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Investigation of the inhibition effect of pitaya (*hylocereus guatemalensis*) bark extracts on some metabolic enzymes

Pitaya (hylocereus guatemalensis) kabuğu ekstraktlarının bazı metabolik enzimler üzerine olan inhibisyon etkisinin araştırılması

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Abstract

Pitaya is a tropical and subtropical plant with various uses. In this study, the maceration method obtained acetone, ethanol, ethyl acetate, dichloromethane, and methanol from the bark parts of pitaya fruit. The *in vitro* effects of fruit peel extracts on the critical metabolic enzyme activities of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), human carbonic anhydrase (hCA I and hCA II), and α-glycosidase, were investigated. Pitaya bark ethanol (PBE) (IC_{50}: 20.16±0.22 µg/mL, r^2:0.9694) for AChE and Pitaya bark acetone (PBA) (IC_{50}:42.10±1.045 µg/mL, r^2:0.9807) extract for BChE showed the most potent inhibition effect. Pitaya bark ethyl acetate (PBEA) extract showed the most potent inhibitory effect for hCA I and hCA II (IC_{50}:53.51±2.22 µg/mL, r^2:0.9562 and IC_{50}:26.29±0.34 µg/mL, r^2:0.9966), respectively. In addition, PBEA showed the most potent inhibition effect on α-glycosidase enzyme (IC_{50}: 24.54±1.80 µg/mL, r^2:0.9199). According to the results, the extracts can be effectively used treatment of diseases such as glaucoma, epilepsy, type 2 Diabetes mellitus (T2DM), and Alzheimer's disease (AD).

Keywords: Enzyme, Enzyme Inhibition, Pitaya.

Öz

Pitaya, çeşitli kullanımları olan tropikal ve subtropikal bir bitkidir. Çalışmada maserasyon yöntemi ile pitaya meyvesinin kabuk kısımlarından aseton, etanol, etil asetat, diklorometan ve metanol ekstraktları elde edilmiştir. Meyve kabuğu ekstraktlarının kritik metabolik enzimler olan asetilkolinesteraz (AChE), butirilkolinesteraz (BChE), insan karbonik anhidraz CA I- II ve α-glikozidaz enzim aktiviteleri üzerine in vitro etkileri araştırıldı. AChE için Pitaya kabuğu etanol (PBE) (IC_{50}: 20.16±0.22 µg/mL, r^2:0.9694) ve BChE için Pitaya kabuğu aseton (PBA) (IC_{50}:42.10±1.045 µg/mL, r^2:0.9807) ekstraktı en güçlü inhibisyon etkisini gösterdi. Pitaya kabuğu etil asetat (PBEA) ekstresi hCA-I ve II için en kuvvetli inhibitör etkiyi gösterdi (sırasıyla IC_{50}:53.51±2.22, r^2:0.9562 µg/mL, IC_{50}:26.29±0.34 µg/mL, r^2:0.9966). Ek olarak PBEA, a-Glikozidaz enzimi üzerinde en güçlü inhibisyon etkisini göstermiştir (IC_{50}:24.54±1.80 µg/mL, r^2:0.9199). Sonuçlara göre ekstraktlar glokom, epilepsi tip 2 Diabetes mellitus (T2DM) ve Alzheimer hastalığı (AH) gibi hastalıkların tedavisinde etkin bir şekilde kullanılabilir.

