Anti-inflammatory, laxative and intestinal motility effects of *Senna macranthera* leaves

Lyvia Guarize, Juliana C. da Costa, Leandro B. Dutra, Renata F. Mendes, Isabel V.A. Lima and Elita Scio*

Departamento de Bioquímica, ICB, Universidade Federal de Juiz de Fora, Juiz de Fora, MG 36036 330, Brazil

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Several species of *Senna* are well known for their therapeutic properties, being used in folk medicine to treat throat inflammation and constipation. In this regard, the anti-inflammatory, laxative and intestinal motility effects of different extracts of *Senna macranthera* (Colladon) H. Irwin and Barneby leaves were determined. The hexane extract significantly reduced carrageenan-induced paw oedema in rats in a manner similar to that of sodium diclofenac. A laxative effect was observed for the methanolic and hexane extracts comparative to the standard drug bisacodyl; however, only the hexane extract increased the intestinal motility in the studied period. Moreover, the extracts were evaluated for their antioxidant activity and the ethyl acetate extract presented a pronounced activity comparable to the standard rutin. Phytochemical screening was also performed and phenolic compounds like flavonoids, tannins and coumarins were observed in all extracts except for the hexane extract, which instead presented anthrones, triterpenes and steroids in its composition. The HPLC profile showed the presence of the anthraquinone emodine in all tested extracts.

**Keywords:** *Senna macranthera*; laxative; intestinal motility; anti-inflammatory; antioxidant

1. Introduction

The genus *Senna* is native throughout the tropics, with a small number of species reaching into temperate regions. Almost all species were at one time or another placed in *Cassia* (Agarkar & Jadge, 1999). Typically, *Senna* species are recognised as ornamental due to the beauty of their yellowish flowers (Viegas et al., 2004).

Several species are well known for their therapeutic properties, being used in the folk medicine of several countries (Corrêa, 1984). The tea of their crushed leaves is used to treat throat inflammation (Viegas et al., 2007) and constipation (Leng-Peschlow, 1993). Also, *Senna* species are known to be a rich source of phenolic
derivatives, most of them with important biological and pharmacological properties (Hazra, Das Saram, & Sanyal, 2004).

Although in recent decades many studies have been published regarding the pharmacological and biological properties of *Senna* extracts (Viegas, Rezende, Silva, Castro-Gamboa, & Bolzani 2006), only a little is known about *Senna macranthera* (Colladon) H. Irwin and Barneby. This species is native to Brazil and also grows in Colombia, Equador, Peru and Venezuela. Phytochemical studies on the endosperm of its seeds have revealed the presence of a galactomannan, a sulphated derivative of which presented anticoagulant activity (Pires, Gorin, Reicher, & Sierakowski, 2001). In another study, Branco et al. (2008) isolated the polyketide rubrofusarin, a human topoisomerase II-α inhibitor.

Due to the widespread medicinal use of *Senna*, the focus of this study was to investigate the anti-inflammatory and laxative activities as well as the effect on intestinal motility of the extracts of *S. macranthera*. Moreover, their antioxidant activity and phytochemical profile were also determined.

2. Results

2.1. *Phytochemical screening*

The results of the phytochemical screening of the extracts are given in Table 1.

2.2. *Antioxidant activity and amount of total phenolic compounds and flavonoids*

The antioxidant activity and the amount of total phenolic compounds and flavonoids are shown in Table 2.

Table 1. Phytochemical constituents of the extracts of *S. macranthera* and commercial senna.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Al</th>
<th>Tr</th>
<th>St</th>
<th>Sa</th>
<th>C</th>
<th>Ph</th>
<th>Ta</th>
<th>F</th>
<th>An</th>
<th>Fan</th>
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<tr>
<td><em>S. macranthera</em></td>
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<td>Commercial senna</td>
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</table>

Note: *Al*, alkaloids; *Tr*, triterpenoids; *St*, sterols; *Sa*, saponins; *C*, coumarins; *Ph*, phenols; *Ta*, tannins; *F*, flavonoids; *An*, anthrones; *Fan*, free anthraquinones.
2.3. **HPLC analysis**

The HPLC profiles of the extracts of *S. macranthera* and also that of commercial senna are shown in Figure 1. Emodine (1,3,8-trihydroxyanthraquinone) was used as the reference standard.

