

## The Determination of Inhibition Effect of Extracts of *Thymbra sintonisii* Bornm. et Aznav. Subsp on Angiotensin Converting Enzyme

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**ABSTRACT:** Angiotensin converting enzyme (ACE, peptidyl dipeptidase A, EC 3.4.15.1) has got a significant role in the arrangement of blood pressure. ACE inhibitors usually play a part in the therapies of hypertension. Hypertension is an significant cardiovascular risk factor. The main purpose of the treatment is to reduce the incidence of hypertension. In this study, the inhibition effect of butanol and water extracts of *Thymbra sintonisii* Bornm. et Aznav. Subsp on angiotensin converting enzyme (ACE) activity in human plasma was investigated. ACE activity was calculated at 345 nm spectrophotometrically. Extracts of *Thymbra sintonisii* Bornm. et Aznav. subsp plant with water and butanol were made. The effectiveness of these extracts on ACE activity was researched. Water and butanol extracts of *Thymbra sintonisii* indicated inhibition impact on ACE. IC<sub>50</sub> values for water and butanol extracts of *Thymbra sintonisii* was measured to be 1.696 mg mL<sup>-1</sup> and 0.395 mg mL<sup>-1</sup> respectively. Inhibition type for water and butanol extracts of *Thymbra sintonisii* from Lineweaver-Burk chart was defined to be non-competitive inhibition. Synthetic ACE inhibitors are utilized in the therapy of hypertension. On the other hand, synthetic ACE inhibitors exhibit a large number of adverse effects. Therefore, ACE inhibitors have been newly researched from native herbs. These conclusions demonstrate that water and butanol extracts of *Thymbra sintonisii* plant may have an ACE inhibition capacity.

**Keywords:** Angiotensin converting enzyme, Inhibition, *Thymbra sintonisii*, Hypertension.

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## INTRODUCTION

Hypertension is a major health problem that concerns more than 25 percent of the world (Kearney et al., 2005). It has got very common and serious condition that can lead to many health problem can be complicated. Hypertension is a serious cause of heart failure, peripheral vascular disease, renal failure, cerebrovascular, angina and cardiovascular diseases. The rate of these diseases increases compared to blood pressure.

Renin-angiotensin system is included in the arrangement of blood pressure and electrolyte metabolism. Renin is synthesized from jukstaglomerular cells of the kidney with a decrease in the blood pressure and volume of the kidney (Onat et al., 2006). Renin acts on the angiotensinogen in the liver and helps in the formation of Angiotensin I. The angiotensin converting enzyme (ACE, EC 3.4.15.1) transforms Angiotensin I into Angiotensin II, which is a direct vasoconstrictor, inactivates bradykinin, which is also a vasodilator. This powerful vasoconstrictor, angiotensin II, increases blood pressure by playing a role in the release of aldosterone by the adrenal cortex, the central nervous system, the sympathetic nervous system and the Na-steroid steroid (Li et al., 2003). High ACE activity causes to increased concentration of angiotensin II and hypertension. ACE is seen as part of the homeostatic mechanism responsible for maintaining blood pressure and electrolyte balance. Two specific ACE inhibitors, lisinopril and captopril, inhibit ACE and inhibit the transformation of angiotensin I to angiotensin II and have important roles in treating hypertension and protecting the electrolyte balance (Carretero and Oparil, 2000).

