Int. J. Adv. Eng. Pure Sci. 2022, 34(1): 147-156
DOI: 10.7240/jeps.1056274

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Potential Application of *Trachystemon orientalis* L. Extracts in the Cosmetic Industries: Skincare, Photoprotective and Antiaging Ingredients

Trachystemon orientalis L. Ekstraktlarının Kozmetik Endüstrisinde Kullanım Potansiyeli: Cilt Koruyucu, Güneş Koruyucu ve Yaşlanma Karşıtı İçerikleri

Gamze KURTULUŞ [1], Orçun TOKSÖZ [2], Nüzhet Cenk SESAL[3]

[1,2]*Marmara University, Institute of Pure and Applied Sciences, Department of Biology, Goztepe Campus, 34722 Istanbul, Turkey*
[3]*Marmara University, Science and Arts Faculty, Department of Biology, Goztepe Campus, 34722 Istanbul, Turkey*

Abstract
In recent years, it has become more preferable for the products to be used in cosmetics to be of herbal origin. In particular, plants with high antioxidant properties are preferred to be the source for cosmetic products. Wild edible plants, which are used only by the local people, will be more profitable economically if they are grown for use in different industries, especially in cosmetics. *T.orientalis* is a wild species that grows in the Black Sea region of Turkey. This study aims to show that these plant extracts have the potential for use in the cosmetic industry. For this purpose, firstly the stem, flower, and leaf parts of *T.orientalis* were extracted with acetone and their antioxidant activities were measured. According to the results, antioxidant activity was especially high in stem extracts (DPPH 57,12 mg L-ascorbic acid/g; total phenol 288,10 ± 8,55 mg/g dry weight). Then, the antibacterial and anti-biofilm activity of the extracts on *S. aureus* was investigated. As a result, no effective activity was found. In addition, SPF values and collagenase, tyrosinase, and elastase inhibiting activities were measured. The best results were observed in the stem (SPF 31,18 ± 0,18%; anti-collagenase 87,25%; anti-tyrosinase 11,47%; anti-elastase 57,64%) and leaf extracts (SPF 31,27 ± 0,24%; anti-collagenase 97,57%; anti-tyrosinase 15,03%; anti-elastase ND). As a result, it has been seen that especially the stem parts of *T.orientalis* can be used as an ingredient for photoprotective and antiaging purposes in cosmetics.
Keywords: Cosmetics, Herbal sources, Sun protection factor, Photoprotective, Antioxidant, Antiaging.

Öz
Son yıllarda kozmetik alanında kullanılan ürünlerin bitkisel kaynaklı olması daha çok tercih edilmektedir. Özellikle antioksidan özelliği yüksek bitkilerin kozmetik ürünlerin içeriğinde yer alması öncelikli istek haline gelmiştir. Genelde sadece yöre halkı tarafından kullanılan yabani bitki türleri, başta kozmetik olmak üzere farklı endüstrilerde kullanılmak üzere yetiştirilirse ülke ekonomisine katkıda bulunacağı aşikârdır. *T.orientalis*, Türkiye'nin Karadeniz bölgesinde yetişen yabani bir türdür. Bu çalışma, bu bitki ekstraktlarının kozmetik endüstrisinde kullanım potansiyeline sahip olduğunu göstermeyi amaçlamaktadır. Bu amaçla öncelikle *T.orientalis*'in gövde, çiçek ve yaprak kısımları aseton ile ekstrakte edilmiş ve antioksidan aktiviteleri ölçülmüştür. Sonuçlara göre antioksidan aktivite özellikle gövde ekstraktlarında (DPPH 57,12 mg L-askorbik asit/g; toplam fenol 288,10 ± 8,55 mg/g kuru ağırlık) yüksek bulunmuştur. Daha sonra ekstraktların *S. aureus* üzerindeki antibakteriyel ve anti-biofilm aktiviteleri araştırılmıştır fakat etkili bir aktivite bulunamamıştır. Ayrıca SPF değerleri ve kollajenaz, tirozinaz ve elastaz inhibe edici aktiviteleri ölçülmüştür. En iyi sonuçlar gövde (SPF 31,18 ± %0,18; anti-kollajenaz %87,25; anti-tirozinaz %11,47; anti-elastaz %57,64) ve yaprak ekstraktlarında (SPF 31, %27 ± 0,24; anti-kollajenaz %97,57; anti-tirozinaz %15,03; anti-elastaz ND) tespit edilmiştir. Sonuç olarak, *T.orientalis*'in özellikle gövde kısımlarının kozmetikte fotokoruyucu ve yaşlanma karşıtı olarak kullanılabileceği ön görülmektedir.
Anahtar kelimeler: Kozmetik, Bitkisel kaynaklar, Güneş koruyucu faktör, Fotokoruyucu, Antioksidan, Yaşlanma karşıtı.

I. INTRODUCTION

The estimated growth of the cosmetics market globally in 2020 is 335.6 billion US dollars [1]. Various sunscreen creams, lotions, oils, and gels are produced in the cosmetics industry to avoid the damaging effects of solar ultraviolet (UV) radiation. These sunscreens absorb (chemical sunblock) or reflect (physical sunblock) UV radiations and help protect against suntan, photoaging, and sunburn [2,3]. Chemical UV filters in sunscreens for example oxybenzone and octinoxate have become disputed because of their potential risks to the environment and human healthiness [4]. Instead of these, inorganic filters are used as an alternative. There are two inorganic filters approved by U.S. Food and Drug Administration (FDA); titanium dioxide (TiO_2) and zinc oxide (ZnO) particles [5]. However, these filters are hazardous to human skin in case of constant application and these

Corresponding Author: Gamze KURTULUŞ, Tel: +90 539 984 50 28, e-posta: gamzekurtulus93@gmail.com
Submitted: 11.01.2022, **Revised:** 07.03.2022, **Accepted:** 07.03.2022

nanoparticles can cause oxidative DNA damage by high activities of Reactive Oxygen Species (ROS) [6]. Moreover, it has been reported in many studies that sunscreens are not completely safe for sun protection due to their ingredients [7,8,9]. Therefore, studies on the use of natural sources for the cosmetic industry will be very important for both environmental and human health.

