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Phytochemical Screening and Assays of Phenolic Compounds in *Senna occidentalis* L. Leaf and Seed Extracts

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Abstract

Phytochemical screening and assay of secondary metabolites, crude extracts with distilled water, aqueous methanol, aqueous acetone and aqueous ethanol of leaves and seeds of *Senna occidentalis* L. were studied in this work. The aim was to verify the distribution of secondary metabolites according to *S. occidentalis* organs. Four leaf samples from four different localities (South, East, West and Central Senegal) and a mixed sample of seeds were used. Functional molecules such as polyphenols, flavonoids and tannins were then assessed in the leaves and seeds using various standard methods. The results show that *Senna occidentalis* L. leaf and seed samples display an identical and homogeneous profile, regardless of locality. They contain secondary metabolites and the polyphenol content of extracts from southern, eastern, western and central leaves is: 0.620 - 0.539 - 0.811 - 0.573 g GAE/100 g DM; flavonoids: 0.064 - 0.074 - 0.130 - 0.101 g CE/100 g DM and tannins: 0.326 - 0.264 - 0.269 - 0.494 g TAE/100 g DM. The efficacy of *S. occidentalis* L. infusions in therapy is thus justified by the presence of these metabolites, whose biological properties are well known. It is then possible to explore isolation of active principles of *Senna occidentalis* L. leaves and even seeds for producing medicines.

Keywords

Senna occidentalis L., Total Polyphenols, Flavonoids, Tannins

1. Introduction

Natural products, such as plant extracts, in the form of pure compounds or standardized extracts, offer unlimited opportunities for the discovery of new drugs due to the availability of chemical diversity [1]. Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic diseases [2]. The active ingredients in plants are chemical compounds [3] which produce a defined physiological action in the human body [4]. Some of the most valuable include alkaloids, tannins, saponins, flavonoids, phosphorus and calcium [4]. Isolating and purifying these principles would make it possible to develop medicines that could be used in primary healthcare at lower cost. *Senna occidentalis* L. is a sub-shrub of the *Caesalpiniaceae* (*Fabaceae*) family native to tropical America and widespread in many tropical countries [5]. It has been used as a laxative, tonic, stomachic, febrifuge and purgative [6]. However, *S. occidentalis* L. is also considered as a toxic legume for several animal species [7]. Nevertheless, it is one of the most widely used medicinal plants in tropical and subtropical regions of the world [8]. In Senegal, it is used in the treatment of numerous pathologies, such as malaria, fever, colds, headaches, stomach aches, flu, muscular pains, coughs, asthma, hypertension, diabetes, inflammation, yellow fever, keratitis, sexual weakness, diarrhea, painful periods, colic, menorrhagia, dermatitis, tooth decay, ulcers, hemorrhoids and vomiting [9]. The aim of this study was to determine the chemical composition of metabolites in the leaves and seeds of *S. occidentalis* L., so as to justify their efficacy in the traditional treatment of a number of diseases in Senegal.

2. Materials and Methods

2.1. Plant Material

Senna occidentalis L. leaves and seeds were collected in December 2021 and January 2022 in the eastern, western, southern and central regions of Senegal (**Figure 1**). Seeds were obtained in the southern region.

2.2. Extraction and Yield Calculation

After harvesting, leaves and seeds were air-dried away from the sun and then ground using an electric grinder (Kenwood, France). The powder, sieved to 1 mm mesh, was stored in airtight jars in the laboratory. The powders were then extracted in a ratio of 10 g/100 mL of solvent in two ways: by infusion with distilled water at 50˚C and maceration at room temperature during twenty-four hours. Maceration was carried out with aqueous ethanol (70% v/v), (96.3%, Alcool Surfin Neutre), aqueous methanol (70% v/v) and aqueous acetone (70% v/v) (99.5%, Scharlab

