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Investigation of Angiotensin-Convertings Enzyme Inhibitory Effects of Medicinal Plants Used in Traditional Persian Medicine for Treatment of Hypertension: Screening Study

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ABSTRACT

Background: Angiotensin converting enzyme (ACE) inhibitors are used widely in the treatment of hypertension and heart failure. These inhibitors such as captopril and enalapril are derived from natural products.
Materials and Methods: In the present study 135 plants used in Traditional Persian Medicine have been investigated for their angiotensin converting enzyme (ACE) inhibitory activity. They were selected on the basis of their usage as antihypertensive, cardiotonics & diuretics. Dried powdered plant material was extracted with mix solution of water and ethanol in ultrasonic bath. The extracts were filtered and concentrated in vacuum except for the water extracts, which were freeze-dried. Test solutions were made by dissolving extract in assay buffer, corresponding to a final concentration of 0.33 mg of crude plant extract in 1 ml test volume. Enzyme assay was performed by HPLC method. Plants exhibiting inhibition levels with more than 50% were further tested for the presence of tannins in order to eliminate possible false positives.
Results: In total, 52 Species out of the 135 (39%) screened, gave more than 50% ACE inhibition. Forty Species were found to possess a high ACE inhibiting ability and were low in their tannin content.
Conclusions: Traditional medicine based on certain plants could be of beneficial effects in hypertension treatment.

Implication for health policy/practice/research/medical education:
Introducig of new antihypertensive medications.

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1. Background

Angiotensin I-converting enzyme activity (ACE, peptidyldipeptide hydrolase, kininaseII, EC 3.4.15.1) plays an important role in regulation of blood pressure (1). ACE is an important blood pressure regulator that catalyzes the release of His-Leu from the carboxyl terminal of angio-

tensin I, which, in turn, generates a potent vasopressor octapeptide, angiotensin II. ACE is also involved in the degradation of vasodilator bradikinin (2). Most if not all commercialized ACE inhibitors have utilized peptides from the venom of the Brazilian viper Bothrops Jararaca as model substances(3). Besides this animal source, microorganisms and plants deliver compounds with ACE inhibitory activity which could serve as model substances in the development of new ACE inhibitors. ACE inhibitors prevent the formation of angiotensin II by ACE and thereby reduce peripheral vascular resistance and blood pressure. However, these synthetic drugs are believed to have certain side effects such as cough, taste disturbances and skin rashes (4). Therefore, for safe and economical use, interest in identifying food sources as ACE inhibitor has increased. More potent ACE inhibitors have also been designed and synthesized to treat hypertension effectively. Oral administration of these drugs frequently results in unwanted side effects; a nutritional approach might be a better medium by which blood pressure in controlled.

Screening for antihypertensive effects in traditional medicines has been performed over many years and certain animal models have been utilized (5). In western medicine, drug development has become increasingly more mechanistic in the focus of excluding unwanted side-effects (5). The rationale behind this approach is to identify a molecular target (receptor or enzyme) which has an essential role both in the regulation of the disease and the search for ligands, substrates or inhibitors of the target. In the treatment of hypertension, inhibition of the ACE is established as one the current therapeutic principles.(6)

In Persian the different traditional medicine systems make use of a number of plants for treating of the hypertension. In order to evaluate the biological activity of Persian medicinal plants, this study was conducted to evaluate the antihypertensive activity of some of these plants based on an in vitro bioassay for ACE inhibition. A number of screening studies have been made on the ACE inhibitory activities of higher plants and different screening methods have been used. To researchers, a variety of methods (e.g. spectrophotometry and fluorometry) are available by which ACE activity can be detected and analyzed (7, 8). High performance liquid chromatography (HPLC) is widely used because of its effective separation of the substrate and product from the ACE reaction mixture in order to ensure accurate measurements. More recently, Braga et al. (2000) investigated 20 species used to treat hypertension in Brazilian flora (9); Somanadhan et al. (1999) investigated 73 plant species used in Indian folk medicine to treat hypertension (10); Duncan et al. (1999) reported the result of screening 20 species from Zulu (11); Nyman et al. (1998) screened 75 species belonging to 42 plants family from Indian traditional medicine (12); 54 plants from Reunion Island with alleged antihypertensive & diuretic effects have been investigated by Anderson et al. (1997) (13), and Hansen et al. (1995) reported the result of screening of 31 species from India, China & Chile in which all of them used HPLC assay method for these screening. Arisawa et al. (1985) investigated some 38 samples of crude drugs by means of spectrophotometric & fluorimetric assay (14); Yun et al. (1981) investigated 27 plant species used to treat hypertension in Korean folk medicine (15).

Furthermore, some studies have been made on single plant species where several classes of ACE inhibitory compounds have been identified, for example proanthocyanidins (16-18), flavonoids (18, 19), xanthones (20), peptides (17), and secoiridoids (21) for a comprehensive review of these compounds (22).