2.4. **Laxative activity**

The results of the laxative effects of the extracts of *S. macranthera* are given in Figure 2. The effects of the hexane and methanol extracts were as potent as the standard drugs.

2.5. **Effect on intestinal motility**

The effects of the extracts on intestinal motility were investigated. The results, as shown in Figure 3, reveal that the hexane extract of *S. macranthera* caused a significant increase in gut motility when compared with the effect produced by normal saline and even with the effect of standard drugs.

2.6. **Anti-inflammatory activity**

The results on the anti-inflammatory activity of hexane and methanol extracts of *S. macranthera* are shown in Figure 4. The hexane extract induced 58% oedema inhibition in the studied period when compared to control. As reference, the non-steroidal anti-inflammatory drug, sodium diclofenac, reduced the oedematous response by 75% in that period.

3. **Discussion**

The active principles of *Senna* were thought to be glycosides of the anthraquinone family. The intestinal bacteria flora account for the reduction of anthraquinone

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**Table 2. Antioxidant activity and amount of phenolic compounds and flavonoids of the extracts of *S. macranthera***

<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH assay IC$_{50}$ (μg ml$^{-1}$)$^a$</th>
<th>Reducing power EC$_{50}$ (μg ml$^{-1}$)$^a$</th>
<th>Total phenolics as TAE (mg g$^{-1}$ of extract)$^a$</th>
<th>Flavonoids as RE (mg g$^{-1}$ extract)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>11.5 ± 1.8</td>
<td>29.18 ± 0.517</td>
<td>270.4 ± 0.014</td>
<td>430.2 ± 0.007</td>
</tr>
<tr>
<td>Hexane</td>
<td>59.1 ± 2.2</td>
<td>88.64 ± 3.514</td>
<td>28.1 ± 0.015</td>
<td>55.0 ± 0.011</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>23.3 ± 2.4</td>
<td>46.64 ± 10.22</td>
<td>382.0 ± 0.013</td>
<td>196.0 ± 0.024</td>
</tr>
<tr>
<td>EtOAc</td>
<td>4.2 ± 3.1</td>
<td>7.68 ± 0.969</td>
<td>547.0 ± 0.025</td>
<td>198.52 ± 0.0065</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>39.4 ± 2.2</td>
<td>24.79 ± 0.124</td>
<td>395.8 ± 0.045</td>
<td>109.18 ± 0.004</td>
</tr>
<tr>
<td>Ascorbic acid$^b$</td>
<td>2.5 ± 0.1</td>
<td>25.2 ± 0.23</td>
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<tr>
<td>Rutin$^b$</td>
<td>0.9 ± 0.06</td>
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<td>Quercetin$^b$</td>
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Notes: $^a$Mean of triplicate assays ± SEM. $^b$Positive controls.
aglycons to their corresponding anthrones. They have been reported to accelerate spontaneous ileal contractions to induce purgative effects (Hara, Leighton, Sharma, & Fleischer, 2008). The HPLC profile of the extracts showed the presence of the anthraquinone emodine in all the extracts of *S. macranthera*, although in different amounts (Figure 1). Interestingly, the Borntraeger reaction used in this study to identify anthracene compounds did not detect anthraquinones in *S. macranthera* extracts, probably because they are lower than the detection limit of the method. On the other hand, anthrones were identified in methanol and hexane extracts. As expected, anthraquinones were found in commercial senna. Although piperidine alkaloids had been identified in several species of *Senna* (Viegas et al., 2004), they were not detected in the extracts of *S. macranthera* and alkaloids were only detected in the CH₂Cl₂ extract of commercial senna (Table 1). Conversely, except for the hexane extract, a great number of phenolics, particularly flavonoids, were found in the extracts (Table 2).
Flavonoids have been used to treat many human diseases, such as diabetes, cancers and coronary heart diseases (Al-Dabbas, Suganuma, Kitahara, Hou, & Fujii, 2006). Moreover, flavonoids have been shown to exhibit the antioxidative, antiviral, antimicrobial and antiplatelet activities (Middleton, Kandaswami, & Theoharides, 2000). The biological activities of these polyphenols in different systems are believed to be due their redox properties, which can play an important role in absorbing and neutralising free radicals, quenching singlet and triplet...

