natural ingredient, volatile oils are a growing market trend, being used in skin care cosmetics (e.g creams, lotions), balms, shampoos, soaps and perfumes [11].

Laurus nobilis (*Lauraceae*), one of the main sources of plant volatile oils, is an evergreen shrub that can grow up to 8 meters tall. It has dark green leaves about 8-14 cm long and 3-4 cm wide [12, 13].

This plant, belonging to *Lauracea* family and unique to the southern Mediterranean region, is widely grown in Europe and USA as an ornamental plant. It is cultivated commercially for aromatic oil, found in its leaves, in Turkey, Algeria, Morocco, Portugal, Spain, Italy, France and Mexico. Turkey is the leader bay leaf exporter of the World. [14].

The volatile oils obtained from the leaves of *L. nobilis* still maintains the importance in both traditional and modern medicine with its pharmacological activities. Studies have shown that *L. nobilis* volatile oil has antioxidant [15], anticonvulsant [16], analgesic, anti-inflammatory [13], antiviral [17], anticholinergic [18], antibacterial [19] and antifungal activities [20]. *L. nobilis*, which is a powerful medicinal and aromatic plant with these pharmacological properties, has been reported in cosmetic uses. *L. nobilis* leaf volatile oil is used for the preparation of hair lotion due to its antidandruff activity and for the treatment of psoriasis [21].

The usage of volatile oils in the production of cosmetics and similar products can both increase the dermo-cosmetic effects of these products and marketing trend for the final product. A great number of usages of plant materials such as volatile oils in cosmetics products provide extra benefits to the skin more than ordinary products. [22].

In this study, the chemical composition of *L. nobilis* leaf volatile oil was examined using GC/MS. The antioxidant activities of different extracts of *L. nobilis* leaves were determined by using DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity, β-carotene-linoleic acid bleaching assay and ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) cation radical decolorization assay. Determination of the total phenolic contents of *L. nobilis* leaf extracts performed using the Folin-Ciocalteau procedure and total flavonoid contents were measured using a spectrophotometric assay. In addition, we prepared skin lotion using the volatile oil obtained with steam distillation. After preparation of lotion, we performed stability tests and organoleptic tests on the final product.

2. MATERIALS AND METHODS

2.1. Standards and Reagents

Ethanol, n-hexane, methanol, ethyl acetate and chloroform were of analytical grade purity were supplied by Merck (Darmstadt, Germany). Essential oil standards were supplied from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Water was HPLC grade (18.2 MΩ), purified by a Milipore Milli-Q (Molsheim, France) system that includes reverse osmosis, ion exchange and filtration steps. β-carotene, tween-40, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS$^{\bullet+}$), potassium persulfate ($K_2S_2O_8$), linoleic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), poly(acrylic acid sodium salt), alpha-tocopherol were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals and solvents were of analytical grade and purchased from usual suppliers.

2.2. Plant Material and Isolation of Volatile Oil

Laurus nobilis leaves were collected in June 2016 from the trees in Fethiye region of Muğla (Turkey). Authentication of the plant was performed by Dr. Ergun KAYA from Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla (Turkey). Collected leaves were dried under ambient temperature (25^{o}C), shadow and airy place. Dry leaves were separated from the vimen.

Air-dried leaves of *L. nobilis* subjected to steam distillation for 120 min to obtain its volatile oil. The resulting volatile oil was dried over anhydrous sodium sulphate, filtered and stored in a dark glass bottle at -21°C until analyze by Gas Chromatography-Mass Spectrometry (GC/MS).

2.3. Determination of Chemical Composition of Volatile Oil Using GC/MS

GC/MS analyses were carried out using an Agilent 6890N Gas Chromatograph equipped with a Multi Mode Inlet (MMI) (280^{o}C), a DB-1 capillary column (30m × 0.25mm; film thickness 0.25μm) and coupled with an Agilent 5975C MS Detector (MSD), operating in the electron impact (EI) mode at 70 eV. Transfer line temperature was set at 250^{o}C. The carrier gas was He (2.1 mL/min), and the oven temperature was held at 60^{o}C for 5 min, then increased up to 220^{o}C at a rate of 2^{o}C/min and held at this temperature for 10 min. The injected volume was 2 μL and the split ratio 40:1.

