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Angiotensin-Converting Enzyme Inhibitory Activity of *Passiflora edulis f. flavicarpa* and *Petroselinum crispum* (Mill) Fuss

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Authors' contributions

This work was carried out in collaboration between all authors. Author NL designed the study and supervised the work. Author RAR wrote the protocol, performed extraction, angiotensin-converting enzyme inhibitory activity and statistical analysis and wrote the first draft of the manuscript. Author MVM performed preliminary phytochemical screening and improved the manuscript. Author PL managed figures presentation and edited the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determinate the angiotensin-converting enzyme inhibitory activity of several extracts from the species *Passiflora edulis* f. *flavicarpa* and *Petroselinum crispum* (Mill) Fuss.
Study Design: Collection of plant material, extraction, preliminary phytochemical screening and evaluation of angiotensin-converting enzyme inhibitory activity of the plant extracts.
Place and Duration of Study: Faculty of Health Sciences, Universidad del Quindío, Armenia, Quindío, Colombia, between November 2009 and August 2010.
Methodology: The fruit juice and the leaves ethanolic extract of *Passiflora edulis* (passion fruit) and the leaves ethanolic extract of *Petroselinum crispum* (Mill) Fuss (parsley) were assessed in vitro regarding their capacity to inhibit ACE. This enzyme's

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activity was determined in serum by the method based on the enzymatic hydrolysis of the Furilacriloil - L - phenylalanyl - glycyl - glycine (FAPGG), by the serum ACE, to Furilacriloil - L - phenyl (FAP) and glycyl – glycine (Gly – Gly). Secondary metabolites families were identified using chemical qualitative assays.
Results: Significant lowering of ACE activity was obtained with *Passiflora edulis* extracts but not with *Petroselinum crispum* (*P* = .05). The fruit juice and the leaves ethanolic extract from passion fruit, as well as the parsley leaves ethanolic extract showed inhibition percentages of 40.0 ± 11.5, 27.4 ± 8.6, and 1.1 ± 11.2%, respectively, by working with 0.1 mg/ml extract concentration in the reaction mixture. Preliminary phytochemical screening revealed the presence of flavonoids in the juice and in the *Passiflora edulis* leaves.
Conclusion: Considerable angiotensin-converting enzyme inhibitory activity was found for *Passiflora edulis* extracts but not for *Petroselinum crispum*. The results suggest that there are potential ACE inhibiting secondary metabolites in the *Passiflora edulis* fruit juice and in the ethanolic extract of its leaves, possibly flavonoids, but not in *Petroselinum crispum* leaves.

Keywords: Hypertension; angiotensin-converting enzyme; Passiflora edulis; Petroselinum crispum.

1. INTRODUCTION

The renin-angiotensin system (RAS) comprises an enzymatic reactions chain that plays a fundamental role in controlling and regulating diverse metabolic processes important in the cardiovascular system [1]. The reaction sequence starts with the proteolytic breakdown of angiotensinogen by renin, an aspartyl protease synthesized in the kidney, resulting in angiotensin I (Ang I). This is the main substrate of the angiotensin-converting enzyme (ACE). The action of ACE on Ang I produces angiotensin II (Ang II), a potent vasoconstrictor implied in the development of important cardiovascular risk factors like hypertension [2,3].

The association of Ang II with cardiovascular disease led to the research and development of inhibitors of the angiotensin-converting enzyme (IACEs), seeking to reduce the concentration of Ang II and, hence, its negative actions upon the cardiovascular system. These inhibitors exist in the market and are widely used in hypertension treatment; however, some undesirable side effects limit their usefulness [4], which has led to the seeking of new safer and more effective drugs, very often based on knowledge of traditional medicine.

