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INVESTIGATION OF BIOACTIVE COMPOUNDS OF WHITE WATER LILY *NYMPHAEA ALBA* LEAVES

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ABSTRACT: Nymphaeaceae is a perennial herbaceous plant with a creeping rhizome that lives in calm waters at the edge of the lake or in the slowly flowing water channel. There are two species, *Nymphaea alba* (white lotus) and *Nuphar lutea* (yellow lily), in Turkey, of which *N. alba* is common found in the Mediterranean region. In the present study, *N. alba* was used to discover novel alternative additives for industrial applications. The leaves of *N. alba* were collected from the Hatay region and dried in room conditions and pulverized, and then powdered. The powdery samples were examined by ICP-MS, GC/MS, LC/MS/MS, SEM and EDX, and XRD. Essential and free fatty acids analysis was conducted by GC/MS and found to be 1.75%. Minerals compounds were scrutinized by ICP/MS, results of minerals were found as Na 12864, Mg 1864.14, K 5144.35, Ca 8262.34 ppm. In addition, the alpha-tocopherol (E vitamin) analysis was examined by LC/MS/MS were found as 5.6 ± 0.4 mg / 100 g. The present analysis showed that *N. alba* extracts can be used a source in pharmaceuticals and cosmetical applications.

INTRODUCTION: Plant extracts and the use of plant parts have been used since ancient times for cosmetic and pharmaceutical applications. Plant products like extracts, oil, and powders have been used in cosmetics as either active part or as excipients ¹. Therefore, most cosmetic products and their applications are defined by active materials that may arise from either synthetic sources or from plant sources. Besides this, no other origin, like human or animal, or genetically modified plant sources, is accepted or allowed in cosmetics.

Applications of plants and plant extracts in cosmetics are extensive and used for aims such as moisturizing, whitening, tanning, color cosmetics, sunscreen, radical-scavenging, antioxidant, immunostimulant, washings, preservatives, thickeners and etc. The range of used plants or parts of plants is broad and ranges from algae, succulents, berries, carnivorous plants, herbs, crops, trees, etc. ² The cosmetic researchers and developed societies are therefore searching for new innovative plant ingredients for cosmetic application.

Nymphaea alba known as the European water lily, White Lotus, is an aquatic flowering plant of the genus Nymphaeaceae. Flower stalks do not come out of the water, and whose leaves usually float on the water. It lives in calm waters at the edge of the lake or in slow-flowing water channels.

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About the structure of leaves, it is rootless, and its leaves are rosette-shaped. This species is distributed mainly in Europe, extending outside the region to North Africa, the Middle East, the Caucasus, and into Jammu and Kashmir in India. There are two species, *Nymphaea alba* (white lotus) and *Nuphar lutea* (yellow lily) in Turkey 3 of which *Nymphaea alba* is common found in the Mediterranean region. *N. alba* was commonly used in Indian folk medicinal products as an antiseptic, an astringent, radical scavenger, in burning and in insomnia while rhizomes are applied externally as a rubefacient⁴. Previously published studies reported the antioxidant, anti-inflammatory as well as hepatoprotective effect of *N. alba* flowers^{5,6}. The purpose of this study was to investigate the leaves of *Nymphaea alba* for the suitability of a raw material source as a novel alternative additive which were used in cosmetic and pharmaceutical applications.

MATERIALS AND METHODS: *Nymphaea alba* leaves were collected from Hatay region. In the laboratory, the samples were washed and separately dried in air. Air-dried and coarsely powdered *Nymphaea alba* were placed in Soxhlet extractor with Hexane. The extracts were then concentrated for dryness under reduced pressure, and its temperature was controlled. Finally, It was analyzed by alpha-tocopherol. In addition, powdered *N. alba* was kept in olive oil for 1 week. Then, olive oil-containing powder was treated in an ultrasonic bath for 2 h and filtered by syringe filter.

Preparation of Extracts: About 30 g of powdered *Nymphaea alba* leaves were transferred into a 2000 ml beaker containing 1500 ml double distilled water, incubated for 2 days. The extract obtained was filtered. Finally, n-butanol was added to the filtered solution and then evaporated.

