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Research Article/Araştırma Makalesi

Investigation of Some Bioactivities and Odor Components of *Jasminum officinale* Linn. (Oleaceae): A Valuable Tool for Cosmetic Product Design

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Abstract: In this study, researches were carried out on the protease enzyme activity of *Jasminum officinale* Linn. flower which grows naturally in Muğla and its surroundings. In addition, fragrance components in the content of jasmine flower were determined. It was aimed to be used in perfume making based on the harmony of white jasmine flower with other flowers and the concept of note. Protease enzyme was purified from *J. officinale* flower using TPP (Three Phase Partitioning) method. Optimal pH and optimal temperature for the enzyme, K_m and V_{max} values for casein, azokazaein, gelatin, hemoglobin, and azoalbumin substrates were determined. SDS-PAGE was used to check the purity of the protease enzyme purified from the *J. officinale*. The molecular weight of the enzyme was calculated as 21.386 kDa using gel filtration chromatography. The phenolic content was also determined. It has been determined that the content of jasmine flower can be used in perfume design which is the most prestigious product of the cosmetic industry.

Keywords: Cosmetics, Jasmine flower, protease enzyme, three phase system.

Jasminum officinale Linn.'nin (Oleaceae) Bazı Biyoaktiviteleri ve Koku Bileşenlerinin Araştırılması: Kozmetik Ürün Tasarımı için Değerli bir Araç

Öz: Bu çalışmada Muğla ve çevresinde doğal olarak yetişen *Jasminum officinale* Linn. çiçeğinin proteaz enzim aktivitesi üzerine araştırmalar yapılmıştır. Ayrıca yasemin çiçeğinin içeriğindeki koku bileşenleri tespit edilmiştir. Yasemin çiçeğinin diğer çiçeklerle olan uyumu ve nota kavramı esas alınarak parfüm yapımında kullanılması amaçlanmıştır. Proteaz enzimi, *J. officinale* çiçeğinden ÜFA (Üç fazlı sistem) yöntemi kullanılarak saflaştırıldı. Enzim için optimum pH ve optimum sıcaklık, kazein, azokazaein, jelatin, hemoglobin, azoalbümin substratları için K_m ve V_{max} değerleri belirlendi. *J. officinale* çiçeğinden saflaştırılan proteaz enziminin saflığını kontrol etmek için SDS-PAGE kullanıldı. Enzimin moleküler ağırlığı jel filtrasyon kromatografisi kullanılarak 21.386 kDa olarak hesaplandı. Fenolik içeriği belirlendi. Kozmetik sektörünün en prestijli ürünü olan parfüm tasarımında yasemin çiçeğinin içeriğinin kullanılabileceği belirlendi.

Anahtar kelimeler: Kozmetik, Yasemin çiçeği, proteaz enzimi, üç fazlı sistem.

1. Introduction

Historically, it is considered that people have benefited from the healing and beautification features of plants and oils for hundreds of thousands of years. It is seen that the understanding of natural life has come to this day by being transferred from generation to generation and has become a lifestyle today. Consuming natural products, doing our personal care with natural cosmetic materials, living by taking vitamins and minerals that we cannot get with food supplements, and many similar examples are important elements for a natural and healthy life (Aşık, 2017). NC (natural cosmetics) aim at the care and prettiness of the human body with the help of active constituents in nature. This can only be achieved with raw materials that are harmless to the skin and the environment. Natural cosmetics help our natural skin functions and rejuvenate the skin. It provides a soft and natural care; and thus, it can be said that it helps the skin of all ages to stay healthy. It is thought that natural cosmetics make the body-spirit harmony more alive. Nature, the source of life, offers us everything necessary to lead a healthy and long life and indigenous people are collecting herbaceous and ligneous species from the wild and other commercial and home gardens to treat a wide range of diseases and satisfy other social conditions. As a result, it is important to be aware of plant availability and use them accordingly (Aşık, 2017; Dossou-Yovo et al., 2021; Dossou-Yovo et al., 2022a; Dossou-Yovo et al., 2022b).

The Oleaceae family is represented by 29 genus and 600 species as trees, shrubs, and rarely climbers in tropical and temperate regions of the World (Yaltırık, 1978; Wallander & Albert, 2000). There are 7 genus (*Ligustrum* L., *Jasminum* L., *Osmanthus* Lour., *Fraxinus* L., *Fontanesia* Labill., *Olea* L., *Phillyrea* L.) belonging to the Oleaceae family in the flora of Turkey. Species belonging to the Oleaceae botanical family have important therapeutical, economic, and horticultural importance and are used in many fields and ways. Among the concerned species, for instance, some are cultivated for adornment and their flowers are extracted (Huang et al., 2019), some are treated as therapeutical wine for the process of joint pain and dried flowers are used as recreational tea (Sõukand et al., 2017). Moreover, some are exploited as antidiabetic (Bai et al., 2010), for treating lowering cholesterol and diarrhea

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(De Feo et al.,1992) and for antioxidant and anticancer effects (Fares et al., 2011). They also have the functions of expelling phlegm and cleaning the eye (Chinese Pharmacopoeia, 2015). Jasminum (*Oleaceae* genus) which consists of more than 2000 plant species all over the world, is one of the important genus of this botanical family (Green, 2003). Jasmine flowers are described by a very beautiful aroma and are of great importance in medical, industrial, and food implementations (Kunhachan et al., 2012). Notably, ethanolic *Jasminum* flower extracts include, CG (cardiac glycosides), phenolics, flavonoids, essential oil, antioxidants, saponins, and coumarins steroids (Reshma et al., 2021). However, it was reported that *Jasminum* contains substances such as Iridoids, secoiridoids, phenolics, essential oils, flavonoids and tannins as its main components, with the isolation and characterization of chemical components (Reshma et al.,2021). Due to their various phytochemical contents, they are the main provenance of raw materials especially for the production of quality perfumes (Joshi, 2000). In general, *Jasminum* has pharmacological features such as angiotensin converting enzyme (Ace) inhibitor, antiulcer, spasmolytic activity, wound healing, anti-acne, anti-inflammatory, antimicrobial, vasodilation, aromatherapy, antioxidant and antiaging, gastroprotective, anti-lipid peroxidative, hepatoprotective, and cytoprotective (Reshma et al.,2021).

There are *J. officinale* L. and *J. fructicans* L. species belonging to the genus *Jasminum* in Turkey. Among the ethnobotanical uses of this genus in Turkey, the essential oil is especially used in the cosmetic industry. The infusion (5%) prepared from its flowers is used as a chest softener, nerve sedative, and constipation. The flowers are also added to tea to give flavor. In addition, infusion (5%) prepared from flowering branches is used as diuretic and worm reducer (Baytop, 1999). In addition to these, the plant *J. officinale*, which constitutes the biological material of our research, has shown various pharmacological actions, anti-viral, anti-spasmodic, and anti-microbial, cytotoxic in addition to wound support (Elhawary et al., 2020). The leaves are responsible for their therapeutic properties such as anti-diabetic, antioxidant, antiseptic, antispasmodic and wound healing (Prachee et al., 2019). The root part is believed to expedite the healing of fractures and is also known to be used to treat headaches and insomnia (Alrashdi et al., 2012). *J. officinale* flowers are conventionally used as a sedative, a mild anesthetic, depressant, and astringent. Its flowers contain a variety of volatile compounds, including farnecene, benzyl benzoate, nerolidol, benzyl alcohol, benzyl acetate, hexenyl benzoate, linalool, jasmine lactone, indole, and jasmine and its usefulness is thought to be due to such phytochemicals (Sahu et al., 2022).

