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RESEARCH ARTICLE

Determination of Some Bioactivities and Chemical Composition of Tulip *(Tulipa armena)* Plant and Investigation of Usability as Homeopathic Drugs

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HIGHLIGHTS

> The protease enzyme was purified from Tulip (*Tulipa armena*) flowers.
> Its phenolic contents were found to be very high.

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ABSTRACT

In this research, the Tulip (*Tulipa armena*) was collected from Muğla/Turkey countryside, defined and content analysis was performed. Moreover, this research includes investigation of protein, phenolic component and protease enzyme activity. Three-phase partitioning method was used for purification of enzyme from flowers of Tulip. The values of optimal pH and optimal temperature were determined for purified enzyme. The SDS-PAGE technique was used to check the purity of the purified enzyme and determine the number of subunits, if any. The molecular weight was also calculated using gel filtration chromatography.
As a result, the protease enzyme was purified from Turkish Tulip flowers and its phenolic components were determined. It has been found that the purified protease enzyme has high activity. The plant extracts were prepared in different organic solvents and water.

Contents

1. Introduction

Tulip (*Tulipa armena*) plant is from family of Liliaceae. It is a perennial herbaceous plant. The tulip has a special place in eastern culture and mythology. Also it has a unique place in our culture. It has name after a historical period. More than 100 tulip cultivars naturally grow in our soil and a large part of them is endemic.

Enzymes are biological catalysts that are produced by living things and increase the speed of chemical reactions in tissues

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[1]. They enable the catalysis of reactions in accordance with cell requirements in a controlled manner. The most important feature that distinguishes enzymes from other protein molecules is their ability to catalyze biochemical reactions and it has been the source of interest for many fields of study [2].

Proteases (3.4.1.1) included in the main group of hydrolases are also known as proteolytic enzymes [3]. Proteases catalyze the hydrolysis of proteins to peptides and amino acids. It is an important enzyme group because of its industrial use potential and has 60% of the world's enzyme market [4–6].

Homeopathy is based on the principle of "heal similar alike". A disease is treated only with the substance that produces an indication similar to the patient's complaints. Homeopathy was found by German physician Samuel Hahnemann in the early 18th century and it is an alternative treatment system that helps the body develop itself naturally. According to the data of the World Health Organization, it is the most commonly used complementary medicine method. In Europe, more than 50% of people are treated with homeopathic treatment. Furthermore, more than 50% of doctors recommend homeopathy together with other treatment modalities.

The tulip, a kind of national flower, therefore it has specially selected in our study and it has been investigated whether it can be used in the production of homeopathic medicines by getting some information from the public [7].

2. Material and Method

2.1. Collection of Herbal Materials

The *Tulipa armena* plant, which grew in April-May 2017, was collected from Mugla countryside. The harvested plant was then stored at -18 °C until used for experimental procedures [8].

2.2. Protein Assay Using Coomassie Brilliant Blue Method

This method has been developed by utilizing Coomassie brillant blue G-250 dye in different concentrations of protein solutions in different violet blue colors. It has been observed that the dye tends to bond with basic amino acids such as arginine, some aromatic amino acids such as tyrosine and tryptophan. Coomassie brillant blue G-250 is conjugated to proteins in phosphoric acid medium and the resulting complex shows maximum absorbance at 595 nm. The sensitivity of the method is between 1 and 100 mg [9].

2.3. Preparation of Homogenate

The tulip flowers were weighed as 10 g thoroughly in the air and thoroughly homogenized by adding 15 ml of sodium phosphate (pH: 7.0, 0.05M) buffer. A -80 ºC cooler was placed in a container and after a few hours it was expected to be removed by dissolution. This was done three times. The homogenate, which had been solubilized by removing it at -80 ° C, was separated from the pulp by filtration. Centrifuged at 6.000 rpm. Protein content determinations were made in the supernatant after centrifugation.

2.4. Purification of Protease Enzyme from Tulip Flowers by Triple Phase Method

The interaction between t-butanol and ammonium sulphate was exploited to efficiently collect the enzymes at the interface in the TPP system. The ratio of the appropriate t-butanol and the amount of ammonium sulphate was determined. In the system, 4 g of ammonium sulphate was added over 10 mL of homogenate and t-butanol was added according to proportions. The reaction was stirred at 200 rpm for 80 min and the mixture was centrifuged at 6000 xg for 20 min to separate the phases. The phases were separated from each other after the process. Upper and lower phases were thrown. Protease activity and protein content were determined in medium phase and it was determined that it had the highest rate of dissolution and purification.

Homeopathic medicines are produced by the original material being stored in water or alcohol followed by a series of dilution and mixing methods**.**

3. Results and Discussion

3.1. Collection and Recognition of Herbal Material

The plant, collected by Prof.Dr. Nazan DEMİR from Mugla University, Faculty of Science, Department of Chemistry, Muğla-Turkey, was diagnosed as *Tulipa armena* plant by Lecturer from Ataturk University, Faculty of Biology Figure 1).

Figure 1 *Tulipa armena* plant.