To authors' knowledge and research in relevant literature, no documents have been reported in Traditional Persian Medicine regarding the systematically screening for this target. Thus, the present study may represent an opportunity to find new non-chemical agents to develop new antihypertensive drugs.

2. Materials and Methods

2.1. Plants Material

In this study 135 plants used in Traditional Persian Medicine have been investigated for their ACE inhibitory activity. They were selected on the basis of their usage as antihypertensive, cardio-tonics, and diuretics.

Plants material was collected throughout the years 2004 – 2005 from different zones of Persia. They were identified according to Herbarium of Tehran University and a voucher specimen of the plants was deposited in the Herbarium of the Faculty of Pharmacy of Tehran University.

2.2. Preparation of Crude Extracts

All plant materials were air dried in the shade before extraction. After grinding, a 1g weight of the dried plant material was extracted with 10 ml water or ethanol (96%) respectively for 2 hours in an ultrasonic bath. The extract were filtered and concentrated in vacuum except for the water extracts, which were freeze-dried. Then the extracts were kept at 2-6°C (refrigerator).

2.3. Chemicals and Buffers

Hippuryl-L-Histidyl-L-Leucine (HHL) and hipuric acid (HA), were purchased from Sigma; all other reagents were analytical grade.

1) Assay buffer: HEPES-NaOH, 50mM, and pH 8.0 contain 300mM NaCL (11.92g of HEPES and 17.52g of NaCL are dissolved in 900 ml bidistilled water, adjusted to pH 8.0 with NaOH and diluted to liter). Before use, buffer is filtered using a filter with a pore diameter of 0.45μm.

2) Substrate (3.5 mM): 5.2 mg of HHL (Hippuryl-L-Histidyl-L-Leucine) are solved in 1ml assay buffer. This solution should be prepared freshly before each assay.

3) Stop solution (0.1N): 11.92g of HPES are dissolved in 800 ml bidistilled water, adjusted to pH 9.0 with NaOH 1 M and diluted to liter). Before use, solution is filtered us-

ing a filter with a pore diameter of 0.45μm.

2.4. Enzyme

For these studies, a commercially available angiotensin-converting-enzyme preparation from rabbit lung (EC 3.4.15.1-purchased from sigma) has been used.1 unit angiotensin-converting enzyme with the specific activity of 3 units/mg protein is dissolved in 2500μL assay buffer. The enzyme solution is highly active and stable for at least 3 months at 2-6°C (refrigerator).

2.5. Preparation of Crude Extracts

Solutions of inhibitors are made by dissolving 1mg of test compound in 1mL of buffer which may contain up to 10% of an organic solvent such as ethanol. This corresponds to a final concentration of 0.33 mg test compound in 1mL assay volume.

2.6. ACE-assay

ACE activity was assayed according to the method described by Horiuchi et al. (1982) and modified by Schnaith et al. (1994) (23, 24) with some modifications for micro assay. This method is based on the ACE-catalyzed cleavage of the HHL (Hippuryl-L-Histidyl-L-Leucine), into Hippuric acid, which is quantitatively measured by High Performance Liquid Chromatography (HPLC). The reaction can be represented as shown in *Figure 1*. 25 μL of inhibitor solution or assay buffer (for control incubation) are placed into a micro tube and mixed with 25 μL enzyme solution. The micro-tube is placed for 5 min in a thermo-mixer at 37°C and the reaction is started by the addition of 25 μL substrate solution (3.5Mm).After incubation at 37°C, the enzyme reaction is stopped by the addition of 50 μL 0.1N Na2EDTA. The incubation time depends on the activation of the applied enzyme batch. No internal standard was used in this procedure. The incubation mixtures are transferred to HPLC tubes. Assays were performed in duplicate. A blank assay was performed for each extracts tested. (*Figure 2*)

2.7. High Performance Liquid Chromatography (HPLC)

The product (HA) and unreacted substrate (HHL) are separated and quantified by reversed phase HPLC with UV detection at 228nm. (1) Instrumentation: Pump: Knauer, K-1001; Injector: Autosampler, Maraton, 20μL loop; Detector: UV-Visible, K-2501, oprated at 228nm; Integrator: Chromogate. (2) Solvent System: Column: Perfectsill target (125 × 4 mm) and ODS-35 Mobile phase: 10 mM Phosphate buffer (pH = 3) - Methanol HPLC grade (60:40), gradient, Flow rate: 1mL/min, Detection wavelength: 228nm. Using this gradient elution system, the total separation is achieved within 5 min.