According to World Cancer Research Fund, skin cancer (melanoma and non-melanoma) is the most common cancer globally [10]. The increase in skin cancers over the past decades is especially related to overexposure to the Sun's rays. Presently, 2 -3 million non-melanoma and 132,000 melanoma skin cancers occur worldwide every year (WHO/World Health Organization) [11]. Exposure to UV radiation is the primary reason for skin cancer. Additionally, photoaging is one of the reasons for skin melanoma, and risk is seen higher in the fair skin people, with about 90% of the European and North American people prevalent to skin photoaging [12]. In order to decrease the risk of skin cancer and to prevent photoaging, many types of research and products are being developed in the field of cosmetics. Natural components have been lately considered as potential sources for agents with sunscreen properties due to their absorption in UV radiation and high antioxidant activities [9, 13]. Phenolic contents (TPC) and flavonoid contents (TFC) in plants can absorb UV and play an essential role against UV radiation [14,15]. The research of cosmetic products containing antioxidant and anti-aging activities and natural sunscreen ingredients has accelerated [1]. Besides, WHO states that accordingly 80% of the world population depends on natural herbal sources as their main treatments [16].

The Solar UV region is divided into 3 subcategories which are, UV-C (200-290 nm), UV-B (290-320 nm), and UV-A (320-400 nm). UV-A and UV-B radiation is the main reason for the generation of ROS and nitrogen centered species and related oxidative stress [17]. Free radicals and oxidative stress caused by UV radiation are important causes of skin photoaging, as they cause oxidation of lipids and proteins in the chemical structure of skin cells. In particular, the effect of UV-induced ROS on the structural proteins of the skin which are collagen and elastin are responsible for preserving the elasticity of the skin, play a significant role in the aging of the skin [18]. Collagen degradation in humans begins with collagenase activation. In addition, oxidative stress causes an increase in collagen destruction with the activation of collagenases, as well as a decrease in collagen synthesis. For this reason, many studies on the avoidance of skin aging focus on the inhibition of collagenase activity [19,20]. Similarly, inhibition of elastase activity induced by UV or ROS can also be applied as a useful method against skin diseases and aging [21,22]. Inhibition of collagenase and elastase activities by natural herbal contents may be a promising approach to prevent skin aging [23].

In Turkey, there are many plants with high antioxidant content used for medical, cosmetic, and food. Among them, *Trachystemon orientalis (L.)* G. Don (early blooming borage), is a plant whose high antioxidant activity, high TPC and TFC have been reported before [24-27]. However, there is no research on the sunscreen or photoprotective activities of *T. orientalis*. This plant, whose flowering branches, leaves and young stems are consumed as a vegetable by the local people, is wild edible in the Black Sea region. The effects of the plant are not fully known since detailed scientific studies have not been done. In this respect, the main purpose of this study is to examine whether these plant extracts have the potential to be used as photoaging and sunscreen in the cosmetic industry.

II. MATERIALS AND METHODS

2.1. Plant Material

The leaf, stem and flower parts of *T. orientalis* were obtained *f*rom local growers of northern Turkey (Sinop Province) was used in this study. In May 2021, plants were obtained and identified using botanical and morphological characteristics. The flower branches, stem, and leave parts were separated, cleaned and then dried for one week at 25 ℃.

2.2. Preparation of Extracts

A total of 20 g of plant parts was soaked for 24 hours in 200 mL of pure acetone (Merck), changing the solvent three times. Then, the solution was filtered with Whatman filter paper. Solvent removal was carried out under reduced pressure with the aid of a rotary evaporator and acetone extracts of the plants were obtained and stored at 4℃.

2.3. Determination of Antioxidant Activity

TPC was measured spectrophotometrically with Cytation-3 (Bıotek) using the Folin-Ciocalteu technique [28]. 10 µl of plant extract (1 mg/ml obtained in methanol (Merck)), 100 µl of Folin-Ciocalteu Reagent (Sigma) diluted in distilled water (1:10), and 75 µl of 7,5% Na_2CO_3 (Merck) were mixed and left in the dark for 1h. The absorbance was read at 750 nm. The tests were repeated ten times. The standard curve was prepared using a 1 mg/ml solution of gallic acid (Merck) in dimethyl sulfoxide (DMSO) (Merck). The concentration of total phenolic content was calculated using the equation derived from the gallic acid standard curve.

TFC was calculated by the aluminum calorimetric method [29]. In order to create a calibration curve, serial solutions of Rutin (Merck) were done starting from 1 mg/mL to 0.0625.(0.0625, 0.125, 0.25, 0.5, 1 mg/mL). The plant extracts of ethanol solution (50 µl, 0.3 mg/mL) was added with 150 µl 2% (w/v) $AlCl_3$

(SIGMA) in 96-well plates. After 15 minutes of incubation, the absorbance was read at 435 nm. The total flavonoid content of the extracts was calculated as rutin equivalent [mg Rutin equivalent (QE) g^{-1} dry extract weight] per dry weight of the extract.

1,1-Diphenyl-2-picryl hydrazyl (DPPH) was used to assay the free radical-scavenging activity of the plant extracts [28]. 20 µl of the plant extracts were diluted with DMSO and the final concentration was obtained as 1 mg/ml. Then, they were mixed with 180 µl of DPPH solution (SIGMA) (40 µg/ml in methanol) in a 96-well plate. After the plates were kept in the dark for 30 minutes, their absorbance was measured at 540 nm with Cytation-3 (Bıotek). DMSO was used as a blank instead of the test sample. L-ascorbic acid, dissolved in DMSO, was used as a standard. The results were calculated according to equation (1) given below and the DPPH scavenging effects of the extracts were expressed as a percentage.

$$\text{DPPH\%} = [(Abs_{control}-Abs_{sample}) / Abs_{control}] \times 100 \quad (1)$$

2.4. In Vitro Determination of Sun Protection Factor (SPF)

SPF values of plant extracts were measured according to Mansur's method [30]. 1 mg of plant extracts was weighed and dissolved in 1 ml of ethanol and then, screened by UV spectrophotometer (Beckman Coulter Du730 Spectrophotometer) from 290 to 320 nm. Absorption spectra of the samples were measured in quartz tubes in 3 repetitions. Ethanol was used as the blank. After the measurements, SPF values were calculated according to the following equation (2) given below:

$$SPF = CF \times \Sigma 320{-}290 \times EE(\lambda) \times I(\lambda) \times ABS(\lambda) \quad (2)$$

- CF = 10 (correction factor)
- EE(λ) = erythematogenic effect
- I(λ) = Sun intensity
- ABS(λ) = absorbance

2.5. Tyrosinase Inhibition Assay

Tyrosinase from mushroom (Sigma) was used as an enzyme and L-DOPA (Cayman Chemicals) was used as a substrate [31]. 150 µl of phosphate buffer (0.05 M, pH=6.8), 10 µl of 1 mg/ml plant extracts, and 20 µl of enzyme solution were added to the wells in the microplate. The microplate was shaken for 3 minutes in the Cytation-3 and the first absorbance was read at 475 nm. And then, this solution was incubated at 37ºC for 10 minutes. After incubation period, 20 µl of the substrate (8.5 Mm, L-DOPA) was added and incubated again at 37ºC for 10 minutes to obtain the final absorbance value at 475 nm. Kojic acid (Cayman Chemicals) was utilized as a positive control in this experiment.

