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Inhibition of Angiotensin I Converting Enzyme (ACE) By Specimens of *Senna* from Brazilian Northeast

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ABSTRACT

Purpose: To screen plant extracts and identify potentially useful health beneficial substances and to increase the knowledge of the chemical composition of Brazilian medicinal plants. **Methods**: The pontential anti-hypentersion capacities of methanol extracts of four *Senna* species from different locations in northeastern Brazil and isolated and identified pure compounds from *Senna georgica* extracts were evaluated in vitro by their ability to inhibit angiotensin I converting enzyme (ACE) using analytical reverse-phase high performace liquid chromatography. **Results**: All tested methanolic extracts of *Senna* species showed appreciable inhibition of ACE, with the exception of *S. georgica* and *S. splendida* leaf extracts which showed only weak inhibition. Root and leaf extracts of *Senna gardneri* displayed high ACE inhibition capacity with IC50 values of 27.82 µg/mL and 12.25 µg/mL respectively. The pure compounds tested all showed ACE inhibition capacity to various degrees, with the exception of resveratrol glucoside. Trans -3,3′,5,5′-tetrahydroxy- 4-methoxystilbene of ACE with an IC50 of 467µM, followed by butein, resveratrol and oxyresveratrol with IC50 values of 624, 750, 951 µM respectively.

INTRODUCTION

The use of angiotensin-converting enzyme (ACE) inhibitors is well established as one of the main therapeutic agents for the treatment of hypertension. ACE is a component of the renin–angiotensin–aldosterone system. It plays a key role in the homoeostatic mechanism of mammals, by contributing to the maintenance of normal blood pressure and to the electrolyte balance, and by being involved in the regulation and control of the arterial pressure. ACE inhibitors are also employed for the prophylactic control of diabetic nephropathy and for the treatment of heart failure. Several ACE inhibitors, such as captopril, enalapril, lisinopril and temocapril, are now in clinical use for the treatment of hypertension. An advantage of the ACE inhibitors over other anti-hypertensive drugs is a reduction of any CNS side effects. However, all the synthetic ACE inhibitor drugs produce some side effects, such as coughing, rashes and taste aversion, thus justifying the search for new natural ACE inhibitors that may be safer and also more economical to use (Chen et al., 2009).

The genus *Senna* m*ill* belongs to the tribe Cassieae Bronn, subtribe Cassinae Irwin & Barneby, together with the genus *Cassia*, are important sources of substances with great structural diversity in the Fabaceae family (Irwin,1982). *Senna* species are used traditionally as a laxative and purgative. Other various relevant activities such as antimicrobial, analgesic, antiparasitic, insecticidal, antitumor and hepatoprotective are proven for several species of *Senna*, which demonstrates the pharmacological potential of this genus (Viegas et al, 2006).

To screen plant extracts and identify potentially useful health beneficial substances and to increase the knowledge of the chemical composition of Brazilian medicinal species of *Senna* were selected namely *Senna gardneri, S. georgica, S. splendida and S. macranthera.* A literature review revealed a complete absence of phytochemical, biological and pharmacological studies performed on this species of *Senna*, is known literature survey on the chemical constituents of the genus *Senna* revealed the presence of secondary metabolites, especially important class of bioactive compounds, such as flavonoids kaempferol, quercetin, rhamnetin, epiafzelechin, catechin, apigenin e luteolin (Vats S., Kamal R., 2014; BAHORUN *et al* 2005) anthracene derivatives, antraquinones (crisofanol, emodine, cassiamine C, cassiamine A, cassiamine B, aloe-emoidne, bianthraquinones) (Koyama et al., 2001; DOS SANTOS et al, 2008), steroids and stilbenoids biologically active.

