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Research Article

Investigation of Bioactive Chemicals of Passion Flower (*Passiflora incarnata* L.)

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Abstract

In this study, a research was conducted to determine the protease enzyme activity and the volatile organic compounds of passion flower (*P. incarnata* L.) to determine the usability of the plant in the pharmaceutical sector. In order to determine the activity of the protease enzyme in passion flower, measurements were taken according to the method of casein digestion in the presence of 1% casein. In the light of the absorbance values, it is determined that the plant has proteolytic activity. In addition, volatile organic compounds of the plant were analyzed by headspace GC/MSD. 5 g fresh plant was taken and put into 20 ml headspace vial. The plant was extracted and analyzed with 6890 GC-5975C MSD using the HP-5 MS UI column. According to the results obtained, it has been scientifically determined that the plant can be used in the pharmaceutical industry.

Key words: Passion flower, *Passiflora incarnata* L, protease enzyme.

Çarkıfelek Çiçeğinin (*Passiflora incarnata L.)* Biyoaktif Kimyasallarının İncelenmesi

Özet

Bu çalışmada, çarkıfelek çiçeğinin (*P. incarnata* L.) proteaz enzim aktivitesi ile uçucu organic bileşenleri belirlenerek bitkinin ilaç sektöründe kullanılabilirliğinin belirlenmesine yönelik bir araştırma yapılmıştır. Çarkıfelek çiçeğinde bulunan proteaz enziminin aktivitesini tayin etmek için %1'lik kazein varlığında kazeinin sindirimi metoduna göre ölçüm alınmıştır. Okunan absorbans değerleri ışığında bitkide proteolitik aktivite olduğu saptanmıştır. Ayrıca bitkinin uçucu organik bileşikleri headspace GC/MSD cihazı ile analiz edilmiştir. 5 g taze bitki alınarak 20 ml headspace vialine konulmuştur. Bitki ekstraksiyon ve HP-5 MS UI kolonu kullanılarak 6890 GC-5975C MSD ile analiz edilmiştir. Elde edilen sonuçlara göre bitkinin ilaç sanayinde kullanılabilir olduğu bilimsel olarak belirlenmiştir.

Anahtar kelimeler: Çarkıfelek çiçeği, *Passiflora incarnata* L., proteaz enzimi.

Introduction

The use of plants as medicine, cosmetics and food has continued for as long as the humankind has been around. The existence of common diseases and the use of plants and each of their specific characteristics for the treatment of these diseases have been discovered by humans (Chandel and Rastogi, 1980; Rao and Sung, 1995; Shao et al., 1996).

Passion flower (*P. incarnata* L.) is one of the rare species of the Passiflora family, which has over 400 species, that is resistant to frost and cold. Known as Maypop, which means 'blooms with the arrival of May', *P. incarnata* L., like its other fast-growing relatives, creeps up to 4 meters in a short time.

Passiflora is traditionally used in combination with other herbs as a mild sedative and there are limited published data relating to the pharmacology of the herbal extract alone (Dhawan et al., 2004; Fiebich et al., 2011). It has been used in the treatment of depression, insomnia and

hemorrhoids. A tranquilizer approved in Germany, the plant is now used against tension-induced agitation and mild insomnia (Dhawan et al., 2001). The mild sedative and relaxing properties of *P. incarnata*'s are attributed to flavonoids and alkaloids, especially to its harmala which repress the oxygen consumption of the brain. It is also considered that these compounds lower circulation and respiratory rates by lowering the pressure in the arteries (Petry et al. 2001; Mowrey 1993; Yuldasheva et al. 2005).

This plant has been used for analgesic, anti-spasmodic, anti-asthmatic, wormicidal and sedative purposes in Brazil; as a sedative and narcotic in Iraq; and for the treatment of disorders such as dysmenorrhoea, epilepsy, insomnia, neurosis and neuralgia in Turkey. In Poland, this plant has been used to treat hysteria and neurasthenia; in America, it has been used to treat diarrhoea, dysmenorrhoea, neuralgia, burns, haemorrhoids and insomnia. *P. incarnata* L. has also been used to cure subjects affected by opiate dependence in India (Miroddi et al., 2013).