The purpose of the present was to investigate some bioactivities of *J. officinale* flower that has limited studies in Turkey and to conduct an analysis on the purification of the protease enzyme as well as to investigate the use of the plant in industry by designing a perfume. To the best of our knowledge, there is no such a detailed research and we believe that this study will not only be informative but it will also motivate many other investigations in many areas serving the industrial sectors.

2. Material and Methods

2.1. Plant material

Specimen of *J. officinale* used in the study was collected from Muğla rural areas (in June and September 2016). The plant was authenticated by Dr. Alevcan Kaplan and given a voucher specimen Muğla/2016/01 before being deposited at the Muğla Sıtkı Koçman University. Since the flowers of the plant would be used in the study, they were separated from the plant and gathered together. It was stored in a deep freezer at -80°C until the experiments were undertaken.

2.2. Extraction of protease enzyme

10 g of *J. officinale* flowers stored in the freezer were weighed first, finely chopped, then thoroughly crushed and homogenized by joining 100 mL of pH 7.05 M sodium phosphate buffer. It was spotted in a -80 ^{o}C cooler and taken out after a few hours and waited to dissolve. This step was performed three times. The samples removed from -80 ^{o}C were thawed and the homogenate was separated from the pulp by filtering with a 3-layer cheesecloth and centrifuged at 6.000 rpm for 25 min and supernatant was gathered carefully. The resulting supernatant was used as the crude enzyme extract (Rawdkuen et al., 2010).

2.3. TPP method

Compared to the traditional protein purification methods; TPP is a fast and effective procedure applied in purification. An important advantage of the TPP technique is that it can be applied to large and small-scale studies. Another reason of TPP importance is the low molecular weight structures, removal of lipids and phenolic structures. The basis of the method is the addition of a high concentration of ammonium sulfate (0.8-2.4M) and a water-restricted aliphatic alcohol (usually *t*-butanol) to the crude enzyme solution. Although alcohols such as 1-propanol, 2-propanol, methanol, *t*-butanol, and ethanol dissolve in water, they do not dissolve in cosmotropic salts thus, two separate liquid phases (alcohol phase at the top and salty aqueous phase at the bottom) are formed (Dennison & Lovrein, 1997). *t*-butanol was used in this study. The relationship between TPP and *t*-butanol can be explained as follows: *t*-butanol imparts buoyancy to the proteins that precipitate during TPP. By redissolving the intermediate phase in the buffer, the specific and total activity is recovered and sometimes increased. To calculate the TPP of *J. officinale* flowers protease enzyme, methods of Rawdkuen et al. (2010) were applied. Since the purification ratio of the middle phase and the activity profit are a high match to the upper and bottom phases, optimization studies were carried out following the middle phase (Rawdkuen et al., 2010).

2.4. SDS-PAGE analysis

Sodium dodecyl sulfate (SDS)-Polyacrylamide gel electrophoresis (PAGE) of the enzyme was implemented according to the method of Laemmli (1970).

2.5. Determination of protease activity

The PA (Protease activity) was determined by using casein as the substrate and determining the amount of protein (casein) that the enzyme breaks down (Fadıloğlu, 2001). 1

EU was determined as the amount of µg of enzyme-cleaved protein per min. 1 g of casein was added to 95 mL (0.05) M phosphate buffer with a pH 7 and the temperature was gradually increased while stirring in a magnetic stirrer. The substrate solution was prepared by stirring for 10 min at a temperature range of 95 °C. The reaction was started by joining 500 µL of pure enzyme solution to 1 mL of casein solution and adding 1 mL buffer, making the total volume to 2.5 mL. This reaction mixture was incubated at 40 °C for 20 min and the reaction was stopped by adding 3 mL of 5% trichloroacetic acid (TCA). Non-degraded proteins were allowed to settle for 30 min and then centrifuged at 6.000 rpm for 20 min. The amount of protein cleaved by the enzyme remaining without precipitation in the supernatant was defined by the Bradford method and the enzyme activity was determined (Bradford, 1976).

2.6. Efficacy of pH and temperature

Optimal pH for *J. officinale*'s flowers protease activity was determined using different buffer system. For measurements, acetate buffer (for pH:4-5), phosphate buffer (for pH:6-7), Tris-HCl buffer (for pH:8-9), and borate buffer was used (for pH:10). The Protease enzyme activity was evaluated at temperature values ranging from 0 to 90 °C to define the optimal temperature of *J. officinale*'s flowers protease activity. The temperature effect was defined by heating the substrate solution in the buffer to the suitable temperature in a water bath; then, the reaction was initiated by adding the protease enzyme extract and incubated for 20 min at each temperature step. Afterwards, by joining 3 mL of 5% TCA, the reaction was stopped and the proteins were allowed to precipitate for 30 min. After this time, centrifuge itself for 15 min at 6.000 rpm. The amount of cleaved products in the supernatant was defined by the Bradford (1976).

2.7. Molecular mass estimation by gel filtration chromatography

140 mL suspension Sepharose 4B was dissolved in ddH_2O (distilled water) and allowed to swell overnight at RT. The gel (1x30) was then fraughted onto the column. Equilibration was performed with 0.05 M Na_3PO_4/1mM dithioerythretol buffer (pH: 7.0) until no absorbance was observed in the column at 280 nm. lysozyme (14.3 kDa), BSA (66 kDa), ß- amylase (200 kDa), albumin EGG (45 kDa), ß-lactalbumin (18.4 kDa), and standard solutions were loaded to be 0.2 mg/mL and eluted with 0.05 M Na_3PO_4/1mM DTT buffer. The standard graphic was drawn. The flow rate of the column was setted to 20 mL/h with the aid of a peristaltic pump. Eluates were collected at 4 mL in each tube. The molecular mass of the enzyme was defined with the aid of the standard graph created (Fig. 5).

2.8. Substrate specificity and enzyme kinetics

Substrate specificity study was performed to define which substrates the enzyme can transform into the product and against which it has higher affinity. The activity of the enzyme against five different (Casein, hemoglobin, azoalbumin, gelatin, azocasein) substrates was determined. In order to determine the enzyme's activity, 100- 800 µL of 1% hemoglobin, gelatin, azoalbumin, and azokazein solutions were taken and the volume was completed to 1 mL using ddH_2O. 0.5 mL of enzyme solution was joined to each prepared tube, and buffer was joined so that the final volume was 2.5 mL. The experiment continued according to the procedure for determining the normal protease enzyme activity and the reaction was terminated. After the supernatant was filtered, the amount of fragmented products in the supernatant was defined by the Bradford method (Bradford, 1976). K_m and V_{max} were calculated by plotting 1/V versus 1/[S] Lineweaver Burk plot. The PA for the enzyme breaks down µg protein/mL calculated in min (Pauling et al., 1973).

2.9. Obtaining Essential Oils

2.9.1. Obtaining EOs (essential oils) by hydrodistillation

Hydrodistillation procedure was implemented 3h using Clevenger. Approximately, 150 g of plant flowers were used in assay. After 3h, the water and EOs mixture harvested was partitioned by liquid-liquid extraction using hexane. Na_2SO_4 was joined to remove any water that may have remained on the EOs. Ultimately, the EOs obtained by lifting the solvent under pressurized nitrogen gas was used in perfume desingning.

2.10. Cosmetic product formulation development with obtained biological materials

In order to appraise the use of flowers of some plants such as jasmine, honeysuckle, lotus, chamomile, lily, primrose, and cedar/sandalwood in the area of cosmetics formulations were prepared with EOs obtained by hydrodistillation procedure and cosmetic product formulations containing protease enzymes were improved. The perfume desingning is shown in Table 1 and Figure 1. Perfume is an odorous liquid obtained by mixing natural or synthetic scented oils (raw materials), water, and alcohol in various proportions (Can et al., 2015).