MATERIAL AND METHODS

Reagents

Angiotensin I converting enzyme (ACE), captopril, trifluoroacetic acid (TFA), HCl, Hippuric acid, *N*-hippuryl-L-histidyl-L-leucine (HHL), sodium chloride and Trizma base, were obtained from Sigma-Aldrich (Deisenhofen, Gemany); acetonitrile from Fluka/Riedel de Haen (Seelze, Germany); acetic acid, DMSO, n-hexane from Merk (Darmstadt, Germany); while resveratrol, oxyresveratrol, resveratrol glucoside and Butein were obtained from Extrasynthese (Lyon nord, Genay, France). Methoxyoxyresveratrol were purified from *Senna georgica* roots extract. All solutions were made up in double distilled water, DMSO or methanol.

Plants (Species of *Senna)*

The plants were collected in several locations of Ceará/Brazil as reported in Table 1, and identified by, Biology Department, Universidade Federal do Ceará (UFC). Voucher specimens of the collected plants are deposited in the Prisco Bezerra Herbarium, Universidade Federal do Ceará, Brazil.

Extract from *Senna* species

The samples were carefully separated to constant weight. The Material (5g) was extracted with hexane in a soxlet apparatus (3 h) to remove lipid. After drying, the solids were extracted with methanol (3 x 3 h) as described by Owen et al. (2000a-d, 2003a,b). Organic solvent was removed by rotary evaporation at 35°C in vacuum.

Identification and isolation of phenolic compounds from methanol extracts of *Senna species*

HPLC-ESI-MS

The identification of phenolic compounds in the methanol extracts of *Senna species,* was conducted on HPLC-ESI-MS Agilent 1100 HPLC, coupled to an Agilent single quadrupole mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Chromatographic separation of solutions in methanol (10 µL) was conducted using a C18, reverse-phase (5µm), column (250 x 4 mm I.D.; Phenomenex, Germany). The mobile phase (1.0 mL/min) consisted of 2 % acetic acid in water (solvent A) and acetonitrile (solvent B) with the following gradient: initially 95 % A for 10 min; to 90 % A in 1 min; to 60 % A in 9 min; to 80 % A in 10 min; to 60 % A in 10 min; to 0 % A in 5 min; and continuing at 0 % A until completion

of the run. Detection of phenolic compounds was by means of UV absorbance (A) at 278, 259 and 340 nm at room temperature. Mass spectra in the negative-ion mode were generated under the following conditions: fragmenter voltage, 100V; capillary voltage, 2500 V; nebulizer pressure, 30 psi; drying gas temperature, 350 °C; and mass range, 100-1500 D. Instrument control and data handling were performed with the same software as for analytical HPLC.

Semi-preparative HPLC

Semipreparative HPLC was conducted on a HP 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a similar C-18 column (10 mm i.d.) as for analytical HPLC. Peaks eluting from the column were collected on a HP 220 Microplate Sampler and subsequently freeze-dried. For the separation of individual compounds in the extracts, the mobile phase consisted of 0.2 % acetic acid in water (solvent A) and acetonitrile (solvent B), utilizing the following solvent gradient over a total run time of 50 min: initially 95 % A for 1 min; to 90 % A in 9 min; to 85 % A in 10 min; to 80 % A in 10 min; to 0 % A in 5 min; and continuing at 0 % A until completion of the run. The flow rate of the mobile phase was 3 mL/min. Peaks eluting from the column were collected on an Agilent HP 220 Microplate Sampler. Each purified fraction was pooled, and the solvent was removed by lyophilization.

Quantitative HPLC determination of hippuric acid

For the analysis of hippuric acid from HHL by analytical HPLC, a similar column was used, and the mobile phase consisted of 0.05 %TFA in deionized water (solvent A) and 0.05 % TFA in acetonitrile (solvent B) with the following gradient: 95 % A to 60 % B in 10 min, maintained for 2 min, followed by a return to 5 % B in one minute and equilibration for 5 minutes. Detection was by UV absorbance (A) at 228 nm with a flow-rate of 1.0 mL/min. Instrument control and data handling were performed with the HP Chemstation software on a PC. In order to determinate the IC_{50} of sample, data were adjusted to a non-linear regression