P. incarnata L. is included in the nine plants for which there is considerable evidence of therapeutic effect, and is marketed in Western countries (Cravotta et al., 2010). The sedative and anxiolytic activities of *P. incarnata* L. have been attributed to benzodiazepine and γ-aminobutyric acid receptor-mediated biochemical processes in the body (Wolfman et al., 1994; Loli et al., 2007). Although the compounds responsible for the therapeutic activity of *P. incarnata* L. are yet to be identified, phytomedicines should be made using plant material characterized by the typical flavonoid profile (Wohlmuth et al., 2010; Aslanargun et al., 2012).

Proteases play an important role in important events such as protein catabolism, blood coagulation, cell growth and migration, tissue regeneration, morphogenesis development, treatment of inflammation, tumor growth, metastasis, zymogen activation, hormone release, obtaining pharmacologically active peptides from precursor proteins, transport of secretory proteins between membranes. In addition, it is used in many areas such as food industry, laundry and dishwashing detergents, pharmacological industry, cosmetics and leather industry (Gupta et al. 2002; El-Safey and Abdul-Raouf 2004). Proteases can be found in a wide variety of sources such as plants, animals and microorganisms. Proteases have a great nutritional importance due to their depolymerizing activities.

In this study, it is aimed to determine the protease enzyme activity and the volatile organic compounds of Passion flower (*P. incarnata* L.) and to investigate the usability of the plant in the pharmaceutical sector.

Figure 1. Passion flower

Material and Methods

Collection of herbal materials

Passion flower (*P. incarnata* L.) was collected from the rural areas of Muğla province in April-May period (Figure 1). The plant, which was collected to purify protease enzyme, was stored at -18 °C in freezer until used.

Preparation of homogenate

25 g of the plant, which was stored in the freezer, was shredded with 75 ml of 1 M KCl and then stirred at room temperature for 30 minutes using a magnetic stirrer. The homogenate was centrifuged at 5000 xg for 30 minutes. Thus, the plant pouch was removed from the protein solution.

Ammonium sulphate precipitation

Ammonium sulphate precipitation was performed in the homogenate as between 10% and 100% (Demir et al., 2007). This sedimentation was carried out in the form of 0-20%, 20-40%, 40-60%, 60-80%, and 80-100%. In which range the highest protease enzyme was been identified grams of

ammonium sulphate used was calculated from the following formula.

$$\text{A. Sulphate Amount (Gram)} = \frac{1.77 \times V \times (S\text{-}So)}{3.54\text{-}S}$$

Protein determination with Coomassie blue method

This method has been developed by utilizing coomassie brillant blue G-250 dye, exhibiting a different violet color in protein solutions at different concentrations. 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 g (Bradford 1976).

DPPH • radical scavenging method

This method was first suggested by Blois (1958) that DPPH • (1,1-diphenyl-2-picryl hydrazyl) radicals could be used in the determination of antioxidant molecules (Blois 1958; Brand-Williams et al., 1995). After extraction with three different solvents, the solvents were removed. The absorbance values were measured separately for each solvent using the DPPH • radical scavenging method and an absorbance against the concentration graph was plotted.

Determination of aromatic volatile organic compounds of passion flower (P. incarnate L.) using headspace GC/MSD

Fresh passion flower (*P. incarnata* L.**)** segments were weighed as 5.00 g for 20 mL headspace vial. Anhydrous magnesium sulphate ($MgSO_4$) was then added and thoroughly stirred with magnetic stirrer. The vial was placed in the headspace sampler and extraction was started at 90 °C for 30 minutes. At the end of 30 minutes, the volatile components at the top of the vial were transferred with helium gas for 1 minute with a transferline in the GC Split/Splitless inlet by the headspace sampler (Dülger et al., 2017).

Results and Discussion

Ammonium sulphate precipitation was performed in the prepared homogenate at a range of 0-100%. The precipitate obtained in each step was dissolved in 0.05M phosphate buffer (pH: 7.6) and activity assay was performed. In addition, the enzymatic activity of the supernatant fraction was monitored at each step. As a result of the determinations made, the highest activity was found in the precipitation in the range of 80-100% and the partial purification results are given in Table 1.