2.8. Quantitative Analysis

It was established that the percentage conversion of the substrate by the enzyme was an accurate method to determine the percentage inhibition of the samples. The following formula was used to calculate the percentage of enzyme activity. This method assumed that the control (without the presence of any inhibitors) would have the highest percentage of the ACE activity. A sample exhibiting high inhibition levels would have low percentage of the ACE activity.Using the following equation, the percentage inhibition of the sample could be calculated: Inhibition (%) = 100-Activity (%)

2.9. Tannins Tes

The tannins test was performed by extracting 5g of dry plant material with 50 mL of water or ethanol (96%). After evaporation of the solvents, the extracts were re-dissolved in 13 mL hot water (90-100°C) and allowed to cool to the room temperature. Two drops of 10% NaCL were added to 'salt' out any non-tannin compounds which could cause a false positive reaction. After vacuum filtration, 3 mL of the filtrate was added to each of four test tubes. The following solutions were added to the test tubes:

1. 4-5 drops of 1% gelatin solution,
2. 4-5 drops of 1% gelatin + 10% NaCL solution,

$$Activity(\%) = \left[\frac{Peak\ area(Hippuric\ acid\ Sample) - Peark\ area(Hippuric\ acid\ Blank)}{Peak\ area(Hippuric\ acid\ \ Control^{+})} \right] \times 100$$

Figure 1. HHL (Hippuryl-L-Histidyl-L-Leucine), is cleaved by ACE into Hippuric acid, which can be quantified (HPLC).

$$\text{Sample: } 25\mu l\ Enzyme + 25\mu l\ Inhibitor \xrightarrow{37^{\circ}C,\ 5'} 25\mu l\ Substrate \xrightarrow{37^{\circ}C,\ 35'} 50\mu l\ EDTA$$

$$\text{Blank: } 25\mu l\ Enzyme + 50\mu l\ EDTA + 25\mu l\ Inhibitor \xrightarrow{37^{\circ}C,\ 5'} 25\mu l\ Substrate \xrightarrow{37^{\circ}C,\ 35'}$$

$$\text{Control: } 25ul\ Enzyme + 25ul\ Buffer \xrightarrow{37^{\circ}C,\ 5'} 25ul\ Substrate \xrightarrow{37^{\circ}C,\ 35'} 50ul\ EDTA$$

Figure 2. The incubation methods.

3. 3-4 drops of 10% ferric chloride,
4. Control (no solution added).

The first and second tubes were observed for the formation of precipitate and the third tube was observed for color produced.

The test would be considered negative if test tubes 1 and 2 showed no precipitation or if 3 showed no color formation, and positive if test tubes 1 and 2 showed precipitation and 3 shows color formation (either blue-black for hydrolysable or brownish-green for condensed tannins) (12).

2.10. Statistical Analysis

For statistical analysis ANOVA (Analysis of variance) and t-test in SPSS ver.11 software were used to determine significant differences between groups and $P < 0.05$ were considered significant.

3. Results

3.1. Hippuric acid Standard Curve (Figure 3)

3.2. Linearity of Enzyme Reaction

The time course action of the enzyme was determined by reacting HHL at a concentration of 3.5mM with 1 units of ACE enzyme at varying incubation times. By plotting the percentage of product (HA) against time, it was possible to determine over what time period the enzymatic activity was constant. *Figure 4* shows that the enzymatic rate was constant over a period of 35 min which it would indicate the optimum incubation time from the bioassay.

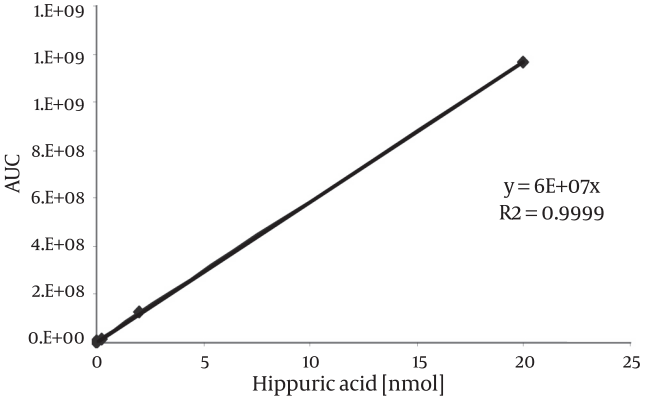

Figure 3. Hippuric acid Standard Curve

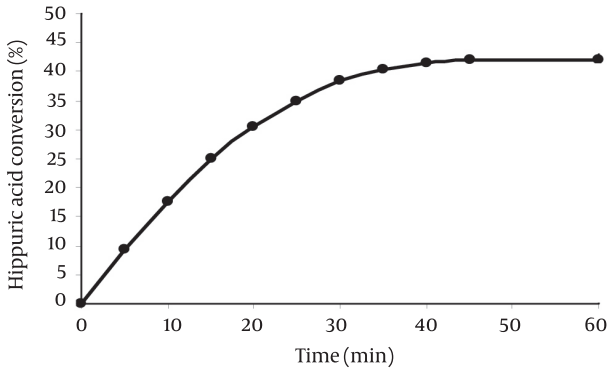

Figure 4. The Time Course of the ACE Reaction

3.3. Establishing the Sensitivity of the ASssay System

The sensitivity of the assay was tested by determination of the IC50 value of the competitive inhibitor captopril and comparing the value cited in the literature (*Figure 5*).The values in the literature were similar to the one determined in this study. From the data, the IC50 value of 20 nM was established. The reference value in the literature for captopril is 23 nM (25).