Anahtar Kelimeler: *Enzim, Enzim İnhibisyonu, Pitaya*

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1.Introduction

The dragon fruit (Cactaceae) belongs to the genus Hylocereus, commonly referred to as Pitaya (Hua et al., 2018). More than 20 tropical and subtropical countries grow it commercially, including Israel, Malaysia, Mexico, Colombia, Philippines, and Taiwan (Wong & Siow, 2015). This fruit contains a high quantity of vitamin C and B group vitamins, as well as crude fiber, protein, carbohydrates, and minerals (Choo & Yong, 2011). Additionally, A red dragon fruit is rich in essential unsaturated fatty acids, linolenic and linoleic acids, and is widely used in cosmetics and therapeutics fields (Al-Mekhlafi et al., 2021). Several studies have demonstrated that phytochemicals obtained from the pulp and peel of red dragon fruit are anti-inflammatory (Kaur et al., 2018), antispasmodic, antimicrobial, diabetes, and anti-cancer (Joshi & Prabhakar, 2020). This food source has been studied extensively due to its rich content. There are several forms in which this fruit may be consumed, such as jam, jelly, fruit juice, and bread (Jalgaonkar et al., 2022). Pitaya fruit peelings account for approximately one-third of the weight of the fruit, which is discarded by fruit and juice businesses. A by-product of pitaya fruit production, the pitaya rind contains abundant bioactive substances, which have been reported to provide dietary fiber and pectin to the food industry (Madane et al., 2020; Zaid et al., 2020). Pitaya peels contain bioactive compounds that can extend the shelf life of foods and food products by containing phytochemicals with different bioactivities, such as betacyanins (Jiang et al., 2021). Pitaya peels benefit from their wealth of content, which allows them to be more widely used.

Carbonic anhydrase enzyme (CA, E.C.4.2.1.1) contains zinc ion (Zn^{2+}) metal inside its structure. In addition, a wide variety of organisms, plant kingdoms, and higher vertebrates contain this enzyme (Hoff et al., 2020; Sever, Türkeş, et al., 2021; Yaşar et al., 2021). The central role of CAs is to catalyze the mutual and reversible conversion reaction between carbon dioxide (CO_2) and water (H_2O) and proton (H^+) and bicarbonate ions (HCO_3^-) (Akıncıoğlu et al., 2021; Burmaoglu et al., 2022; Gul et al., 2016). The CO_2 hydration reaction, on the other hand, is of fundamental importance in various physiological and biochemical reactions based on acid-base balance, ion transport, and gas Exchange (Nar et al., 2013). CAs consist of eight different subdivisions, which include the α-, β-, γ-, δ-, ζ-, η-, θ-, and t-CA families. Several diseases are associated with alterations in hCA activities, including epilepsy, glaucoma, obesity, osteoporosis, ulcer, and cancer (Öztaşkın et al., 2019).

Alzheimer's disease (AD) is mainly related to changes and deterioration of the memory and behavior of people in their middle and older years. Clinically, this disorder is characterized by progressive degeneration of brain tissue as a result of a deficiency in acetylcholine (ACh) (Atmaca et al., 2018; Burmaoglu et al., 2021). Acetate and choline are two molecules converted by the enzyme AChE, a critical molecule in the central and peripheral nervous system (Aksu et al., 2018). The nonspecific cholinesterase BChE is found in vertebrate serum, blood plasma, pancreas, heart, liver, and central nervous system. BChE can hydrolyze different esters in addition to choline esters and is also known as pseudo-ChE (Anil et al., 2022; Biçer et al., 2019; Demir et al., 2018).

A chronic disease, diabetes mellitus (DM), affects approximately 4% of the world's population. T2DM, one of the types of diabetes, is becoming more common both in developed and developing countries (Sever, Altıntop, et al., 2021; Sever et al., 2020). α-Glucosidase (E.C.3.2.1.20) is one of the enzymes that can hydrolyze polysaccharides and oligosaccharides up to glucose and fructose units in small intestinal cells (Gulçin et al., 2018; Taslimi et al., 2018). The use of inhibitors of the carbohydrate glucosidase enzyme is proven to be essential for controlling hyperglycemia and T2DM (Gondolova et al., 2018). In order to minimize the quantity of glucose in T2DM, α-glucosidase inhibitors (α-GIs) effectively compete with oligosaccharides for binding to the enzyme's active site (Demir et al., 2019, 2020).

The present study aims to investigate the inhibitory effects of pitaya peels, which are considered out of consumption as waste and have very little use, on AChE, BChE, hCA I, hCA II, and α-glucosidase, which are associated with common and global health diseases.

