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Research Article

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Biochemical properties and urease, α-amylase inhibitory effects of *Ocimum basilicum* L. (Reyhan)

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ABSTRACT

With the understanding of the role of antioxidants in preventing degenerative and age-related diseases caused by oxidative stress, and taking into account the multitude of pharmacological applications such as antidiabetic, antifungal, cardioprotection, immunostimulant, interest in plants rich in this respect has increased. *Ocimum basilicum* L. (purple) is a one-year, fragrant spice herb with its own aroma. In this study, chemical characterization of *Ocimum basilicum* plant was performed and inhibition effects on urease and a-amylase were investigated. Total phenolic content of *Ocimum basilicum* leafs ethanolic and water extracts were 320.08±2.03, 388.15±1.05 mg GAE/100g; total flavonoids were 282.57±1.12, 307.75±0.89 mg QE/100g; antioxidant capacity of samples were 0.46±0.01 and 0.52±0.02 mM Fe^{+2}/mg extract, 0.46±0.01; IC_{50} values of urease were 18.77±0.22, 20.19±0.15 μg/mL and IC_{50} values of α-amylase were 0.47±0.01, 0.42±0.01 μg/mL, respectively. It is determined that ethanolic extract of leaves is rich in linalool, linolenin, phytol and α-humulene. The data show that the leaves of the plant may be effective on two important diseases such as Diabetes mellitus and *H. pylori*.

KEYWORDS: Inhibition, Urease, α-amylase, Phenolics

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INTRODUCTION

Medicinal and aromatic plants have been used from ancient times. These plants, which people benefit from, have a certain appearance, color, smell, use as food and spice, and consume as a medicine. It is very old that people can cure diseases with plants. The vast majority of new chemicals discovered by today's modern sciences are of vegetable origin. Drugs that allow treatment of many diseases in the medical world are being produced and developed from herbal substances [1]. The basil, called *Ocimum basilicum* is a one-year fragrant spice herb with its own aroma. This plant, originating from Iran, South Asia, and especially India, grows naturally in places with a Mediterranean climate and a warm climate, but is also cultivated in France, Italy and Spain [2-3]. *Ocimum basilicum* has a wide morphological and chemical variation within the species. Therefore, it is divided into many subspecies and varieties and examined. In some regions, especially in eastern provinces, purple types are common and are called purple basil. Green varieties known as 'sweet basil' in the foreign literature, which is more common in western provinces, are called basil (*Ocimum basilicum*) [4].

Essential oil obtained from the flowering branches of basil plant, soothing in medicine, diuretic, degassing, urinary tract antiseptic, pain reliever, expectorant, worm reducer, calming, cough suppressant, mouth and dental complaints, diarrhea and chronic dysentery, respiratory diseases and it is effective in the treatment of fungal disease [5]. It is also used as a repellent to protect against invigorating baths, toothpastes, mouthwashes and insects, has an antimicrobial, antifungal effect [6,7]. It is known to be gastric, diuretic and anthelmintic and beneficial in heart and brain related disorders. These properties of basil can make it an important material in traditional and complementary medicine applications.

Inhibition effects on urease and α-amylase enzymes have been investigated lately. *H. pylori* infection is usually an important factor causing chronic gastritis, gastro duodenal ulcer, and lymphoid tissue lymphoma due to low-grade gastric mucosa. Epidemiological data show that the high H. pylori infection rate causes gastric cancer and adenocarcinoma incidence [8,9]. Urease catalyzes the hydrolysis of urea into ammonium and carbon dioxide, and its most important role is to protect bacteria in the acidic environment of the stomach [10]. Also, ammonia and monochloramine, a reaction product of ammonia and hypochloric acid, have been reported to exhibit strong toxicity in the gastric epithelium [11]. In addition, *H. pylori* lacking urease activity has been shown to not cause infection in animal models. Therefore,

inhibition of urease is important. Diabetes, obesity and oxidative stress are metabolic and degenerative disorders that have long-term effects. Diabetes occurs when not enough insulin is produced (type-1). Diabetes is the main cause of a number of complications such as blindness, heart attack, lower limb amputation and kidney failure [12]. Type-2 diabetes is the dominant form of diabetes. Inhibition of pancreatic amylase can be effective in controlling postprandial sugar levels in patients with diabetes [13].

In this study, biochemical characterization of water and ethanolic extracts prepared from purple *Ocimum basilicum* was determined. The inhibition effect of the extracts on urease and α-amylase enzymes was also studied.