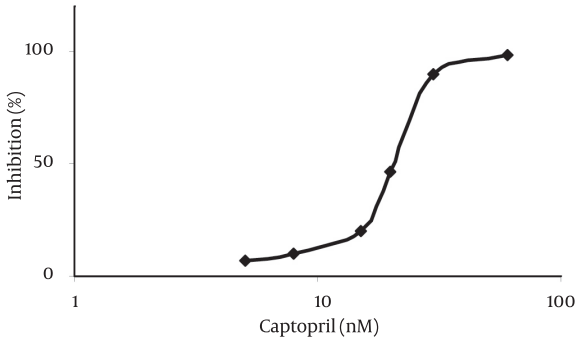

Figure 5. Inhibition of ACE by Captopril at varying concentrations. The IC50 value was determined to be 20 nM

3.4. ACE Inhibitory Activity of Medicinal Plants

The results of the screening are presented in Table 1. In total, 52 Species out of 135 (39%) are screened, have resulted in an ACE inhibition around or above 50%, redering them suitable for closer investigation and isolation of active constituents. these species are further screened for their content of tannins and results are summarized in Table 1. In total, 40 species (29% of total investigated) have been found to possess a high ACE inhibiting ability, not depending on the presence of tannins.

4. Discussion

To obtain the active substances for ACE inhibition the screening was performed on 135 species which were selected on the basis of their usage as antihypertensive, cardio-tonics & diuretics, using water and ethanol (96%) extraction.

As far as possible, the traditionally used part of the plant was employed for the screening. However, in certain cases other parts were also tested. A literature survey on pharmacological activity of all the plants screened for ACE inhibitory activity was carried out and summarized in *Table 1*.

Table 1. Ethnomedical information & Result of the ACE screening.

No.	Scientific Name	Family Name	Plant part used	Traditional use (literature Source)*			ACE Inhibition (%)		Presentce of tannins
				Antihypertensive	Diuretic	Cardiotonic	Water Extract	Ethanol Extract	
1	Achillea millefolium L.	Compositae	Aerial part			1-3	-	-	
2	Achillea santolina L.	Compositae	Flower			1-3	-	-	
3	Adiantum copillus-veneris L.	Polypodiaceae	Leaf			2-5	-	-	
4	Ailanthus altissima(Mill.) Swingle	Simarubaceae	Fruit & Seed			3-8	-	-	
5	Ailanthus altissima (Mill.) Swingle	Simarubaceae	Leaf			3-8	-	1	
6	Alcea digitata (Boiss.)Alef.	Malvaceae	Flower			2-3	63	-	-
7	Alhagi persarum Boiss.8 Buhse.	Papilionaceae	Dried gum resin	9	9		-	51	-
8	Allium cepa L.	Liliaceae	Bulb	1-3-5-6	1-3-5-6	1-2-3-4-6	52	50	-
9	Allium hirtifolium Boiss.	Liliaceae	Bulb	9	8		-	-	
10	Allium porrum L.	Liliaceae	Leaf	6-8	6-8	1-3-6-8	52	62	-
11	Allium sativum L.	Liliaceae	Bulb	1-4-6-7	1-4-6-7	1-3-7	76	68	-
12	Aloe vera L.	Liliaceae	Resin	6-8	6-8		10	3	
13	Aloe vera L.	Liliaceae	Leaf & Gel	6-8	6-8		39	32	
14	Anethum graveolens L.	Umbelliferae	Seed			1-2-3-8	-	-	
15	Apium graveolens L.	Umbelliferae	Seed	6-8	6-8	1-2-3-6-8	-	-	
16	Arctium lappa L.	Compositae	Root			1-3-4-6	-	-	
17	Armeniaca vulgaris L.	Rosaceae	Leaf			1	-	-	
18	Artemisia dracunculus L.	Compositae	Aerial part			1-6	31	50	+
19	Asparagus officinalis L.	Asparaginaceae	Root			1-3	-	68	+
20	Asperugo procumbens L.	Borraginaceae	Aerial part	2	2	2	-	52	+
21	Avena sativa L.	Gramineae	Whole plant	4	4	1-3	2	-	
22	Berberis integerrima Bge.	Berberidaceae	Fruit	3	3	4	-	81	-
23	Berberis vulgaris L.	Berberidaceae	Fruit	3	3	4	65	23	-
24	Brassica napus L.	Cruciferae	Seed			3-5-8	-	-	
25	Brassica oleracea L.	Cruciferae	Leaf	6	6	3-5-8	-	-	
26	Bunium persicum (Boiss.)B. Fedtsch.	Umbelliferae	Seed			1-3-5-8	-	51	-