In this study, the identification of the compounds was based on the comparison of their retention times (RT) and mass spectra with those from the NIST 2008, Wiley 2008 and Flavor2

libraries. Relative percentages of compounds were calculated based on the peak areas from the MS data.

2.4. Determination of Antioxidant Activities

2.4.1. Preparation of Extracts

In the determination of antioxidant activities of *L. nobilis* leaf, four different extracts, hexane, ethyl acetate, ethanol and water, were used. The sample was extracted five times for 24 hours at room temperature with hexane, ethyl acetate, ethanol, water and then the combined extracts were filtered through Whatman No 4, separately. The combined extracts of hexane, ethyl acetate, and ethanol were evaporated (rotary evaporator Heidolph, Hei-VAP Precision) to dryness in vacuum, and the combined water extracts were lyophilized (Christ Freeze Dryer, Alpha 1-4 LD plus, Germany). The extracts were stored in deepfreeze until the time of study. The results were given as 50 % inhibition concentration (IC_{50}). The sample concentration ($\mu g.mL^{-1}$) inhibiting 50 % antioxidant activity (IC_{50}) was calculated from the graph of activity percentage against sample concentration.

2.4.2. $DPPH^{\bullet}$ Free Radical Scavenging Assay

The free radical scavenging activity of *L. nobilis* leaf extracts was determined using the method, $DPPH^{\bullet}$ free radical protocol [23] with slight modifications. The extract solutions prepared in different concentrations (40 µL) and ethanolic solution (120 µL) containing $DPPH^{\bullet}$ radicals (0.4 mM) were incubated in darkness at room temperature for 30 min. Absorbance was measured at 517 nm in SpectraMax 340 PC, Molecular Device (USA). The radical-scavenging activity (RSA) was calculated as a percentage of $DPPH^{\bullet}$ decolorization using the following equation:

$$\%RSA=[(A_{DPPH}-A_S)/A_{DPPH}]\times 100$$

Where A_S is the absorbance of the solution containing the sample and A_{DPPH} is the absorbance of the $DPPH^{\bullet}$ solution.

2.4.3. β-Carotene-Linoleic Acid Bleaching Assay

The total antioxidant activity was determined using β-carotene-linoleic acid test system based on the detection of inhibition of conjugated dien hydroperoxides due to oxidation of linoleic acid [24, 25]. β-Carotene (0.5 mg), dissolved in 1mL of chloroform, was mixed with linoleic acid (25 µL) and Tween 40 emulsifier (200 mg). Chloroform was evaporated under low pressure, 50mL of distilled water was added by vigorous shaking. Aliquots (1.60 µL) of this emulsion were added to 40 µL of the extract solutions at different concentrations. As soon as the emulsion was added to each tube, the zero time absorbance was initially measured at 470 nm, and then the absorbance measurements were done for every 30 min until 120 min.

2.4.4. $ABTS^{\bullet+}$ Cation Radical Decolorization Assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the previously described method [26]. The $ABTS^{\bullet+}$ (7 mM) in water and potassium persulfate (2.45 mM) reacted to give $ABTS^{\bullet+}$, stored in the dark at room temperature for 12 h, and oxidation of $ABTS^{\bullet+}$ appeared immediately, however, the stability of absorbance was gained after 6 h. Then, the sample solution (40 µL) in ethanol at different concentrations were mixed with $ABTS^{\bullet+}$ solution (160 µL), giving the absorbance at 734 nm by using a 96-well microplate reader in 10 minute. The scavenging capability of $ABTS^{\bullet+}$ was calculated using the following equation:

$$ABTS^{\bullet+}\text{ scavenging effect }\%=[(A_{ABTS^{\bullet+}}-A_S)/A_{ABTS^{\bullet+}}]\times 100$$

Where A_S is the absorbance of remaining concentration of $ABTS^{\bullet+}$ in the presence of sample and $A_{ABTS}{}^{\bullet+}$ is the initial concentration of the $ABTS^{\bullet+}$.