3.2. Purification of Protease Enzyme Using Three phase partitioning Method

The data related to all purification steps are presented in Table 1. In the first step of the enzyme purification by TPP, different t-butanol ratios were applied (1: 0.25; 1.0: 0.5, 1.0). For a constant 20% (w / v) ammonium sulfate saturation: 1.0, 1.0: 1.5 and 1.0: 2.0. It was observed that T-butanol provided maximum folding and maximum recovery (%) of the protease enzyme (5%) and maximum recovery with 1.2 and

50.3%. As the butanol content increased, the amount of the treatment layer and the recovery of the protease enzyme activity were determined to decrease [10, 11].

In the second step of the TPP, the best saturation of the ammonium sulfate concentration was observed for purification of protease from *Tulipa armena* in the aqueous subphase. For this purpose, ammonium sulphate (between 20% and 80%) in different proportions was applied for constant 1: 0.25 (v / v) n-butanol saturation. It was observed that the recovery and purification layer of the protease enzyme was 8.41% and 1.35. When the ammonium sulfate concentrations increased, the recovery of protease enzyme (%) decreased in the aqueous phase [12, 13].

Table 1 *Tulipa armena* the specific activity and purification rate results of protease enzyme purified from flowers.

Samples	Homogenate	Middle phase	Subphase	Dialysis
Activity (EU/ml)	1.354	0.681	0.114	0.108
Total aktivity (EU/mL)	135.4	68.1	11.04	10.08
Total protein (mg)	165.25	81.125	10.25	9.5
Specific activity (U/mg)	0.82	0.84	1.11	1.136
Purification coefficient	1	1.2	1.35	1.38
Yield (%)	100	50.3	8.41	7.96

The molecular mass of the purified protease enzyme from *Tulipa armena* was 93.890 kDa in the present study. It was observed single protein bands at about 93.890 kDa. The molecular weight of the enzyme was determined as 43 kDa by using the gel filtration chromatograph and comparing with known standard proteins. This result shown that purified protease enzyme has single subunits [9, 14].

4. Conclusions

As a result, the protease enzyme was purified from tulip flowers and its phenolic components were determined. It has been found that the purified protease enzyme has high activity. The plant extracts were prepared in different organic solvents and water. Furthermore, dilution grades of extracts were determined for animal experiments. The molecular weight of the enzyme was determined as MA = 93890 Da.

As a result of preliminary surveys and content analyzes, it is understood that the plant is a flower that can be used in homeopathy. It is planned that it is submitted to the approval of The Ministry of Health as a traditional and complementary product by optimizing usability as a medicine.

References

[1] Altan N. *Biyokimya Olgu Yaklaşımı*. Ankara: Palme Yayıncılık (2000). 69–74.
[2] Wolfson D, Olmstead S, ve Ralston J, Meiss D. *Making Sense of Digestive Enzymes*: Klaire LabsTM, A division of ProTheraR Inc.
[3] Demir N, Demir Y, Kaya E, Aydın B. Cysteine Protease from Primrose (Primula vulgaris). *Asian Journal of Chemistry* (2012) **24**(4):1479–1482.
[4] Çalkı Ş. Bazı Su Ürünlerinde Proteolitik Enzim Aktiviteleri, Tr. *Turkish Journal of Veterinary and Animal Sciences* (1999) **23**:385–390.
[5] Kalisz HM. Microbial Proteinases. *Advances in Biochemical Engineering/Biotechnology* (1988) **36**:1-65.
[6] Rao MB, Tanksaie AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Review* (1998) **62**:597–635.
[7] Huq M, Aziz-ul Huq AM. Experimental Measurements of Heat Transfer in An Internally Finned Tube. *International Communications in Heat* (1998) **25**(5):619–630.
[8] Uçkaya F. *Citrus sinensis (L.) Osbeck (Portakal) ve Citrus limon (L.) Burm.F. (Limon) Çiçeklerinden Proteaz ve Peroksidaz Enzimlerinin Saflaştırılması ve Endüstride Kullanımlarının Araştırılması [Purification of Protease and Peroxidase Enzymes from Citrus sinensis (L.) Osbeck (Orange) and Citrus lemon (L.) Burm.F. (Lemon) Flowers and Their Use in Industry]*. Muğla Sıtkı KoçmanUniversity (2015).
[9] Bradford MM. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analitical Chemistry* (1976) **72**:248–253.
[10] Nadaroglu H, Onem H. Preparation and properties of purified phytase from oakbug milkcap (Lactarius quietus) immobilised on coated chitosan with iron nano particles and investigation of its usability in food industry. *Journal of Food and Nutrition Research* (2014) **2**(12):938–945.
[11] Nadaroglu H, Sonmez Z. Purification of an endo-beta 1,4-mannanase from Clitocybe geotropa and immobilization on chitosan-coated magnetite nanoparticles: application for fruit juices digest. *Digest Journal of Nanomaterials and Biostructures* (2016) **11**(2):685–697.
[12] Nadaroglu H, Dikbas N. Purification and Characterization of a β-Mannanase from Lactobacillus plantarum (ATCC® 14917TM). *International Journal of Innovative Research and Reviews* (2018) **2**(1):1–6.
[13] Onem H, Nadaroglu H. Immobilization of purified phytase enzyme from Tirmit (Lactarius volemus) on coated chitosan with iron nanoparticles and investigation of its usability in cereal industry. *Iranian jour-nal of science and technology* (2017) **42**(3):1063–1075. doi:10.1007/s40995-016-0111-y.
[14] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* (1970) **22**:680–685.