Figure 2. Laxative effect of the extracts of *S. macranthera* in rats. Difference of means of the number of faeces between controls and treatment values ± SD (n = 7). Variation compared to the control animals. ANOVA followed by Dunnett’s test: *p < 0.05. Group I, bisacodyl; group II, saline; group III, MeOH extract of commercial senna; group IV, MeOH extract of *S. macranthera*; group V, hexane extract; group VI, EtOAc extract; group VII, CH₂Cl₂ extract; group VIII, *n*-BuOH extract.

Figure 3. Effect of the extracts of *S. macranthera* on intestinal motility in rats between control and treatment values ± SD (n = 7). Variation compared to the control animals. ANOVA followed by Dunnett’s test: *p < 0.01 and **p < 0.05. Group I, bisacodyl; group II, saline; group III, MeOH extract of commercial senna; group IV, MeOH extract of *S. macranthera*; group V, hexane extract; group VI, EtOAc extract; group VII, CH₂Cl₂ extract; group VIII, *n*-BuOH extract.
oxygen, or decomposing peroxides (Osawa, 1994). DPPH assays evaluate the ability of antioxidants to scavenge free radicals. Hydrogen-donating ability is an index of primary antioxidants. These antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to the inhibition of the propagation of lipid oxidation (Lugasi, Dworschá, Blázovics, & Kéry, 1998). The reducing power property indicates that these compounds are electron donors, and can reduce the oxidised intermediates of lipid peroxidation processes, so they can act as primary and secondary antioxidants (Yen & Chen, 1995).

Several studies have evaluated the relationships between the antioxidant activities of extracts and their phenolic contents. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no relationship. Fabri, Nogueira, Braga, Coimbra and Scio (2009) reported a high correspondence between phenolic content and antioxidant activity using DPPH and reduction power assays in *Mitracarpus frigidus*. On the other hand, in this work, the findings did not show a relationship between antioxidant activity and phenolic contents (Table 2). These results agree with those of Conforti, Rigano, Menichini and Houghton (2009), who reported that the differences in antioxidant activities of plant extracts could be due to the different qualitative and quantitative compositions of their phenolic constituents.

In this study, an oral administration of the hexane and methanol extracts of *S. macranthera* presented a laxative effect on rats. These extracts significantly increased the stool number 0–8 h after their administration. The laxative activities of these extracts were similar to that of commercial senna, used popularly due to its strong laxative effect, and were greater than the reference drug, bisacodyl. Bisacodyl was used as positive control as it is a stimulant laxative drug commonly used for the treatment of constipation (Flig, Hermann, & Zabel, 2000).

The animals treated with the hexane extract produced wet or unformed faeces. After 16 h, no difference in the amount of faeces was observed between the different treatments, including saline and standard drugs (Figure 2). This has probably occurred because after 8 h of treatment, the animals were allowed free access to food, and the extracts and standard drugs had already acted in the first 8 h of treatment.
Interestingly, during this study period, only the hexane, but not the methanol extract, presented an increase on the intestinal motility of rats, when compared to saline and even with the standard drugs (Figure 3). This is probably due to a difference in the chemical structure or concentration of the relaxant constituents of the two extracts, which can be confirmed by the HPLC chromatogram of both (Figure 1). Certainly, emodine contributed to the observed laxative and intestinal motility activities, but this compound was not the only one responsible for those activities as it was found in a greater amount in the inactive extracts (Figure 1). The effect of bisacodyl on the intestinal motility was not significant when compared to saline, probably because when given orally, this drug stimulates the colon to empty within 6–12 h after administration (Adams, Meagher, Lubowski, & King, 1994).