2.5. Determination of Total Phenolic Concentrations

The concentrations of total phenolic content in *L. nobilis* leaf were expressed as microgrammes of pyrocatechol equivalents (PEs), determined with Folin-Ciocalteu reagent (FCR) [27]. The sample solution (1 mL) dissolved in methanol was added to distilled water (46 mL) and FCR (1 mL), and mixed thoroughly, 2% sodium carbonate (3 mL) were added to the mixture in 3 min and shaken intermittently for 2 h at room temperature. The absorbance was measured at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard pyrocatechol graph: Absorbance = 0.0073x - 0.1665, $r^2 = 0.9976$

2.6. Determination of Total Flavonoid Concentrations

Measurements of total flavonoid concentration of the extracts were based on the previously reported method [24], and results were expressed as quercetin equivalents (QEs). An aliquot of the sample solution (1 mL) extracts in methanol was mixed with 10% aluminum nitrate (0.1 mL), 1 M potassium acetate (0.1 mL) and 80% methanol (3.8 mL) in test tubes, and then the absorbance was measured at 415 nm in 40 min, and stayed at room temperature. The concentrations of flavonoid compounds were calculated according to following equation that was obtained from the standard quercetin graph: Absorbance = 0.0082x + 0.0073, $r^2 = 0.9998$.

2.7. Preparation of Lotion

The lotion was prepared by following steps. The ingredients; oils, purified water and poly(acrylic acid sodium salt) as emulsifier polymer blend, were weighed separately. 6 g of lilac-flavored hazelnut oil and 6 g of bay leaf volatile oil were added dropwise to 87 mL of purified water in a glass beaker at room temperature under magnetic stirring.

After the mixture was stirred with a magnetic stirrer for 10 min, the formulation is completed by addition of the homogenized emulsifier polymer blend. After lotion preparation was completely homogenized, it was poured gently (to avoid the presence of bubbles) into a container.

2.8. Stability Tests of Cosmetic Preparation

2.8.1. Determination of Viscosity

The rheology analysis of the lotion formulation containing *L. nobilis* leaf volatile oil was performed using Brookfield LVDV -I+ viscometer at 10 rpm, 25°C. Rheological analyses were repeated 1, 5, 30 and 90 days after lotion preparation was completed.

2.8.2. Centrifugation Assay

The centrifuge test, which is often used for having preliminary information in the stability tests, was performed as described in [28]. The lotion sample was centrifuged twice; each was 15 min at 3000 rpm and under ambient temperature. A 10 mL centrifuge tube was used for centrifugation and it was observed whether there was phase separation.

2.8.3. pH Measurements

pH change during storage is one of the indicator for chemical stability of cosmetic preparations. The pH of the skin care lotion was measured according to the method given in [29]. The measurement was performed at room temperature with a Sartorius pH meter after 1/10 (v/v) dilution of the sample with purified water and filtration. Before the measurement, the pH meter was calibrated with the standard buffer solution (pH = 4, 7 and 10).

2.9. Organoleptic Tests of Cosmetic Preparation

Cosmetic preparation was evaluated for appearance, color, odor and spreadability. A visual evaluation was made by adding the sample to a glass container, placed over a white background, and compared it to the previous observations. Spreadability of cosmetic preparation was evaluated according to the expressions of user. All of the organoleptic analyzes were carried out in the same light, temperature and packaging conditions to avoid variations in appearance, color, odor and spreadability parameters. All tests were repeated on 1, 5, 30 and 90 days after the product was prepared.

3. RESULT AND DISCUSSION

3.1. Chemical Composition of Volatile Oil

In this study, forty-eight components were detected in the volatile oil obtained from *L. nobilis* leaves by steam distillation (Table 1). Volatile oil analyzed by GC/MS instrument and 1,8-cineol (46.16%), *alpha*-terpinyl acetate (10.62%), *alpha*-pinene (6.27%), terpinen-4-ol (5.07%), sabinene (4.99%) and *beta*-pinene (4.47%) detected as major compounds among forty-eight components. The other compounds such as 3-hexen-1-ol (0.03%), *cis*-*beta*-ocimene (0.05%) and 2-methylprop-1-enyl-cyclohexa-1,3-diene (0.07%) were minor compounds.

The chemical composition of *L. nobilis* volatile oil from different locations has been studied by different researchers. In all studies, 1,8-cineole was the major component with percentages ranging between 26.70% and 68.48% [14, 30]. Moreover, *alpha*-terpinyl acetate with percentages ranging between 0.65-25.70% [31, 32] and terpinen-4-ol with percentages ranging between 1.50-4.56% [32, 33] were found as major components. According to the our result, we found average amount of 1,8-cineol but terpinen-4-ol was found to be more than average amounts reported by previous researches (Figure 1).