No.	Scientific Name	Family Name	Plant part used	Traditional use (literature Sorce)*			ACE Inhibition (%)		Presentce of tannins
				Antihypertensive	Diuretic	Cardiotonic	Water Extract	Ethanol Extract	
27	Calendula officinalis L.	Compositae	Whole plant	1	6	6	33	72	-
28	Camellia thea Link.	Theaceae	Leaf		1	9	-	-	
29	Camellia thea Link.	Theaceae	Leaf		1	9	-	-	
30	Cannabis Sativa L.	Cannabaceae	Seed		3-5		3	18	
31	Capsicum annuum L.	Solanaceae	Fruit		1-3		-	1	
32	Cerasus avium (L.) Moench	Rosaceae	Fruit tail	3-9	1-2-3-4-6	9	77	70	-
33	Cerasus vulgaris Miller.	Rosaceae	Fruit tail	3-9	2-3	9	-	-	
34	Cerasus vulgaris Miller	Rosaceae	Fruit	3-9	1-3-8	9	-	60	-
35	Cichorium intybus L.	Compositae	Leaf	3-8	1-2-3-4-6-8	5	7	11	
36	Cichorium intybus L.	Compositae	Root	3-8	1-2-3-4-6-8	5	51	62	+
37	Cichorium intybus L.	Compositae	Seed	3-6-8	1-2-3-4-6-8	5	19	-	
38	Citrullus vulgaris Schard.	Cucurbitaceae	Seed	6	3		19	-	
39	Citrus aurantifolia (Christm.) Swingle	Rutaceae	The peel	1-6	1-6	1-3-6	67	35	-
40	Citrus aurantium L.	Rutaceae	The peel	9		3-6	2	-	
41	Citrus aurantium L.	Rutaceae	Fruit		2-3	3-6	60	56	-
42	Citrus Medica L.	Rutaceae	The peel	3		3-6	-	-	
43	Citrus sinensis(L.) Osbeck	Rutaceae	Leaf		1-6	1-3-6	-	11	
44	Coriandrum sativum L.	Umbelliferae	Seed	6	1	3	-	-	
45	Crocus sativus L.	Iridaceae	Flower	7	3-7	7	64	57	-
46	Cucumis melo L.	Cucurbitaceae	Seed	5	1-3-5		-	-	
47	Cucumis sativus L.	Cucurbitaceae	Fruit	6	1-3-5		-	13	
48	Cucumis sativus L.	Cucurbitaceae	Seed	6	1-3-5		62	13	-
49	Cucurbita pepo Dc.	Cucurbitaceae	Seed		1-3-4	3	-	-	
50	Cydonia oblonga Mill.	Rosaceae	Seed		5-8	9	-	-	
51	Cydonia oblonga Mill.	Rosaceae	Fruit		5-8	9	42	64	+
52	Daucus carota L.	Umbelliferae	Seed	1	2-3-4-5-7	3	-	-	
53	Dracocephalum moldavica L.	Labiatae	Leaf			2	16	-	
54	Echium amoenum Fisch.et Mey.	Boraginaceae	Flower		1	3-6	-	-	
55	Elaeagnus angustifolia L.	Elaeagnaceae	Fruit	3	5	3	10	40	

No.	Scientific Name	Family Name	Plant part used	Traditional use (literature Source)*			ACE Inhibition (%)		Presentce of tannins
				Antihypertensive	Diuretic	Cardiotonic	Water Extract	Ethanol Extract	
56	Equisetum arvense L.	Equisetaceae	Aerial part		1		-	-	
57	Faba vulgaris Moench.	Papilionaceae	Seed		9		50	-	+
58	Ficus carica L.	Moraceae	Fruit	7	3	3-6	53	7	-
59	Foeniculum vulgare Miller.	Umbelliferae	Seed		1-3-5-7	6	56	7	-
60	Foeniculum vulgare Miller.	Umbelliferae	Root		1-2-3-6	6	50	61	-
61	Fumaria parviflora Lam.	Fumariaceae	Aerial part		3-4-8		-	-	
62	Glycyrrhiza glabra L.	Papilionaceae	Root	9	3-6		-	53	-
63	Helianthus annus L.	Compositae	Seed		1	9	61	-	-
64	Helianthus annus L.	Compositae	Flower		4	9	-	-	
65	Helianthus tuberosus L.	Compositae	Bulb	9			71	-	-
66	Hibiscus gossypifolius Mill.	Malvaceae	Fruit	2-9			-	73	-
67	Humulus lupulus L.	Cannabaceae	Flower		1-4		11	40	
68	Hypericum Perforatum L.	Hypericaceae	Aerial part		1-2-3-5		-	66	-
69	Hyssopus officinalis L.	Labiatae	Aerial part	1	1		-	-	
70	Juglans regia L.	Juglandaceae	Fruit		5		-	42	
71	Juglans regia L.	Juglandaceae	Seed		5		-	-	
72	Lactuca sativa L.	Compositae	Seed	6	3-5-6-7	3-6	28	-	
73	Lallemantia royleana (Wall.)Bth	Labiatae	Seed		2	6	-	-	
74	Laurus nobilis L.	Lauraceae	Leaf		1		-	51	+
75	Lavandula officinalis L.	Labiatae	Whole plant	5-6		3	-	-	
76	Lawsonia inermis L.	Lythraceae	Leaf	3-6-7	3		55	68	+
77	Lepidium sativum L.	Curciferae	Leaf	1-6	1-2-3-6-7		21	-	
78	Lippia citriodora H.B et K.	Verbenaceae	Aerial part		2	1-6	-	-	
79	Lippia citriodora H.B. et K.	Verbenaceae	Aerial part		2	1-6	-	-	
80	Malus orientalis Ugl.	Rosaceae	The peel	9	1-3-6	3-5-6	-	63	+
81	Malva sylvestris L.	Malvaceae	Fruit		1-2-3-5-6		-	-	
82	Malva sylvestris L.	Malvaceae	Whole plant		1-2-3-5-6		-	72	-
83	Matricaria chamomilla L.	Compositae	Flower	3-6-7	1-3-5-6-7		5	1	
84	Medicago sativa L.	Papilionaceae	Seed		9		-	69	-