There are also other ACE inhibitors used in addition to drugs such as lisinopril and captopril. However, the drugs used as these inhibitors have many side effects. Therefore, the economic, safe and without adverse effects of ACE inhibitors from medicinal plants can provide an remarkable opportunity to find new potential pathways in the development of antihypertensive drugs. The plants are usually used as they are safe and preventable with a little adverse effects. For this reason, more efficient, scientifically complementary treatment pathways with the minimum repercussions are being researched (Leong et al., 2013). For example, the inhibition effects on ACE activity of *n*-hexane, methanol, and chloroform of *Asperul glomerata*, *Prangos asperula*, *Calamintha origanifolia*, *Satureja thymbra*, *Sideritis perfoliata*, *Hyssopus officinalis*, *Marrubium radiatum*, *Erythraea centaurium*, and *Salvia acetabulosa* were studied. Some of these plants, *Marrubium radiatum* and *Salvia acetabulosa* methanol extracts showed a powerful inhibitory effect to ACE with  $IC_{50}$  of 72.7 and 52.7  $\mu\text{g mL}^{-1}$ , respectively (Loizzo et al., 2008). In one work, the impact of fatty acids isolated from *Nigella sativa* on pure ACE in human plasma was examined and the five isolated fractions indicated inhibition effect on ACE. The  $IC_{50}$  constants of the fractions were determined to be 1.597  $\text{mg mL}^{-1}$  (Fr 1), 0.053  $\text{mg mL}^{-1}$  (Fr 2), 0.527  $\text{mg mL}^{-1}$  (Fr 4), 0.044  $\text{mg mL}^{-1}$  (Fr 5), and 0.136  $\text{mg mL}^{-1}$  (Fr 6) (Basi and Turkoglu, 2018).

One of the plants used for medical purposes is *Thymbra Sintonisii* Bornm. et Aznav. The family Labiatae has got an significant role as a origin of medical and fragrant herbs of merchant significance. *Thymbra L.* is located within these aromatic genus. *Thymbra* (Labiatae) is demonstrated by the following 2 species and 4 taxa in the Flora of Turkey: *T. spicata* L. (var. *spicata*, var. *intricata* P.H.Davis) and *T. sintonisii* Bornm. & Aznav. (subsp. *sintonisii*, subsp. *isaurica* P.H.Davis). Type of TS is a Mediterranean plant known for many years, with its hirsute purple flowering, shrub appearance. Tea or oil is used for analgesic effect in stomach pains. Regarding antiparasitic, antiseptic and blood circulation effects the *Thymbra Sintonisii* plant is used. In South Eastern Anatolia the plant's dried leaves and inflorescences are named as Zahter or Sater and are consumed as an antiseptic and inducing plant tea. It contains a variety of aromatic compounds mainly are carvacrol and thymol. In many of studies carvacrol,

antioxidant, antibacterial, insecticides, anthelmintic, cholesterol-lowering, analgesic, antifungal, liver protective, and anti-carcinogenic properties such as has been shown (Fakilli and Özgüven, 2012; Prasanth et al., 2014; Akkol et al., 2009; Koparal and Zeytinoğlu, 2003).

Our aim in the work is to research and evaluate antihypertensive properties *Thymbra Sintonisii* Bornm. et Aznav via ACE inhibition. In the study, it was performed inhibition of ACE enzyme activity from human plasma. For this, it was prepared water and butanol extractions of *Thymbra Sintonisii* Bornm. The impact of these extracts on ACE inhibition was researched. To date, there is no study on the ACE inhibitory effect of water and butanol extracts of *Thymbra Sintonisii* Bornm. et Aznav. Therefore, we believe that this study contributes to the literature about this plant.

## MATERIALS AND METHODS

### Chemical Materials

HepesNa, N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), were purchased from Sigma-Aldrich. Butanol, methanol, chloroform, and hexane were obtained from VWR Chemicals. Entire other chemicals used were obtained from either Merck or Sigma-Aldrich.

### Plant Materials

*Thymbra sintonisii* plant used in this study was purchased a local herbal store located in Mardin, Turkey. (August, 2018). The plant was identified and deposited at the Yüzüncü Yıl University, Faculty of Science, Department of Chemistry, Van, Turkey.

### Preparation of the human plasma

Blood samples used to prepare human specimens were supplied from the Van Red Crescent Blood Center of Turkey. The blood specimens were put into tubes including EDTA, then the tubes were centrifuged 15 minute and 1500 rpm. The plasma was meticulously taken from the blood. And then plasma was again centrifuged for 1 hour (4°C, 8500 rpm) to uproot the ghosts and intact cells. The resulting plasma was then stored for using in the experiment.