Tanriverdi *et al.* [34] found that leaves of *L. nobilis* of Turkish origin contained 1,8-cineole (62.64%), *alpha*-pinene (3.14%), and terpinen-4-ol (3.11%). Riaz *et al.* [35] established that leaf oil of *L. nobilis* contained 1,8-cineole (44.12%). Florini *et. al.* [36] from France reported that *L. nobilis* leaves volatile oil contained 1,8-cineole (39.10%), linalool (10.00%), β-caryophyllene (1.60%), *alpha*-terpinyl acetate (18.20%) and terpinene-4-ol (1.40%) while Yalçın *et. al.* [37] found 1,8-cineole (58.59%), *alpha*-terpinyl acetate (8.82%), and terpinene-4-ol (4.25%) as the main components of the essential oil isolated from the leaves of the *L. nobilis* plant (from the Northern Cyprus Mountains) by hydrodistillation.

Volatile oils have been used as antioxidants for the prevention of skin disorders such as skin cancer and wrinkles which caused by oxidative stress on skin surface [38].

Table 1. GC/MS analysis of volatile oil composition of *Laurus nobilis* leaf

No	RT (min.)	Compound	Concentration (%)	Identification method
1	3.656	3-Hexen-1-ol	0.03	b, c
2	5.707	*alpha*-Thujene	0.50	a, b, c
3	5.893	*alpha*-Pinene	6.27	a, b, c
4	6.089	2,4(10)-Thujadien	0.08	b, c
5	6.262	Camphene	0.75	a, b, c
6	7.116	Sabinene	4.99	a, b, c
7	7.209	*beta*-Pinene	4.47	a, b, c
8	7.902	*beta*-Myrcene	0.30	a, b, c
9	8.301	*alpha*-Phellandrene	0.27	a, b, c
10	8.627	3-Carene	0.08	b, c
11	8.849	*alpha*-Terpinene	0.82	a, b, c
12	8.984	o-Cymene	2.61	a, b, c
13	9.394	1,8-Cineol	46.16	a, b, c
14	9.429	*alpha*-Limonene	1.90	a, b, c
15	10.369	cis-*beta*-Ocimene	0.05	a, b, c
16	10.732	*gamma*-Terpinene	1.48	a, b, c
17	10.883	trans-Sabinene hydrate	0.16	b, c
18	11.885	Unknown	0.08	a, b, c
19	12.190	*alpha*-Terpinolene	0.33	a, b, c
20	12.349	cis-Sabinene hydrate	0.12	b, c
21	12.733	Linalool	1.43	a, b, c
22	13.598	p-Ment-2-en-1-ol	0.18	b, c
23	14.305	trans-Pinocarveol	0.41	b, c
24	15.115	(E)-Sabinen hydrate	0.26	b, c
25	16.630	Terpinen-4-ol	5.07	a, b, c
26	16.861	Myrtenal	0.26	b, c
27	17.052	*beta*-Phellandren-8-ol	0.12	b, c
28	17.174	p-Mentha-1(7),8-dien-2-ol	0.10	b, c
29	17.308	*alpha*-Terpineol	2.17	a, b, c
30	17.553	Estragole	0.06	b, c
31	17.669	Myrtenol	0.41	a, b, c
32	18.428	cis-Piperitol	0.08	b, c
33	22.663	4-Thujen-2-alpha-YL	0.26	b, c
34	23.120	Bornyl acetate	0.63	a, b, c
35	25.067	Unknown	0.67	
36	25.533	1-ethyl-3,5-dimethyl-benzene	0.09	b, c
37	26.600	2-Methylprop-1-enyl cyclohexa 1,3-diene	0.07	b, c
38	26.817	Eugenol	1.18	b, c
39	27.176	*alpha*-Terpinyl acetate	10.62	a, b, c
40	28.230	Unknown	0.09	
41	29.758	Eugenol methyl ether	3.24	b, c
42	30.106	*beta*-Elemene	0.10	b, c
43	31.395	*beta*-Caryophyllene	0.12	a, b, c
44	31.785	Cinnamyl acetate	0.28	b, c
45	35.047	Isoeugenyl methyl ether	0.17	b, c
46	39.688	Spathulenol	0.19	b, c
47	39.842	Caryophyllene oxide	0.22	a, b, c
48	42.754	*beta*-Eudesmol	0.10	b, c

