Chemical Science International Journal

Volume 32, Issue 6, Page 21-30, 2023; Article no.CSIJ.108321
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

Determination of Polyphenols and Flavonoids and Evaluation of Antioxidant Activity of Crude Extraction from the Leaves, Seeds, Stems and Roots of *Cassia occidentalis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2023/v32i6867

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108321

Original Research Article

Received: 20/08/2023
Accepted: 27/10/2023
Published: 01/11/2023

ABSTRACT

In this article, we studied four organs of *Cassia occidentalis* namely leaves, seeds, stems and roots. *Cassia occidentalis* is a plant used in traditional Senegalese medicine to treat several pathologies including asthma, malaria, high blood pressure, bronchitis, anemia, stubborn stomach aches, etc.

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This study focuses on the determination of the antioxidant activity of these extracts. This study focuses on the determination of total polyphenols and flavonoids of different organ extracts (leaves, seeds, stems and roots) of *Cassia occidentalis* and the determination of the antioxidant activity of these extracts. Total polyphenols are assayed using the Folin-Ciocalteu reagent and total flavonoids using aluminum trichloride ($AlCl_3$). The antioxidant activity of the extracts is evaluated by the DPPH method using TROLOX as reference antioxidant. The results of this study show that leaves and roots are the richest organs in polyphenols with contents ranging from 0.4941 ± 0.0633 mg EAG/g to 11.5114 ± 0.0791 mg EAG/g for leaves and 0.1235 ± 0.0220 mg EAG/g to 4.1968 ± 0.0481 mg EAG/g for roots. These same organs are richer in flavonoids with contents ranging from 1.6645 ± 0.0215 mg EQ/g to 7.2215 ± 0.1728 mg EQ/g for the leaves and 0.0813 ± 0.0022 mg EQ/g to 3.4839 ± 0.0538 mg EQ/g for roots. These contents are higher in the methanolic and aqueous extracts. Total antioxidant capacities are significantly correlated with polyphenol and flavonoid contents. This is because the leaves and roots have more antioxidants that can react with the DPPH radical. This quantity is more noted in the methanolic extracts (14.1502 ± 0.3781 mg ETr/g for the leaves and 7.7042 ± 0.0619 mg ETr/g for the roots). Only the methanolic extracts show remarkable antiradical activities with IC_{50} values of 0.2409 ± 0.0006 mg/mL for the leaves and 0.1415 ± 0.0005 mg/mL for the roots. IC_{50} values for other extracts are not available, due to the low total antioxidant capacity. In short, the methanolic extracts of the roots and leaves therefore have an appreciable antioxidant power. This antioxidant power is more significant at the level of the methanolic root extract with the lowest IC_{50} value. This would justify the use of *Cassia occidentalis* in traditional Senegalese medicine to fight against several pathologies. This plant would therefore be a good source of natural antioxidants.

Keywords: Cassia occidentalis; polyphenols; flavonoids; antioxidant capacity.

1. INTRODUCTION

The peoples of the world in general and those of Africa in particular have recourse to traditional methods, sometimes empirical, to treat certain pathologies. This is partly due to the fact that plants have shown a certain effectiveness in the treatment of certain tropical pathologies against which modern medicine has remained powerless. On the other hand, the difficult economic situation of several countries in Africa through the weak development of infrastructures and health personnel. The low purchasing power of the populations as well as the prohibitive price of pharmaceutical products are also a cause of this recourse to traditional pharmacopoeia which is even less expensive and diversified [1]. Currently, many works carried out in the field of ethnopharmacology show that most of the plants used in traditional medicine and tested in the laboratory, have interesting biological properties and are not often toxic [2]. These biological properties are related to their chemical composition, in secondary metabolites, which are bioactive molecules most of which evolve as defenders against several pathologies. Indeed, alkaloids have local anesthetic properties, tannins are healing, antibacterial and antiseptic, flavonoids have anti-inflammatory and antibacterial properties, saponosides have anti-inflammatory, anti-edematous and analgesic properties, terpenes and steroids are analgesic and anti-inflammatory and quinone derivatives are antibacterial [3].

Phenolic compounds or polyphenols are attracting considerable interest in food, chemistry and medicine due to their promising antioxidant potential [4].They are widely distributed in the plant kingdom and are the most abundant secondary metabolites in plants. These metabolites include many classes ranging from simple phenolic acids to complex flavonoids [5].

Antioxidants are free radical scavengers. These free radicals, when overproduced, are implicated in a number of disease processes such as asthma, cancer, cardiovascular disease, cataracts, diabetes, inflammatory disease, liver disease and degenerative diseases [6]. The reduction of free radicals by antioxidants would reduce the oxidative stress associated with these pathologies.