Inhibition % of the tyrosinase enzyme was determined using equation (3):

$$\text{Inhibition \%} = (A_{control} - A_{sample}) / A_{control} \times 100 \quad (3)$$

2.6. Collagenase Inhibition Assay

Collagenase from Clostridium histolyticum (ChC) (Sigma) was dissolved in the 50 mM Tricine buffer (10 mM $CaCl_2$ and 400 mM NaCl, pH 7.5) (Fisher Scientific) for use at an initial concentration of 0.8 units/ml. The synthetic substrate, N-[3-(2-furil) akriloil]-Leu-Gly-Pro-Ala (FALGPA), (Sigma) was dissolved in the Tricine buffer to 2 mM. The tests were performed in 96- well microplates. Sample extracts (25 µl Tricine buffer, 25 µl extracts (1 mg/ml)) were incubated with the enzyme (25 µl ChC) in the buffer for 15 minutes before adding substrate. After the incubation, 50 µl of the substrate (FALGPA) was added to the wells, and absorbances were measured at 340 nm for 20 minutes. Measurements were made for each sample in triplicate. The positive control in this experiment was -(-) Epigallocatechin gallate (EGCG) (Acros). The enzyme inhibition value was determined according to the following equation (4) [32].

$$\text{Inhibition\%} = [(A340_{control} - A340_{sample})/ A340_{control}] \times 100 \quad (4)$$

2.7. Elastase Inhibition Assay

For the measurement of elastase activity, elastase obtained from pig pancreas was used as the enzyme source and N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) (Sigma) was used as a substrate. A concentration of 3.33 mg/ml was obtained by dissolving the enzyme in 0.2 mM Tris-HCl (Sigma) buffer at pH 8.0. The substrate was also dissolved in the same buffer solution at a concentration of 1.6 mM. plant extracts, 10 µl of the enzyme, 10 µl of extract, and 120 µl of Tris-HCl were incubated with the enzyme at 25ºC for 20 minutes, then 10 µl of substrate solution was added. The absorbance at 410 nm was measured in comparison to the control. EGCG, 250 mM (0.115 mg/ml) was used for positive controls [22].

Calculations for elastase activity determination were made as follows equation (5):

$$\text{Enzyme inhibition activity (\%)} = [A_{control}-A_{sample} / A_{conrol}] \times 100 \quad (5)$$

2.8. Determination of Antibacterial Activity and Biofilm

For the determination of antibacterial activity, *Staphylococcus aureus* ATCC 25923 was incubated overnight at 37 ºC in the Tryptic Soy (TS) (Merck) broth. The antibacterial activity tests were performed in 96- well microplates. TS broth and serial dilutions of the plant extracts were added to each well. The final

concentrations of all plant extracts were 240, 120, and 60 µg/ml. The tests were performed in four replicates. The ratios of bacterial growth were measured at an optical density (OD) of 450-600 nm with the Cytation 3 microplate reader.

For the biofilm experiment, *S. aureus* ATCC 25923 was incubated at 37ºC for 24 hours in the Tryptic Soy (TS) broth. The potential anti-biofilm activities of the plant extracts at the concentrations of 120, 60, 30 µg/ml were analyzed in 96 well microplates. The tests were performed in four replicates. To screen biofilm formations and possible anti-biofilm efficacy of the tested extracts, the microplates were stained with 0.1% crystal violet (Sigma) and measured at OD 590 nm by using Cytation 3-Biotek microplate reader.

2.9. Statistical analyses

The statistical analyses were performed in triplicate using One-way Anova analysis of variance followed by Tukey's test. The significance of the applications was designated at the $P < 0.05$ level. The data presented are means ± Standard Deviation (SD).

III. RESULTS AND DISCUSSION

3.1. Determination of Antioxidant Activity

It is known that ROS caused by environmental factors such as UV rays cause skin problems such as wrinkles and hyperpigmentation. For this reason, most of the plants are used in the cosmetics industry because they have natural antioxidant content [33]. *T.orientalis*, which is a wild edible plant in the Black Sea Region of Turkey, is only used as food by the local people and there are not many studies other than antioxidants and TPC/TFC of this plant [24-27]. In previous studies, it has been reported that *T. orientalis* extract is a natural antioxidant source due to its flavonoid, phenolic, and anthocyanin compounds.

DPHH radical scavenging assay was used to determine the antioxidant activities of acetone crude extracts of *T.orientalis* as shown in Table 1. According to our results, the highest DPPH activity was found in the stem parts of *T. orientalis* (57,12 mg/ml-ascorbic acid/g), while the medium activity was observed in the leaf parts (13,21 mg/l ascorbic acid/g) and the least activity was observed in the flower parts (0,98 mg/L ascorbic acid/g). In a previous study, Saçan [26] reported that the leaves of *T. orientalis* showed a high radical scavenging potential with an 81.99 ± 7.45% DPPH inhibition percentage. In another study, radical removal activities of Butylated hydroxytoluene (BHA), butylated hydroxyanisole (BHT), leaf and stem extracts were determined as 85.2 ± 3.2%, 79.3 ± 2.9%, 65.1 ± 2.4% and 59.4 ± 2.7%, respectively [27]. When the results obtained in this study were compared with the literature, the acetone extract of the stem of *T.orientalis* showed higher activity than the leaves.