2.11. Determination of aromatic volatile organic compounds of *J. officinale* flowers by Headspace GC/MSD

The aromatic volatile organic compounds of *J. officinale* flowers were determined by Headspace GC/MSD according to Daşdemir (2017). Fragmented fresh jasmine flowers were weighed at 5.00g into a 20 mL headspace vial. Then, $MgSO_4$ (anhydrous magnesium sulfate) was joined and it was completely mixed with the magnetic. The vial was placed in the headspace sampler and the extraction process was started, that would take 30 min at 90°C. After 30 min, the volatile components at the top of the vial were transferred by the headspace sampler with a GC Split/Splitless inlet transferline for 1 min with helium gas. Headspace GC/MSD instrument analysis parameters are shown in Table 2.

Figure 1. Perfume studies

Table 1. Perfume formulation

Formulation	Percentage of substances in perfume (%)
EtOH	65
Plant extract	10
Natural essential oil	5
Pure water	15
Odor stabilizer	5

Table 2. Headspace GC/MSD instrument analysis parameters

Device Parameters	
Balancing Time	2 min
Maximum Temperature	300°C
Device Program	60°C for 1min 10°C/min; 100°C for 1min 10°C/min; 260°C for 8 min
Operation time	30 min
MMI Input Parameters	
Method	Divide
Heater	250°C
Thermal Aux (Tranfer Line)	
Heater	On
Temperature	250°C
Column	
Name	Agilent J&W 19091S-431UI HP-5MS UI (15µmx250µmx0.25µm)
Pressure	21.801 psi
Flow	1.8 mL/min
MS Acquire Parameter	
Acquisition Mode	View
EM voltage	1200
Low mass	35.0
High mass	400.0
Threshold	150
MS Source	230°C max 250°C
MS quadrupole	150°C max 200°C
GC-MSD Parameters	
Device Temperature	95°C
Cycle Temperature	110°C
Transfer Line Temperature	120°C
Bottle Balance	30 min
Injection Time	1 min
GC Turnover Time	40 min
Bottle Size	20 mL
Fill Mode and Pressure	Pressure / 14psi
Cooldown	0.5 min
Extraction Method	Multiple extraction

2.12. Determination of phenolic components

2.12.1. Extraction conditions of samples

For sample analysis, MeOH extracts were arranged at RT for 24 h using a magnetic stirrer, and the solutions were filtered with the help of blue band filter paper in order to get rid of possible solid particles and dirtiness and to ensure further homogeneity. After defining the final concentration of the obtained extracts, the extract solvent was lifted in a RE at 60°C and the residue was dissolved in 10 mL of distilled water with a pH of 2. Thereafter, diethylether and then ethylacetate extraction of 5 mL was performed 3 times each. The extracts obtained at the end of the extraction treat were taken from evaporator balloons and their solvents were removed in a RE at 60°C. The phenolic component analyzes of the extracts, the contents of which were dissolved with 2 mL of MeOH, were performed by HPLC-UV (Can et al., 2015).

2.12.2. Definition of phenolic compounds by HPLC-UV

HPLC-UV study was carried out on an HPLC system equipped with a UV-Vis detector (Elite LaChrom Hitachi, Japan) at 280 nm. Gradient program with 2% acetic acid (pure water) in A reservoir and 70-30% acetonitrile-pure water in B reservoir is given in Table 3. Additionally, working optimization was achieved by adjusting the injection volume of samples and standards to 25 µL, the mobile phase flow rate to 1.2 mL.min^{-1} and the column temperature to 30 °C in the column furnace (Can et al., 2015).

Table 3. RP-HPLC-UV gradient program

Time (min)	A (2% acetic acid in pure water)	B (70-30% acetonitrile-pure water)
0.01	95.00	5.00
3.00	95.00	5.00
8.00	85.00	15.00
10.00	80.00	20.00
12.00	75.00	25.00
20.00	60.00	40.00
30.00	20.00	80.00
35.00	95.00	5.00
50.00	95.00	5.00

3. Results and Discussion

The world of cosmetics, which has reached the present day from the prehistoric times and now is a sector, has attracted the attention of people in every period. The emergence of cosmetics on the stage of history begins with the point where the perception of beauty and aesthetics begins; that is, almost with the birth of humanity. Along with the rapid developments in the chemical industry, the production of synthetic chemicals from the end of the 19th to the beginning of the 20th century has also positively affected the issue of odor (Aşık, 2017). On the other hand, the rapid and great increase in the world population and the increase in the cultural level have led to the production and consumption of a wide variety of personal cleaning, cosmetic products, and domestic and industrial cleaning products. Parallel to this increase, the need for fragrance substances has increased (Aşık, 2017). According to the literature review, no study has been conducted in which protease enzyme is purified and characterized from *J. officinale* flowers and these parameters are combined with perfume design, which is the most prestigious product in the cosmetic world.

The protease enzyme was purified by TPP, one of the biodegradation techniques. In the TPP method used in this study, ammonium sulfate saturation 30% (w/v) and

homogenate: *t*-butanol rate 1:1.5, the protease enzyme remained predominantly in the middle phase. Therefore, identification was started using these ratios. Purification results are summed up Table 4. From Table 4, it can be shown that, the protease enzyme was purified with a purification fold of 1.052 and a yield of 50.30%. SDS-PAGE was performed to check the purity of the protease enzyme purified from *J. officinale* flowers (Laemli, 1976) (Fig. 2). Rawdkuen et al. (2010) reported that they purified *Calotropis procera* (Aiton) Dryand. latex protease enzyme 6.92 times with 132% efficiency using TPP systems. Chaiwut et al. (2010) performed the extraction of proteases from papaya peels by TPP method and noted that they achieved a recovery rate of 10.1 times with a recovery of about 89.4%. Then, these authors also performed the optimization by joining up to 55% $(NH4)_2SO_4$ at the lower phase of the first step. These results were generally consistent with our findings. In the TPP system, the phase in which the enzyme will be collected differs depending on the characteristics of the amino acids contained in the enzyme and their isoelectric points. For this reason, the interphase distributions of the same enzyme may vary in extracts obtained from different sources (Kat & Yılmazer Keskin, 2013). In the current study, it has been shown that TPP which is a simple, cost-effective, bioseparation technique that can be used in large volumes and can be performed at room temperature compared to other multi-step, costly chromatographic purification techniques can be efficiently used for the extraction of proteases from *J. officinale* flowers. Thus, we can suggest to used it effectively in industrial purposes.