2. Material and method

2.1 Preparation of extracts

Pitaya fruit was purchased from local vendors from the province of Antalya in the Mediterranean region of Turkey. The fruit's skin was dried and powdered separately and stored in the refrigerator until study time. Extracts were prepared using 2.5 g of dried red dragon fruit peel and 50 mL of solvent. Amounts of extract

obtained for methanol, ethyl acetate, ethanol, acetone, and dichloromethane extracts were determined at 200 mg.

2.2 Enzymes inhibition assay

2.2.1 Cholinesterase activity method

A partial modification of the method of Ellman et al. was used for the purpose of determining the activity of the AChE and BChE enzymes (Ellman et al., 1961; Topal & Gulcin, 2022).

2.2.2 hCA activity determination method

Esterase activities of hCA I and hCA II enzymes were determined based on the method developed by Verporte et al. (Verpoorte et al., 1967). A method based on the maximum absorbance of the *p*-nitrophenol molecule is used, which is formed as a result of the enzyme decomposition of the *p*-nitrophenyl acetate molecule at 340 nm (Biçer et al., 2020).

2.2.3 The α-glycosidase activity determination method

The activity of the *α*-glycosidase enzyme was measured based on the method described by Tao et al. (Tao et al., 2013).

2.3 Kinetic studies

IC_{50} values were calculated by examining the extracts' effects on AChE, BChE, hCA I and hCA II, and *α*-glycosidase enzyme activities. For this purpose, enzyme activities at 5 different concentrations were measured spectrophotometrically for all extracts. The activity (%)-[extract] graph was drawn using the obtained data. IC_{50} values were calculated using the graph (Biçer et al., 2019; Kazancı et al., 2021).

3. Results and discussion

The enzyme inhibition effects of the extracts obtained from the peel of the Pitaya fruitc peels against AChE, BChE, hCA I -II, and α-glycosidase were determined. Pharmaceutical, food, and cosmetic industries have studied enzyme inhibition as a therapeutic medium extensively. Furthermore, enzyme inhibitors are currently being used clinically to treat glaucoma, AD, DM, and obesity (Ökten et al., 2019). Some synthetic inhibitors have adverse effects, including hepatotoxicity and gastrointestinal disturbances. As a result, new and natural inhibitors that are consumed daily and have no side effects are becoming increasingly popular (Soto-Hernandez et al., 2017).

3.1 hCA I and hCA II inhibition results

The CA family members are fascinating due to their monomeric structure and the absence of disulfide bonds in polypeptide chains (Göksu et al., 2014). It was determined that PBE (IC_{50}: 53.51±2.22 µg/mL, r^2: 0.9562) was the extract with the most potent inhibition effect on hCA-I activity among the prepared extracts. The IC_{50} value was PBA (IC_{50}: 64.26±3.01 µg/mL, r^2: 0.9448), PBEA (IC_{50}: 65.8±0.95 µg/mL, r^2: 0.9349), and PM (IC_{50}: 72.5±2.67 µg/mL, r^2: 0.9647), respectively, and PBDCM (IC_{50}: 90.43±2.43 µg/mL, r^2: 0.9866) had the least inhibitory effect. Moreover, PBE (IC_{50}: 26.29±0.34 µg/mL, r^2: 0.9966) was the extract with the strongest inhibitory effect on hCA-II activity. The IC_{50} value was PBA (IC_{50}: 33.06±0.33 µg/mL, r^2: 0.9448), PBEA (IC_{50}:40.23±1.09µg/mL, r^2: 0.9455), and PBM (IC_{50}: 46.85±2.1µg/mL, r^2: 0.9887), respectively, and PBDCM (IC_{50}: 49.63±0.74 µg/mL, r^2: 0.9809 µg/mL) had the least inhibitory effect (Table 1, Figure1). In light of various studies conducted on CA inhibitors, these inhibitors have been found to be effective in treating diseases such as glaucoma and epilepsy (Taşkın et al., 2022), and identifying new natural inhibitors is vital. A study suggested that the ethyl acetate extract of the L. acutangula plant showed an inhibitory effect on CA, which was proportional to the total amount of flavonoid and phenolic substances contained in the extracts (Chanda et al., 2019).