Currently the use of antioxidant molecules of synthetic origin is called into question because of the toxicological risks they can cause. This is why new natural antioxidants are actively researched at the plant level which show a good source of antioxidants [7] and with less adverse effects.

In this present, we undertook to study the antioxidant activity of crude extracts of organs of *Cassia occidentalis*, a plant of the Senegalese pharmacopoeia endowed with various pharmacological properties [8]. It is therefore necessary to confirm this study and make a comparison with the study of three other organs, namely seeds, stems and roots. This study is combined with the dosage of total polyphenols and flavonoids of the different extracts.

2. MATERIALS AND METHODS

2.1 Plant Material

The various *Cassia occidentalis* organs studied (Fig. 1) were harvested in April 2021 in FISSEL (14° 33'N, 16° 37'W), a town in the MBOUR department of the THIES region (Senegal). After harvesting, the different organs (leaves, seeds, stems and roots) were spread out separately on drying mats sheltered from the sun and water in our laboratory. After drying, the different organs were ground using an electric grinder. The powders obtained were used as plant material for the various analyzes carried out.

2.2 Preparation of Extracts

The extraction was done using the method described by Gaye et al. which results from an optimization of the extraction process (temperature, duration and ratio) [9].

Crude organic and aqueous extracts were prepared by maceration. The organic solvents used are hexane, ethyl acetate and methanol.

- For organic extracts, 1g of plant material was macerated in 50 mL of organic solvent for 24 hours at room temperature.
- The aqueous extracts were prepared by maceration of 0.5g of plant material in 50 mL of distilled water for 20 min at a temperature of 70°C.

After filtration, the solutions were used for the different assays.

2.3 Assay of Total Polyphenols

The reagent used is the "Folin-Ciocalteu" reagent; it is a mixture of complexes of phosphotungsten ($H_3PW_{12}O_{40}$) and phosphomolybdenum ($H_3PMo_{12}O_{40}$) yellow acids. The principle of the method is based on the oxidation of phenolic compounds by this reagent, it leads to the formation of a new molybdenum-tungsten complex of blue color [10].

The method used is that described by Gaye et al. [9] which is the following: 50 µL are taken from each extract and made up to 200 µL with distilled water. A volume of 150 µL of Folin Ciocalteu's reagent, 600 µL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water are added to it further. In parallel, a range of gallic acid from a 0.1 mg/mL stock solution was prepared. After 30 minutes of incubation in the dark and at room temperature, the absorbance is read by UV-visible spectrophotometry at 760 nm.

The total polyphenol content is calculated from the calibration range regression equation and the results are expressed in milligrams of gallic acid equivalent per gram of extract (mg EAG/g). All analyzes were carried out in triplets (n=3) and the results were expressed as mean ± standard deviation.

2.4 Assay of Total Flavonoids

The quantification of total flavonoids was carried out by the aluminum trichloride ($AlCl_3$) method, described by Gaye et al. [9].

2.5 mL of each extract (dilutions are made for some extracts) are added to 2.5 mL of a 2% $AlCl_3$ ethanolic solution. In parallel, a range of quercetin from a 0.1 mg/mL stock solution was prepared. After 1 hour of incubation in the dark and at room temperature, the absorbance is read by UV-visible spectrophotometry at 425 nm.

The total flavonoid content is calculated from the quercetin calibration range regression equation and the results are expressed as milligrams of quercetin equivalent per gram of extract (mg EQ/g). All analyzes were carried out in triplets (n=3) and the results were expressed as mean ± standard deviation.

2.5 Determination of Antioxidant Activity by the DPPH Method

The DPPH. (2,2-Diphenyl-1-picryl-hydrazyl) is generally the most widely used substrate for the rapid and direct assessment of antioxidant activity due to its stability in free radical form and the simplicity of the assay. It absorbs in the visible at a wavelength of 517 nm [2]. It is dark purple in color and its reaction with an

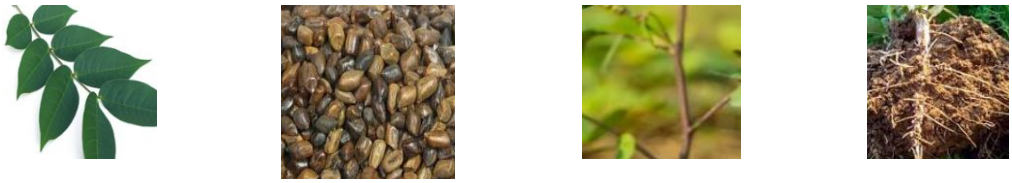

Fig. 1. Leaves, Seeds, Stems and Roots of *Cassia occidentalis*

antioxidant is accompanied by a color change from purple to yellow depending on the antioxidant efficiency. This results in a decrease in absorbance. Indeed, a low absorbance reflects a strong inhibition of the DPPH radical and therefore a strong antioxidant activity.