[a]Comparison with standard compounds, [b]GC/MS analysis, [c]Literature comparison

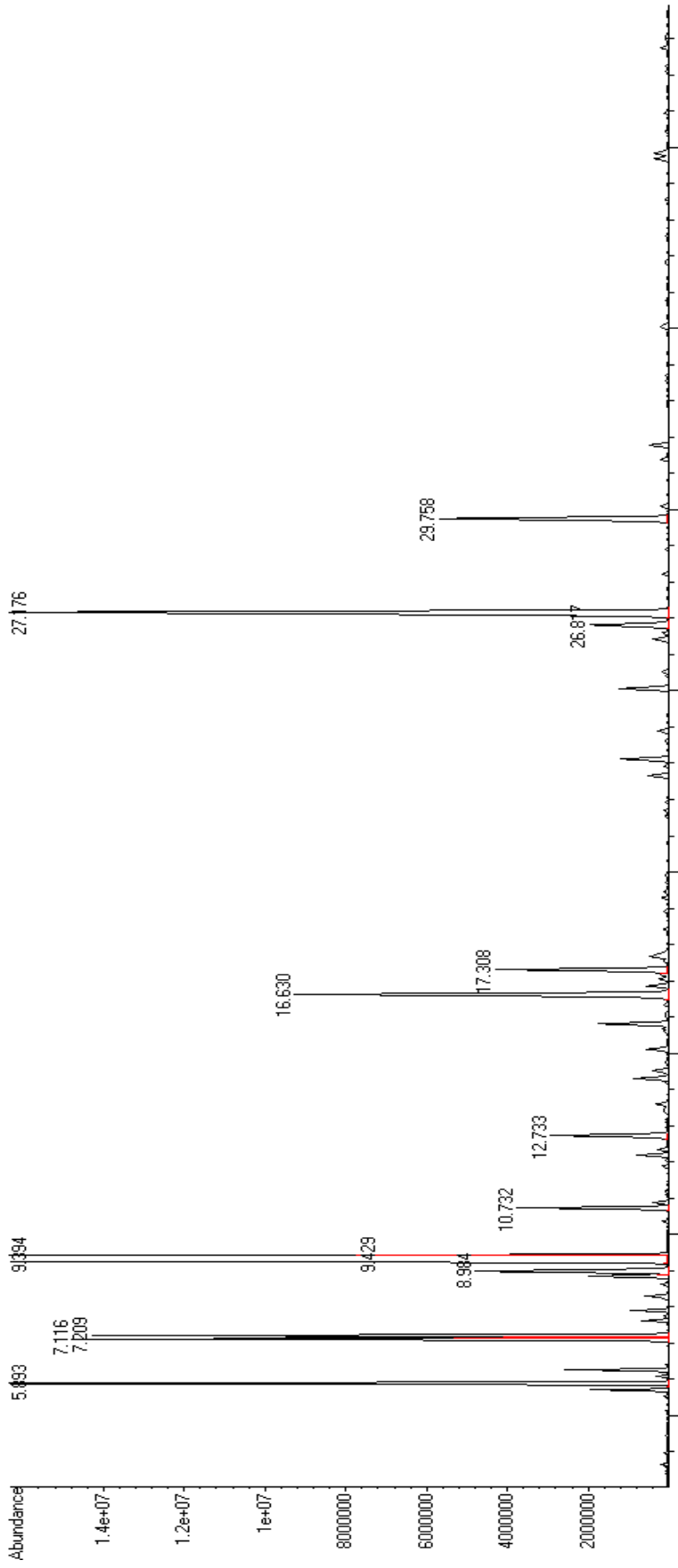

Figure 1. GC/MS chromatogram of *Laurus nobilis* volatile oil. (Retention times of the components above the concentration of 1% are given). *alpha*-pinene (5.893), sabinen (7.116), *beta*-pinene (7.209), o-cymene (8.984), 1,8-cineol (9.394), *alpha*-limonene (9.429), *gamma*-terpinene (10.732), linalool (12.733), terpinen-4-ol (16.630), *alpha*-terpinol (17.308), eugenol (26.817), *alpha*-terpinyl acetate (27.176), eugenol methyl ether (29.758)

High amount of 1,8-cineol (46.16%) and terpinen-4-ol (5.07%) makes volatile oil a potent of cosmetic ingredient due to their high antioxidant activity [39]. However, these compounds described as potent of antifungal agent by previous researches [40] and with this reason they can be considered as self-preserving ingredient for cosmetic products.