No.	Scientific Name	Family Name	Plant part used	Traditional use (literature Source)*			ACE Inhibition (%)		Presentce of tannins
				Antihypertensive	Diuretic	Cardiotonic	Water Extract	Ethanol Extract	
85	Mentha longifolia (L.) Hudsom	Labiatae	Aerial part		1-5	1	-	-	
86	Mentha piperita L.	Labiatae	Leaf	6	3-6	1-3	-	-	
87	Morus alba L.	Moraceae	Leaf	1	3-5-7		26	53	-
88	Morus nigra L.	Moraceae	Leaf	2	5		67	50	-
89	Nigella sativa L.	Ranunculaceae	Seed		3-4-5		-	-	
90	Ocimum Basillicum L.	Labiatae	Seed	6	3-6-7	3-5-6	-	-	
91	Olea europaea L.	Oleaceae	Leaf	1-2-6	6-7	1	-	-	
92	Origanum vulgare L.	Labiatae	Aerial part		1-2-3	3-6	-	-	
93	Papaver somniferum L.	Papaveraceae	Seed		9	9	28	7	
94	Peganum harmala L.	Zygophyllaceae	Seed	3-9	3-5		72	84	-
95	Pelargonium roseum L.	Geraniaceae	Aerial part	1		1	-	-	
96	Persica vulgaris Miller.	Rosaceae	Leaf		1		-	-	
97	Petroselinum hortense Hoffm.	Umbelliferae	Leaf	6	2-4-6		68	-	-
98	Petroselinum hortense Hoffm.	Umbelliferae	Seed	6	2-4-6		18	-	
99	Piminella anisum L.	Umbelliferae	Seed		1-2-5-7	1	-	-	
100	Pistacia vera L.	Anacardiaceae	The peel			3	-	-	
101	Pisum sativum L.	Papilionaceae	Seed		1-3-7		-	-	
102	Polygonatum orientale Desf.	Liliaceae	Root	1-3	1-2-7	3-6	-	-	
103	Portulaca oleracea L.	Portulacaceae	Seed	6-5	1-3-6-7		5	32	
104	Punica Granatum L.	Punicaceae (Granata-ceae)	Flower	5	3-5-6-7	7	55	-	-
105	Pyrus communis L.	Rosaceae	Fruit	6	1-6	6	-	-	
106	Pyrus communis L.	Rosaceae	Leaf	6	1-6	6	-	-	
107	Rheum ribes L.	Polygonaceae	Steam & Flower			6	-	40	
108	Rhus coriaria L.	Anacardiaceae	Fruit	9	3		-	-	
109	Rosa damascena Mill.	Rosaceae	Flower	5	9	3-5-6	-	-	
110	Rosmarinus officinalis L.	Labiatae	Aerial part		1-3-4	6	-	-	
111	Rubia tinctorum L.	Rubiaceae	Root	6	4		32	69	-
112	Rubus hyrcanus Juz.	Rosaceae	Leaf		1-2-3-6-7		-	60	-
113	Ruta graveolens L.	Rutaceae	Leaf	3-4	1-3-7	3-4	-	-	