Table 1. Enzyme inhibition results of pitaya bark extracts against hCA I and hCA II enzyme

	IC_{50} (µg/mL)			
	hCA I	**r^2**	**hCA II**	**r^2**
PBEA	65.8±0.94	0.9349	40.23±1.09	0.9455
PBA	64.26±3.01	0.9448	33.06±0.32	0.9544
PBE	53.51±2.21	0.9562	26.28±0.34	0.9966
PBDCM	90.43±2.42	0.9866	49.63±0.74	0.9809
PBM	72.50±2.67	0.9646	46.85±1.40	0.9887

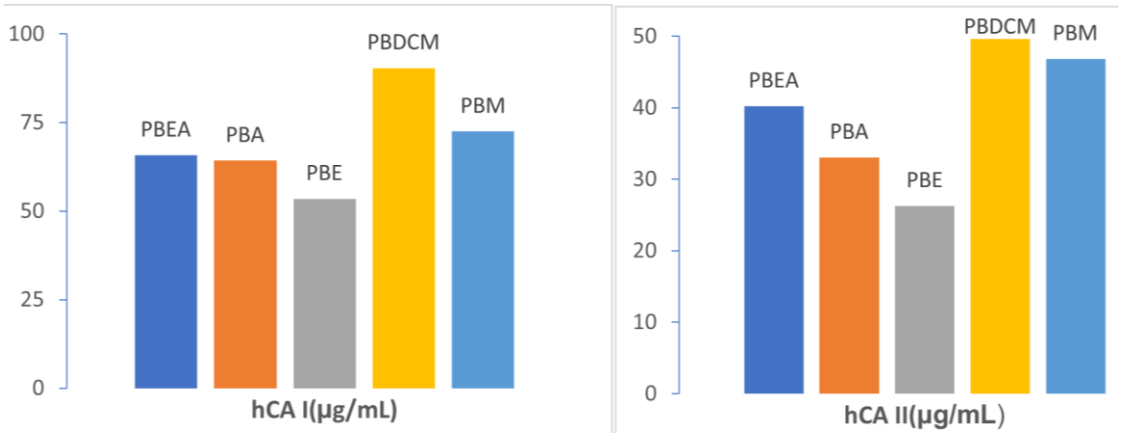

Figure 1. IC_{50} values for hCA I and hCA II of pitaya bark extracts

3.2 Cholinesterase inhibition results

Several clinical trials have been carried out using AChEIs (AChE inhibitors) generated from natural products. AChEIs identified as phenolic compounds are important for treating AD and serve as precursor molecules (Gülçin et al., 2016; Gülçin et al., 2019). According to Ellman's procedure, the extract's AChE and BChE inhibitory properties were determined (Ellman et al., 1961). It was determined that PBE (IC_{50}: 20.16±0.22µg/mL, r^2:0.9694) was the extract having the strongest inhibitory effect on AChE activity among the prepared extracts. The IC_{50} value was PBA (IC_{50}: 23.57±0.71 µg/mL, r^2: 0.9611), PBDCM (IC_{50}: 24.04±0.29 µg/mL, r^2: 0.9991), and PM (IC_{50}: 24.15±0.36 µg/mL, r^2: 0.9827), respectively, and PBEA (IC_{50}: 30.81±0.71 µg/mL, r^2: 0.9611) had the least inhibitory effect. Moreover, PBA (IC_{50}: 42.10±1.04 µg/mL, r^2: 0.9807) was the extract with the strongest inhibitory effect on BChE activity. The IC_{50} value was PBM (IC_{50}: 48.47±0.58 µg/mL, r^2: 0.9848), PBEA (IC_{50}: 59.23±0.51µg/mL, r^2: 0.9721), and PBE (IC_{50}: 68.29±0.78 µg/mL, r^2: 0.9966), respectively and PBDCM (IC_{50}: 76.44±0.48 µg/mL, r^2: 0.9723) had the least inhibitory effect (Table 2, Figure 2). Flavanones, flavonols, and derivatives of hydroxycinnamic acid are the major phenolic compounds found in the red dragon fruit and its peel. In addition, the presence of phenolic acids such as syringic acid, p-coumaric acid, gallic acid, and caffeic acid was characterized in white and red dragon fruits (Castro-Enríquez et al., 2020; Chen et al., 2021; García-Cruz et al., 2017; Zain et al., 2019). Therefore, in this study, it is thought that extracts containing important phytochemicals have an inhibitory effect on cholinesterase enzymes and that the peels of pitaya fruits may be essential in eliminating cholinergic deficiency in treating neurodegenerative disorders such as AD.