Angiotensin I Converting Enzyme assay

The activity of ACE was determined using the substrate HHL (5mM-2.15mg/mL) dissolved in 50 mM Tris-HCl (pH 8,3) containing 0,3 M NaCl. Inhibitor solutions (25 µL dissolved methanol) were added to the substrate solution (100 µL) and incubated at 37°C for 10 minutes. ACE solution (10 µL, 200 mU/mL) in 50 mM Tris-HCl, pH 8.3 containing 0.3 M NaCl was

added and the mixture was incubated at 37°C for 30 min. with continuous agitation at 450 rpm. The reaction was stopped by addition of HCl (100 µL). The reaction was analysed by analytical reverse phase HPLC. The antihypertensive agent Captopril was used as positive control at concentrations of 2 ng/mL where either methanol was used as negative control. The IC_{50} value was defined as the concentration of inhibition required to inhibition 50 % of the ACE activity under the assay conditions and was determined by regression analyses of the ACE inhibition (%) versus the log of the inhibitor concentration.(Lahogue et al., 2010).

RESULTS AND DISCUSSION

Identification of phenolic of *Senna*

The species of *Senna* was identified, fractionated and was separated by semi-preparative HPLC. The UV spectra of the purified compound (Figure 1 and 2) were confirmed by nano-ESI-MS. The structure was confirmed unambiguously by NMR experiments at 600 mHz.

Inhibition of ACE by extracts and phenolics of *Sennas*

The potential antihypertensive activity of plant *Sennas* species occurring in Brazil was evaluated by the inhibition of the angiotensin converting enzyme (ACE), using a HPLC assay, the assay system was calibrated with captopril, a positive control and it showed ACE inhibitory activity with IC_{50} value of 6.85 nM.

Of the 12 methanol extracts (Table 2) from the four *Senna* species which were investigated the methanol extract of *S. gardneri* leaves displayed the highest percentage of ACE inhibitory activity (99 ± 2.89% at a concentration of 10 mg/mL) and exhibited a significant IC_{50} value of 12.25 µg/mL, followed by the extracts of *S. gardneri* root (98 ± 3.76% at a concentration of 10 mg/mL) with a significant IC_{50} value of 27.82 µg/mL, *S. macranthera* leaves (98 ± 1.06 % at a concentration of 10 mg/mL) with a IC_{50} value of 37.61 µg/mL and *S. macranthera* roots (98 ± 3.32 % at a concentration of 10 mg/mL) IC_{50} value of 80.38 µg/mL.

S. macranthera bark (96 ± 4.13% at a concentration of 10mg/mL), with a IC_{50} value of 93.53 µg/mL, *S. splendida* flowers (90 ± 2.05% at a concentration of 10mg/mL) IC_{50} value of 113.58 µg/mL, *S. georgica* Bark (97 ± 3.72% at a concentration of 10mg/mL) IC_{50} value of 86.94

μg/mL, *S. splendida* root (84 ± 2.43% at a concentration of 10mg/mL) IC_{50} of 301.96 μg/mL, *S. splendida* bark (89 ± 3.79% at a concentration of 10mg/mL) IC_{50} value of 55.49 μg/mL.

The extracts of *S. georgica* and, *S. splendida* leaves showed only weak inhibition activity with percentage ACE inhibitory activities lower than 60% at a concentration of 10 mg/mL.

These data show the methanolic extracts of leaves and root of *Senna gardneri* have a significantly higher ACE inhibitory activity than the seed extract *Cassia tora* (34.21μg/mL). (Sook et al, 2009). Although the ACE inhibitory activities of the extracts were significantly less than that of captopril, it was still evident that contents of the *Senna* extracts had potential ACE inhibitory activities.

The ACE inhibitory activity of the pure compounds was tested by hippuric acid HHL using HPLC monitoring and evaluated for their IC_{50} values. The stilbene trans -3,3′,5,5′-tetrahydroxy-4-methoxystilbene showed good ACE inhibitory capacity with an IC_{50} of 128μg/mL (468μM), followed by the chalcone butein with an IC_{50} of 170 μg/mL (624 μM), resveratrol with an IC_{50} of 171μg/mL (745μM) and oxyresveratrol with an IC_{50} of 232μg/mL (951μM) whereas resveratrol glucoside showed activity lower than 60%, at a concentration of 1mg/mL (Table 3).