Passiflora edulis, commonly known as passion fruit, and *Petroselinum crispum* (Mill) Fuss, common name parsley, are broadly used in traditional medicine in many human communities around the world to treat various diseases, including hypertension [5,6,7]. Several *in vivo* studies have confirmed an antihypertensive effect of the ethanolic extracts from the *Passiflora edulis* leaves and from the fruit juice and its corresponding peel in rats with induced and spontaneous hypertension [8,9,10]; however, it is not known if this antihypertensive effect is a consequence of an inhibition on the ACE or if it implies some other mechanism. *Petroselinum crispum* has shown calcium-channel-blocker activity in intestine and uterus muscle [11]. Various fractions of its methanolic extract have been studied in order to determinate its properties to reduce blood pressure in vivo. Although every fraction showed hypotensive effect, non-polar fractions seemed to be more effective [12]. More recently, strong hypotensive effect of the ethanolic and aqueous extracts of its

leaves has been reported when administrated to normotensive rats [13]. In addition, a hypotensive effect of the aqueous extract of its seeds was found in rats after its administration [14]. Regarding to its hypotensive mechanism, it was described that the aqueous extract from *Petroselinum crispum* leaves showed high ACE inhibition [15]. Nevertheless, to date, there is no work exploring the hypotensive mechanism of the ethanolic extract. Hence, the objective of this work was to determine the effect of the *Passiflora edulis* ethanolic extract from the leaves and its fruit juice and the *Petroselinum crispum* leaves ethanolic extract on the ACE activity.

2. MATERIALS AND METHODS

2.1 Plant Material

Passiflora edulis leaves and fruit were collected at the La Miranda farm, in the township of La Herradura, Km 7 on the road to La Tebaida - Valle del Cauca, municipality of La Tebaida, Department of Quindío-Colombia. *Petroselinum crispum* was purchased from local market. The collection was conducted between January and March 2010. Both specimens were identified by the HUQ Herbarium, Universidad del Quindío (collection numbers for *Passiflora edulis* and *Petroselinum crispum* are 33976 and 33975, respectively).

2.2 Preparation of Extracts

The *Passiflora edulis* and *Petroselinum crispum* leaves were first washed, then dried in an air furnace at 40ºC, and finally pulverized. Treatment of the dried and ground plant material was done according to that described by Bilbao [16].

A total of 298 g of dried and ground parsley leaves and 320 g of dried and ground passion fruit leaves were leached independently for 8 days with rectified ethanol (EtOH) at 96%. The chlorophyll was separated by adding a 1:7 mixture of EtOH/water. The extracts were concentrated at reduced pressure at 40ºC and then dried. The solid obtained was weighed and resuspended in a 20% solution of EtOH until reaching the appropriate concentration for the assay. The final solution was stored at 4ºC until use.

Ripe *Passiflora edulis* fruit were initially washed and peeled. The juice was extracted by mechanical methods, strained to eliminate seeds, and centrifuged to achieve homogeneity. Then, it was evaporated until dry at 40ºC under reduced pressure in a rotavapor evaporator and the solid obtained was weighed and resuspended in distilled water until reaching the appropriate concentration for the assay. The final solution was stored at 4ºC until use.

2.3 Preliminary Phytochemical Screening

A preliminary phytochemical screening was conducted on the *Passiflora edulis* fruit juice extract, on the ethanolic extract of its leaves, and on the ethanolic extract of the *Petroselinum crispum* leaves. Qualitative tests were made to characterize secondary metabolites like tannins, flavonoids, quinones, sterols, saponins, cardiac glycosides, terpene lactones, coumarins, and carotenoids, as well as to identify reducing sugars, ketoses, and deoxy sugars according to that described by Sanabria and Bilbao [17,16].