No.	Scientific Name	Family Name	Plant part used	Traditional use (literature Source)*			ACE Inhibition (%)		Presentce of tannins
				Antihypertensive	Diuretic	Cardiotonic	Water Extract	Ethanol Extract	
114	Salix babylonica L.	Salicaceae	Leaf		3-6-7	3-6	-	-	
115	Solanum Melongena L.	Solanaceae	Fruit		1-3-6	9	-	-	
116	Solanum nigrum L.	Solanaceae	Fruit		6		19	5	
117	Solanum tuberosum L.	Solanaceae	Bulb		9	9	4	-	
118	Spinacia oleracea L.	Chenopodiaceae	Leaf		2-36		-	-	
119	Spinacia oleracea L.	Chenopodiaceae	Seed		2-3-6		50	64	-
120	Taraxacum officinale Weber	Compositae	Whole plant		1-5-6	9	-	28	
121	Teucrium polium L.	Labiatae	Flower	9		9	-	52	-
122	Tribulus terrestris L.	Zygophyllaceae	Fruit		1-3		56	37	-
123	Trifolium pratense L.	Papilionacea	Seed			3-6	-	-	
124	Trigonella Foenum-graecum L.	Papilionacea	Leaf	1-6	9		55	23	-
125	Trigonella Foenum-graecum L.	Papilionaceae	Seed	1-6	6		-	-	
126	Urtica pilulifera L.	Urticaceae	Seed	2-6			-	66	+
127	Vaccinium Arctostaphylos L.	Ericaceae	Seed	2-6			-	51	-
128	Valeriana officinalis L.	Valerianaceae	Root		9	4-6	-	55	+
129	Viola odorata L.	Violaceae	Flower	5	3-4		3	-	
130	Vitis vinifera L.	Vitaceae (Ampelidaceae)	Leaf	1-6	1-4-6	6	-	35	
131	Zataria multiflora Boiss.	Labiatae	Aerial part		1-3-5		-	-	-
132	Zea mays L.	Gramineae	Fruit	4	1-2-6	1-4	60	75	
133	Zingiber officinale Rocs.	Zingiberaceae	Root		6-7		31	10	
134	Ziziphus vulgaris L.	Rhamnaceae	Fruit	2-6	1-6	3	-	68	+
135	Ziziphus vulgaris L.	Rhamnaceae	Leaf	2-6		3	50	62	-

Literature Source:

1. Zargari, a., Herbal Medicine, vol. 1 to 5, Tehran, University, 1373
2. Amin,GH., Popular Herbal Medicine in Iran, Tehran University,Tehran,1384
3. Hosseini, M., Tofeie Hakim, Mahmoodi book store, Tehran, 1234
4. Velag,J.,Stodola,J.,Herbal Medicine.Ghoghnoos,Tehran,1379
5. Avesina, sh., Ghanoon dar Teb, Soroosh, Tehran, 1364
6. Sajadi, A., 5000 Herbal Prescription, vol.1-2, Tehran, 1374
7. Razi, Z., Alhavi, vol. 21, Medical Science Academy of Iran, Tehran, 1384
8. Razi,Z.,Alhavi, vol. Medical Science Academy of Iran,Tehran,1384
9. Traditional Physician

This study showed that the water extract didn't give the same result as ethanol extract in each spices and this two groups showed significant differences, like the study of Adseren et al. (1997) (13). On the other hand, this is of considerable magnitude, no less when it is taken into account that the alleged effects are not necessarily medicated by ACE inhibition.

We have found a rather high positive hit rate (29%) among this figure which is based on crude extracts and inhibits the enzyme by more than 50% when there is 0.33 mg test compound in 1 ml assay volume, as described by Elbi and Wagner (1991) (17). However, the limit of 50% is a hypothetical value which is difficult to justify since we do not know anything about the compounds present in the crude extracts.

Several crude extracts in the present investigation have given an inhibition in the interval from 35 to 50% and should thus be regarded as negative. Our suggestion is that sample with an inhibition in this interval could well be worth a reinvestigation but this should be based upon a new collection of the plant material.

In some cases, identical plant materials were collected in different localities which also resulted in differences in ACE inhibition (Hibiscus gossypifolius Mill.). Nyman et al. (1998) reached the same conclusion (12); these contradictory results probably arise from differences in the ages of the plants specimens assayed and/or from variations in environmental conditions.

The presence of a strong ACE inhibitory activity, however, does not necessarily imply that the species could provide powerful antihypertensive drugs. As discussed by Wagner et al. (1991) (17), some flavonoids may show an in vitro activity due to the generation of chelate complexes within the active center of ACE. It must be remembered that if the plant exhibits low levels of ACE inhibitory activity, it may act on a different mechanism causing hypertension; as a result, it could be used as an antihypertensive agent.

In conclusion, the screening report in this paper has led to the identification of two species from the Persian flora with high potential antihypertensive activity by inhibition more than that of 80% of ACE activity: Peganum harmala L. and Berberis integrrima Bge. Berberis integrrima Bge is used traditionally in Persian as antihypertensive and, then, our results corroborated its popular use.

Further studies on the pure compounds isolated from the active extracts are necessary, and work in this area is presently going on in our laboratory.

Due to the abundant of plants in Persian, there lies an untapped reservoir of potentially useful chemical compounds; unique templates that could serve as the starting points for analog preparation by chemist, as well as interesting tools that can be applied to achieve a better understanding of biological processes involved in hypertension.