### Activity Determination

ACE activity was gauged spectrophotometrically at 345 nm by the decrease in the absorbance at 35°C accordingly the procedure of Holmquist, Bünning and Riordan (1979). 50 mM HepesNa buffer, 0.3 M NaCl, 10 µM ZnCl<sub>2</sub> and 1 mM FAPGG added to the assay cuvette. One unit of activity was defined as the quantity of ACE that produces a  $\Delta A_{345} / \text{min.}$  of 1.0 (Andújar-Sánchez et al., 2003; Holmquist et al., 1979).

### Preparation of Plant Extracts

*Thymbra sintonisii* Bornm. et Aznav. Subsp was dried in the shade. *Thymbra sintonisii* was levigated with a blender. 37 g of the powder of *Thymbra sintonisii* was weighed and extracted by chloroform and methanol (CHCl<sub>3</sub>: MeOH 1:1) for 3 days and the CHCl<sub>3</sub>: MeOH was evaporated. Subsequently, A two-phase mixture was attained by adding 200 mL of bidistilled water: hexane (1:1) to the attained dry specimen. This biphasic mixture was poured to the division funnel and shaken and allowed to stand for division of the phases and these phases were meticulously separated from the tap of the division funnel. This procedure has been recurrent for three times.

Thereafter the hexane phase was evaporated, the resulting water phase was fractionated by adding 100 mL of ethyl acetate and the phases were splitted with a division funnel. This method has been recurrent for three times. The ethyl acetate phases were then assembled and evaporated in the evaporator and a dry sample was attained. After that, the ethyl acetate phase was removed, the resulting water phase

was fractionated by adding 100 mL of butanol and the phases were separated with a separatory funnel. This method has been recurrent for three times. The butanol phases were then assembled and evaporated in the evaporator and a dry specimen was attained 38 mg was received from the resulting dry sample and dissolved in 3 mL of bidistilled water. This manner the butanol extract was attained.

For all that the solvent was removed in the last water phase and a dry sample was attained. 45 mg was received from the resulting dry specimen and dissolved in 1 mL of bidistilled water. The water extract was provided in this way. The extracts were kept at 4 °C then the extracts were used in the evaluation.

### ACE Inhibition Assay

In this study, human plasma was preferred to evaluate ACE activity. Two tubes were received as blind and specimen and 100 µL of plasma containing ACE was incorporated to each tube. Then, 900 µL of HEPES buffer (50 mM HEPES, 0.3 M NaCl, 10 µM ZnCl<sub>2</sub> pH 7.5) was incorporated to the blind tube without substrate (FAPGG) and stirred. The spectrophotometer was reset with a blind cuvette. To the specimen tube, 50 mM HEPES buffer (pH 7.5), inhibitor (plant extracts) and 1 mM substrate (FAPGG) were added to be total volume to 1000 µL and stirred and absorbance at 345 nm was measured. The specimen tube was incubated for 30 minutes at 35 °C. Then 30 minutes, the absorbance of the tubes at 345 nm was determined and the quantity of reduce in absorbance was measured.

$$A (\text{ACE Activity}) = (\Delta\text{OD}/0.517) \times (V_c/V_e) \times f,$$

where  $\Delta\text{OD}$  is the reduce in the absorbance at 345 nm per minute,  $V_c$  is total reaction volume,  $V_e$  is the volume of enzyme solution (plasma hemolysate),  $f$  is the dilution coefficient, and 0.517 is the millimolar absorption coefficient of FAPGG.