Table 4. Purification results of *J.officinale* flower's protease enzyme by TPP method

Samples	Activity (EU/mL)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (folds)	Yield (%)
Homogenate	0.66	66	78.5	0.841	1	100
Medium phase	0.332	33.2	37.5	0.885	1.052	50.30

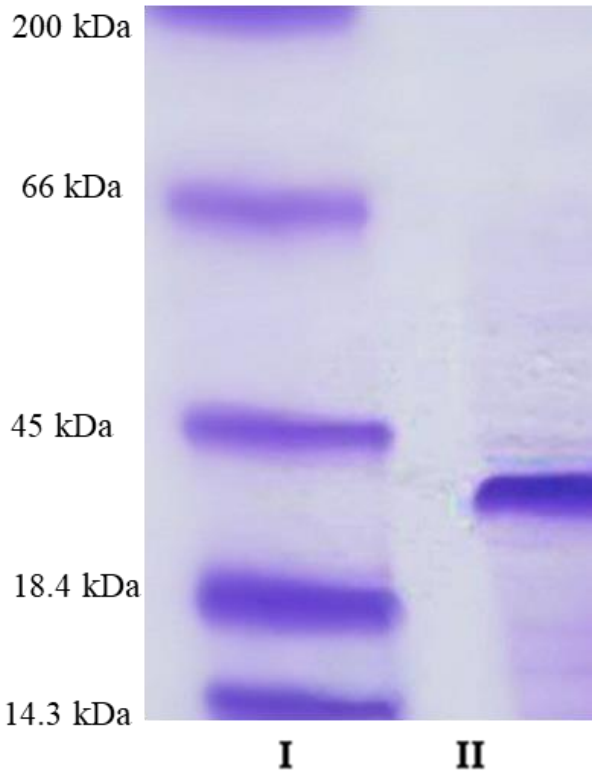

Figure 2. SDS-PAGE image of the purified *J. officinale* flower's protease enzyme (I, standard protein mix: BSA (66 kDa), Albumin EGG (45 kDa), ß-Amylase (200 kDa), ß-lactalbumin (18.4 kDa), Lysozyme (14.3 kDa); II, Protease enzyme purified from *J. officinale* flower)

In order to define the optimal pH of the protease enzyme purified from the *J. officinale* flower, activity measurements were made by various buffer systems. The amount of proteolytic activity against pH change for the protease enzyme is depicted in Figure 3. It was defined that the optimal pH of the enzyme was 5 and it showed activity in the pH:4-9 range. To define the optimal temperature of the protease enzyme purified from *J. officinale* flowers, activity measurements were made at 10-100°C. Activity - temperature graphic is given in Figure 4. It was defined that the optimal temperature was 30°C and the enzyme was active in the range of 20-60°C. Thermal factors such as increase in temperature or prolongation of the incubation period cause denaturation in the three-dimensional structure of the enzyme in the protein structure, leading to results such as failure to ensure the enzyme-substrate relationship, structural deterioration of the active center and failure to function and leading to losses in activity. Hereby, in industrial applications temperature ranges in which the studied enzyme can maintain its stability are preferred and it is important to define this range. The stability of the enzyme, that is, its ability to maintain its activity over time, increases its applicability in processes and significantly affects the cost (Karkaş, 2009). In addition, Banik et al. (2018) determined the optimal pH and temperature of the protease enzyme obtained from the leaves of *Moringa oleifera* L. as 8 and 37 °C, respectively. In another study, Prabhu et al. (2018), determined the pH of the protease enzyme obtained from pod, seed, and leaf samples of *Vicia faba* L. as 6. Parlak et al. (2008) determined that the optimal pH of the protease enzyme purified from the flowers of Anatolian orchid (*Orchis anatolica* Boiss.) is 6 and the pH range in which it is active is 4-9. The temperature range at which the enzyme is active was determined as 10-70°C and the temperature at which it showed maximum activity was determined as 60°C. In Atrooz & Alomari's (2020) study on the protease enzyme of *Mentha piperita* L. and *Thymus capitatus* (L.) Hoffmanns. & Link plants, the pH of *Thymus* was determined as 2.0 and 3.5, the maximum relative activity was 65%, between pH 6.0-8.0, the maximum relative activity was 100%, Mentha's was 100% and 70%, respectively. They calculated that it has two optimal pH at 3.0 and 9.0 with relative activity. In the same work, the calculated maximum relative activity for the protease enzyme for *Thymus* and *Mentha* to be 40°C (100%) and 35°C (100%), respectively. Considering the importance of the enzyme, it is clear that the protease enzyme has been broadly studied in different plant and food products by various researchers (Cheng et al., 2016; Mandujano - González et al., 2016; Niemer et al., 2016; Sequeiros et al.,

2016). It is thought that the enzyme purified in the present study will also be advantageous as a preferable source for cosmetic products to be active at the appropriate temperature and pH.

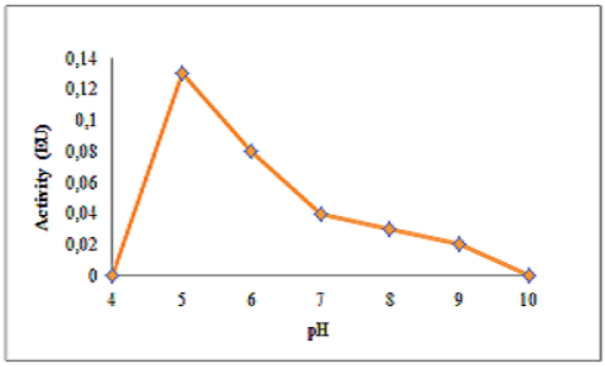

Figure 3. The efficacy of pH on the activity of enzyme

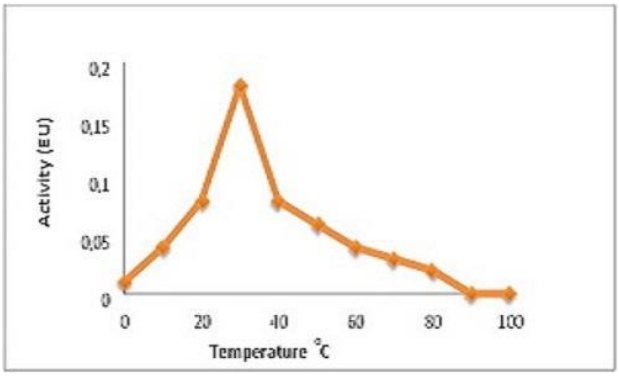

Figure 4. The efficacy of temperature on the activity of enzyme

The molecular mass of the enzyme was defined by GFC (gel filtration chromatography). The values of Ve/Vo and LnMW were calculated by determining which tube the standard proteins were in according to their molecular mass (Table 5). Based on the data calculated in Table 5, the standard protein graph to be used in the molecular mass calculation was created and given in Figure 5. The absorbance evaluation at 280 nm of the eluted proteins were taken as a result of gel filtration chromatography of the purified enzyme. Tubes with protein were identified. The enzyme activity was determined in these tubes and the molecular mass was calculated by determining which tube the protease enzyme in the white jasmine flower was in (Table 6). The molecular mass of the protease enzyme purified from the flowers of *J. officinale* was defined as 21.386 kDa using gel filtration chromatography. Prabhu et al. (2018) determined the molecular weight of protease enzyme from pod, seed and leaf samples of *V. faba* L. leaf supernatant (100kDa), leaf pellet (100kDa), seed supernatant (60kDa, seed, pellet (60 kDa), pod supernatant. (85 kDa), and pod pellet (100 kDa). Jinka et al. (2009) prufied Cysteine protease from *Horse gram*. Asif-Ullah et al. (2006) isolated Serine protease from the *Cucumis trigonas* Roxb. In the study by Parlak et al. (2008), the molecular mass of the protease enzyme purified from *O. anatolica* flowers was calculated as 8.4 kDa using SDS-polyacrylamide gel electrophoresis and sephadex G-100 gelfiltration chromatography. Previous literature studies have noted the presence of proteases in different plant sources. The protease enzyme can be classified into four types, namely serine, aspartate, metallo, and cysteine proteases, respectively. Among these groups, cysteine proteases are predominantly found in plant sources (Domsalla & Melzig, 2008; Rawlings et al., 2010; González et al., 2011). It was observed that the molecular mass of jasmine flower has a value among the molecular weights of other plants.