CONCLUSION

In conclusion, our survey disclosed plant species of *Senna* from Brazilian Northeast which are potential sources of angiotensin-converting enzyme inhibitors. Among the assayed species, the methanolic extracts of *Senna gardneri* presented in this study are promising compounds with antihypertensive activity. Thus study suggests the search for bioactive compounds in the most promising species.

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Table 1: Families and species selected for the present study

Species	Collection	Voucher number
Senna georgica H.S. Irwin & Barneby	Pico Alto Guaramiranga/Ceará-Brazil	41630/41631.
Senna gardneri H. S. Irwin & Barneby	Viçosa do Ceará/Ceará-Brazil	47385
Senna splendida (Vogel) H. S. Irwin & Barneby	Tianguá/Ceará-Brazil	47388
Senna macranthera var pudibunda (Benth.) H.S. Irwin & Barneby	Crato/Ceará-Brazil	54170

Table 2: Inhibition of Angiotesin I converting enzyme (ACE) by extracts of *Senna* purified compounds and identified compounds.

Extract of Sennas	IC_{50}(µg/mL)
Captopril (positive control)	6.85nM
S. georgica leaf	**
S.georgica root	159,6
S. georgica bark	86,94
S. splendid flowers	113,58
S.splendida bark	55,49
S. splendid leaf	**
S. splendida root	301,96
S. gardneri root	27,82
S.gardneri leaf	12,25
S. macranthera leaf	37,61
S. macranthera root	80,38
S. macranthera bark	93,53

** Percentage of ACE inhibitory activity lower than 60%at a concentration of 10mg/mL.

Table 3: Inhibition of Angiotesin I converting enzyme (ACE) by purified compounds from *Senna*.

Pure compounds	IC50 (µg/mL) and (µM)
Captopril (positive control)	6.85nM
Resveratrol	171,11µg/mL (749,62µM)
Methoxyoxyresveratrol	127,9µg/mL (467µM)
Oxyresveratrol	232,16µg/mL (951,48µM)
Resveratrol glucoside	
Butein	169,91µg/mL (624,1µM)