The amount of TPC in acetone extracts of *T. orientalis* was ranged from 67,93 ± 4,86 to 288,10 ± 8,55 mg GAE/g (Table 1). The stem parts of *T. orientalis* extracts showed the highest TPC values of 288,10 ± 8,55 mg GAE/g. In literature, the maximum level of TPC in the water extract of edible body parts of *T. orientalis* was reported to be 90 mgGA/g [25]. In another study, it was reported that *T. orientalis* leaf extract contains 36.00 ± 0.59 µg pyrocatechol/mg TPC [26]. Moreover, Ayhan [27] reported that, TPC of methanolic leaf and stem extracts of *T. orientalis* as 67.01 mg GAE/g and 54.04 mg GAE/g, respectively. Lastly, Demir [34] reported that TPC of methanol, ethanol and pure water extracts of *T. orientalis* as 0.749 mg GAE/g, 1.81 mg GAE/g and 3.62 mg GAE/g, respectively. When the results obtained in this study were compared with the literature, a much higher TPC was observed, especially in the acetone extract of the stem of T.*orientalis*.

The amount of TFC in acetone extracts of *T. orientalis* was ranged from 28,45 ± 1,98 to 406,65 ± 9,59 mg Rutin/g (Table 1). The leaf parts of extracts from *T. orientalis* showed the highest TFC values of 406,65 ± 9,59 mg Rutin/g. In a previous study, TFC of dry weight of *T. orientalis* were determined as 82.1±1.5 mg pyrocatechol/g [24]. In another study, the maximum level of TFC in the water extract of edible body parts of *T. orientalis* was reported to be 56.88 mg catechin/g [25]. Moreover Saçan [26] reported that *T. orientalis* leaf extract contains 29.34 ± 0.62 µg TFC. Finally, Ayhan [27] reported a phenol concentration of 68.9 mg pyrogallat/g and 17.5 mg pyrogallat/g respectively in water and ethanolic extracts. When the results obtained in this study were compared with the literature, much higher TFC was observed specially in the acetone extract of the leaf of *T.orientalis*.

It is reported that in many plants antioxidant activities correlate positively with TPC [35]. According to our results, we observed that there is a correlation between antioxidant activity and the TPC of the extracts. The presence of high TFC/TPC in the plant extracts may attribute to their antioxidant activities. From this point of view, extracts obtained from the roots of *T.orientalis* can be used as a potential sunscreen as they give more successful results than extracts obtained from other parts of the plant. Therefore, it can be suggested that the use of *T.orientalis* has advantages for the cosmetics sector, as it is not only consumed as a vegetable by the local people but also an easily grown plant.

Table 1. Antioxidant activity of acetone extracts from *T.orientalis*

	DPPH% (mg L-ascorbic acid /g)	**Total phenolics (mg Gallic acid/g)**	**Total flavonoids (mg Rutin/g)**
L-Ascorbic Acid	74,20		
T. orientalis (Leaf)	13,21[b]	79,70±4,93[a]	406,65±9,59[c]
T. orientalis (Stem)	57,12[a]	288,10±8,55[b]	141,49±1,02[b]
T. orientalis (Flower)	0,98[c]	67,93±4,86[a]	28,45±1,98[a]

The results were shown as mean ± SD of the assays. The data which are shown as a, b, c are significantly different ($p < 0.05$).

3.2. Photoprotective activity/ Sun Protection Factor (SPF)

The sunscreen activities of plants are determined by measuring their SPF values. According to FDA, SPF is a data of how much UV radiation is required to cause sunburn on protected skin [36]. Additionally, SPF is related to the amount of solar energy required to produce sunburn on unprotected skin. Therefore SPF number is important data for quantifying the effectiveness of sunscreen.

The SPF values of the leaf, stem, and flower parts of *T.orientalis* are shown in Table 2. The results showed that the leaves and stems of *T. orientalis* had the highest SPF value (about 31 mg/ml). The SPF values of the leaf parts of *T.orientalis* were found to be approximately 12 mg/ml.

Table 2. Photoprotective activity/ Sun Protection Factor (SPF) of *T.orientalis*

	Sun Protection Factor (SPF 1 mg/ml)
Rutin	32,11 ± 0,04[b]
T. orientalis (Leaf)	31,27 ± 0,24[b]
T. orientalis (Stem)	31,18 ±0,18[b]
T. orientalis (Flower)	12,03 ± 0,01[a]

The results were shown as mean ± SD of the assays. The data which are shown as a, b are significantly different ($p < 0.05$).

SPF values are considered as a minimum (2–12), moderate (12–30), and high (≥30) [37]. According to our results, the plant extracts had both high and moderate sunscreen activities. Whereas we observed the highest SPF activity in leaf extracts of *T.orientalis* (31.27 ± 0,24 mg/ml), the lowest SPF activity was observed in flower extracts of *T. orientalis* with a medium protection effect by a value of 12,03 ± 0,01 mg/ml.

The possible correlation between SPF values with TPC and TFC was indicated in the literature [38,39,13]. There are several studies investigating the correlation between SPF values and the amount of antioxidant and TFC/TPC of medicinal plants [9]. Researchers reported that there was a link between SPF and TPC, but no correlation between flavonoid and antioxidant activity and SPF. However, our results suggest that there is a correlation between antioxidant activity and the total flavonoid/phenolic compounds and also SPF values of the extracts.

3.3. Tyrosinase Inhibition Activity

Tyrosinase inhibitors reduce hyperpigmentation in human skin, especially caused by environmental factors. Tyrosinase inhibitory activities of various agents such as kojic acid are reported [40]. In this study, potential tyrosinase inhibitory activities of the acetone extracts of *T. orientalis* were investigated and kojic acid was utilized as a positive control. Tyrosinase inhibitory activity of many plant extracts was carried out to find new sources of anti-tyrosinase compounds [41]. Due to the increasing use of herbal resources in cosmetic products, tyrosinase inhibitory activities of different parts of the herbal material have been reported by several studies [42-44]. In particular, medicinal plants from traditional medicine systems have the potential to be used for the treatment of hyperpigmentation and as tyrosinase inhibitors [45].

The anti-tyrosinase activities of the leaf, stem, and flower parts of *T.orientalis* are shown in Figure 1. Our results demonstrated that the leaf extracts of *T.orientalis* showed more successful inhibition compared to stem and flower extracts. On the other hand, the lowest inhibition was observed in the extracts obtained from flower parts of *T.orientalis*.

Skin pigmentation is an important mechanism for preventing UV radiation damage. Melanin absorbs UV radiation, thus protecting skin cells from UV radiation damage. Tyrosinase plays a role in catalyzing skin pigmentation and is directly related to pigmentation disorders in human [41]. Our data obtained from the experiments for in vitro SPF values and tyrosinase inhibition activity show that leaf and stem extracts of *T. orientalis* have a potential to be used effectively in the cosmetic industry.