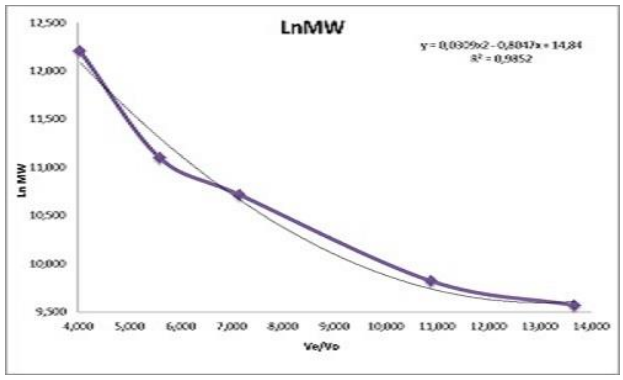

Figure 5. Gel filtration chromatography standard curve

The PA of the enzyme was used to define the V_{max} and K_m values of the enzyme. The substrate specificity of the enzyme purified from *J. officinale* flowers was evaluated according to the K_m and V_{max} results obtained from the Lineweaver burk plot using different substrates and are given in Table 7. It shows that, the substrate affinity of the enzyme was determined as casein (K_m and V_{max} values; 1.16 µM and 1.27 µg/mL.min)>, azoalbumin (K_m and V_{max} values; 1.90 µM and 0.70 µg/mL.min)>, hemoglobin (K_m and V_{max} values; 2.14 µM and 1.42 µg/mL.min)>, gelatin (K_m and V_{max} values; 2.82 µM and 1.03 µg/mL.min, respectively. It was defined that the enzyme did not show affinity for azocasein substrate. Parlak et al. (2008), investigated the substrate specificity of the protease enzyme purified from the flowers of *O. anatolica* and found that it hydrolysed hemoglobin, albumin, azoalbumin and casein, but did not hydrolyze gelatin. The same researcher, using the casein of the purified protease enzyme purified from the flowers of *O. anatolica*, plotted the Linewear-Burk plot and calculated the V_{max} and K_m values as 0.16 µg/L.min and $2.8x10^{-3}$g/mL, respectively. Atrooz & Alomari (2020) determined that the most suitable substrate for the protease enzyme obtained from *M. piperita* and *T. capitatus* plants was egg albumin. It was found that the egg albumin has more affinity toward *Mentha* proteases (K_m 1.7 mg/mL) than *Thymus* proteases (K_m 3.33 mg/mL). Working on the kinetics of an enzyme is considered to be of crucial value in determining the appropriate affinity and conditions for optimal activity, especially if these enzymes are to be used for industrial and biochemical applications (Atrooz & Alomari 2020).

Aroma substances of *J. officinale* flowers were determined using Headspace GC/MSD (Table 8 and Fig. 6). The dominant compound was determined to be 64.87 % Linalool. Linalool ($C_{10}H_{18}O$) is a monoterpene naturally found in more than 200 oils derived from different parts of plants (Peana & Moretti, 2008). Linalool is defined as a light and refreshing, floral-woody, specific fragrance with a slight citrus note (Arctander, 1994). Linalool is also the main ingradient of many essential oils known to exhibit a variety of biological abilities such as antiplasmodial and antibacterial effects (Van Zyl et al., 2006). Further, the

antihyperalgesic anti-inflammatory and antinociceptive efficacy of linalool have been studied by various researchers by establishing experimental setups to examine the effects in different animal models (Peana et al., 2002; Peana et al., 2006a; Peana et al., 2006b). Roughly, 95% of synthetic linalool, and practically all by volume from natural sources, is used in formulations to add a specific scent to cosmetics, soaps, perfumes, and household cleaning products, while only about 1% is joined to flavor and flavor foods and beverages (Kamatou & Viljoen, 2008). Before the 1950s, nearly all linalool used in perfumery was isolated from EOs, especially rosewood oil (Kamatou & Viljoen, 2008). Nowadays, with the expansion of the range of products in the sectors and the increase in demand, the tendency to seek new sources has increased. The high amount of this component in our study shows us that the *J. officinale* flower can be used in perfume design. Moreover, the perfume products we obtained in our studies were produced very successfully (Fig. 1).

Phenolic components of *J. officinale* flowers were defined by HPLC-UV and are detailed in Figure 7 and Table 9. As a result of the phenolic component study, it was observed that the amount of *p*-OH benzoic acid and protocatechuic acid was high. These components are the ones with high antioxidant capacity. Coumaric acid, coumarin, and their derivatives are widely found in nature. Many natural and synthetic coumarin derivatives are components that can be used in different applications in the fields of chemistry, biology, medicine, and physics. Coumarin and its derivatives are components used in food, perfume, cosmetics, and pharmaceuticals (Angelescu et al., 2006). It can be said that the coumaric acid found in the *J. officinale* flower is an important phenolic component for perfume. Ferulic acid is a property that is exceedingly helpful in creating anti-aging cosmetics products. Ferulic acid is one of the most powerful natural antioxidants. Ferulic acid is a phenolic component that neutralizes free radicals such as superoxide and nitric oxide that damage a cell wall and DNA. Ultraviolet rays can increase the antioxidant effect of ferulic acid. Ferulic acid can be included in the content of products that prevent aging in the field of cosmetics (Aşık, 2017). In addition to the use of essential oil from the flowers of *J. officinale*, it is important to report the phenolic components giving an opportunity to use the species in medicine. Such a medicinal exploitation will no doubt positively impact the country's economy.

Table 5. Calculations for the Standard Protein chart

Standard protein mixture	MW(Dalton)	Tube sequence	Ve/Vo	LnMW
Lysozyme	14300	44	13.681	9.568
ß-Lactoglobuline	18400	35	10.882	9.820
Albumin, EGG	45000	23	7.151	10.714
Albumin, Bovine	66000	18	5.597	11.097
ß- Amylase	200000	13	4.042	12.206

Table 6. Calculations for molecular mass determination of protease enzyme purified from *J. officinale* flowers by GFC

Proteins	MW (Dalton)	Tube sequence	Ve/Vo	LnMW
1. Protein	559591	7	2.1765	13.235
2. Protein	178478	13	4.0420	12.092
3. Protein	109262	16	4.9748	11.602
4. Protein	81164	18	5.5967	11.304
5. Protein	61751	20	6.2185	11.031
6. Protein	24256	29	9.0169	10.096
7. Protein	21386	53	16.4791	9.970
8. Protein	49537	62	19.2774	10.810
9. Protein	156906	70	21.7648	11.963

Table 7. Substrate specificity results of purified protease enzyme

Substrate	Km (µM)	Vmax (µg/mL.min)
Casein	1.16	1.27
Hemoglobin	2.14	1.42
Azoalbumin	1.90	0.70
Gelatin	2.82	1.03
Azocasein	nd	nd

*nd: not determined

Table 8. Percentage of aromatic volatile organic compounds of *J. officinale* flowers

