Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF FOUR SPECIES OF *SENNA* MILL. FROM NORTHEAST BRAZIL

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Received: 09 Mar 2014 Revised and Accepted: 11 Apr 2014

ABSTRACT

Objective: The present investigation evaluated the antioxidant and anticancer properties and total phenolic contents of four species of *Senna*: *S. gardneri*, *S. macranthera*, *S. splendida* and *S. trachypus* from northeast Brazil.

Methods: Ethanolic extracts of leaves and roots of the four *Senna* species were screened for phytochemical procedures. An *in-vitro* antioxidant study was conducted by means of DPPH and ABTS radical scavenging assays. Anticancer activity was evaluated using the MTT 3-(4,5-dimethylthiazole-2-yl)-2,5- diphenyltetrazolium bromide) method against the HCT-116, SF-295 and OVCAR-8 cancer cell lines. Total phenolic contents were determined using a Folin-Ciocalteau colorimetric assay.

Results: Anthraquinones, flavonoids, phenolics, steroids, tannins, triterpenoids, xanthones, and glycosides were detected in all *Senna* extracts. The results for the antioxidant activity showed that the highest percentage of scavenger radicals was present in the extract from roots of *Senna trachypus* (StR), which showed higher levels than the two standards used. The highest percentage of inhibition of the cancer cell lines tested was obtained with the leaf extracts of *S. gardneri* (SgL) and *S. splendida* (SsL).

Conclusion: The ethanolic extracts of the four species of *Senna* showed antioxidant activity in both assays, and this activity can be attributed to the presence of phenolic compounds such as flavonoids and anthraquinones. *S. trachypus* showed a higher antioxidant potential than the standards, as well as a higher total phenolic content. Only two of the plants showed promising results for anticancer activity.

Keywords: *Senna gardneri*, *Senna. macranthera*, *Senna splendida, Senna trachypus*, Antioxidant, Anticancer, Phenolic compound.

INTRODUCTION

Brazilian traditional and folk medicine has long made use of plants and plant-derived phytomedicines to treat a wide range of illnesses [1]. Some studies have investigated the potential of plant products to serve as antioxidants and anticancer agents [2]. Antioxidants are natural or synthetic molecules that even in low concentrations can prevent or reduce the extent of oxidative damage. Antioxidant agents act through different mechanisms, such as by complexing metal ions, the capture of free radicals, and the decomposition of peroxides [3]. Cancer is responsible for a significant and growing number of fatal cases, representing the second leading cause of death of the world's population. As an alternative to chemotherapy, plants offer the possibility of finding substances of therapeutic interest for the treatment of cancer. About 60% of anticancer drugs used in current therapy have their origins in natural products [4].

The genus *Senna* Mill. belonging to the tribe Cassieae Bronn, subtribe Cassiinae Irwin & Barneby is one of the most species-rich of the family Fabaceae. Its species occur as trees, shrubs or subshrubs [5] in great diversity and wide distribution, with several species of economic importance for the production of medicinal substances. The members of this genus characteristically contain anthraquinones and alkaloids, which are substances with antioxidant and anticancer activities [6,7]. This study involved testing the antioxidant activity and cytotoxicity *in-vitro* and the total phenolic content of four native species of *Senna* from northeast Brazil.

MATERIALS AND METHODS

Plant materials

Specimens of the *Senna* species were collected in Chapada Ibiapaba, Ceará, Brazil. Voucher specimens of *S. gardneri* H.S. Irwin & Barneby (47.385), *S. macranthera* H.S. Irwin & Barneby (47.384), *Senna splendida* H.S. Irwin & Barneby (47.387) and *S. trachypus* H.S. Irwin & Barneby (47.377) have been deposited in the Herbarium of the Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil.

Extraction and Phytochemical screening

The plant material was separated into its various parts, and the roots and leaves were dried at room temperature, crushed, and extracted sequentially with hexane and ethanol. The solvents were removed and the residues were dried and stored at 27 °C. Phytochemical screening was performed with the hexanic and ethanolic extracts, using procedures described by Harborne (1973) [8] and Matos (2009) [9]. Antioxidant capacity, anticancer activity and total phenolic content were evaluated for the ethanolic extracts.