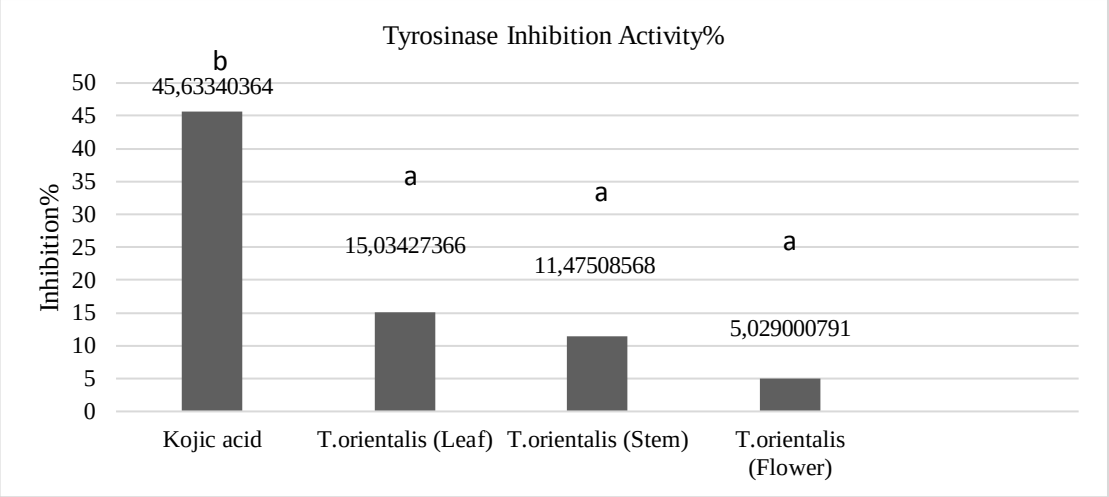

Figure 1. Tyrosinase inhibition activity of *T.orientalis*
The results were shown as mean ± SD of the assays. The data which are shown as a, b are significantly different ($p < 0.05$).

3.4. Anti-Collagenase Activity

It is known that UV radiation promotes ROS production and increases collagen degradation. As a result of collagen degradation, skin aging occurs. [46]. For this reason, the effects of inhibitors that will prevent collagen degradation are very important. The inhibitory effect of acetone extracts of different parts of *T.orientalis* (leaf, stem, flower) on collagenase activity was evaluated using collagenase enzyme obtained from *Clostridium histolyticum* (Figure 2). The best inhibition was observed in the leaf extracts of *T. orientalis*. Moreover, the stem extracts also had considerably high collagenase inhibitory activity with the inhibition ratio of 87.25%. The high efficiency (54.99%) was also recorded for the acetone extracts from flower parts of *T. orientalis*. The results obtained are higher than the positive control, indicating that the inhibition activities are very high. The reason for the results to be higher than the positive control is thought to be due to the synergistic effect of the substances in *T.orientalis* extracts.

To our best of knowledge, there is no study evaluating the anti-collagenase activity of the extracts from different parts of *T.orientalis*. In a study, collagenase and elastase activities were investigated by extracting different parts of plants belonging to Asphodeline with different solvents, which is endemic in Turkey and consumed as a local food [47]. According to the results of the researchers, they observed that theese activities varied depending on the different parts of the plant. They reported that the plants consumed as food on a local basis may have potential importance for the cosmetic sector. According to the our results, the anti-collagenase activity of *T.orientalis* was noticeably higher especially in leaf extracts as well as stem extracts. Similarly, this plant is thought to have the potential for use in the cosmetic industry.

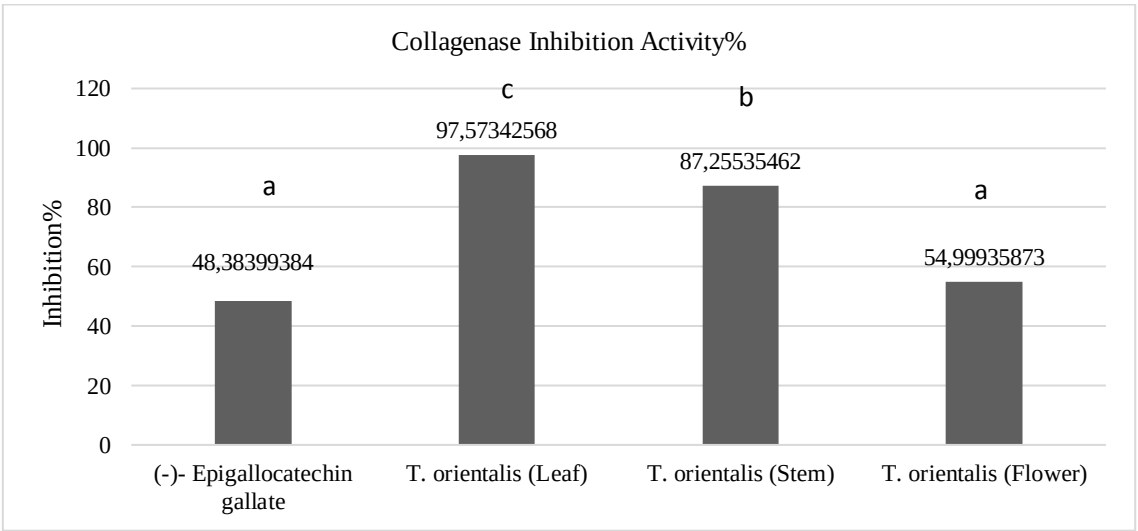

Figure 2. Anti-collagenase activity of *T.orientalis*
The results were shown as mean ± SD of the assays. The data which are shown as a, b, c are significantly different ($p < 0.05$).

3.5. Elastase Inhibition Activity

Elastase is usually found in connective tissue and disrupts the elastic fibers that give the skin its elasticity, and therefore elastic fiber degeneration is thought to be related to the formation of wrinkles [48]. In recent studies, it has been shown that especially some local endemic and wild plant species have inhibitory efficiencies on elastase activities [49,50].

The anti-elastase activities of the acetone extracts of leaf, stem, and flower parts of *T.orientalis* are shown in Figure 3. The best anti-elastase activity was observed in the stem extracts of *T. orientalis* with the inhibition ratio of 57.64%. Moreover, the flower extracts also had considerably high elastase inhibitory activity with an inhibition ratio of 42.11%.