Table 2. Enzyme inhibition results of of pitaya fruit peel extracts against AChE and BChE enzymes

	IC_{50} (µg/mL)			
	AChE	**r^2**	**BChE**	**r^2**
PBEA	30.81±0.72	0.9611	59.23±0.51	0.9721
PBA	23.57±0.32	0.9648	42.10±1.04	0.9807
PBE	20.16±0.22	0.9694	68.39±0.78	0.9968
PBDCM	24.04±0.29	0.9991	76.44±0.48	0.9723
PBM	24.15±0.36	0.9827	48.47±0.58	0.9848

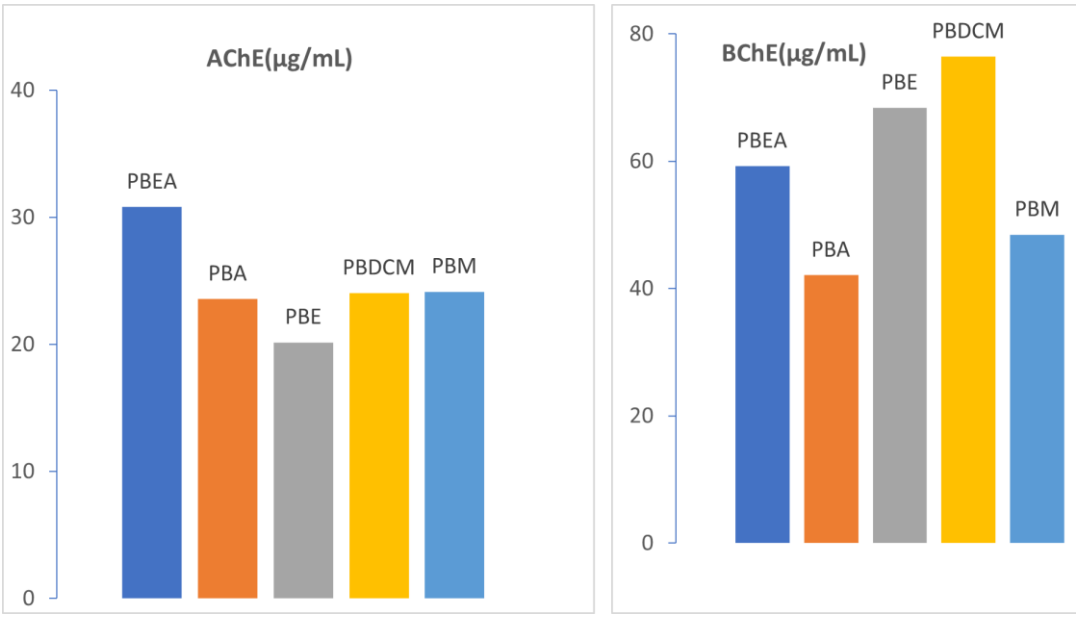

Figure 2. IC_{50} values for AChE and BChE of pitaya bark extracts

3.3 α- Glycosidase inhibition studies

DM is a serious health problem that has a significant impact on the quality of life of patients across the globe. In addition to being a metabolic and chronic disease, it is also accompanied by cardiovascular, neuropathy, and retinopathy symptoms (Kocyigit et al., 2018; Takim et al., 2021). It was determined that PBEA (IC_{50}: 24.54±1.80 µg/mL, r^2: 0.9199) was the extract with the most potent inhibition effect on *α*- glucosidase activity among the prepared extracts. The IC_{50} value was PBDCM (IC_{50}: 34.39±1.07 µg/mL, r^2: 0.9236), PBA (IC_{50}: 34.42±1.73 µg/mL, r^2: 0.9442), and PBM (IC_{50}: 58.74±1.77 µg/mL, r^2: 0.9362) respectively, and PBE (IC_{50}: 66.72±2.98 µg/mL, r^2: 0.9305) had the least inhibitory effect (Table 3, Figure 3). It has been stated that *α*-glycosidase inhibitors, one of the target enzymes in the treatment of T2DM, may be a preferred treatment method compared to the side effects of traditional treatments (Ranilla et al., 2010). Gülçin and his friends in the study of the phenolic compounds of the garden of the gardenin A (238,06 nm), usnic acid (281,13 nm), phloretin (295.27 nm), vulpinic acid (626,58 nm) *α*-glycoside enzyme. They have been reported to have better IC_{50} values than the control with control (Gulçin et al., 2018). The recent study showed that the hot water extract of Cola Nitida, one of