Antioxidant capacity

DPPH radical scavenging assay

The antioxidant capacity was evaluated using the method of scavenging the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, which is based on a photometric assay of DPPH, which forms a purple solution in alcohol and when reduced in the presence of antioxidant molecules, forms 1,1-diphenyl-2-picrylhydrazyl, which is colorless. The assays were performed according to the methodology described by Brand-Williams (1995) [10]. The sample solutions were prepared at a concentration of 1.0 $mg.mL^{-1}$in MeOH. Five concentrations ranging from 100 to 500 $mg.mL^{-1}$ were tested. Aliquots of 0.1 mL of solutions of the samples were placed in individual labeled eppendorf vials, and 0.9 mL of the DPPH solution was added to each (100 $\mu mol.L^{-1}$). The absorbance of this solution was measured in triplicate at 515 nm in an Elisa Thermoplate Reader. Readings of at least ten concentrations (10 to 1000 $\mu g.mL^{-1}$)

were obtained 30 min after the start of the test. BHT and quercetin were used as standard solutions. The IC_{50}, the concentration of sample or standard causing 50% inhibition of the initial concentration of DPPH, was determined by linear regression. For the plot points, the mean values obtained from three replicates of each test were used.

ABTS radical scavenging assay

The ABTS+• method [2,2-azino-bis (ethylbenzothiazoline-6-sulfonic acid) diammonium salt] is based on the generation of ABTS+•, which forms a blue-green color by the reaction of ABTS+•, with potassium persulfate, with maximum absorption at 645, 734 and 815 nm. With the addition of an antioxidant, ABTS+• is reduced to ABTS, causing loss of color from the reaction medium. With the loss of color, the percentage inhibition of ABTS+• is determined from Trolox, a standard subject to the same conditions of antioxidant analysis. The ABTS+• assay was described by Re et al. (1999) [11]. A solution of the extract was prepared at a concentration of 600 $mg.L^{-1}$, and then in the dark, an aliquot was transferred to 30 µL of the diluted extract in a tube with 3.0 mL of ABTS • + radical, and homogenized. The reading was performed at 765 nm and the assay was performed in triplicate. Curves were prepared with Trolox standard solutions, and the results were expressed as TEAC (Trolox equivalent antioxidant activity) in µmol TEAC g^{-1} sample.

Total phenolic content

The phenolic content was determined by the colorimetric method described by Bonoli (2004) [12]. Extracts (30 mg) were dissolved in methanol and transferred to a 50 mL volumetric flask, and then the final volume was completed with methanol. A 100 mL aliquot of this solution was stirred with 500 µL of Folin-Ciocalteau reagent and 5 mL of distilled water for 1 min. After this period, 2 mL of 15% Na_2CO_3 was added and stirred for 30 s. The volume was adjusted to 10 mL with distilled water. Readings were taken at 765 nm in a UV/VIS spectrophotometer. For quantification, a standard curve was used with a solution of gallic acid at concentrations of 10 to 350 mg/L, and the absorbance of the samples was compared to the values obtained for gallic acid. The total polyphenol content was expressed as mg of gallic-acid equivalent (GAE), using a derived equation from the calibration curve: $y = 0.0102x - 0.0202$ and $R^2 = 0.9997$.

Anticancer activity assay

The tumor cell lines OVCAR-8 (ovarian carcinoma), HCT-116 (human colon) and SF-295 (human glioblastoma) used in this experiment were provided by the National Cancer Institute (USA). The leaf and root extracts of *Senna* species were diluted in DMSO at a concentration of 50 $mg.mL^{-1}$, plated, and soon after the cells were added, at a concentration of 0.1 x 106 $cells.mL^{-1}$ for OVCAR-8 and 0.7 x 105 $cells.mL^{-1}$ for the HCT-116 and SF-295 lineages. These were incubated with the extracts for 72 h in 5% CO_2 at 37°C. Following this treatment, the cells were washed and fresh medium was added. The MTT dye solution (150 µL) was added to each well for 3 h. The absorbance was measured after the precipitate was dissolved with 150 mL of pure DMSO, in a plate spectrophotometer at 595 nm [13]. The experiments were repeated independently three times. The results are expressed as percentage of cell viability.