GC/MS Analyses: Air-dried leaves were treated to water distillation for 4 hrs using a Clevenger-type apparatus to produce the essential oils. The oils were dried over anhydrous CaCl₂ and stored in sealed vials at low temperature in the refrigerator before the analysis. The essential oils were analyzed by triple quad GC-MS using Agilent (7000 series) and compared with GC-MS Hewlett Packard GCD (model 6890) and (model 5972) equipped with a mass selective detector (MSD).

ICP/MS: 1 g ground *Nymphaea alba* leaves were dissolved at the temperature of 100 °C in a mixture of nitric acid / perchloric acid (8/2) (v / v) and filtered to analyze in ICP. Measuring elemental content of *Nymphaea alba* leaves was made by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) AGILENT 7500ce ICP-MS, Cetac ASX-520 model instrumentation.

XRD Analyses: X-ray diffraction (PAN analytical EMPYREAN XRD) is used to find the thickness of thin (5-200 nm depending on mean atomic number) crystalline films on polished, flat surfaces. It is possible to deal with multiple films to a limited extent. Amorphous films can be measured.

Powder samples were done at standard angular resolutions by XRD. The crystallographic texture and composition can be determined using XRD Phase Analysis. The crystallographic orientation of samples up to 20 × 20 mm can be determined.

LC-MS/MS Analyses: About 12.6 g of powder *N. alba* was transferred into a 500 ml beaker containing 200 ml olive oil, incubated for 1 week. It was then treated in an ultrasonic water bath for 1.5 h. It was then treated in an ultrasonic water bath for 1.5 h. 0.5281 g of powdered white water Lilly leaves were placed in Soxhlet extractor with Hexane to get oil.

The liquid extract obtained was filtered. THERMO SCIENTIFIC Quantum Access Model LC/MS/MS was used to analyze alpha-tocopherol values of the *N. alba* samples.

SEM and EDX Analyses: Measuring elemental content of *Nymphaea alba* leaves were made by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) AGILENT 7500ce ICP-MS, Cetac ASX-520 model instrumentation. PAN analytical EMPYREAN XRD is used.

RESULTS AND DISCUSSION: The hexane-extracted oil content of *Nymphaea alba* cultivated at coastal sites of Hatay was found to be 1.75%. In addition, the alpha-tocopherol analysis value was found to be 5.6 ± 0.4 mg / 100 g **Table 1**. Saleem et al.,⁷ noted that the extract of *Nymphaea lotus* flowers collected from Pakistan is a stronger antioxidant than alpha-tocopherol and contains relatively higher phenolic contents.