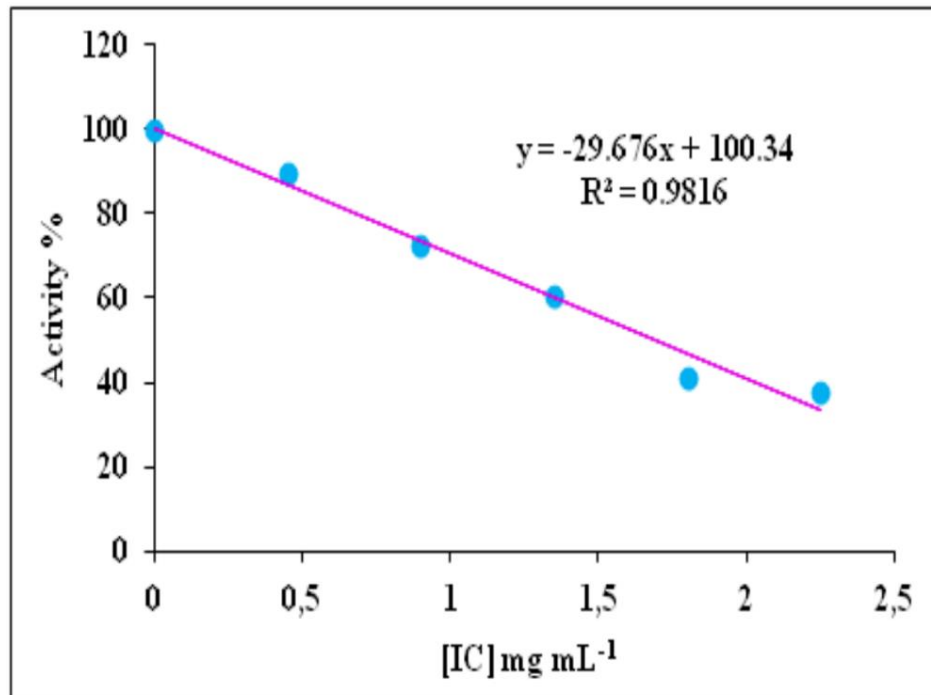
The ACE inhibitory activity (inhibition %) was found from the calibration curve and computed by the following equation:

$$\% \text{ ACE inhibition} = \text{Uninhibited activity} - \text{Inhibited activity} / \text{Uninhibited activity} \times 100$$

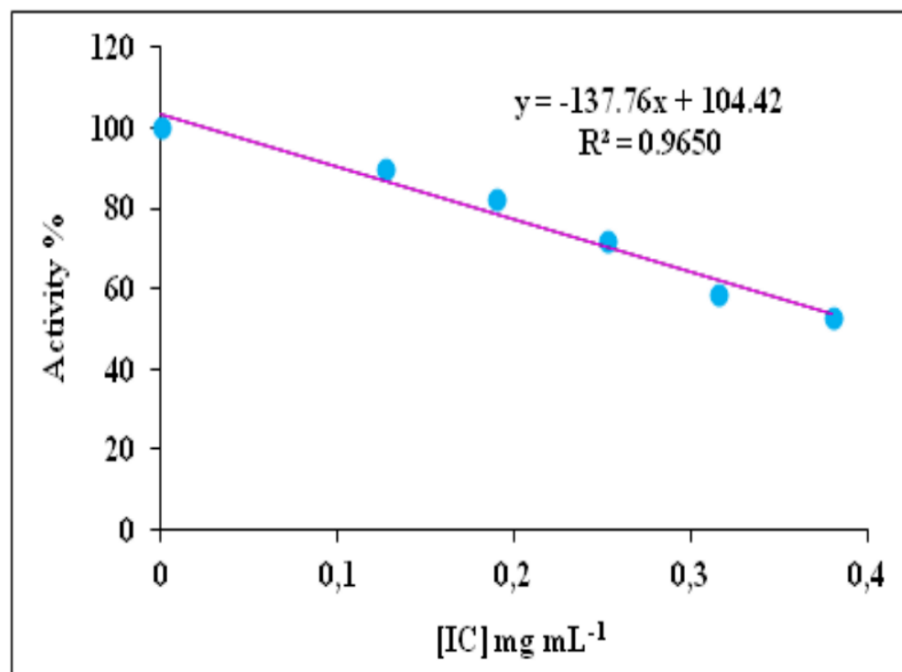
## RESULTS AND DISCUSSION

The *Thymbra sintonisii* plant material was successively extracted by maceration using methanol and chloroform (CHCl<sub>3</sub>:MeOH 1:1). The crude extract was obtained from solvent evaporation under vacuum. And then water and butanol extracts were composed by using this crude material. We investigated the inhibitory activities of water and butanol extracts of *Thymbra sintonisii* Bornm. et Aznav on ACE in human plasma.