2.4 ACE Inhibition Assay

Human blood serum was used as ACE source. Samples were taken from five apparently healthy volunteer males who accepted to participate in the research through an informed signed consent. The determination of the ACE activity in serum was carried out *in vitro* by using the method by Simonetta Ronca – Testoni [18] modified by the Biochemistry Laboratory at Universidad del Quindío, plus some considerations proposed by Serra *et al.* (2005) [19] for an ACE inhibition assay using plant extracts. The method is based on enzymatic hydrolysis of the Furilacriloil - L - phenylalanyl - glycyl - glycine (FAPGG), by the serum ACE, to Furilacriloil - L - phenyl (FAP) and glycyl – glycine (Gly – Gly): Briefly, two tubes with 25 µl of serum each received the addition of 225 µl of distilled water, 250 µl buffer (0.8 mM FAPGG, 400 mM NaCl, 50 mM HEPES pH 8.2), and plant extract at a concentration of 0.1 mg/ml in reaction mixture. As a blank, another tube was used containing exactly the same, plus EDTA 3.3 mM as an ACE inhibitor. Distilled water was used as a negative control and Captopril (80 nmol/l), as positive control. The tubes were incubated at 37 ℃ for 20 minutes and left to stand on ice to halt the enzymatic reaction. Finally, absorbance was read for each at 345 nm by using a Milton Roy Genesis 5 spectrophotometer. Five assays were run per extract, one with each serum sample. During each assay ACE activity with plant extract was measured in triplicate. The activity was obtained by applying the following equation:

$$ACE\ activity = (\Delta A \times V_f \times 1000/t)/(0.5 \times Vs) \quad (1)$$

Where **ΔA** is the absorbance difference between the samples and the blank, $\mathbf{V_f}$ is the test final volume, **1000** converts ml into liter, ***t*** is incubation time, 0.5 is the hydrolysis absorbance of 1mM of FAPGG under test conditions, and ***Vs*** is the volume of the serum sample (0.025 ml). ACE activity is expressed in ACE Units per liter (**U/L**). An ACE unit (1U) is the amount of the enzyme that converts 1 µmol of FAPGG in FAP and Gly – Gly per minute at 37ºC.

2.4.1 Inhibition percentage

The percentage of inhibition (% I) of each extract on ACE was determined by using the equation:

$$\%\ I = [(A_c - A_s)/ A_c] \times 100\% \quad (2)$$

Where A_c is ACE activity for negative control and A_s is the ACE activity in the presence of the plant extract or Captopril. Values are expressed as the average of the inhibition obtained in the five repetitions.

2.5 Statistical Analysis

The statistical analysis was performed with the SPSS Statistics 18.0 software. Enzyme activities and percentages of inhibition between negative control and samples were compared via one-way ANOVA followed by a Post Hoc test (Duncan, Tukey), establishing a degree of significance when $P < .05$ and these are expressed as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

This study investigated the *in vitro* effect of the ethanolic extract of the leaves and the fruit juice from *Passiflora edulis* and the ethanolic extract of the leaves from *Petroselinum crispum* on ACE activity. Both species were selected based on their use as antihypertensives in traditional medicine in different communities around the world and also because of their properties to reduce blood pressure observed in some *in vivo* studies [8,9,10,13].

ACE activity in the presence of each of the extracts studied is shown in Fig. 1. The results reveal significant decrease of the activity with *Passiflora edulis* (juice and leaves extract), (*P* = .05) but not with *Petroselinum crispum.*