Considering the proven effects of *L. nobilis* leaf volatile oil and previous studies, the use of *L. nobilis* leaf essential oil in cosmetic formulations can result in high added value to the product and contribute to product functionality.

3.2. Antioxidant Activities

Antioxidant activities of extracts from *L. nobilis* leaves were investigated using three different methods (β-carotene-linoleic acid, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$). The antioxidant activities of *L. nobilis* leaf extracts were measured by using spectrophotometric methods after they were dissolved in appropriate solvents. When the total antioxidant activity values were examined, the 50% inhibition concentration (IC_{50}) of ethyl acetate extract was found to be 19.32±1.04 μg mL^{-1} in the β-carotene-linoleic acid assay. Compared to the other extracts and standards, ethyl acetate extract seems to be quite active.

In addition, according to the results of $ABTS^{\bullet+}$ cation radical and $DPPH^{\bullet}$ free radical scavenging assays, ethyl acetate extract was found to be the most active extract, respectively (24.98±0.87 μg mL^{-1} and 75.65±0.77 μg mL^{-1}). $DPPH^{\bullet}$ free radical inhibition IC_{50} value of ethyl acetate extract of *L. nobilis* leaves was found to be 75.65 ± 0.77 μg mL^{-1}, whereas butylated hydroxytoluene (BHT) IC_{50} value, used as standard, was 68.27 ± 0.67 μg mL^{-1} (Table 2).

Table 2. Antioxidant activity of the extracts of *L. nobilis* by β-carotene-linoleic acid, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays

		Antioxidant activity		
	Extracts	*β*-Carotene-linoleic acid assay IC_{50} (μg mL^{-1})	$ABTS^{\bullet+}$ assay IC_{50} (μg mL^{-1})	$DPPH^{\bullet}$ assay IC_{50} (μg mL^{-1})
Laurus nobilis	Hexane	47.08±0.20	55.34±1.21	201.47±0.60
	Ethyl acetate	19.32±1.04	24.98±0.87	75.65±0.77
	Ethanol	36.23±1.01	43.74±0.57	129.10±0.66
	Water	124.01±1.65	99.75±1.41	203.55±0.98
Standards	BHA	2.18±0.09	6.28±0.10	67.40±0.41
	BHT	2.05±0.11	6.37±0.08	68.27±0.67
	α-Tocopherol	3.87±0.15	6.97±0.13	10.41±0.19

IC_{50} values represent the means ± SD of three parallel measurements
BHA; Butylated hydroxyanisole, BHT; Butylated hydroxytoluene

Number of studies have been done to evaluate the antioxidant properties of different extracts of *L. nobilis* leaves. Previous researchers have reported that ethanolic extract of *L. nobilis* leaves exhibited an IC_{50} value of 22 ± 0.531 μg.mL^{-1} using by $DPPH^{\bullet}$ assay and 1 ± 0.315 μg.mL^{-1} according to β-carotene-linoleic acid assay [41]. In another study, ethyl acetate extract and water extract of *L. nobilis* leaves exhibited an IC_{50} value of 83.24 μg.mL^{-1} and 161.83 μg.mL^{-1}, respectively, using by $DPPH^{\bullet}$ assay [42]. More studies in the literature indicated that leaf extracts of *L. nobilis* provide significant antioxidant effect [43,44]. Our results are compatible with the literature with respect to antioxidant effect.

3.3. Total Phenolic and Flavonoid Concentrations

Amounts of total phenolic and flavonoid contents in extracts of *L. nobilis* leaves are given in Table 3. The highest amount of total phenolic content with the amount of 54.42±0.14 µg PEs/mg extract was determined in ethyl acetate extract of *L. nobilis* leaves and the minimum total phenolic content with the amount of 11.04±0.20 µg PEs/mg extract was measured in water extract of leaves. According to the results of total flavonoid content assessments of extracts, the highest total flavonoid content was found in ethanol extract (8.60±0.12 µg QEs/mg extract) and the lowest flavonoid content was determined in the hexane extract (1.01±0.10 µg QEs/mg extract) of *L. nobilis* leaves.