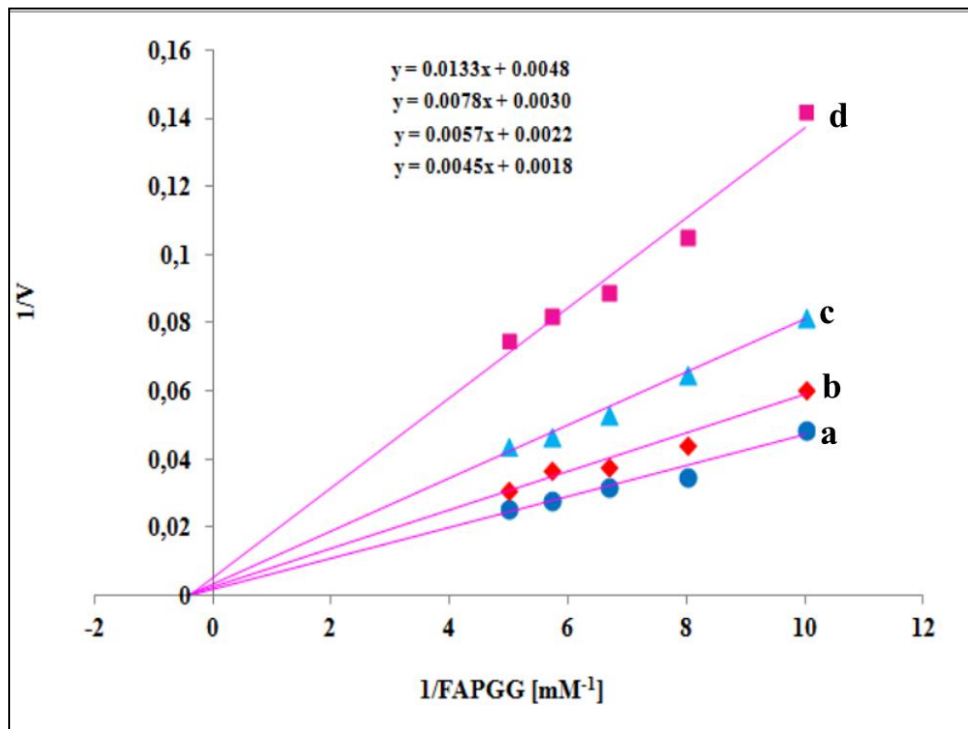
Herein, the impact of water and butanol extracts of *Thymbra sintonisii* on ACE activity were researched. Water and butanol extracts of *Thymbra sintonisii* plant demonstrated inhibition effect on Angiotensin Converting Enzyme. IC<sub>50</sub> valuations for water and butanol extracts of *Thymbra sintonisii* were designated as 1.696 mg mL<sup>-1</sup> and 0.395 mg mL<sup>-1</sup>, respectively (Figure 1, 2). The inhibition type for inhibitors was found as non-competitive with Lineweaver-Burk chart (Figure 3, 4).