** Percentage of ACE inhibitory activity lower than 60% at a concentration of 1mg/mL.

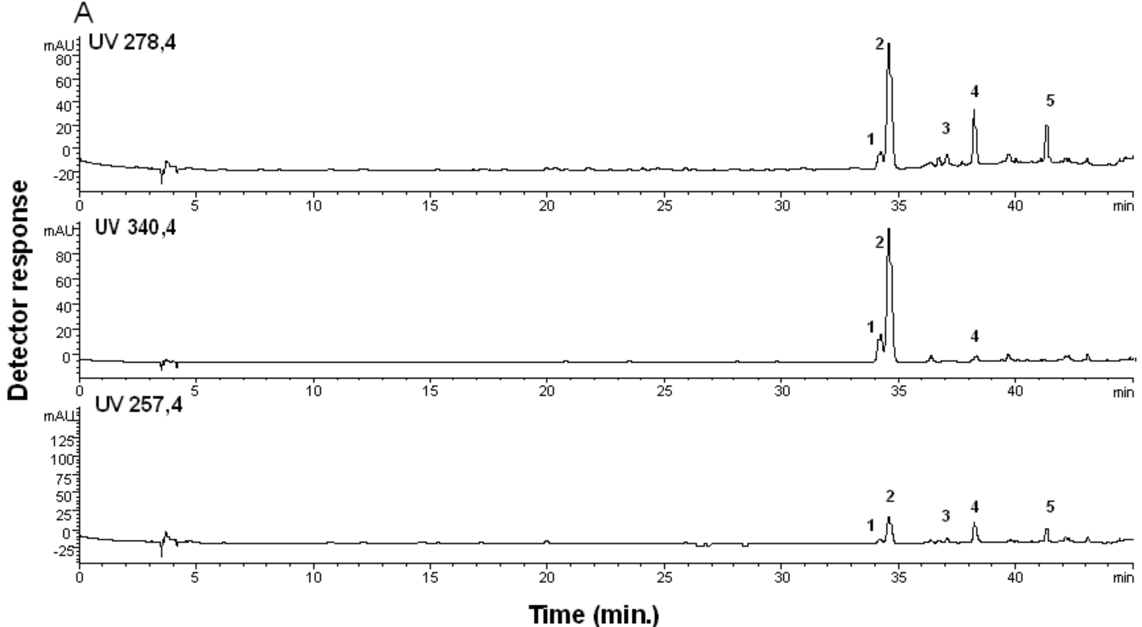

Figure 1: Analytical HPLC chromatogram of a methanolic extract of *Senna georgica* root at (278, 340 and 257 nm). The identity of the peaks is (1) Oxyresveratrol; (2) trans - 3,3′,5,5′-tetrahydroxy- 4-methoxystilbene; (3) Cis-3,3′,5,5′-tetrahydroxy- 4-methoxystilbene; (4) Butein diglycoside isomer; (5) Butein glucoside isomer

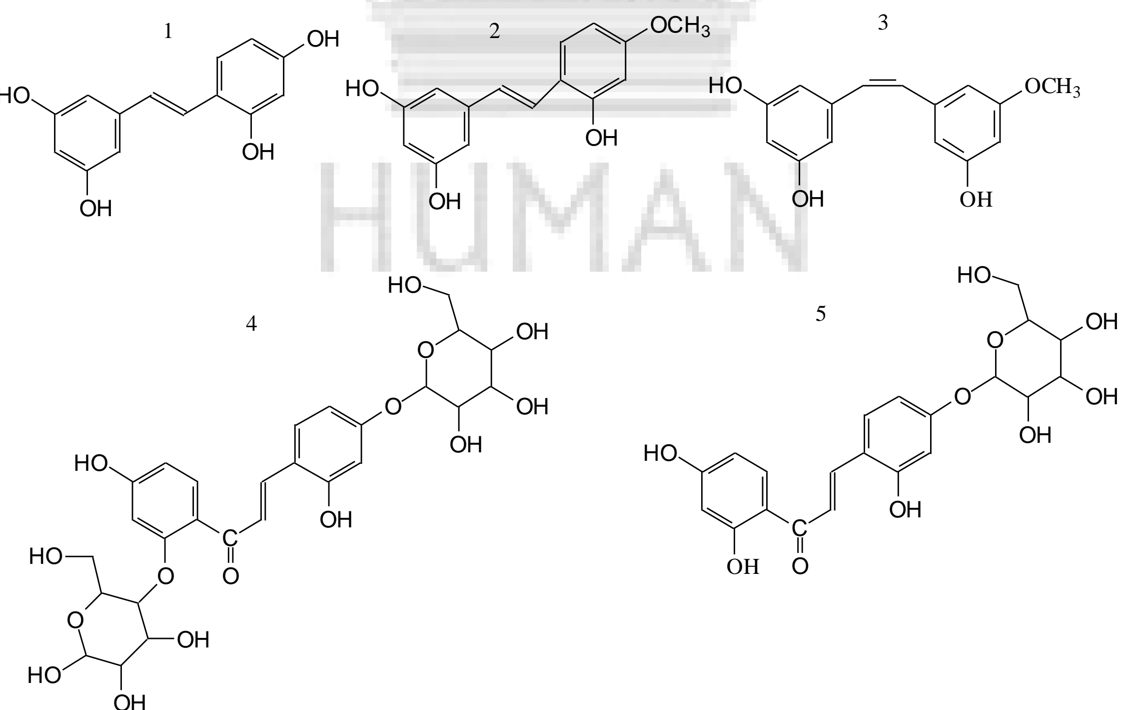

Figure 2: Purified compounds from *Senna georgica*