No	Component Name	Percentage (%)
1	Linalol	64.87

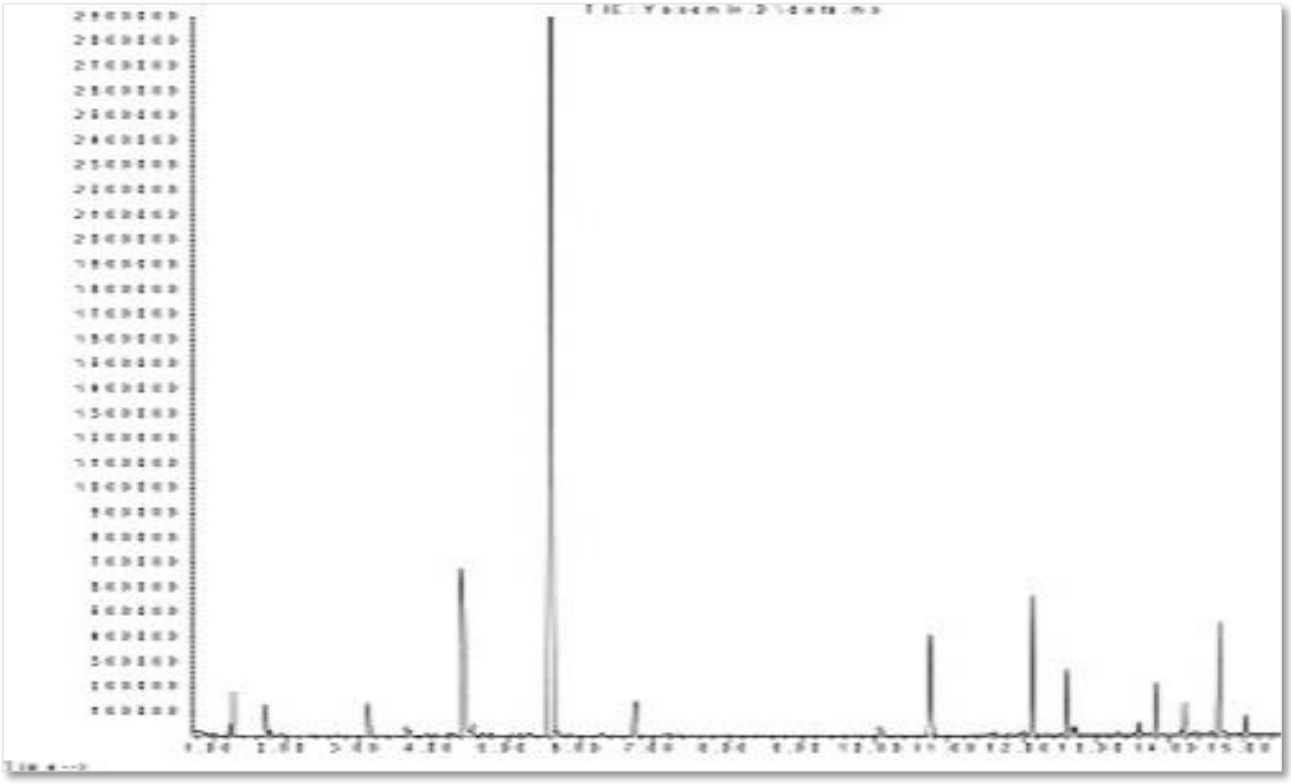

Figure 6. Agilent 7890 GC/ 5975C MSD device measurement results by using Head-space solid-phase microextraction (HS-SPME) methods for flavoring volatile organic compounds of *J. officinale* flowers

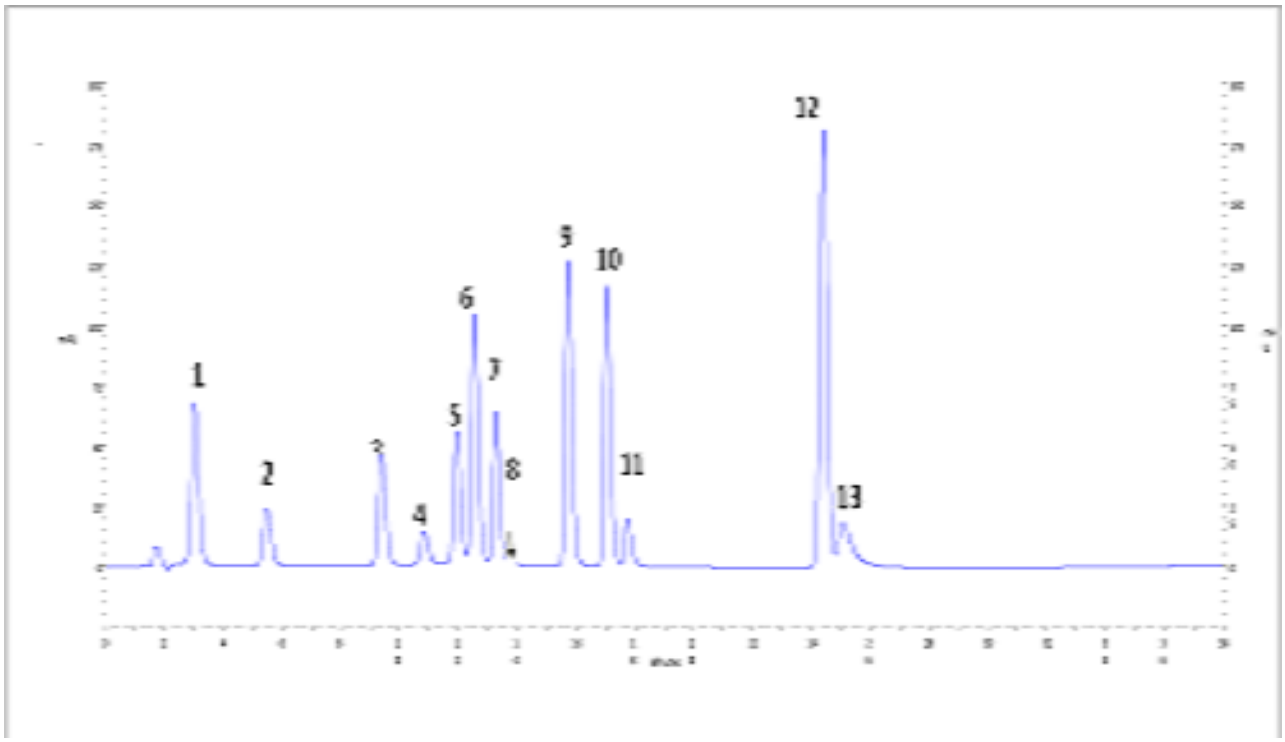

Figure 7. Phenolic acid standard chromatogram 1. Gallic acid, 2. Protocatechuic acid, 3. *p*-OH benzoic acid, 4. Catechin, 5. Vanillic acid, 6. Caffeic acid, 7. Syringic acid, 8. Epicatechin, 9. *p*-Coumaric acid, 10. Ferulic acid, 11. Rutin, 12. Daizein, 13. *t*-Cinnamic acid, 14. Luteolin

Table 9. Phenolic ingradients of *J. officinale* flowers

No	Standarts	Samples (µgextract/g sample)
1	Gallic acid	T.E
2	Protocatechuic Acid	108.92±0.31
3	*p*-OH Benzoic Acid	73.97±0.21
4	Catechin	T.E

Table 9. (Continued)

No	Standarts	Samples (µgextract/g sample)
5	Vanillic Acid	43.81±0.17
6	Caffeic Acid	5.69±0.02
7	Syringic Acid	8.39±0.05
8	Epicatechin	T.E
9	*p*-Coumaric Acid	5.76±0.02
10	Ferulic Acid	1.42±0.01
11	Rutin	38.15±0.11
12	Daidzein	T.E
13	*t*-Cinnamic Acid	T.E
14	Luteolin	T.E

*T.E: It means that it is below the analysis limit

4. Conclusions

To summarize the present work, different properties of the *J. officinale* plant have been investigated and its use in the cosmetics and pharmaceutical sectors has been examined and given in detail. The effect of the raw materials used in perfume making, the characteristic of the smell and its naturalness can be taken into consideration. The harmony of fragrances with flowers, fruits or spices can be taken into account. An idea can be presented based on the scent characteristics of flowers. In this study, perfume formulations, the most prestigious product of the cosmetic factor, were developed and fragrant perfumes were designed and it was predicted that the flower of *J. officinale* could be used in the perfume industry.