The results obtained are similar to the positive control, indicating that the inhibition activities are high. When the leaf extract was compared with the positive control, it was determined that there was no inhibition activity.

According to our results, elastase inhibition activity of *T.orientalis* was observed in the stem and flower extracts, but not in the leaf extracts. From this point of view, it can be suggested that stem and flower extracts of *T. orientalis* can be used in the cosmetic industry due to their potential elastase inhibitory activities.

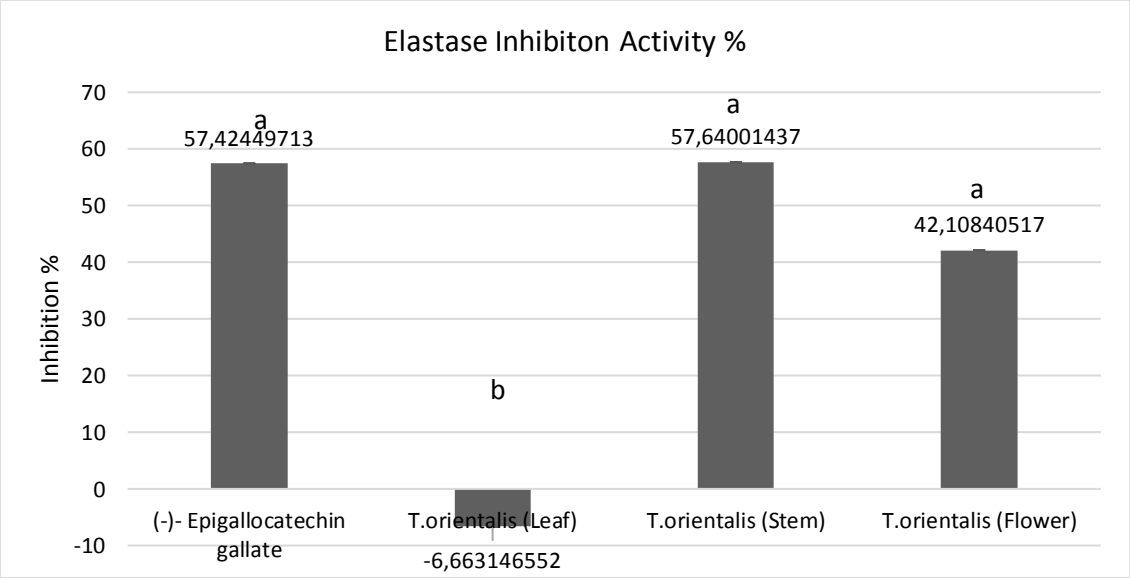

Figure 3. Elastase inhibition activity of *T.orientalis*

The results were shown as mean ± SD of the assays. The data which are shown as a, b, c are significantly different ($p < 0.05$).

3.6. Determination of Antibacterial Activity and Biofilm

In the present study, we also tested antibacterial activities of the acetone extracts of leaf, stem, and flower parts of *T.orientalis* against *S.aureus* ATCC 25923, but we observed no antibacterial efficacy at tested concentrations of 60, 120, and 240 µg/ml. Similar to our results, either very low or no antibacterial activity was reported against *S.aureus* ATCC 25923 in previous studies [27,51]. In a study evaluating the antimicrobial activities of ethanol and petroleum ether extracts of various plants collected from the Sakarya region on different microorganisms by disc diffusion methods, it was demonstrated that both *T.orientalis* extracts had no activity against *S.aureus*. However, they reported that only *T.orientalis* ethanol extract was effective against *E.coli* [51]. In another study, researchers tested the antimicrobial activities of leaf and stem extracts of *T.orientalis* against some Gram-negative bacteria and Gram-positive bacteria. They reported that the highest antimicrobial activity was against *E.coli* and *B. subtilis*. On the other hand, the lowest antimicrobial activity was detected against *S.aureus* [27].

Inhibition of biofilm formation was conducted on the acetone extracts of leaf, stem, and flower parts of *T.orientalis*. There is no inhibition was recorded for extracts. Since there were no previous antibiofilm studies, no comparison could be made with *T.orientalis.*

The reason why we could not detect any antimicrobial activity and biofilm inhibition for the extracts of *T.orientalis* against *S.aureus* may be due to the insufficient concentrations of the extracts or the variability of antibacterial and biofilm activity according to the bacterial species.

It has been demonstrated that stem extracts of *T. orientalis* have the highest anti-elastase, SPF and antioxidant activities. The leaf extracts had the highest anti-collagenase activity. Moreover, leaf extracts also

had considerably high SPF and antioxidant activities. Finally, flower extracts had effective anti-collagenase and anti-elastase activities. Our data obtained from the experiments show that especially leaf and stem extracts of *T. orientalis* have a potential to be used effectively in the cosmetic industry.

IV. CONCLUSION

In conclusion, total phenolic content of *T. orientalis* indicates that it can be used as a natural source of natural antioxidants and sunscreen in pharmaceutical or cosmetic formulations. Due to the high total phenolic content (TPC) and total flavonoid content (TFC) of *T. orientalis*, it can be suggested that absorption of UV radiation and prevention of skin damage can be successfully achieved by using this plant in formulations. The TPC and TFC in plant extracts degrade ROS damage caused by exposure of skin to UV radiation and reduce the negative effects of sunburn. Considering the potential inhibitory efficacies of *T. orientalis* for collagenase, elastase, and tyrosinase activities, it could be suggested that *T. orientalis* may be a potential source of anti-aging in the field of cosmetics. The fact that this plant grows easily in nature as wild species is an important positive aspect. It is predicted that if it is cultured, it can be used as a potential resource for the cosmetics industry. In summary, *T. orientalis* extracts, especially obtained from stem parts of the plant, has the potential for use as photoaging and sunscreen in the cosmetic industry.

REFERENCES

[1] Khuanekkaphan, M., Noysang, C., & Khobjai, W. (2020). Anti-aging potential and phytochemicals *of Centella asiatica*, *Nelumbo nucifera*, and *Hibiscus sabdariffa* extracts. *Journal of Advanced Pharmaceutical Technology & Research*, *11*(4), 174.

[2] Hubbard, G., Kyle, R. G., Neal, R. D., Marmara, V., Wang, Z., & Dombrowski, S. U. (2018). Promoting sunscreen use and skin self-examination to improve early detection and prevent skin cancer: quasi-experimental trial of an adolescent psycho-educational intervention. *BMC Public Health*, *18*(1), 1-15.