MATERIAL AND METHODS

Preparation of Plant Extracts

Ocimum basilicum L. was freshly supplied by commercial from Malatya, Turkey in the cultivation season of 2019. Leaves were air dried in a shady place. Ten grams of dried samples of leaves were separately placed in a flask with 100 mL ethanol (99%) and stirred at room temperature for 24 h, then sonicated for 2 h with an ultrasonicator (ultrasonic Elma Schmidbauer GmbH, Germany). The mixtures were filtered with filter paper (Whatman) and concentrated in a rotary evaporator (IKA-Werke, Staufen, Germany) at 40 °C. Water extract of *Ocimum basilicum* L. was obtained as same method. The residues were then individually resolved with a minimal volume of ethanol and kept at 4 °C until used.

Determination of Total Phenolics

The total phenolic content of *Ocimum basilicum* L. extract was determined by using the Folin- Ciocalteu method [14,15]. A calibration plot was prepared using the Gallic acid (GA) standard and the results were expressed in mg GAE / 100 g in Gallic acid equivalent.

Determination of Total Flavanoid

Determination of total flavonoid content was made according to [16]. Quercetin (QE) was used as standard and total flavanoid content was expressed in mg QE / 100g.

Determination of Antioxidant Activity

Antioxidant activity of *Ocimum basilicum* L exracts was determined by using ferric reducing antioxidant power (FRAP) [17].

GC-MS Analysis

Main chemical composition of ethanolic *Ocimum basilicum* extract was determined with Gas chromatography coupled with mass spectrometry. Derivatization of extracts was carried out by using N-Methyl-N-(trimethylsiliyl)-trifluoroacetamide (MSTFA). Shortly, *Ocimum basilicum* extract was dried by using rotary evaporator and 5mg of dried residue was mixed with 50 μL of dry pyridine and 75 μL of MSTFA. This reaction mixture was heated at 80°C for 20 min. GC-MS analysis was applied with an Agilent 7890A GC system equipped with HP5-MS capillary column (30 m* 0.25 mm * 0.5 mm). The oven temperature was programmed from 75 to 325°C at a rate of 5°C/min, and a 15 min hold at 325°C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:50, the injector temperature 300°C, and the ionization voltage 70 eV [18]. Identification of the compounds was performed using commercial libraries as Wiley.

Determination of α-amylase Inhibition

α-amylase activity was assayed according to the modified DNS method described by [19]. Reaction mixture containing 300 μL of 1% soluble starch and 300 μL of enzyme solution was incubated for 30 min at 35 °C. Equal volume of DNS reagent was added into tubes and kept in a boiling water bath to quantify generated reducing sugars as glucose equivalent. All characterization assays were performed in triplicate. IC_{50} value of the extracts was determined at five different extract concentrations at standard assay condition and dose response curve was generated [20] . Acarbose was used as reference inhibitor.

Determination of Urease Inhibition

Urease activity was determined according to [21]. This method is based on the determination of the amount of ammonia released from urea by the action of urease enzyme by using the indo phenol method that has an absorbance of 625 nm (Weatherburn). Shortly, reaction mixture contained 5 μL of urea solution (100mM), 40 μL of jack bean urease and 5 μL of buffer (0.01M KH_2PO_4, 1mM EDTA and 0.01M LiCl; pH 8.2). After incubation at 35 °C 15min, 750 μL of phenol reagent (1%w/v phenol and 0.005% w/v sodium nitroprusside) was added, vortexed and then, 750 μL of alkali reagent (0.5% w/v NaOH and 0.1% v/v NaOCl) was added and vortexed. This mixture was incubated for 15 min more at 35 °C and optical densitywas measured at 625 nm against a blank solution including distilled water instead of enzyme. For the determination of the IC_{50} value of the extracts, activity assays were conducted at five different extract concentrations and dose response curve was generated [20]. Thiourea was used as standard inhibitor.

RESULTS

Total phenolic content of *Ocimum basilicum* leaf ethanolic and water extracts were 320.08±2.03, 388.15±1.05 mg GAE/100g; total flavonoids were 282.57±1.12, 307.75±0.89 mg QE/100g; antioxidant capacity of samples were 0.46±0.01 and 0.52±0.02 mM Fe^{+2}/mg extract, IC_{50} values of urease were 18.77±0.22, 20.19±0.15 μg/mL and IC_{50} values of α-amylase were 0.47±0.01, 0.42±0.01 μg/mL, respectively (Table 1). It is determined that ethanolic extract of leaves is rich in linalool, linolenin, phytol and α-humulene (Table 2).