Carrageenan-induced rat paw oedema is a well-accepted and popular model for evaluating anti-inflammatory activity. It is known to produce a biphasic response, where the early phase is related to the production of histamine, leukotrienes, PAF and possibly cyclooxygenase products, while the delayed phase is linked to the neutrophil infiltration, eicosanoid release, production of free radicals and also release of other neutrophil-derived mediators (Kumar et al., 2008). This study evaluates the anti-inflammatory effect of the hexane and methanolic extract of S. macranthera. The results demonstrate that the hexane extract possesses a significant anti-inflammatory activity on an acute inflammatory process comparable to the standard drug, sodium diclofenac, being more potent in the first hour. In agreement with data from the literature, the paw oedema induced by the carrageenan injection is associated with three different phases from alterations of the vascular permeability induced by mediators. During the first 60 min, possibly histamine and serotonin release occurs. A second phase is characterised by the release of bradykinin and prostaglandins (mainly PGE2) (Khalil, Sperotto, & Manfron, 2006; Pramanik, Bhattacharya, Chatterjee, & Mandal, 2005). The anti-inflammatory effect observed for the hexane extract may be due to the presence of triterpenoids and steroids in its composition (Table 1).

In conclusion, this work reveals that S. macranthera is an interesting source of anti-inflammatory, laxative and antioxidant principles.

4. Experimental

4.1. Plant material

Senna macranthera leaves were collected in Juiz de Fora, Minas Gerais, Brazil, in July 2007. The plant was identified by Dr Fatima Regina Salimena. A voucher specimen (CESJ 46159) was deposited at the Herbarium Leopoldo Krieger of the Federal University of Juiz de Fora. Senna angustifolia (Vahl) Batka, named here commercial senna (Pharmacopeia®), was purchased from local market.

4.2. Drugs and chemicals

Bisacodyl (Dulcolax®) and sodium diclofenac were obtained from Boehringer Ingelheim (Switzerland) and Medquimica (Brazil), respectively. Emodin, carrageenan and 1-1diphenyl-2-picrylhydrazyl (DPPH) were from Sigma Chemical Co.
Acetonitrile, of HPLC grade, was from Tedia Company, Inc. (Fairfield, OH, USA), and all other chemicals used were of analytical grade.

4.3. Preparation of the extracts

The oven-dried and powdered leaves of the plant (2.5 kg) were extracted by maceration with methanol (3 x 3 L) for 5 days at room temperature. The crude extract (400 g), after removal of solvent, was dissolved in MeOH–H2O (8 : 2) and partitioned with hexane, dichloromethane (CH2Cl2), ethyl acetate (EtOAc) and n-butanol (n-BuOH) successively to give hexane (28 g), CH2Cl2 (12 g), EtOAc (16 g) and n-BuOH (28 g) extracts. The solvents were evaporated and kept in tightly stoppered bottles under refrigeration until used for biological testing and phytochemical analysis.

4.4. Phytochemical screening

A portion of each sample that was subjected to biological screening was used for the identification of the major secondary metabolites, employing the protocols described by Matos (1997). Briefly, the samples were dissolved in methanol (1 mg mL\(^{-1}\)) and submitted to the following identification reactions: the characterisation for tannins was performed by gelatin, iron salt and lead acetate reactions. Triterpenoids and steroids were investigated by Liebermann–Burchard reagent and the alkaloid analysis was done by precipitation reactions with the reagents of Dragendorff, Bouchardat, Mayer and Bertrand. For the study of flavonoids, the reactions of Shinoda and aluminium chloride were employed. The characterisation of antraquinones and anthrones was done using Borntraeger reaction. The analysis for the presence of saponins was done by the formation of foam, and coumarins were characterised using KOH and phenols with FeCl3.