When we compared the results obtained, there is a good correlation between high antioxidant activity and high amount of phenolic compounds in extracts [45]. In previous studies, some researchers have found such positive correlations between antioxidant activity and the amount of phenolic compounds of plants [46, 47].

Table 3. Total phenolic and flavonoid concentrations of *L. nobilis* leaf extracts

	Extracts	Total Phenolic Content (µg PEs/mg extract)	Total Flavonoid Content (µg QEs/mg extract)
Laurus nobilis	Hexane	12.80±0.35	1.01±0.10
	Ethyl acetate	54.42±0.14	5.48±0.65
	Ethanol	25.32±0.10	8.60±0.12
	Water	11.04±0.20	1.07±0.10

Results expressed as mean ± standard deviation.

The ethanol and ethyl acetate extracts obtained by the subsequent extraction appear to be rich in phenolic and flavonoid content in both extracts. Ethyl acetate extracts rich in phenolic compounds were found to have highest antioxidant activity in three different methods (β-carotene-linoleic acid, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$).

Based on the results, it can be said that phenolic compounds have an important effect on the antioxidant activities of extracts. In addition to the phenolic concentrations of extracts, flavonoid concentrations of *L. nobilis* leaf extract were investigated. The total amount of flavonoid contents in the ethyl acetate and ethanol extracts with high amounts of phenolic contents were found to be 5.48 ± 0.65 and 8.60 ± 0.12 µg QEs/mg extract, respectively.

3.4. Stability and Organoleptic Tests of Cosmetic Preparation

The results obtained from the stability tests and the organoleptic analyses of the cosmetic formulation made by the cold process following the analysis are given in Table 4. No phase separation was observed in both 15 min analyzes performed on the days 1, 5, 30 and 90 in centrifugation tests. The pH measurements made on the formulation showed that there was no significant change in the pH of the lotion even after 90 days. Only very small changes due to time have been identified. The viscosity of the product has changed to negligible level due to very small pH changes and it maintains product stabilization after 90 days.

The product was homogeneous and easy to spread when it was prepared. Lotion left a soft, non-greasy feel after it was subjected. There was no change in the product's spreadability and appearance after 90 days.

Table 4. Results of stability tests and organoleptic analysis of formulation

Test / Time	1st day	5th day	30th day	90th day
Stability Tests				
pH	5.86	5.87	5.90	5.95
Viscosity(cPs)	14400	14460	14465	14502
Centrifuge	Stable	Stable	Stable	Stable
Organoleptic Tests				
Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Color	Greeny white	Greeny white	Greeny white	Greeny white
Odor	Laurel odor	Laurel odor	Laurel odor	Laurel odor
Spreadability	Easy	Easy	Easy	Easy

4. CONCLUSION

Nowadays, it is clear to see that the escape from artificial substances will further increase the importance of natural products. With this reason, investigation of composition and biological activities of natural substances is important. In this study, the volatile oil composition of *L. nobilis* leaves was accurately determined by comparative methods using GC/MS instrument. According to the our results, the amounts of 1,8-cineol, *alpha*-terpinyl acetate and *alpha*-pinene are similar to the previous studies made by different researchers. However, the amount of terpinen-4-ol in this study found to be higher than other studies.

The antioxidant potential of the extracts was determined using three complementary methods. The extracts investigated in this study have significant antioxidant activity. The highest antioxidant activity in all assays (DPPH, ABTS, β-carotene) was measured in ethyl acetate extract of *L. nobilis* leaf extract.

L. nobilis volatile oil has potency in cosmetic products due to its numerous antioxidant components. At present, the cosmetic formulation formed within the scope of the study has passed successfully the stability and organoleptic tests that were carried out. At the end of 90 days, there was no significant change in the pH and viscosity parameters of the formulation, and no change in organoleptic properties, such as product appearance, color, odor, spreadability were observed. Good stabilization of the formulation proves that the volatile oil has successfully performed its mission of acting as a preservative in the cosmetic formulation.

In the next step of the study, various dermo-cosmetic effects can be examined of the prepared formulation, and the obtained oil can be used on the formulations in different types of cosmetic products.

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