TABLE 1: LC/MS/MS RESULTS OF ALPHA TOCOPHEROL FROM NYMPHAEA ALBA

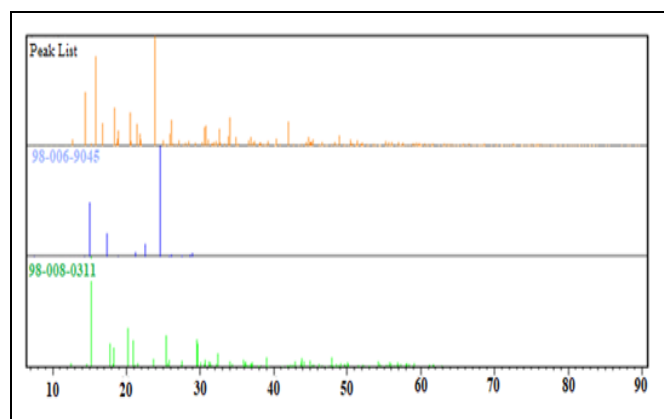
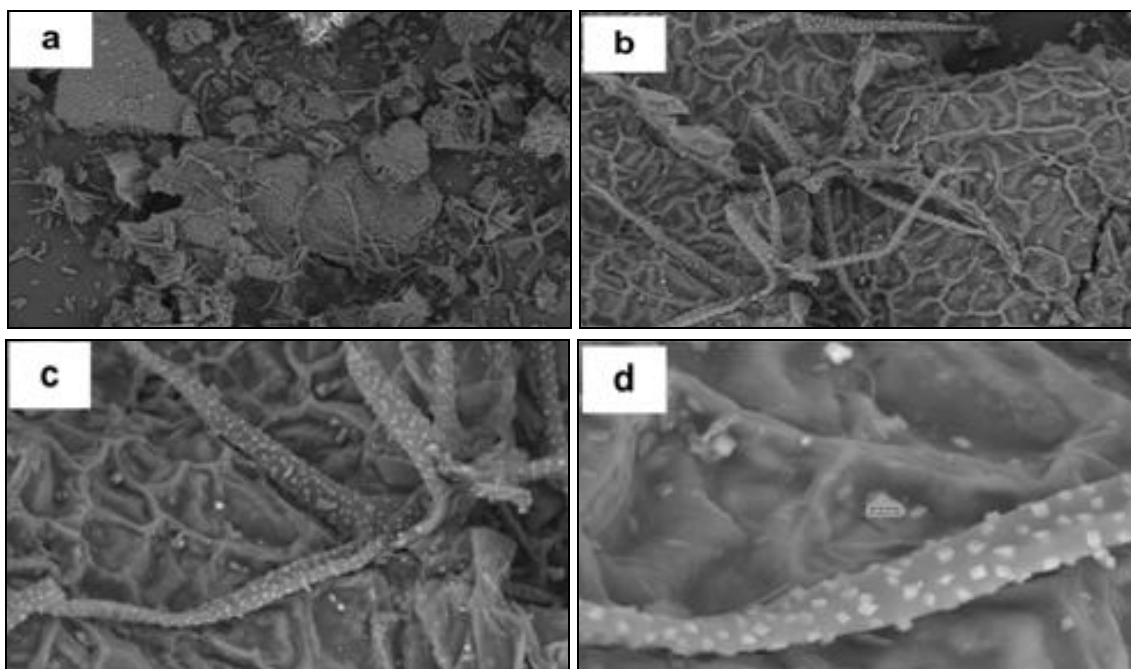
Sample Name	Result
Olive oil	147, 17 µg/ml
<i>N. alba</i> (12.6 g) in olive oil (200 mL)	478, 27 µg/ml
0.5281 g <i>N. alba</i> in Hexane	7457, 26 µg/ml

The results of moisture content value are found to be 8.68%. In comparison with other plants, Min *et al.*,⁸ studied the Chemical Composition of *Salicornia herbacea* and found moisture content found as 9.09%. In addition, the alpha-tocopherol analysis value is found to be 5.6 ± 0.4 mg / 100 g. The results of minerals were Na 12864, Mg 1864.14, K 5144.35, Ca 8262.34 ppm, found by ICP/MS. Cudalbeanu *et al.*,⁹ studied exploring new anti-oxidant, and mineral compounds from *Nymphaea alba* leaf, and results of minerals were Mg, K, Ca, 2057.3, 4931.3, 8103.1 ppm, respectively. Thermo scientific quantum access model LC/MS/MS was used to analyze alpha-tocopherol values of the *N. alba* samples. The rate of alpha-tocopherol in powdered *N. alba* leaves left in olive oil was increased to 3 times more than that olive oil without *N. alba* leaves. In addition, approximately 12.6 g of *N. alba* powder was treated with heptane, and this ratio was increased by 50 times more than that olive oil without *N. alba* powder. In order to reduce the use of chemicals, olive oil was preferred instead of heptane. The heptane is not preferred because of its irritant property.