Table 1. The enzyme unit in the protease enzyme homogenate obtained from the flowers passion flower (*P. incarnata* L.) the specific activity and enzyme unit in the protease enzyme purified from homogenate, the specific activity and the purification results.

Steps	Volume mL	Activity EU/mL	Total Activity EU %		Amount of protein (mg/mL)	Specific activity EU/mg	Purification rate
Raw extract	100	1.23	123	100	3.53	0.34	-
%80-100 $(NH_4)_2SO_4$	90	0.87	78.3	63.66	0.98	0.88	2.58

Table 2. Ammonium sulphate precipitation and absorbance values obtained as homogenate activity.

% Range	$(NH_4)_2SO_4$ (g)	Absorbance (λ)
0-20	4.24	0.026
20-40	4.28	0.018
40-60	4.45	0.027
60-80	4.65	0.036
80-100	4.87	0.042
Precipitate		0.031

In addition, the table of absorbance values obtained as a result of ammonium sulfate precipitation is given in Table 2.

For antioxidant determination after extracting with three different solvents, the solvents were removed. Using the DPPH • radical scavenging method, the absorbance values were determined for each solvent separately and the absorbance values were determined against the concentration.

The absorbance values of hexane, acetone and methanol, which are used as solvents in the determination of antioxidant activity, at different concentrations are given in Table 3. Comparison of the results from the above data with α-tocopherol is shown in the graphic Figure 2.

Table 3. Absorbance values in 3 different solvents resulting from DPPH • radical scavenging activity determination.

Concentration (g/ml)	Hexane (Absorbance)	Acetone (Absorbance)	Methanol (Absorbance)
10	1.954	1.746	1.248
20	1.841	1.582	0.975
30	1.728	1.512	0.725

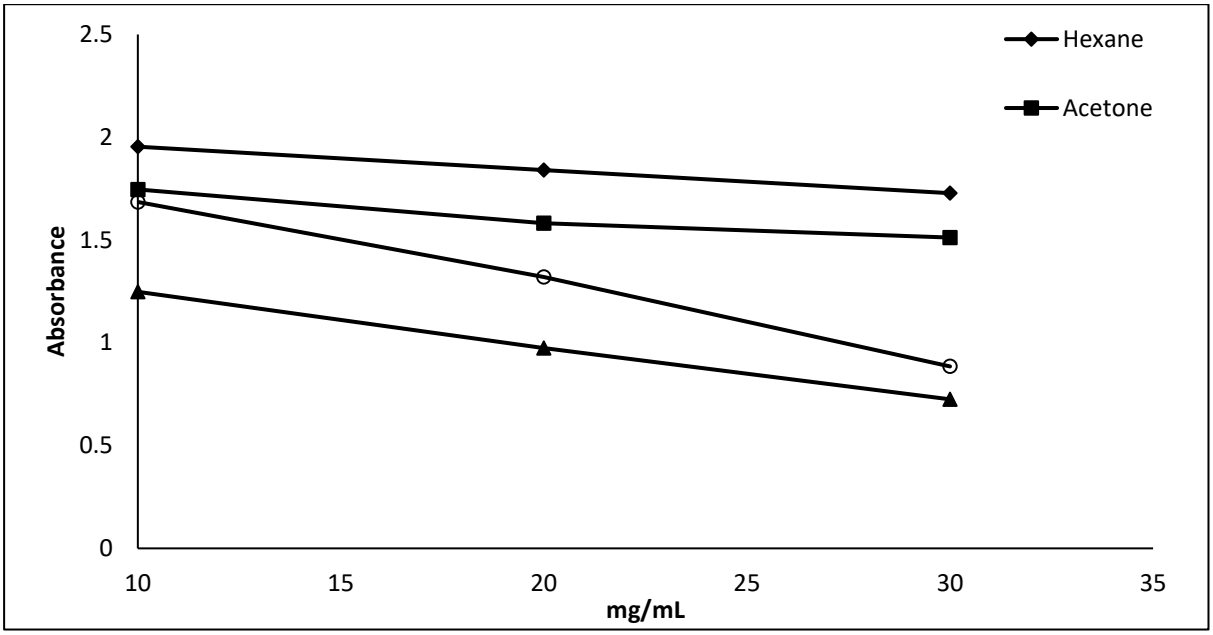

Figure 2. Comparison of DPPH • free radical scavenging activities of passionflower with methanol, hexane and acetone extracts at different concentrations with α-tocopherol, a standard antioxidant.