[3] Sharma, T., Tyagi, V., & Bansal, M. (2020). Determination of sun protection factor of vegetable and fruit extracts using UV–Visible spectroscopy: A green approach. *Sustainable Chemistry and Pharmacy*, *18*, 100347.

[4] Schneider, S. L., & Lim, H. W. (2019). A review of inorganic UV filters zinc oxide and titanium dioxide. *Photodermatology, Photoimmunology & Photomedicine*, *35*(6), 442-446.

[5] Osmond, M. J., & Mccall, M. J. (2010). Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. *Nanotoxicology*, *4*(1), 15-41.

[6] Tyagi, N., Srivastava, S. K., Arora, S., Omar, Y., Ijaz, Z. M., Ahmed, A. G., ... & Singh, S. (2016). Comparative analysis of the relative potential of silver, Zinc-oxide and titanium-dioxide nanoparticles against UVB-induced DNA damage for the prevention of skin carcinogenesis. *Cancer letters*, *383*(1), 53-61.

[7] Vainio, H., Miller, A. B., & Bianchini, F. (2000). An international evaluation of the cancer–preventive potential of sunscreens.

[8] Westerdahl, J., Ingvar, C., Måsbäck, A., & Olsson, H. (2000). Sunscreen use and malignant melanoma. *International journal of cancer*, *87*(1), 145-150.

[9] Ebrahimzadeh, M. A., Enayatifard, R., Khalili, M., Ghaffarloo, M., Saeedi, M., & Charati, J. Y. (2014). Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. *Iranian journal of pharmaceutical research: IJPR*, *13*(3), 1041.

[10] Clinton, S. K., Giovannucci, E. L., & Hursting, S. D. (2020). The world cancer research fund/American institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions. *The Journal of nutrition*, *150*(4), 663-671.

[11] Skin cancers. World Health Organization. https://www.who.int/uv/publications/en/primaryteach Date accessed: October 21, 2021

[12] Shah, H., & Mahajan, S. R. (2013). Photoaging: New insights into its stimulators, complications, biochemical changes and therapeutic interventions. *Biomedicine & Aging Pathology*, *3*(3), 161-169.

[13] Hashemi, Z., Ebrahimzadeh, M. A., & Khalili, M. (2019). Sun protection factor, total phenol, flavonoid contents and antioxidant activity of medicinal plants from Iran. *Tropical Journal of Pharmaceutical Research*, *18*(7), 1443-1448.

[14] de Oliveira-Junior, R. G., Souza, G. R., Guimarães, A. L., de Oliveira, A. P., de Souza Araújo, C., Silva, J. C., ... & da Silva Almeida, J. R. G. (2015). Photoprotective, antibacterial activity and determination of phenolic compounds of *Neoglaziovia variegata* (Bromeliaceae) by high performance liquid chromatography-diode array detector (HPLC-DAD) analysis. *African Journal of Pharmacy and Pharmacology*, *9*(22), 576-584.

[15] Oliveira, M. B., Valentim, I. B., Santos, T. R., Xavier, J. A., Ferro, J. N., Barreto, E. O., ... & Goulart, M. O. (2021). Photoprotective and antiglycation activities of non-toxic *Cocos nucifera* Linn.(Arecaceae) husk fiber ethanol extract and its phenol chemical composition. *Industrial Crops and Products*, *162*, 113246.

[16] Shukri, S. M., Pardi, F., & Sidik, N. J. (2021).

In Vitro anti-collagenase activity and total phenolic content of five selected herbs: a review. *Science Letters*, *15*(1), 117-127.

[17] Piccinino, D., Capecchi, E., Tomaino, E., Gabellone, S., Gigli, V., Avitabile, D., & Saladino, R. (2021). Nano-Structured Lignin as Green Antioxidant and UV Shielding Ingredient for Sunscreen Applications. *Antioxidants*, *10*(2), 274.

[18] Era, B., Floris, S., Sogos, V., Porcedda, C., Piras, A., Medda, R., ... & Pintus, F. (2021). Anti-Aging Potential of Extracts from *Washingtonia filifera* Seeds. *Plants*, *10*(1), 151.

[19] Ganceviciene, R., Liakou, A. I., Theodoridis, A., Makrantonaki, E., & Zouboulis, C. C. (2012). Skin anti-aging strategies. *Dermato-endocrinology*, *4*(3), 308-319.

[20] Nantarat, N., Mueller, M., Lin, W. C., Lue, S. C., Viernstein, H., Chansakaow, S., ... & Leelapornpisid, P. (2020). Sesaminol diglucoside isolated from black sesame seed cake and its antioxidant, anti-collagenase and anti-hyaluronidase activities. *Food Bioscience*, *36*, 100628

[21] Lee, J. H., & Kim, T. Y. (1999). Relationship between constitutive skin color and ultraviolet light sensitivity in Koreans. *Photodermatology, photoimmunology & photomedicine*, *15*(6), 231-235.

[22] Chompoo, J., Upadhyay, A., Fukuta, M., & Tawata, S. (2012). Effect of *Alpinia zerumbet* components on antioxidant and skin diseases-related enzymes. *BMC complementary and alternative medicine*, *12*(1), 1-9.

[23] Deniz, F. S. S., Orhan, I. E., & Duman, H. (2021). Profiling cosmeceutical effects of various herbal extracts through elastase, collagenase, tyrosinase inhibitory and antioxidant assays. *Phytochemistry Letters*, *45*, 171-183.

[24] Özen, T. (2010). Antioxidant activity of wild edible plants in the Black Sea Region of Turkey. *Grasas y aceites*, *61*(1), 86-94.

[25] Çol Ayvaz, M. (2015). Antioxidant activity of *Trachystemon orientalis* (L.) G. Don (Borage) grown and eaten as food in Ordu, Turkey. *Herba Polonica*, *61*(4).

[26] Sacan, O. (2018). Antioxidant Activity, Total Phenol and Total Flavonoid Contents of *Trachystemon orientalis* (L.) G. Don. *European Journal of Biology*, *77*(2), 70-75.

[27] Ayhan, B. S., Yalçın, E., Çavuşoğlu, K., & Acar, A. (2019). Antidiabetic potential and multi-biological activities of *Trachystemon orientalis* extracts. *Journal of Food Measurement and Characterization*, *13*(4), 2887-2893.