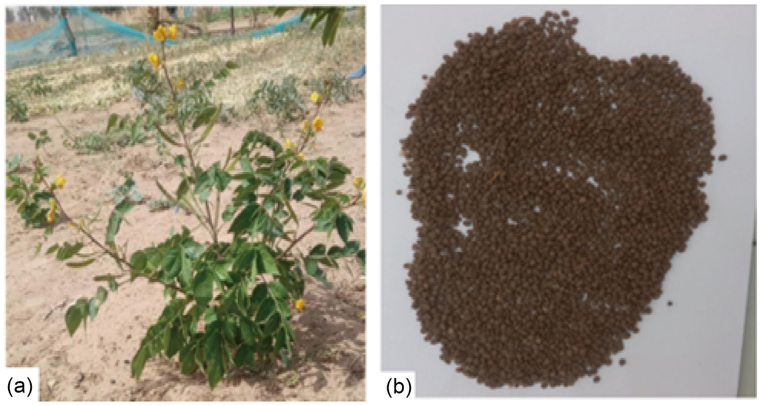

Figure 1. The aerial parts of *Senna occidentalis* L. (a) Leaves; (b) Seeds.

S.L., SPAIN). After cooling, the mixture was filtered under vacuum on Wattman No.1 paper. Traces of solvent were removed from the extracts using an oven (SalvisLAb) at 105˚C for 2 hours. Extraction yields (R) were calculated using the following formula:

$$Y(\%) = \frac{M1 - M2}{MP} \times 100$$

M1: mass of the balloon with test sample after dehydration; M2: mass of the empty balloon; MP: mass of the test portion.

2.3. Phytochemical Screening

The main families of secondary metabolites were sought in the leaves and seeds of plants from the different zones. Qualitative phytochemical analysis was carried out using standard methods and several tests: flavonoid by NaOH test [10]; tannins through stiasny reaction with ferric chloride reaction; alkaloids by Dragendorff and Mayer sterols by Liebermann-Buchard reaction and saponosides through foam index [11] [12]. Once the chemical compounds had been identified, the total polyphenols, flavonoids and tannins were quantified.

2.4. Determination of Total Polyphenols

The total polyphenol content was determined using the Folin-Ciocalteu method [11]. The results are expressed in milligram equivalents of gallic acid per gram of dry matter (mg GAE/g DM) using a calibration curve.

2.5. Determination of Total Flavonoids

The total flavonoid content of the extracts was determined using the colorimetric method [12]. The results are expressed as milligram equivalents of catechin per gram of dry matter (mg CE/g DM) using a calibration curve.

2.6. Dosage of Tannins

Condensed tannin content is determined using the colorimetric method of Folin Denis [13]. The result is expressed as milligram equivalent of tannic acid per gram of dry matter (mg TAE/g DM) using a calibration curve.

2.7. Statistical Analysis

The analytical results obtained from three independent trials were subjected to analysis of variance (ANOVA) using STATISTICA 7.1 software. Statistical differences with a probability value of less than 0.05 are considered significant.

3. Results and Discussion

3.1. Extraction Yields

Extracts' yields of various solvent are shown in **Figure 2**. The results of the leaves show that infusion gives the best yield (23.75%) compared with maceration with hydro-ethanol (12.37%), hydro-acetone (11.99%) and hydro-methanol (11.21%). At the same time, seed extracts also gave the same conclusion: infusion (12%), hydro-methanolic (11.47%), hydro-acetone (9.92%) and hydro-ethanolic (9.07%) macerates.

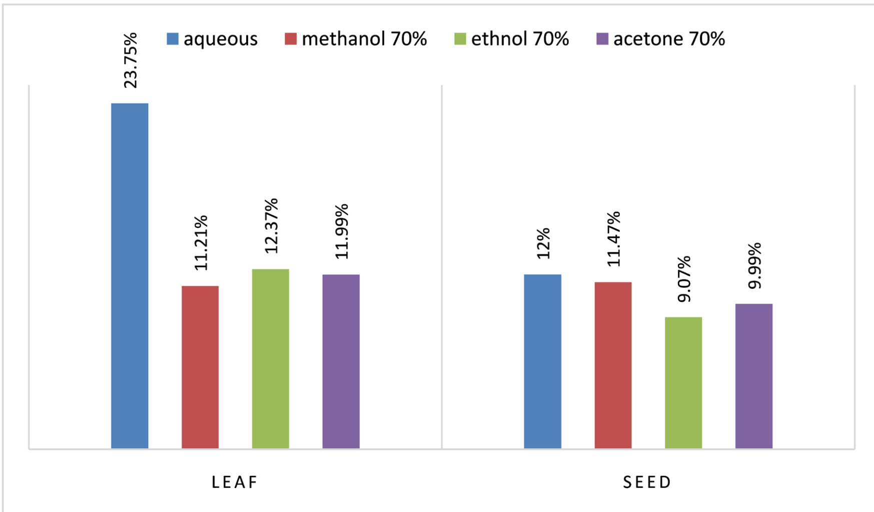

Figure 2. Average extraction yields obtained with leaves and seeds.