RESULTS

Extraction and Phytochemical screening

The percentage yield (w/w) of the ethanol extraction was determined: *SgL: Senna gardneri*-leaves (39.66%); *SgR: Senna gardneri*-roots (32.53); *SmL: Senna macranthera*-leaves (46.97); *SmR: Senna macranthera*-roots (44.79); *SsL: Senna splendida*-leaves (40.75); *SsR: Senna splendida*-roots (50.13); *StL: Senna trachypus*-leaves (42.57); *StR: Senna trachypus*-roots (29.38). The secondary metabolites anthraquinones, flavonoids, phenolics, steroids, tannins, triterpenoids, xanthones, and glycosides were detected in all *Senna* extracts. The phytochemical analyses found no alkaloids, anthocyanins or anthocyanidins in the extracts.

Antioxidant capacity and Total phenolic content

The results obtained for antioxidant activity using two different methods (DPPH and ABTS radical scavenging assays) and the total content of phenolic compounds are shown in Table 1 and Figure 1.

Table 1: Antioxidant activity and total content of phenolic compounds of *Senna* extracts.

Ethanolic extracts	ABTS TEAC[a]	DPPH - IC_{50}* ($mg.mL^{-1}$)	TPC mg GAE[b]. g^{-1} dry extract
SgL	47.91 ± 5.25	0.089 ± 0.057	338.76 ± 7.09
SgR	57.13 ± 1.15	0.396 ± 0.057	214.25 ± 4.42
SmL	26.72 ± 5.30	0.424 ± 0.032	207.71 ± 4.63
SmR	53.72 ± 1.30	0.534 ± 0.041	122.09 ± 9.62
SsL	29.63 ± 4.95	0.286 ± 0.044	148.24 ± 4.90
SsR	36.02 ± 3.14	0.502 ± 0.033	146.60 ± 4.63
StL	30.71 ± 8.60	0.401 ± 0.018	322.09 ± 2.83
StR	64.47 ± 2.35	0.253 ± 0.001	1277.34 ± 79.54
BHT	-	0.371 ± 0.031	-
Quercetin	-	0.349 ± 0.048	-

SgL: Senna gardneri-leaves; *SgR: Senna gardneri*-roots; *SmL: Senna macranthera*-leaves; *SmR: Senna macranthera*-roots; *SsL: Senna splendida*-leaves; *SsR: Senna splendida*-roots; *StL: Senna trachypus*-leaves; *StR: Senna trachypus*-roots., [a]TEAC: Trolox Equivalent Antioxidant Capacity (mmol Trolox equivalent/g dry extract); [b]IC50: Concentration of DPPH radical-scavenging activity in 30 min; [c]TPC: Total Phenolics compounds; [d]GAE: Gallic-Acid equivalents.

Table 2: Cytotoxic activity of *Senna* extracts against human cancer cells (% inhibition ± SD)

Ethanolic extracts		HTC-116*	SF-295*	OVCAR-8*
Senna gardneri	Leaves	62.91 ± 7.31	59.91 ± 5.44	36.93 ± 1.64
	Roots	58.55 ± 6.16	63.23 ± 1.71	38.49 ± 5.33
Senna macranthera	Leaves	42.73 ± 8.66	43.90 ± 1.55	11.07 ± 0.49
	Roots	15.07 ± 4.94	17.26 ± 2.56	0.00 ± 0.01
Senna splendida	Leaves	60.59 ± 2.37	63.15 ± 4.05	45.86 ± 0.66
	Roots	5.86 ± 4.62	13.98 ± 1.76	0.00 ± 0.01
Senna trachypus	Leaves	16.43 ± 3.55	22.91 ± 13.96	23.40 ± 2.36
	Roots	21.60 ± 5.84	0.00 ± 0.01	0.00 ± 0.01

*HCT-116 (colon - human), SF-295 (glioblastoma - human) and OVCAR-8 (ovarian carcinoma)