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References

Alrashdi, A.S., Salama, S.M., Alkiyumi, S.S., Abdulla, M.A., Hadi, A.H., Abdelwahab, S.I., Taha, M.M., Hussiani, J., & Asykin, N. (2012). Mechanisms of Gastroprotective Effects of Ethanolic Leaf Extract of *Jasminum sambac* against HCl/Ethanol-Induced Gastric Mucosal Injury in Rats. *Evidence-Based Complementary and Alternative Medicine*, 2012, 786426, 1-15. https://doi.org/10.1155/2012/786426

Angelescu, E., Pavel, O.D., Bırjega, R., Zavoianu, R., Costentin, G., & Che, M. (2006). Solid base catalysts obtained from hydrotalcite precursors, for Knoevenagel synthesis of cinamic acid and coumarin derivatives. *Applied Catalysis A: General*, 308, 13-18. https://doi.org/10.1016/j.apcata.2006.04.011

Arctander, S. (1994). Perfume and Flavor Chemicals (Aroma Chemicals). Allured Publishing Corporation; Carol Stream, Illinois.

Asif-Ullah, M., Kim, K.S., & Yu, Y.G. (2006). Purification and characterization of a serine protease from *Cucumis trigonus* Roxburghi. *Phytochemistry*, 67(9), 870-875.

Aşık, S. (2017). Beyaz Yasemin Çiçeğinin (*Jasminum officinale*) Bazı Biyoaktiviteleri ile Koku Bileşenlerinin Araştırılması ve Parfüm Tasarımında Kullanılması. Retrieved from: https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=YMYx1FtU5Irj-u1RU7QajQ&no=sHf1HfMghp84EAgyB0b6HQ

Atrooz, O.M., & Alomari, F.N. (2020). Determination of the activity and kinetics parameters of proteases in the crude plant extracts of *Mentha piperita* L. and *Thymus capitatus* L. *Journal of Applied Biology & Biotechnology*, 8(6), 33-37. https://doi.org/10.7324/JABB.2020.80606

Bai, N., He, K., Ibarra, A., Bily, A., Roller, M., Chen, X., & Rühl, R. (2010). Iridoids from *Fraxinus excelsior* with adipocyte differentiation-inhibitory and PPARα activation activity. *Journal of Natural Products*, 73, 2-6. https://doi.org/10.1021/np9003118

Balkrishna, A., Sharma, G., Sharma, N., Rawat, N., Kumar, A., & Arya, V. (2022). Transition of Indian Agriculture from Glorious Past to Challenging Future: A Serious Concern. *Indian Journal of Ecology*, 49(3), 977-986. https://doi.org/10.55362/IJE/2022/3625.

Banik, S., Biswas, S., & Karmakar, S. (2018). Extraction, purification, and activity of protease from the leaves of *Moringa oleifera* [version 1; referees: 2 approved, 1 approved with reservations]. *F1000Research*, 7, 1151. https://doi.org/10.12688/f1000research.15642.1

Baytop, T. (1999). *Türkiye'de Bitkiler İle Tedavi*, Baskı II, Nobel Tıp Kitabevleri LTD., İstanbul, 234 ss.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254. https://doi.org/10.1006/abio.1976.9999

Can, Z., Yildiz, O., Sahin, H., Turumtay, E.A., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chemistry,180, 133-141. https://doi.org/10.1016/j.foodchem.2015.02.024

Chaiwut, P., Pintathong, P., & Rawdkuen, S. (2010). Extraction and three-phase partitioning behavior of proteases from papaya peels. *Process Biochemistry*, 45, 1172-1175. https://doi.org/10.1016/j.procbio.2010.03.019

Chaturverdi, A.P, Kumar, M. & Tripathi, Y.B. (2013). Efficacy of *Jasminum grandiflorum* L. Leaf extract od dermal wound healing in rats. *International Wound Journal*, 10(6), 675-82. https://doi.org/10.1111/j.1742-481X.2012.01043.x

Cheng, Z., Li, J.F., Niu, Y., Zhang, X.C., Woody, O.Z., Xiong, Y., Djonović, S., Millet, Y., Bush, J. & McConkey, B.J. (2015). Pathogen-secreted proteases activate a novel plant immune pathway. *Nature*, 521(7551), 213-216.

Chinese Pharmacopoeia Commission. (2015). Pharmacopoeia of the People's Republic of China, vol. 1, China Medical Science Publisher, Beijing, 45-46 (&271).

Daşdemir, S.N. (2017). Müge Çiçeğinin (*Convallaria majalis*) Bazı Biyoaktivitelerinin Belirlenmesi Ve Diğer Bazı Çiçeklerle Birlikte Parfüm Tasarımında Kullanılabilirliğinin Araştırılması. Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=4bBx1zmuywZwb8LkKyUN-w&no=0EfeptaMkhl8uE62XxAb4A

De Feo, V., Aquino, R., Menghini, A., Ramundo, E. & Senatore, F. (1992). Traditional Phytotherapy in the peninsula Sorrentina, Campania, Southern Italy. *Journal of Ethnopharmacology*, 36, 113-125. https://doi.org/10.1016/0378-8741(92)90010-O

Dennison, C., & Lovrein, R. (1997). Three phase partitioning: concentration and purification of proteins. *Protein Expression and Purification*, 11, 149-161. https://doi.org/10.1006/prep.1997.0779

Domsalla, A., & Melzig, M.F. (2008). Occurrence and properties of proteases in plant latices. *Planta Medica*, 74(07), 699-711. https://doi.org/10.1055/s-2008-1074530

Dossou-Yovo, H.O., Kindomihou, V., Vodouhè Fifanou, G., & Sinsin, B. (2021). Assessment of the diversity of medico-magic knowledge on four herbaceous species in Benin. *The Scientific World Journal*, 6650704, 1- 11. https://doi.org/10.1155/2021/6650704

Dossou-Yovo, H.O., Kindomihou, V., Vodouhè Fifanou, G., & Sinsin, B. (2022a). Investigating the use profile of *Kigelia africana* (Lam.) Benth. through market in Benin. *Conservation*, 2(2), 275-285. https://doi.org/10.3390/conservation2020019

Dossou-Yovo, H.O., Vodouhè Fifanou, G., Kaplan, A., & Sinsin, B., (2022b). Application of ethnobotanical indices in the utilization of five medicinal herbaceous plant species in Benin, West Africa. *Diversity*, 14, 612. https://doi.org/10.3390/d14080612

Elhawary, S., EL-Hefnawy, H., & Mokhtar, F.A. (2020). Green synthesis of silver nanoparticles using extract of *Jasminum officinale* L. leaves and evaluation of cytotoxic activity towards bladder (5637) and breast cancer (MCF-7) cell lines. *International Journal of Nanomedicine*, 15, 9771-9781. https://doi.org/10.2147/IJN.S269880

Fadıloğlu, S. (2001). Immobilization and characterization of ficin. *Nahrung/Food*, 45 (2), 143-146. https://doi.org/10.1002/1521-3803(20010401)45:2<143::AID-FOOD143>3.0.CO;2-8

Fares, R., Bazzi, S., Baydoun, S.E. & Abdel-Massih, R.M. (2011). The antioxidant and antiproliferative activity of the Lebanese *Olea europaea* extract. *Plant Foods for Human Nutrition*, 66, 58-63. https://doi.org/10.1007/s11130-011-0213-9

González, R.N., Badillo, J.A., Aranda, A.J.S., & Carmen O.S.M. (2011). Production of plant proteases *in vivo* and *in vitro*-a review. *Biotechnology Advances*, 29(6), 983-996. https://doi.org/10.1016/j.biotechadv.2011.08.017

Green, P.S. (2003). Synopsis of the Oleaceae from the Indian sub-continent. *Kew Bulletin*, 58(2), 257-295. https://doi.org/10.2307/4120616

Huang, Yan-Li., Oppong, M.B., Guo, Y., Wang, Li-Zhi., Fang, Shi-Ming., Deng, Yan-Ru., & Gao, Xiu-Mei. (2019). The Oleaceae family: A source of secoiridoids with multiple biological activities. *Fitoterapia*, 136,104155. https://doi.org/10.1016/j.fitote.2019.04.010

Jinka, R., Ramakrishna, V., Rao, S.K., & Rao, R.P. (2009). Purification and characterization of cysteine protease from germinating cotyledons of horse gram. *BMC Biochemistry*, 10(1), 1-11. https://doi.org/10.1186/1471-2091-10-28

Joshi, M.C., Raju, A., Arulanandham, A., & Saraswathy, G.R. (2008). Hepatoprotcetive activity of *Jasminum angustifolium* Linn against CCl_4 induced hepatic injury in rat. *Pharmacologyonline*, 3, 197-205.