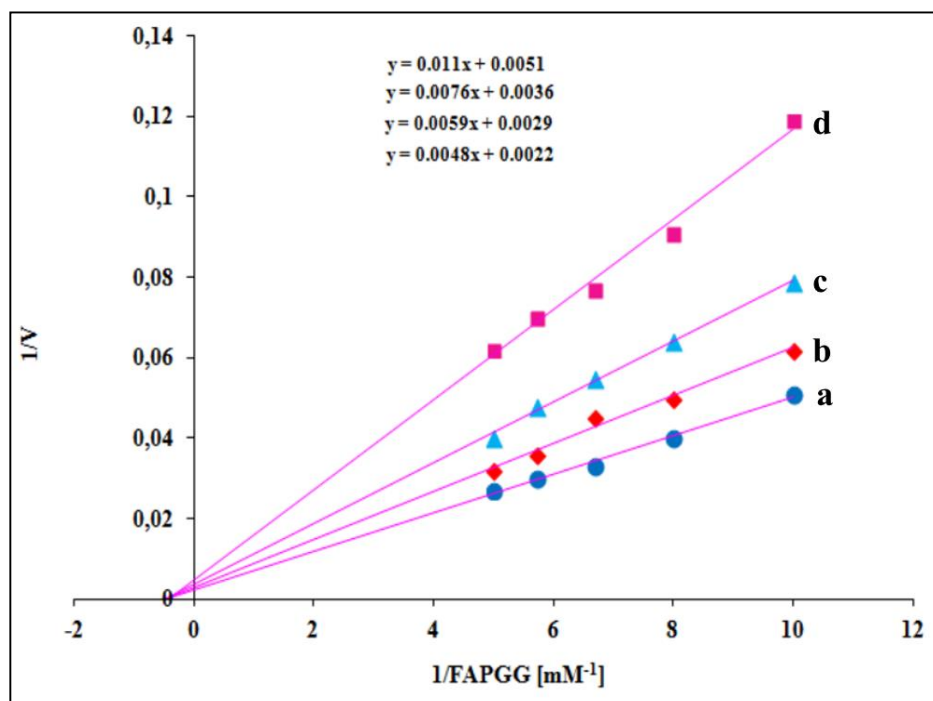
**Figure 1.** The inhibition impact of *Thymbra sintonisii* water extracts on the ACE. Five dissimilar *Thymbra sintonisii* water extracts (from 0.45 to 2.25 mg mL<sup>-1</sup>) on ACE activity were designated.



**Figure 2.** The inhibition impact of *Thymbra sintonisii* butanol extracts on the ACE. Five dissimilar *Thymbra sintonisii* butanol extracts (from 0.13 to 0.38 mg mL<sup>-1</sup>) on ACE activity were designated.



**Figure 3.** Lineweaver-Burk chart by five distinct substrate concentrations (FAPGG) and three different *Thymbra sintonisii* water extract concentrations (control (a), 0.45 mg mL<sup>-1</sup> (b), 1.35 mg mL<sup>-1</sup> (c), 2.25 mg mL<sup>-1</sup> (d) used for the definition of inhibition sort.



**Figure 4.** Lineweaver-Burk chart by five dissimilar substrate concentrations (FAPGG) and three dissimilar *Thymbra sintonisii* butanol extract concentrations (control (a), 0.13 mg mL<sup>-1</sup> (b), 0.25 mg mL<sup>-1</sup> (c), and 0.38 mg mL<sup>-1</sup> (d) used for the definition of inhibition sort.

Plants and bioactive materials that obtained of herbs were employed as conventional medication to treat diseases throughout history. In these days, treatment with plants still continues significantly in various cultures. According to World Health Organization datas, almost 80 % of people around the world uses plants for primary health care. The herbal substances even have got a significant place in health care for remaining 20 % (Ranilla et al., 2010). Hypertension is a widespread progressive disrupt causing to some chronic diseases like that cardiovascular disease, stroke, renal disease and diabetes. ACE inhibitory medicines are the first class therapeutics since decades. However, exposure to drugs for a certain period of time may lead to repercussions such as lack of power, tiredness, sleepiness, inability, cool hands and feet, crisis, sleeplessness, unusual heartthrobs, skin itching, dried mouth, dizziness, coughing, and angioneurotic edema (Israili and Hall, 1992). Some new options have been discovered obviously that can replace of these medicines. Many plants like Garlic (*Allium sativum*), Lime blossom (*Tilia europea*), Kudzu (*Pueraria lobata*), Mistletoe (*Viscum album*), Valerian (*Valeriana officinalis*), and Saffron (*Crocus sativus*) could be used as a natural source for developing newer therapeutic agents. Nowadays, native substances and their reproductions contain more than 50 % of all drugs in clinical application (Raji et al., 2012).