Analysis of the results shows that the metabolite content depends not only on the solvent used but also on the organ studied. The extraction efficiency of the four solvents was greater with the aqueous extract in both leaves and seeds. A comparative study of the extraction yield between the leaves, seeds and roots of *Senna occidentalis* L. in methanol revealed that the leaves had the highest extraction yield (16.92%) compared to the seeds and roots [14].

In general, the best contents for leaves were obtained with aqueous acetone (70%) while aqueous ethanol was the best for seeds. The aim of using this hydro-ethanol mixture was to extract polar compounds as well as medium and low polarity compounds. The ability of ethanol to increase the permeability of cell walls facilitates the extraction of a greater number of compounds [15]. Aqueous extracts were prepared by infusion. Water is not the ideal solvent for several bioactive plant constituents. In fact, it is preferentially used to extract polar compounds. At high temperatures, water can also extract some amphiphilic compounds [16]. The effectiveness of acetone and methanol as solvents relates to their intermediate polarities, which enable them to extract low molecular weight organic compounds with functional groups [17].

3.2. Phytochemical Screening

Phytochemical screening of *Senna occidentalis* L. extracts revealed the presence of polyphenols, tannins, flavonoids, alkaloids and saponosides in both leaves and seeds, as summarised in **Table 1**.

Table 1. Results of phytochemical screening of extracts of *Senna occidentalis* L.

Organs	Areas	Polyphenols	Flavonoids	Tannins	Alkaloids		Saponosides
					DR	MR	
Leaves	South	+	+	+	+	+	+
	East	+	+	+	+	+	+
	West	+	+	+	+	+	+
	Center	+	+	+	+	+	+
Seeds	South	+	+	+	+	+	+

Note: DR: Dragendorff's reagent; MR: Mayer's reagent; +: presence of secondary metabolites in leaves and seeds.

The present study highlights the presence of various secondary metabolites in the leaves and seeds of *S. occidentalis* L. These results are similar to studies carried out in Madagascar and Gabon, which revealed a qualitative richness in secondary metabolites in *S. occidentalis* L. [14] [18]. The richness of the chemical composition of extracts is also noted according to the type of solvent used for extraction [18]. Preliminary phytochemical examination revealed the presence of alkaloids, saponins, tannins, reducing sugars, phenols, anthraquinones, glycosides and resins in some of the extracts (hexane, ethyl acetate, methanol) of *Senna occidentalis* L. leaves [19]. Thus, the variation in metabolite levels in the areas sampled may depend on the quality of the soils and many other abiotic factors. Although climatic and geological conditions are relatively homogeneous, numerous soil classes are represented in Senegal, including sub-arid soils, ferruginous soils and outcrops [20]. The levels of secondary metabolites (certain sesquiterpene lactones, total polyphenols and flavonoids) vary according to the season and are higher in the site with the most restrictive hydric conditions (draining soil, higher temperature and presence of a dry period in summer) [21]. Similarly, one study highlighted the differences in polyphenol content between the powders and pulps of wild and domesticated *Ceratonie silique* L. trees [22], showing that variation in secondary metabolites can depend on the environment in which the plant is located. Thereby, carob dry powders from wild trees show total polyphenols, flavonoids and condensed tannins contents reaching 22.75 mg GAE/g, 1.02 mg QE/g and 1.02 mg PE/g, respectively. Also, pulps from wild trees show total polyphenols, total flavonoids and condensed tannins contents by one fresh carob pulp reaching 118.04 mg GAE, 4.83 mg QE and 7.36 mg PE, respectively, while domesticated trees show lower contents for powders. Thus, total polyphenols, flavonoids and condensed tannins contents reach 18.06 mg GAE/g, 0.73 mg QE/g and 0.68 mg PE/g, respectively [22].