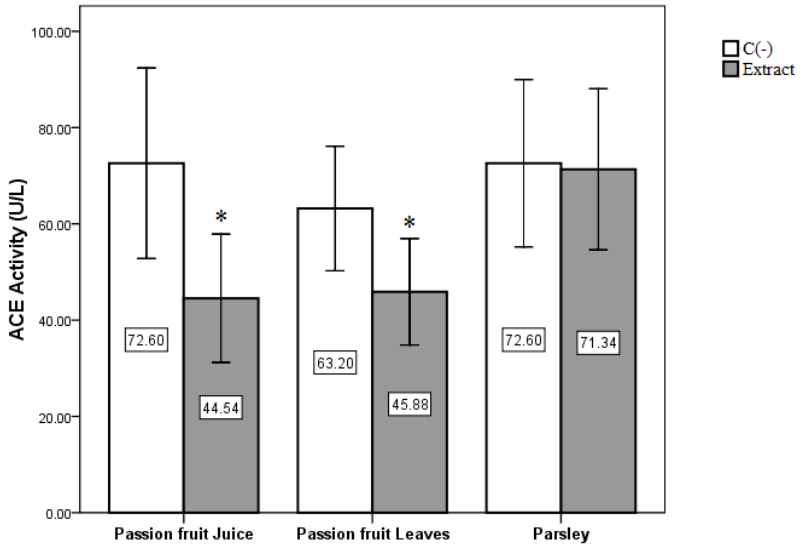

Fig. 1. Average activity of the enzyme (ACE) at the presence of each plant extract. Where *C(-)* is the activity for the negative control (white)

*One-way ANOVA followed by a Post Hoc test: Extracts: Significant difference from negative control, *P = .05.*

Mean ± S.D = Mean values ± Standard deviation of five experiments.

The percentages of ACE inhibition for the *Passiflora edulis* fruit juice and the leaves ethanolic extract and *Petroselinum crispum* leaves ethanolic extract are shown in Table 1. Contrary to that described in this study, it has been previously reported that passion fruit leaves extract has no inhibition effect [20]. It is well known that certain environmental factors such as sunlight exposure, temperature and soil composition can affect the chemical composition and the quality of crops [21], so this disparity may be substantiated, on the one hand, by differences in geographic location and, thereby, by the environmental conditions of the collection sites. On the other hand, *Passiflora edulis* is mainly a commercial species, which possibly means that unregistered genetic modifications may have taken place, from region to region, in order to favor certain properties of the plant [22]. It is likely that the distinct environmental conditions and the potential genetic variations affect metabolic pathways that favor or inhibit metabolite biosynthesis in the plant. It is also possible that the

differences between the two studies are due to the use of different methods to extract metabolites and to determine ACE activity. We know of no studies, besides that reported by Braga et al. [20] or ours, about the inhibitor potential of *Passiflora edulis* over the angiotensin-converting enzyme which permit settling this controversy.

Table 1. Percentages of inhibition of the plant extracts

Species	Part used	Extract	% of ACE inhibition*
Passiflora edulis	Fruit	Juice	40.0 ± 11.5**
Passiflora edulis	Leaves	Ethanolic	27.4 ± 8.6***
Petroselinum crispum	Leaves	Ethanolic	1.1 ± 11.2

**Values are expressed as mean ± SD of the percentages of inhibition of each extract obtained for each of the 5 assays.*

*One-way ANOVA followed by a Post Hoc test: Extracts with significant difference from negative control, **P < .001, ***P = .004.*

Antihypertensive properties of the *Passiflora edulis* fruit have been previously reported. Considerable decrease of blood pressure by extracts of its peel [9,10] and juice [8] has been described when administrated to hypertensive rats. In addition, a clinical trial has reported that the *Passiflora edulis* fruit juice works as an effective and safe co-adjuvant of Enalapril in diminishing blood pressure [23]. To date, there is no evidence of the mechanism through which this antihypertensive effect occurs, nevertheless, our results suggest that the antihypertensive activity described by them may be due to an ACE inhibition; although, it is necessary to conduct more studies to confirm this appreciation.

The preliminary phytochemical screening revealed the presence of several secondary metabolites in *Passiflora edulis* fruit juice and leaves ethanolic extract. Besides tannins and sterols, similar chemical profiles were found for both extracts. Unlike *Passiflora edulis* fruit juice and leaves, Parsley leaves ethanolic extract did not reveal the presence of flavonoids, quinones and carotenoides (Table 2).

Table 2. Results of preliminary phytochemical screening

Metabolite	Passion fruit juice	Passion fruit leaves	Parsley leaves
Tannins	-	+	+
Flavonoids	+	+	-
Quinones	+	+	-
Sterols	+	-	+
Saponins	-	-	-
Cardiotonic Glycosides	+	+	+
Terpene Lactones	-	-	-
Coumarins	-	-	-
Carotenoids	+	+	-

Results are expressed as: +, presence of the metabolite and -, lack of the metabolite.