TABLE 2: XRD ANALYSIS RESULTS AND GRAPHICS

%	Ref. Code	Compound Name
*56.4	98-006-9045	Hexaamminechromium Hexaaquanickel Chloride Ammonium Chloride
*45.6	98-008-0311	Ammonium Aquadioxomolybdenum Phosphate

According to the XRD results, the amount of Ammonium Chloride was found as 56.4% in *N. alba* powder. XRD analysis results and graphics are given in **Table 2**. Ammonium Chloride is an alkaline salt used as a pH balancer in skin-care products. The crystalline system is found Monoclinic **Fig. 1**. The surface morphology of the prepared sample was characterized by SEM (FEI Quanta 650 Field Emission SEM). **Fig. 2** shows the SEM image of *N. alba*. *N. alba* in the SEM figures reveals a hexagonal shape in the structure.

**FIG. 1: GRAPHS OF NYMPHAEA ALBA BY XRD****FIG. 2: SEM MICROGRAPHS OF N. ALBA POWDER**

The occurrence of elemental was determined by using EDX Zeiss Evo 50. The samples were dried at room temperature and then analyzed for its NPs

composition. The results of EDX analysis is given in **Fig. 3**.

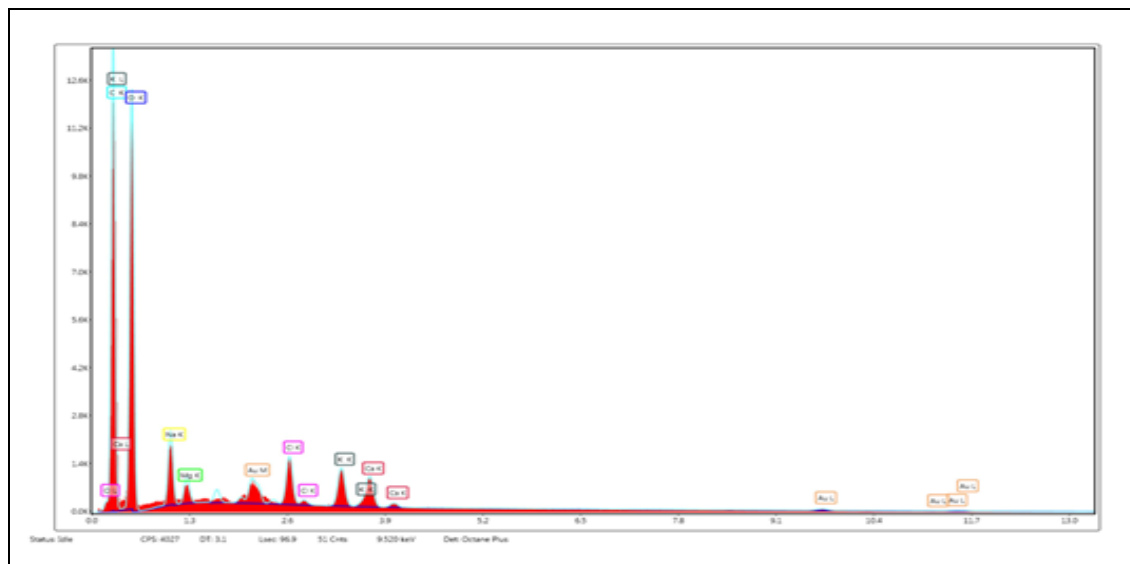


FIG. 3: EDX GRAPH OF *N. ALBA* SAMPLES AND THE CONDITIONS OF EDX

CONCLUSION: The interest in cosmetic products with natural ingredients is increasing. Therefore, the use of extracts obtained from plants in cosmetic products has become widespread. Plant extracts can protect the skin against harmful agents and may help to eliminate many skin disorders due to their activities, such as anti-aging, antibiotics, and anti-malarial activities.

High levels of E vitamin are found in some plants and seeds, and they vary in the number of methyl substituents on the hydrophilic 6-chromanol head group, and the number of double bonds in the side chain Tocopherols are potent antioxidants and function as radical scavengers^{10, 11, 12}. In the light of this information, *Nymphaea alba* samples are analyzed to be a source for pharmaceuticals and cosmetics. The present analysis revealed that *N. alba* extracts can be used as a source in pharmaceuticals and cosmetical applications.

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CONFLICTS OF INTEREST: No conflict of interest declared.

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