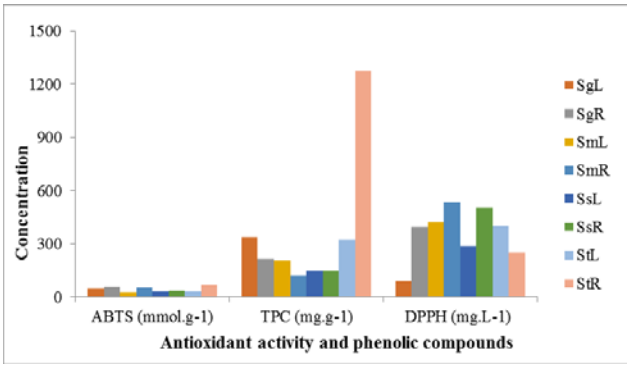

Fig. 1: Antioxidant activity and phenolic compounds of ethanolic extracts of *Senna* species

SgL: Senna gardneri-leaves; *SgR: Senna gardneri*-roots; *SmL: Senna macranthera*-leaves; *SmR: Senna macranthera*-roots; *SsL: Senna splendida*-leaves; *SsR: Senna splendida*-roots; *StL: Senna trachypus*-leaves; *StR: Senna trachypus*-roots.

Anticancer activity

The cytotoxic activity of *Senna* extracts against human cancer cells is presented in Table 2.

DISCUSSION

We studied the antioxidant activity and cytotoxicity *in-vitro* as well as the total phenolic content of four native species of *Senna* from northeast Brazil. Both activities are well known, but research on important therapeutic agents from plant sources is increasing because of the roles of these agents in the prevention or treatment of various human diseases.

The results for antioxidant activity of the *Senna* extracts showed that especially the root extract of *Senna trachypus* (StR) showed higher levels of free radical scavenging activity than the two standards used (BHT and quercetin). The linear correlation between antioxidant activity and total phenolic content found for *Senna trachypus* suggests that these compounds are mainly responsible for the strong antioxidant potential of this plant. The leaf extract of *Senna gardneri* (SgL) exhibited strong antioxidant activity in the DPPH assay, with the lowest IC_{50} of the four species. These data show that the compounds in the bioactive extracts can act as radical scavengers because of their ability to act as hydrogen donors [14, 15]; this activity probably results from the presence of the anthraquinones and flavonoids detected in the phytochemical screening. These compounds contain hydroxyl groups that are able to donate hydrogen, stabilizing the DPPH radical, thus forming substances with excellent antioxidant properties. Other species of the genus also contain compounds with proven antioxidant activity, such as resveratrol, which was isolated from the root acetone extract of *Senna italica* [6].

The MTT method is based on the ability of the mitochondrial enzyme succinate-dehydrogenase, present in the strains tested, to transform the MTT tetrazolium salt to a blue-colored product called formazan, which is proportional to the number of viable cells present [16]. The experiments of cytotoxicity *in-vitro* for the species of *Senna* yielded only modest results, given that only percentages above 75% are considered sufficient to justify performing more-detailed studies of cytotoxic activity of the extracts. Reports confirm the anticancer activity of various anthraquinones such as aloe-emodin, isolated from *Aloe vera* leaves, which exhibits an anticancer effect against colon carcinoma [17]. This class of compound was detected in the phytochemical screening, although probably the concentrations in the extracts were too low to give satisfactory results in the assay. The leaf extracts of *S. gardneri* (SgL) and *S. splendida* (SsL) showed the highest percentage of inhibition of the cancer cell lines tested, indicating that the isolation of metabolites may lead to potential anticancer agents.

CONCLUSION

Although several chemical and pharmacological studies are published for species of the genus *Senna*, none has evaluated the species selected for this investigation. The classes of secondary metabolites present in these plants have important pharmacological activities, as reported in the literature. The ethanolic extracts of the four species of *Senna* showed antioxidant activity as assayed by both methods, and this activity can be attributed to the presence of phenolic compounds such as flavonoids and anthraquinones. *Senna trachypus* exhibited greater antioxidant potential than the standards, as well as higher total phenolic content. In the study of anticancer properties, only two of the species showed promising results.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENTS

This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

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