the leaf-free plants specific to West Africa, has inhibited the α-glucosidase enzyme and has promising in vivo antidiabetic properties in T2DM rats (Erukainure et al., 2019). Consequently, it is possible to propose that including products derived from extracts of Pitaya shells in the diet may be beneficial in preventing and treating T2DM according to the results of the study.

Table 3. Enzyme inhibition result of pitaya fruit peel extracts against the α-glycosidase enzyme

	IC_{50} (µg/mL)	
	***α*- glycosidase**	**r^2**
PBEA	24.54±1.80	0.9199
PBA	34.42±1.73	0.9442
PBE	66.72±2.98	0.9305
PBDCM	34.39±1.07	0.9236
PBM	58.74±1.77	0.9362

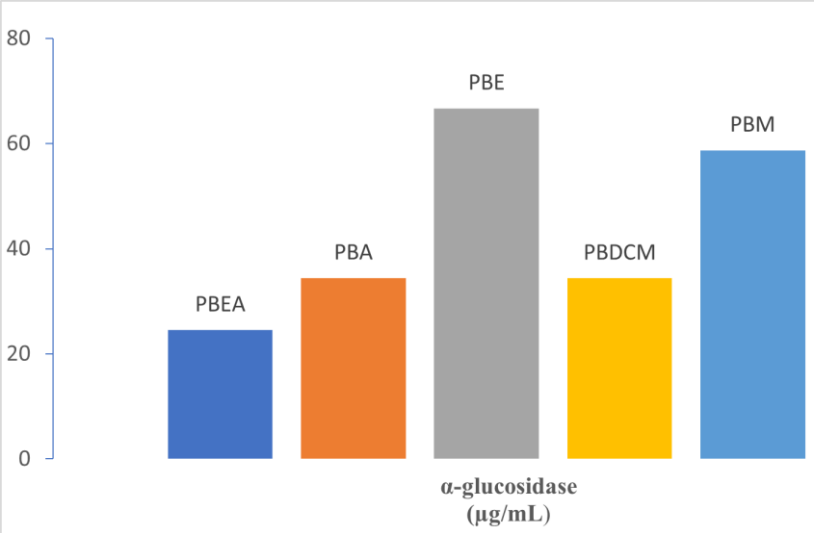

Figure 3. IC_{50} values for *α*- glycosidase enzyme of pitaya bark extracts

As a result, our study, the in vitro effects of ethanol, ethyl acetate, methanol, dichloromethane, and acetone extracts prepared from the peels of pitaya fruit, known as a waste product, on AChE, BChE, hCA-I and hCA-II, and α-glucosidase activities were investigated. The results revealed the inhibition effects of the extracts on the enzymes. Therefore, Pitaya peels may be effective in treating diseases such as AD, glaucoma, epilepsy, and T2DM when included in daily consumption. In addition, according to the results obtained, it is thought that pitaya shells, which are used as waste and whose consumption is insufficient compared to fruit, can contribute quite a lot if they are included in daily consumption with this study.

Author contribution

The manuscript was written by the corresponding author.

Declaration of ethical code

The author declares that the materials and methods used in this study do not require ethical committee approval or legal-specific permission.

Conflict of interest

The authors declare no conflict of interest.

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