4.5. Amounts of total phenolic compounds and flavonoids

The amounts of phenolic compounds in the plant extracts were determined by the Folin–Denis method (Duh & Yen, 1997). Tannic acid was used as the standard for a calibration curve. One millilitre of the samples resuspended in ethanol (0.5 mg mL\(^{-1}\)) was mixed thoroughly with 1 mL of the Folin–Denis reagent and 8 mL of Na2CO3 2% in 0.1N NaOH. After a 60 min incubation period at 30°C, the absorbance was read against a blank at 730 nm. All determinations were performed in triplicate. The total content of phenolic compounds was expressed in milligram per gram of plant extracts in tannic acid equivalents (TAE).

The amount of flavonoids was determined as previously described by Miliauskas, Venskutonis and Van-Beek (2004), with slight modifications. Rutin was used as the standard for a calibration curve. One millilitre of the plant extracts was resuspended in methanol (0.5 mg mL\(^{-1}\)) and mixed with 1 mL of 2% aluminium trichloride in ethanol. The mixture was diluted with ethanol to 25 mL and allowed to stand for 40 min at 20°C. Thereafter, its absorbance was measured at 415 nm. The blank was prepared from 1 mL of the plant extracts and one drop of acetic acid, diluted to 25 mL in ethanol. All determinations were performed in triplicate. The total
amount of flavonoids was expressed in milligram per gram of plant samples in rutin equivalents (RE).

4.6. **HPLC analysis**

Liquid chromatography was performed using an Agilent 1200 machine (Waldbronn, Germany). The extracts were applied using an automatic injector and separated on a Zorbax SB-C18 column, 250 × 4.6 mm² at 25°C. For MeOH and hexane extracts, the mobile phase was a gradient system with acetonitrile/water (pH 3.8) with acetic acid, as follows: 0–20 min, 90 : 10; 20–30 min, 70 : 30; 30–40 min, 20 : 80. For CH₂Cl₂, EtOAc and n-BuOH extracts, a gradient system from 10 : 90 to 50 : 50 of acetonitrile/water (pH 3.0) with phosphoric acid, 0–35 min was used. The flow rate was 1 mL min⁻¹ and for the detection a diode-array detector (DAD) was used. Emodine was used as a standard reference.

4.7. **DPPH assay**

The free radical scavenging activity of the extract solutions in methanol was determined based on their ability to react with stable DPPH free radical (Govidarajan et al., 2003). The plant samples at various concentrations (7.8–250 μg mL⁻¹) were added to a 152 μmol solution of DPPH in methanol. After incubation at 37°C for 30 min, the absorbance of each solution was determined at 517 nm. The antioxidant activity of the samples was expressed as an IC₅₀ value, which was defined as the concentration (in microgram per millilitre) of sample required to inhibit the formation of DPPH radicals by 50%. All tests were performed in triplicate. Rutin and quercetin were used as positive controls.

4.8. **Reducing power**

The reducing power was determined by the method of Oyaku (1986). Ten milligrams of each sample were mixed with 2.5 mL of potassium phosphate buffer (0.2 mol, pH 6.6) and 2.5 mL of potassium ferricyanide (10 g L⁻¹). The mixture was incubated at 50°C for 20 min. A 2.5 mL aliquot of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 × g for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃ and absorbance was measured at 700 nm. Ascorbic acid was used as a reference material. All tests were performed in triplicate. An increase in the absorbance of the reaction indicated the reducing power of the samples. A higher absorbance indicated a higher reducing power. EC₅₀ values (μg mL⁻¹) were calculated and they indicated the effective concentration at which the absorbance was 0.5 for reducing power.

4.9. **Animals**

Male Wistar rats, 60 days in age, weighing 160–200 g, obtained from the Reproduction Biology Centre of the Federal University of Juiz de Fora, were housed in a room kept under controlled conditions with temperature maintained at 23°C ± 2°C, on a 12 h light : 12 h dark cycle and free access to water and complete
commercial chow (Nuvital™, Colombo, PR, BR). Throughout the experiments, the animals were processed according to the suggested ethical guidelines for the care of laboratory animals. This study was approved by the Brazilian College of Animal Experimentation (COBEA).