Table 1: Biochemical properties of *Ocimum basilicum* extract

Ocimum basilicum	Total phenolic content mg GAE/100g	Total Flavanoid content mg QE/100g	Antioxidant capacity FRAP mM Fe^{+2}/mg extract	Urease IC_{50} μg/mL	α-amylase IC_{50} μg/mL
Leaf EE	320.08±2.03	282.57±1.12	0.46±0.01	1.877±0.22	0.47±0.01
Leaf WE	388.15±1.05	307.75±0.89	0.52±0.02	2.019±0.15	0.42±0.01
Acarbose					0.31±0.04
Thiourea				1.00±0.01	

E: Ethanolic exract WE: Water extract

Table 2: Chemical characterization of Ocimum basilicum extract

Compounds	Leaf EE Area %
Ocimene	1.02
Neophytadiene	2.11
n-Butylphthalate 0.88	0.72
2-Hexadecanoic acid	6.25
6-Octadecenoic acid	0.98
2,3-Beta quinoline	0.11
Oxirane	2.16
Linolenin	10.27
Linalool	18.52
Tetradecanal	0.88
1,2-Benzenedicarboxylic acid	1.73
Farnesol	2.07
Trans-alpha-bergamotene	1.04
Alpha-humulene	2.62
Caryophyllene	0.21
12,15- Octadecanoic acid	1.09
Pentadecanoic acid	0.86
Phytol	10.45
Octadecanal	2.19
Hexadecanal	0.81
Geraniol	0.66
Pentadecanal	1.23
Palmitic acid	1.40

DISCUSSION

Plants have high antioxidant effect thanks to their secondary metabolites. With the increasing interest in traditional and complementary medicine practices, the rate of benefiting from these features of plants has increased. Purple basil is a pleasant fragrance and colorful plant used in daily life. Studies show that this plant has a high antioxidant effect and inhibits α-amylase and urease enzymes.

Gülçin *et al.* [22] reported that the possible radical scavenging and antioxidant activity of basil's water (WEB) and ethanol extracts (EEB) were investigated using different antioxidant methodologies. Total antioxidant activity was found at a concentration of 50 μg / mL made according to the ferric thiocyanate method, and the inhibition effects of WEB and EEB on peroxidation of linoleic acid emulsion were 94.8% and 97.5%, respectively. Additional total phenolic content of basil extracts was determined as gallic acid equivalent and found to be equivalent.

Rezzoug *et al.* [23] investigated the antioxidant and antimicrobial activities of the ethanolic extracts (EE) and essential oils (EO) of two species from the Lamiaceae family, *Ocimum basilicum* and Thymus algeriensis Boiss. Phenolic compounds found in ethanol extracts from both plants were analyzed by HPLC and showed a rich flavonoid content. Chemical analysis of essential oil from Ocimum basilicum revealed 26 unique compounds with linalool (52.1%) and linalyl acetate (19.1%) as main compounds.

Khair-ul-Bariyah *et al.* [24] compared some phytochemical parameters of *Ocimum*, *O. basilicum* and O.sanctum genus in their study. Both plants showed good urease inhibitory activity and *O. basilicum* extracts showed a greater urease activity than O. sanctum extracts. *O. basilicum's* alpha-amylase inhibitory activity is also higher than that of *O. sanctum*.

Nabati *et al.* [25] worked with 137 raw extracts and found that *Ocimum basilicum* (Reyhan-e banafsh) leaves showed 19.61 ± 0.05 (inhibition %) and *Ocimum basilicum* (Reyhan) leaves showed 0.41 ± 0.01 (inhibition %) urease inhibitor activity. Ahmet *et al* [26] investigated the inhibitory activities of the polar and non-polar extracts of *Ocimum basilicum L.* leaves and flowers on amylase and lipase (PPL). They determined the chemical components of essential oils and hexane extract by GC/MS (Gas Chromatography-Mass Spectrometry). For amylase inhibition, the IC_{50} (g / mL) of the extracts was found to be 0.27-0.37, which was found to be close to acarbose.

CONCLUSION

Ocimum basilicum L. (purple), used as food in daily life, has a powerful antioxidant effect thanks to the secondary metabolites it contains. In this study, the total amount of phenolic content, total flavanoid content and antioxidant capacity of ethanolic and water extracts were determined. It was determined that plant extracts had an inhibition effect on α-amylase and urease enzymes. The chemical composition of the ethanolic extract was clarified using GC / MS. The data obtained shows that the leaves of the plant may be effective on two important diseases such as Diabetus mellitus and H. pylori.

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