Flavonoids have been reported as the major constituents of *Passiflora edulis* in several studies. In the fruit, flavonoids schaftoside, isoschaftoside, isoorientin, orientin, isovitexin, luteolin-6-C-chinovoside and luteolin-6-C-fucoside have been previously identified [24]. This last two luteolin-glycosides, luteolin-6-C-chinovoside and luteolin-6-C-fucoside, have been also identified in the fruit juice [25]. Flavonoid luteolin and luteolin-6-C-glucoside from *Passiflora edulis* rind were suggested to have an antihypertensive effect when administrated

to hypertensive rats [9]. A more recent work has described that the anthocyanin fraction of the passion fruit peel extract may has been one of the main responsible for the antihypertensive effect observed for this extract when administrated to spontaneously hypertensive rats [10]. This evidence suggests that flavonoids from the fruit juice of *Passiflora edulis* may have played an important role in lowering blood pressure in previous studies where this extract was used.

The preliminary phytochemical screening revealed the presence of some types of flavonoids in *Passiflora edulis* leaves and fruit, unlike *Petroselinum crispum* where there was no evidence of the presence of these types of metabolites. Several authors have shown that certain flavonoids have ACE inhibitor properties. Some flavan-3-ols [26] and anthocyanins [27] described high percentages of ACE inhibition when evaluated in vitro. Although the mechanisms by which the antihypertensive effect of *Passiflora edulis* might occur have not been clarified, from our results and those from previous works it is possible to suggest that reduced ACE activity in the presence of *Passiflora edulis* fruit juice and leaves ethanolic extract may be due to an inhibition mainly caused by flavonoids, or rather, due to the synergistic effects of different compounds.

In contrast with *Passiflora edulis*, considerably low percentage of inhibition was shown by *Petroselinum crispum* at assay conditions. High ACE inhibition has been previously described for the aqueous extract of this species. Over 50% inhibition was observed when evaluated at 0.33 mg/mL [15]. It is very likely that considerable differences between results from the two studies are due to the type of extract used in each method and to the difference in assay concentrations of the extract. This means, higher extract concentration will imply higher quantity of its constituents, which may contribute to increase ACE inhibition. In addition, environmental conditions of the collection sites, as described previously for *Passiflora edulis*, may also explain this disparity. Despite that in this study no ACE inhibition by *Petroselinum crispum* was found, evidence of ACE inhibition by its aqueous extract would allow to consider this species as a potential source of ACE inhibitors, and, on the other hand, it does not discard this plant as an antihypertensive, given that it can contain in its composition secondary metabolites that may intervene in other hypertension mediating processes. Studies to this regard are limited; however, strong diuretic properties [28] as well as properties as a calcium channel blocker [11] have been reported for this plant. It might be possible that the presence of these compounds is responsible for the antihypertensive effects observed in this plant in traditional medicine. However, it is necessary to broaden *in vitro* and *in vivo* studies to discard it or confirm it as an antihypertensive.

4. CONCLUSION

In conclusion, this study shows a high percentage of ACE inhibition by *Passiflora edulis* juice and leaves extract which leads to consider this species as a potential source of ACE inhibitor secondary metabolites. The juice and the leaves ethanolic extract contained flavonoids, possible secondary metabolites responsible for the inhibition of the enzyme. The potential ACE inhibitor found for *Passiflora edulis* in the current study may well support the antihypertensive properties observed in both the juice and the leaves ethanolic extract from this species in ethnopharmacological and *in vivo* studies; however, complementary studies are needed to demonstrate this relationship. Also, the low percentage of inhibition registered by the ethanolic extract of *Petroselinum crispum* may suggest that it is not an important source of ACE inhibitors although metabolites with ACE inhibition properties may still be present in this species. In a similar way, this does not discard *Petroselinum crispum* as an

antihypertensive, which is why this species is proposed for further studies seeking to determine over what other hypertension mediating mechanism it could act.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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