3.3. Evaluation of Quantitative Analysis

The results of the quantitative analyses of the leaves show that the contents of total polyphenols, total flavonoids and condensed tannins can vary significantly but not depending on the sampling areas (**Table 2**).

Table 2. Essential secondary metabolites of different leaf extracts.

Raws extracts	**Areas**	**Polyphenols (g GAE/100 g DM)**	**Flavonoids (g CE/100 g DM)**	**Condensed tannins (g TAE/100g DM)**
Aqueous	South	0.620 ± 0.084^{ab}	0.064 ± 0.003^{b}	0.326 ± 0.061^{a}
	East	0.539 ± 0.077^{b}	0.074 ± 0.003^{b}	0.264 ± 0.041^{a}
	West	0.811 ± 0.177^{a}	0.130 ± 0.030^{a}	0.269 ± 0.024^{a}
	Center	0.573 ± 0.136^{b}	0.101 ± 0.008^{ab}	0.494 ± 0.253^{a}
Methanol 70%	South	0.502 ± 0.153^{b}	0.120 ± 0.007^{b}	0.492 ± 0.025^{b}
	East	0.516 ± 0.028^{b}	0.148 ± 0.004^{a}	0.552 ± 0.05^{ab}
	West	0.748 ± 0.029^{a}	0.090 ± 0.002^{c}	0.540 ± 0.044^{ab}
	Center	0.548 ± 0.050^{b}	0.125 ± 0.006^{b}	0.570 ± 0.028^{a}
Acetone 70%	South	0.867 ± 0.056^{a}	0.502 ± 0.006^{b}	0.435 ± 0.011^{b}
	East	0.766 ± 0.220^{a}	0.556 ± 0.005^{a}	0.473 ± 0.008^{a}
	West	0.406 ± 0.028^{b}	0.302 ± 0.006^{c}	0.440 ± 0.030^{b}
	Center	0.548 ± 0.005^{b}	0.269 ± 0.004^{d}	0.394 ± 0.005^{c}
Ethanol 70%	South	0.524 ± 0.059^{ab}	0.179 ± 0.034^{b}	0.129 ± 0.021^{a}
	East	0.597 ± 0.030^{a}	0.233 ± 0.03^{a}	0.125 ± 0.024^{a}
	west	0.442 ± 0.056^{bc}	0.246 ± 0.019^{a}	0.080 ± 0.004^{b}
	Center	0.407 ± 0.029^{c}	0.198 ± 0.016^{ab}	0.084 ± 0.015^{b}

Note: The letters a and b show the statistical difference between contents if there is an exponent a/b; no significant difference between exponent a and exponent b figures.

Thus, it more expressive to consider the average of metabolites in *S. occidentalis* L.'s leaves showed in **Table 3**. The average of the contents of the four extracts gives respectively 0.647 g EAG/100 g DM, 0.636 g EAG/100g DM, 0.579 g EAG/100g DM and 0.493 g EAG/100g DM for the hydro-acetone, aqueous, hydro-methanol and hydro-ethanol extracts. However, in the hydro-acetone extract, the quantification model shows that the total polyphenol content is less than the sum of flavonoids and tannins. The flavonoid contents of the different extracts are as follows, with a higher content in the hydro-acetone extract (0.407 g EC/100g DM), followed by the hydro-ethanol extract (0.214 g EC/100g DM) and the hydro-methanol and aqueous extracts with 0.121 g EC/100g DM and 0.092 g EC/100g DM respectively. It appears that the best solvent for polyphenols and flavonoids is acetonic's one except the hydro-methanolic for tannin (0.538 g EAT/100g DM).

Table 3. Average content of the four leaves samples in secondary metabolites.

Raws extracts	Polyphenols (g GAE/100 g MS)	Flavonoids (g CE/100 g MS)	Condensed tannins (g TEA/100 g MS)
Aqueous	0.636 ± 0.121	0.092 ± 0.030	0.338 ± 0.108
Methanol 70%	0.579 ± 0.115	0.121 ± 0.024	0.539 ± 0.033
Acetone 70%	0.647 ± 0.209	0.407 ± 0.143	0.436 ± 0.032
Ethanol 70%	0.493 ± 0.085	0.214 ± 0.031	0.105 ± 0.026

In seeds, ethanolic solvent gave better results for total polyphenol, while acetonic solvent was for flavonoids and tannins (**Table 4**).