4.10. Effect on intestinal motility
The method described by Jansen and Jageneau (1957) and Wong and Wai (1981) was used to test the effect of the extracts on intestinal motility. The animals were divided into eight groups \( (n = 7) \) as follows:

- **Group I**: reference group received bisacodyl in saline (0.25 mg kg\(^{-1}\));
- **Group II**: negative control received normal saline orally;
- **Group III**: received MeOH extract of commercial senna;
- **Group IV**: received MeOH extract of *S. macranthera*;
- **Group V**: received hexane extract;
- **Group VI**: received CH\(_2\)Cl\(_2\) extract;
- **Group VII**: received EtOAc extract;
- **Group VIII**: received \( n \)-BuOH extract.

The animals were starved for 12 h prior to the experiment, but were allowed free access to water. All extracts were resuspended in saline (400 mg kg\(^{-1}\) mL\(^{-1}\)) and administered orally by gavage (1 mL). After 40 min, 1 mL of 10% charcoal suspension in 5% acacia solution was administered to each animal orally. The animals were sacrificed after 20 min and the abdomens were opened. The small intestines were dissected out and placed on a clean surface. The distance travelled by the charcoal meal from the pylorus was measured. The entire length of the small intestine was measured and then the percentage distance travelled by the charcoal plug along the small intestine (from the pylorus to the caecum) was estimated for the extracts, saline (negative control) and the reference drugs (commercial senna and bisacodyl).

4.11. Laxative activity
The test was performed according to Capasso, Mascolo, Autore and Romano (1986) on rats fasted for 8 h before the experiment, but with water provided *ad libitum*. The animals were divided into eight groups \( (n = 7) \), as mentioned above (Section 4.10).

All extracts were resuspended in saline (400 mg kg\(^{-1}\) mL\(^{-1}\)) and administered orally by gavage (1 mL). Immediately after dosing, the animals were separately placed in cages suitable for the collection of the faeces. After 8 h of drug administration, the faeces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 h.

4.12. Anti-inflammatory activity
A carrageenan-induced hind paw oedema model was used for the determination of anti-inflammatory activity (Saha et al., 2007; Winter, Risley, & Nuss, 1962). The rats
fasted for 8 h before the experiment, but with water provided ad libitum. The animals were divided into four groups \((n = 7)\) as follows:

- **Group I:** negative control received normal saline orally;
- **Group II:** reference group received standard drug sodium diclofenac \((10 \text{ mg kg}^{-1})\);
- **Group III:** received MeOH extract of *S. macranthera* \((400 \text{ mg kg}^{-1})\);
- **Group IV:** received hexane extract of *S. macranthera* \((400 \text{ mg kg}^{-1})\).

The control saline, extracts and standard drug were administered 60 min prior to the administration of carrageenan \((0.1 \text{ mL of 1% w/v solution in physiological saline})\) into subplantar tissue of the left hind paw. As the control, 1 mL saline solution was injected into the tissue of the right paw. The paw sizes were measured using a pachimeter immediately and thereafter at hourly intervals, for 4 h, following the administration of carrageenan. Results were determined as the percentage inhibition of oedema and were compared to the control.

### 4.13. Statistical analysis

The values of *in vivo* tests were presented as means ± SD \((n = 7)\). The statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the Dunnett test using the GraphPad Prism 4 statistic computer program. A difference in the mean values of \(p < 0.05\) was considered to be statistically significant. The IC\(_{50}\) and EC\(_{50}\) values for antioxidant activity and reducing power, respectively, the contents of phenolics and flavonoids were all expressed as means ± SEM \((n = 3)\).

### Acknowledgements

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and to Universidade Federal de Juiz de Fora (UFJF) for financial support, to Dr Fatima Regina Salimena for the botanical identification of the species and to the Reproduction Biology Center of the Federal University of Juiz de Fora for providing the rats.

### References