[28] Clarke, G., Ting, K. N., Wiart, C., & Fry, J. (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants*, *2*(1), 1-10.

[29] Yang, Y. C., Lii, C. K., Lin, A. H., Yeh, Y. W., Yao, H. T., Li, C. C., ... & Chen, H. W. (2011). Induction of glutathione synthesis and heme oxygenase 1 by the flavonoids butein and phloretin is mediated through the ERK/Nrf2 pathway and protects against oxidative stress. *Free Radical Biology and Medicine*, *51*(11), 2073-2081

[30] Mansur, J. D. S., Breder, M. N. R., Mansur, M. C. D. A., & Azulay, R. D. (1986). Determinação do fator de proteção solar por espectrofotometria. *An. Bras. Dermatol*, 121-4.

[31] Chiari, M. E., Joray, M. B., Ruiz, G., Palacios, S. M., & Carpinella, M. C. (2010). Tyrosinase inhibitory activity of native plants from central Argentina: Isolation of an active principle from *Lithrea molleoides*. *Food chemistry*, *120*(1), 10-14.

[32] Van Wart, H. E., & Steinbrink, D. R. (1981). A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase. *Analytical biochemistry*, *113*(2), 356-365.

[33] Kusumawati, I., & Indrayanto, G. (2013). Natural antioxidants in cosmetics. In *Studies in natural products chemistry* (Vol. 40, pp. 485-505). Elsevier.

[34] Demir, E. Profile of fatty acids, vitamins, phytosterols and phenolic acids in *Trachystemon orientalis* L. and evaluation of their antioxidant activity. *Avrupa Bilim ve Teknoloji Dergisi*, (33), 112-118.

[35] Kızıl, G., Kızıl, M., Yavuz, M., Emen, S., & Hakimoğlu, F. (2008). Antioxidant Activities of Ethanol Extracts of *Hypericum triquetrifolium* and *Hypericum scabroides*. *Pharmaceutical Biology*, *46*(4), 231-242.

[36] Sun Protection Factor (SPF). Center for Drug Evaluation and Research. Food and Drug Administration https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/sun-protection-factor-spf Date accessed: October 21, 2021

[37] Ratnasooriya, W. D., Pathirana, R. N., Dissanayake, A. S., Samanmali, B. L. C., & Desman, P. K. (2016). Evaluation of invitro sun screen activities of salt marshy plants *Suaeda monoica*, *Suaeda maritima* and *Halosarcia indica*. *International Journal of Pharmaceutical Research & Allied Sciences*, *5*(2)

[38] Khazaeli, P., & Mehrabani, M. (2010). Screening of sun protective activity of the ethyl

acetate extracts of some medicinal plants. *Iranian Journal of Pharmaceutical Research*, (1), 5-9.

[39] Yasmeen, S. H. A. G. U. F. T. A., & Gupta, P. R. O. M. I. L. A. (2016). *In vitro* demonstration of *Dalbergia sissoo* (Indian rosewood) methanolic extracts as potential agents for sunscreening and DNA nick prevention. *Int J Pharm Pharm Sci*, *8*(6), 175-81.

[40] Saidi, I., Nimbarte, V. D., Schwalbe, H., Waffo-Téguo, P., Harrath, A. H., Mansour, L., ... & Jannet, H. B. (2020). Anti-tyrosinase, anti-cholinesterase and cytotoxic activities of extracts and phytochemicals from the Tunisian *Citharexylum spinosum* L.: Molecular docking and SAR analysis. *Bioorganic Chemistry*, *102*, 104093.

[41] Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of enzyme inhibition and medicinal chemistry*, *34*(1), 279-309.

[42] Chatatikun, M., Supjaroen, P., Promlat, P., Chantarangkul, C., Waranuntakul, S., Nawarat, J., & Tangpong, J. (2020). Antioxidant and Tyrosinase Inhibitory Properties of an Aqueous Extract of *Garcinia atroviridis* Griff. ex. T. Anderson Fruit Pericarps. *Pharmacognosy Journal*, *12*(1).

[43] Bakhouche, I., Aliat, T., Boubellouta, T., Gali, L., Şen, A., & Bellik, Y. (2021). Phenolic contents and *in vitro* antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte *Limonium delicatulum*. *South African Journal of Botany*, *139*, 42-49.

[44] Chelly, S., Chelly, M., Occhiuto, C., Cimino, F., Cristani, M., Saija, A., ... & Antonio, S. (2021). Evaluation of antioxidant, antinflammatory and antityrosinase potential of extracts from different aerial parts of *Rhanterium suaveolens* from Tunisia. *Chemistry & Biodiversity*.

[45] Mukherjee, P. K., Biswas, R., Sharma, A., Banerjee, S., Biswas, S., & Katiyar, C. K. (2018). Validation of medicinal herbs for anti-tyrosinase potential. *Journal of herbal medicine*, *14*, 1-16.

[46] Iwamoto, A., Yamauchi, R., Oogai, S., Tsuruta, Y., Keisuke, T., Nagata, Y., & Yanagita, T. (2022). Lotus root extract inhibits skin damage through suppression of collagenase production *in vitro*. *Cytotechnology*, 1-9.

[47] Ilhan, M., Zengin, G., Küpeli Akkol, E., Aktümsek, A., & Süntar, I. (2016). The importance of Asphodeline species on enzyme inhibition: anti-elastase, anti-hyaluronidase and anti-collagenase potential. *Turk. J. Pharm. Sci*, *13*, 323-327.

[48] Kim, S. Y., Go, K. C., Song, Y. S., Jeong, Y. S., Kim, E. J., & Kim, B. J. (2014). Extract of the mycelium of T. matsutake inhibits elastase activity and TPA-induced MMP-1 expression in human fibroblasts. *International journal of molecular medicine*, *34*(6), 1613-1621.

[49] Chiocchio, I., Mandrone, M., Sanna, C., Maxia, A., Tacchini, M., & Poli, F. J. I. C. (2018). Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest. *Industrial crops and products*, *122*, 498-505.

[50] Gangadharan, A. (2021). Free radical scavenging and elastase inhibitory activity of different extracts of *Leucas aspera* (Willd.) Link-An *in vitro* study. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]*, *12*(1), 61-67.

[51] Uzun, E., Sariyar, G., Adsersen, A., Karakoc, B., Ötük, G., Oktayoglu, E., & Pirildar, S. (2004). Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. *Journal of ethnopharmacology*, *95*(2-3), 287-296.