The volatile substances contained in the passion flower were determined using Headspace GC / MSD. The results obtained from the analysis are shown in Table 4.

Analysis of volatile organic compounds by Headspace GC/MSD showed the following compounds: hexanal (1.4%), benzyl alcohol (4.1%), linalool (3.2%), 2-phenylethyl alcohol (1.2%), 2-hydroxy benzoic acid methyl ester (1.3%,) carvone (8.1%), trans-anethole (2.6%), eugenol (1.8%), isoeugenol (1.6%), β-ionone (2.6%), α-bergamotol (1.7%), palmitic acid (7.2%) and oleic acid (6.3%) (Figure 3).

Table 4. Percentage of aromatic volatile organic components of passion flower (*P. incarnata* L.).

No	Component Name	Percentage (%)
1	Hexanal	1.4
2	Benzylalcohol	4.1
3	Linalool	3.2
4	2-phenylethyl alcohol	1.2
5	2-hydroxy benzoic acid methyl ester	1.3
6	Carvone	8.1
7	Trans-anethole	2.6
8	Eugenol	1.8
9	İsoeugenol	1.6
10	β-ionone	2.6
11	α-bergamotol	1.7
12	Phytol	1.9
13	Palmitic acid	7.2
14	Oleic acid	6.3

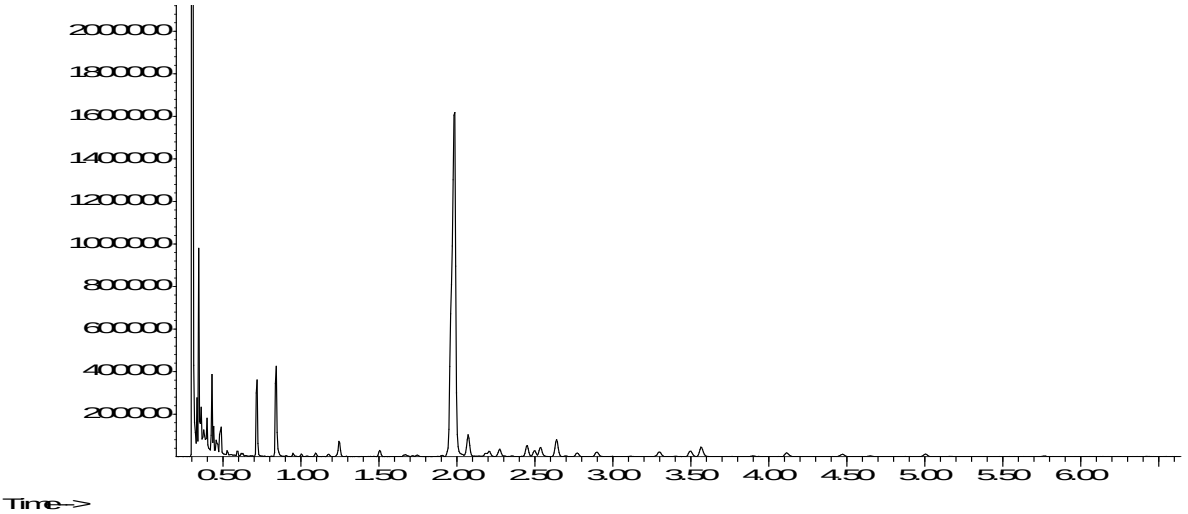

Figure 3. Chromatogram obtained as a result of examination of passion flower with Headspace GC/MSD device.

Today, proteases (serine proteases, cysteine proteases, etc.) derived from different sources, detergent and food industry, textile, leather, photo, silk and feed industries and various clinical applications have a wide use in pharmaceutical and cosmetic fields (Kalisz, 1988; Dülger et al., 2017).

Conclusions

According to the results obtained in this study, passion flower showed high enzyme activity and methanol extracts of flowers had high activity in antioxidant capacity. Based on this result, it has been demonstrated that the plant can be used for active pharmaceutical ingredient isolations.

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