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References

1. Gohlke P, Linz W, Scholkens BA, Kuwer I, Bartenbach S, Schnell A, et al. Angiotensin-converting enzyme inhibition improves cardiac function. Role of bradykinin. *Hypertension*. 1994;**23**(4):411-8.
2. Skeggs LT, Jr., Kahn JR, Shumway NP. The preparation and function of the hypertensin-converting enzyme. *J Exp Med*. 1956;**103**(3):295-9.
3. Cushman DW, Ondetti MA. History of the design of captopril and related inhibitors of angiotensin converting enzyme. *Hypertension*. 1991;**17**(4):589-92.
4. Atkinson AB, Robertson JI. Captopril in the treatment of clinical hypertension and cardiac failure. *Lancet*. 1979;**2**(8147):836-9.
5. Villar A, Paya M, Terencio M. Plants with antihypertensive action. *Fitoterapia*. 1986;**52**:131-45.
6. Hansen K, Nyman U, Smitt UW, Adsersen A, Gudiksen L, Rajasekharan S, et al. In vitro screening of traditional medicines for anti-hypertensive effect based on inhibition of the angiotensin converting enzyme (ACE). *J Ethnopharmacol*. 1995;**48**(1):43-51.
7. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol*. 1971;**20**(7):1637-48.
8. Tsuzuki W KY, Shinokara M. Nippon Shokuhin Kogya Gakkaishi. *Nippon Shokuhin Kogya Gakkaishi*. 1992;**39**:188-92.
9. Castro Braga F, Wagner H, Lombardi JA, de Oliveira AB. Screening the Brazilian flora for antihypertensive plant species for in vitro angiotensin-I-converting enzyme inhibiting activity. *Phytomedicine*. 2000;**7**(3):245-50.
10. Somanadhan B, Varughese G, Palpu P, Sreedharan R, Gudiksen L, Smitt UW, et al. An ethnopharmacological survey for potential angiotensin converting enzyme inhibitors from Indian medicinal plants. *J Ethnopharmacol*. 1999;**65**(2):103-12.
11. Duncan AC, Jager AK, van Staden J. Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors. *J Ethnopharmacol*. 1999;**68**(1-3):63-70.
12. Nyman U, Joshi P, Madsen LB, Pedersen TB, Pinstrup M, Rajasekharan S, et al. Ethnomedical information and in vitro screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). *J Ethnopharmacol*. 1998;**60**(3):247-63.
13. Adsersen A, Adsersen H. Plants from Reunion Island with alleged antihypertensive and diuretic effects--an experimental and ethnobotanical evaluation. *J Ethnopharmacol*. 1997;**58**(3):189-206.
14. Arisawa M NM, Ikeda A, Ueno H, Hayashi T, Morita N. Studies on inhibitory activity of medicinal plants on angiotensin converting enzyme (ACE). *Shoyakugaku Zasshi*. 1985;**39**(3):246-9.
15. Yun H, Chung S, Han B. Screening of plant materials for the inhibitory activities against angiotensin-converting enzyme. *Korean Journal of pharmacognosy*. 1981;**20**:51-4.
16. Inokuchi J, Okabe H, Yamauchi T, Nagamatsu A, Nonaka G, Nishioka I. Antihypertensive substance in seeds of Areca catechu L. *Life Sci*. 1986;**38**(15):1375-82.
17. Wagner H EG, Lotter H, Guinea M. Evaluation of Natural products inhibitors of the angiotensin converting enzyme (ACE). A review between 1980 - 2000. *Pharmaceutical and Pharmaceutical letters ; 1:15-18*. 1991.
18. Wagner H, Elbl G. ACE-inhibitory procyanidins from Lespedeza capitata. *Planta Med*. 1992;**58**(3):297.
19. Hansen K NU, Smitt UW, Adseren A, Christensen SB, Schwantner C, Wagner H. Angiotensin converting enzyme (ACE) inhibitory flavonoids from Erythroxylum laurifolium. *Phytomedicine*. 1996;**2**:313-7.
20. Chen CH, Lin JY, Lin CN, Hsu SY. Inhibition of angiotensin-I-converting enzyme by tetrahydroxyxanthones isolated from Tripterospermum lanceolatum. *J Nat Prod*. 1992;**55**(5):691-5.
21. Hansen K AA, Christensen SB, Jensen SR, Nyman U, Smith UW.

Isolation of an angiotensin converting enzyme (ACE) inhibitor from olea eurapaea and olea lancea. *Phytomedicine*. 1996;**2**:319-25.
22. Hansen K. Invitro Screening for the Detection of angiotensin converting enzyme (ACE) inhibitors in selected Medicinal plants and Identification of the Active principles [PhD]. Copenhagen: The Royal School of pharmacy; 1995.
23. Horiuchi M, Fujimura K, Terashima T, Iso T. Method for determination of angiotensin-converting enzyme activity in blood and tissue by high-performance liquid chromatography. *J Chromatogr*. 1982;**233**:123-30.
24. Schnaith E BR, Buckner B, Klein RM, Rick W. 1994; 227:145-158. Optimized determination of angiotensin I-Converting enzyme activity with hippuril-L-histydyl-L-leucine as substrate. *Clin Chim Acta*. 1994;**227**:145-58.
25. Ondetti MA. Structural relationships of angiotensin converting-enzyme inhibitors to pharmacologic activity. *Circulation*. 1988;**77**(6 Pt 2):I74-8.