In this study, the impact of *Thymbra sintonisii* plant extracts on ACE was researched. Water and butanol extracts of *Thymbra sintonisii* plant demonstrated inhibitor impact on ACE. *Thymbra sintonisii* Bornm et. Aznav is used a medically plant in Anatolia, Turkey. Meanwhile, this herb has germicidal, and stimulative impacts. In many studies conducted with plants, the inhibitory impact was shown on ACE enzyme. According to a study was realized the inhibition impact of hydroalcoholic extracts of *N. jatamansi*, *P. ferulacea* and *M. vulgare* on ACE activity. IC<sub>50</sub> values of these plants were found as 2.147 mg mL<sup>-1</sup>, 4.057 mg mL<sup>-1</sup> and 0.791 mg mL<sup>-1</sup>, respectively. The minimum value of IC<sub>50</sub> was *M. Vulgare* (Namjoyan et al., 2015). In our study IC<sub>50</sub> values for water and butanol extracts of *Thymbra sintonisii* plant were found as 1.696 mg mL<sup>-1</sup> and 0.395 mg mL<sup>-1</sup>, respectively According to our study, inhibitory impact of water and butanol extracts of *Thymbra sintonisii* is more than their plants extracts. So, the inhibitor values of our study are the best compared to other studies.

The drugs was used in hypertensive treatment has side effects so, investigations was turned into biological sources such as herb extracts. It has been demonstrated that the methanolic extracts of *Musa X paradisiaca* inhibited ACE activity by 68.63%–98.3% (Acharya et al., 2016). In other work, the ACE-inhibitory effect of citrus leaf extracts were showed in rats fed with palm oil heated five times (Siti et al., 2017). Besides herb extracts, food sourced ACE-inhibitory peptides as using an option to synthetical medicines and are regarded ideally accepted class of bioactive peptides (Jimsheena and Gowda, 2011). In South Africa, twenty traditional plants were investigated against to ACE for their antihypertensive properties. Among these plants, *Adenopodia spicata* leaves were showed the most inhibitory effect (97%) (Duncan et al., 1999). In another work, butanol and water extracts of *Juniperus excelsa* Bieb. fruits inhibited ACE activity purified from human plasma. IC<sub>50</sub> values for water and butanol extracts of *Juniperus excelsa* fruits were designated as 5.790 mg mL<sup>-1</sup> and 2.858 mg mL<sup>-1</sup> respectively (Basi et al., 2019). In a study examining the efficacy of both GSSG and GSH on human plasma ACE activity, GSSG peptide demonstrated activator impact. GSH demonstrated inhibitor impact on ACE efficiency and lisinopril was utilized as specific inhibitor. IC<sub>50</sub> values for lisinopril and GSH were calculated as 0.781 nM and 16.2 μM, respectively (Basi and Turkoglu, 2019).

At the same time, many recent studies have investigated the inhibition effect on ACE activity of peptides from natural sources. For example, in one study, Three ACE inhibitor peptides were isolated from broccoli protein hydrolysates and identified as IPPAYTK, LVLPGELAK and

TFQGPPHGIQVER. The IC<sub>50</sub> values of these peptides were 23.5, 184.0, and 3.4 μM, respectively (Dang et al., 2019). The Gly-Val-Gly-Ser-Pro-Tyr hexapeptide obtained from the pearl oyster (*Pinctada fucata*) shell protein hydrolyzate showed an inhibition effect on ACE and the IC<sub>50</sub> value was found to be 5.82 μg mL<sup>-1</sup> (Sasaki et al., 2019). SAGGYIW and APATPSFW peptides isolated from wheat gluten had a significant inhibition effect on ACE activity with IC<sub>50</sub> values of 0.002 mg mL<sup>-1</sup> and 0.036 mg mL<sup>-1</sup>, respectively (Zhang et al., 2020). In other words, natural sources and natural compounds obtained from these sources constitute an important research area as ACE inhibitors.

Enzyme efficiency is determined in the tampon system. The polarity has a big significance in terms of creation a homogeneous solution in the tampon medium. In our work, water and butanol phase extracts inhibited the enzyme efficiency. At the same time, because of the nanostructures of butanol, the polar molecules have a great affinity for this solvent. Therefore, butanol extract had a significant effect on ACE activity.

## CONCLUSION

Traditional herbal teas are usually used by the people in the water by boiling or brewing method is prepared. The polar phase was more effective in our study. This situation is explained above. This study may be a preliminary study with this plant. In later times, the active substance isolation of *Thymbra sintonisii* Bornm. et Aznav plant can be made and their effects on this enzyme can be investigated.

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