Kamatou, G.P.P., & Viljoen A.M. (2008). Linalool - A Review of a Biologically Active Compound of Commercial Importance. *Natural Product Communications*, 3(7), 1183-1192. https://doi.org/10.1177/1934578X0800300727

Karkaş, T. (2009). İnvertaz Enziminin Ekstraksiyonu ve Saflaştırılması için Sulu İkili-Faz Afinite Sistemlerinin Geliştirilmesi. Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=H8tgyFc-BcxsxgrIA0Gz2g&no=7EkHfFxCHwM0PT6-p1vR-Q

Kat, B., Yılmazer Keskin S. (2013). Üçlü faz sistemi ile saflaştırılan invertaz enziminin termal kararlılığının incelenmesi. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 17 (2), 291-294.

Kunhachan, P., Banchonglikitkul, C., Kajsongkram, T., Khayungarnnawee A., & Leelamanit, W. (2012). Chemical Composition, Toxicity and Vasodilatation Effect of the Flowers Extract of *Jasminum sambac* (L.) Ait. *Evidence-Based Complementary and Alternative Medicine*, 2012, 471312, 1-7. https://doi.org/10.1155/2012/471312

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, 227, 680-85.

Mandujano Gonzalez, V., Téllez, J.A., Anducho, R.M.A., Arana, C.A., & Mercado, F.Y. (2016). Purification and characterization of the extracellular aspartyl protease APSm1 from the phytopathogen fungus *Stenocarpella maydis*. *Protein Expression and Purification*, 117,1-5. https://doi.org/10.1016/j.pep.2015.09.017

Niemer, M., Mehofer, U., Verdianz, M., Porodko, A., Schähs, P., Kracher, D., Lenarcic, B., Novinec, M., & Mach, L. (2016). *Nicotiana benthamiana* cathepsin B displays distinct enzymatic features which differ from its human relative and aleurain-like protease. *Biochimie*, 12(2), 119-125. https://doi.org/10.1016/j.biochi.2015.06.017

Parlak, B., Güngör, A. A., Demir, Y., & Demir, N. (2008). *Anadolu Orkidesi (Orchis anatolica) Çiçeklerinden Proteaz Enziminin Saflaştırılması ve Peynir Üretimindeki Kullanılabilirliğinin Araştırılması*. Türkiye 10. Gıda Kongresi, Mayıs 21-23, Erzurum, Türkiye.

Pauling, L. (1973). Die Natur der chemischen Bindung, Chemie-Varlag, Weinheim, 523 pp.

Peana, T.A., & Moretti, M.D.L. (2008). Linalool in Essential Plant Oils: Pharmacological Effects. Botanical medicine in clinical practice, 716-724, CAB International, Wallingford, UK. https://doi.org/10.1079/9781845934132.0716

Peana, A.T., D'Aquila, P.S., Panin, F., Serra, G., Pippia, P., & Moretti, M.D.L. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine*, 9, 721-726. https://doi.org/10.1078/094471102321621322

Peana, A.T., Marzocco, S., Popolo, A., & Pinto, A. (2006a). (−)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sciences*, 78, 719-723. https://doi.org/10.1016/j.lfs.2005.05.065

Peana, A.T., Rubattu, P., Piga, G.G., Fumagalli, S., Boatto, G., Pippia, P., & De Montis, M.G. (2006b). Involvement of adenosine A1 and A2A receptors in (−)-linalool-induced antinociception. *Life Sciences*, 78, 2471-2474. https://doi.org/10.1016/j.lfs.2005.10.025

Prabhu, S., D., Apoorva, S., Nandita, J., Palani, C., & Devi Rajeswari, V. (2018). Purification of protease enzyme from the leaf, seed and pod samples of *Vicia faba* L. *International Food Research Journal*, 25(5), 1904-1911.

Prachee, D., Ayushi, T., Praveen, T., Shikha, A., A.K, R., & Watal, G. (2019). Phytochemical and Phytoelemental Profile of *J. officinale*. *International Journal of Pharmacognosy and Phytochemical Research*, 11(1), 5-9.

Rawdkuen, S., Chawut, P., Pntathong, P., & Benjakul, S. (2010). Three-Phase Partitioning of Protease from *Calotropis procera* latex. *Biochemical Engineering Journal*, 50, 145-149.

Rawlings, N.D., Barrett, A.J., & Bateman, A. (2010). MEROPS: The peptidase database. *Nucleic Acids Research*, 38(1), 227-233. https://doi.org/10.1093/nar/gkp971

Reshma, D., Anitha, C.T., & Tharakan, S.T. (2021). Phytochemical and Pharmacological Properties of Five Different Species of *Jasminum*. *Plant Archives*, 21(2), 126-136. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.022

Sabharwal, S., Aggarwal, S., Vats, M., & Sardana, S. (2012). Preliminary phytochemical investigation and wound healing activity of *Jasminum sambac* (linn) ait. (Oleaceae) Leaves. *International Journal of Pharmacognosy and Phytochemical Research*, 4(3), 146-150.

Sahu, R., Arya, V., Joshi, R., Kaushik, P., & Chauhan, M. (2022). Pharmacological and Therapeutic Properties of *Jasminum officinale*. L: A Review. *Indian Journal of Ecology*, 49(3) (SI), 1122-1128. https://doi.org/10.55362/IJE/2022/3640

Sequeiros, C., Torres, M.J., Nievas, M.L., Caffini, N.O., Natalucci, C.L., López, L.M., & Trejo, S.A. (2016). The Proteolytic Activity of *Philibertia gilliesii* Latex. Purification of Philibertain g II. *Applied Biochemistry and Biotechnology*, 179(2), 322-346. https://doi.org/10.1007/s12010-016-1997-8

Sõukand, R., Hrynevich, Y., Vasilyeva, I., Prakofjewa, J., Vnukovich, Y., Paciupa, J., Hlushko, A., Knureva, Y., Litvinava, Y., Vyskvarka, S., Silivonchyk, H., Paulava, A., Kõiva, M., & Kalle, R. (2017). Multi-functionality of the few: current and past uses of wild plants for food and healing in Liubań region, Belarus. *Journal of Ethnobiology and Ethnomedicine*, 13(10), https://doi.org/10.1186/s13002-017-0139-x

Van Zyl, R.L., Seatlholo, S.T., van Vuuren, S.F., &Viljoen, A.M. (2006). The biological activities of 20 nature identical essential oil. *Journal of Essential Oil Research*, 18, 129-133. https://doi.org/10.1080/10412905.2006.12067134

Wallander, E., & Albert, V.A. (2000). Phylogeny and Classification of Oleaceae Based on rps 16 and trnL-F Sequence Data, *American Journal of Botany*, 87(12),1827-1841. https://doi.org/10.2307/2656836

Yaltırık, F. (1978). *Olea* L., In: Flora of Turkey and East Aegean Islands (Ed: P.H. Davis), Vol 6, Edinburgh, 155-156.