Table 4. Secondary metabolite contents of seeds according to the solvent.

Raws extracts	Seeds		
	Polyphenols (g GAE/100 g DM)	Flavonoids (g CE/100 g DM)	Condensed tannins (g TAE/100 g DM)
Aqueous	0.358 ± 0.016^b	0.279 ± 0.076^b	0.119 ± 0.006^b
Methanol 70%	0.443 ± 0.084^b	0.273 ± 0.012^b	0.113 ± 0.002^b
Acetone 70%	0.398 ± 0.064^b	0.542 ± 0.018^a	0.139 ± 0.020^a
Ethanol 70%	0.596 ± 0.100^a	0.254 ± 0.004^b	0.118 ± 0.005^b

Note: The letters a and b show the statistical difference between contents.

The hydro-ethanolic and hydro-acetone extracts of *Senna occidentalis* L. seeds respectively had the highest polyphenol content (0.596 ± 0.016 g GAE/100g DM) and flavonoid (0.542 ± 0.018 g CE/100g DM) and condensed tannin (0.139 ± 0.020 g CE/100g DM).

The assay results show that the total polyphenol content varies significantly between the different extracts and the different organs. This disparity is a phenomenon reported by several authors, such as [23] [24]. The polyphenol content of ethanolic extracts of *Senna occidentalis* L. seeds is different from that found by other methods ; (0.079 ± 0.021 g/100g), flavonoids (0.027 ± 0.01 g/100g), tannins (0.087 ± 0.03 g/100g) [25]. *Senna occidentalis* L. leaves flavonoids recorded a higher percentage yield (sample of 2, 45 mg/g sample) compared to alkaloids (1.56 mg/g sample), tannins (0.21 mg/g sample), and phenols (0.16 mg/g sample) in the aqueous extract [25].

Secondary metabolites play both a defensive role against herbivores, pathogen attacks and inter-plant competition, and an attractive role towards organisms such as pollinators or symbionts [26]. The flavonoids present in the plant could be responsible for its anti-inflammatory properties [27]. Alkaloids are various groups of secondary metabolites with antimicrobial properties [28]. Alkaloids are also known to lower blood pressure, balance the nervous system in cases of mental illness, and have antimalarial properties [28]. The leaves contain tannins, steroids, alkaloids, glycosides, phlobatanins and flavonoids related to apigenin or vitexin, anthraquinones such as chrysophanol, emodin, phycsion and their derivatives, triterpenes and saponins [29].

The seeds contain numerous anthracene derivatives and a toxalbumin. It also contains sugars and fatty acids, phytosterols, an alkaloid (nitrogen derivative: N-Methyl-morphine) [30], chrysophanic acid and emodin. It also contains tannins and anthraquinones (rhein and physcion) which are thought to exist in three forms: dianthrone, anthronic and anthraquinonic [31]. The efficacy of using *Senna occidentalis* L. in traditional medicine would therefore be justified by the presence of these secondary metabolites, as with *Bauhinia rufescens* Lam, for which the results of phytochemical screening showed the presence of secondary metabolites known for their medicinal activities. The results of the total polyphenols and antioxidant activity of *Bauhinia rufescens* Lam extracts in three tests showed that the hydro-alcoholic extracts of the bark were more active [32].

4. Conclusion

In the present study, the leaves and seeds of *Senna occidentalis* L. revealed the same phytochemical compounds and showed a slight non-significant variation in the concentration of secondary metabolites between sampling localities. Qualitative and quantitative analyses identified polyphenols, flavonoids, tannins, alkaloids and saponosides. In both leaves and seeds, secondary metabolites are distributed between the various organs. These secondary metabolites are a potential source of antioxidants that can have a positive impact on human health. Investigations are needed into the anti-free radical and therapeutic activities of the various extracts from the different organs of *Senna occidentalis* L., as well as the safety effects of effective, accessible molecules or solutions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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