The method used is that of Akhtar and *al.* with some modifications [11].

The evaluation of the antioxidant activity begins with the determination of the total antioxidant capacity and then the 50 % inhibitory concentration (IC_{50}).

► Determination of total antioxidant capacity

To determine the antioxidant capacity, 200 µL of each extract (dilutions were made for some extracts giving a total volume of 200 µL) were mixed with 3.8 mL of a methanolic solution of DPPH at 0.1014 mM and incubated for 30 minutes in the dark at room temperature. A range of TROLOX (519 µmol/L), used as a standard, was prepared in parallel. Absorbance is read at 517 nm. The amount of antioxidant likely to react with the DPPH radical was determined using the equation of the TROLOX calibration line. The analyzes were carried out in triplets and the results are expressed in milligrams of TROLOX equivalent per gram of extract.

► Determination of the 50 % inhibitory concentration (IC_{50})

In order to determine the IC_{50} of the different extracts, six dilutions were carried out for each extract, thus giving six solutions. 200 µL of each solution was mixed with 3.8 mL of DPPH. at 0.1014 mM. After 30 minutes of incubation in the dark and at room temperature, the absorbance is read at 517 nm with a UV-visible spectrophotometer.

The results are expressed as percentage inhibition (PI) according to the formula:

$$PI = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

The curve corresponding to the percentage inhibition as a function of the concentrations was plotted for each extract and the IC_{50} was determined from this curve. IC_{50} results were expressed in mg/mL.

3. RESULTS AND DISCUSSION

3.1 Total Polyphenol Content

The following gallic acid calibration curve was used to determine the total polyphenol content. This content is listed in Table 1 and illustrated in Fig. 2.

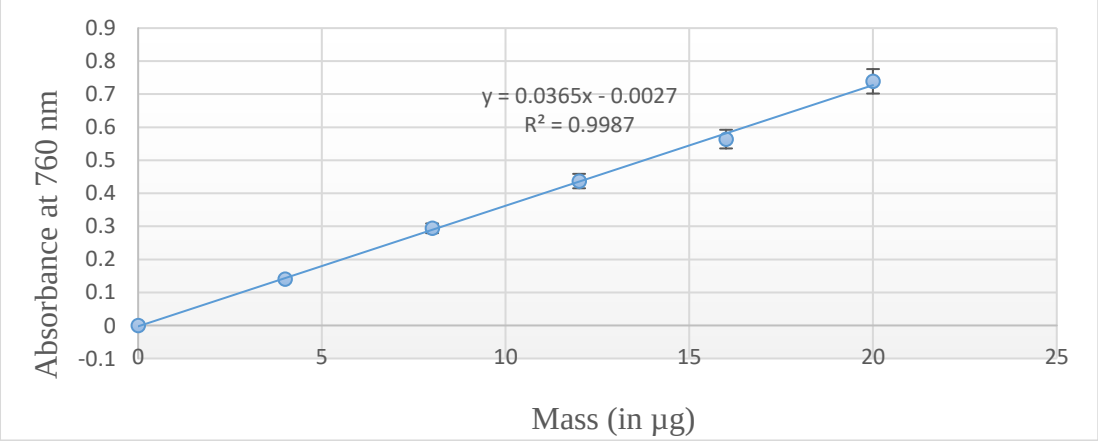

Fig. 2. calibration curve of gallic acid (0,1 mg/mL)

Table 1. Content of total polyphenols in crude extracts of the different organs studied of *C. occidentalis*

	Content of total polyphenols (in mg EAG/g)			
Extracts	**Aqueous extract**	**Methanolic extract**	**Ethyl acetate extract**	**Hexane extract**
Leaves	11.5114 ± 0.0791	10.1164 ± 0.3425	1.5808 ± 0.0274	0.4941 ± 0.0633
Seeds	3.4904 ± 0.0548	3.8151 ± 0.2055	0.6306 ± 0.0209	0.7721 ± 0.0345
Stems	2.9639 ± 0.0209	3.2379 ± 0.0618	0.3747 ± 0.0068	0.0870 ± 0.0068
Roots	4.1968 ± 0.0481	3.4479 ± 0.0274	0.7925 ± 0.0068	0.1235 ± 0.0220

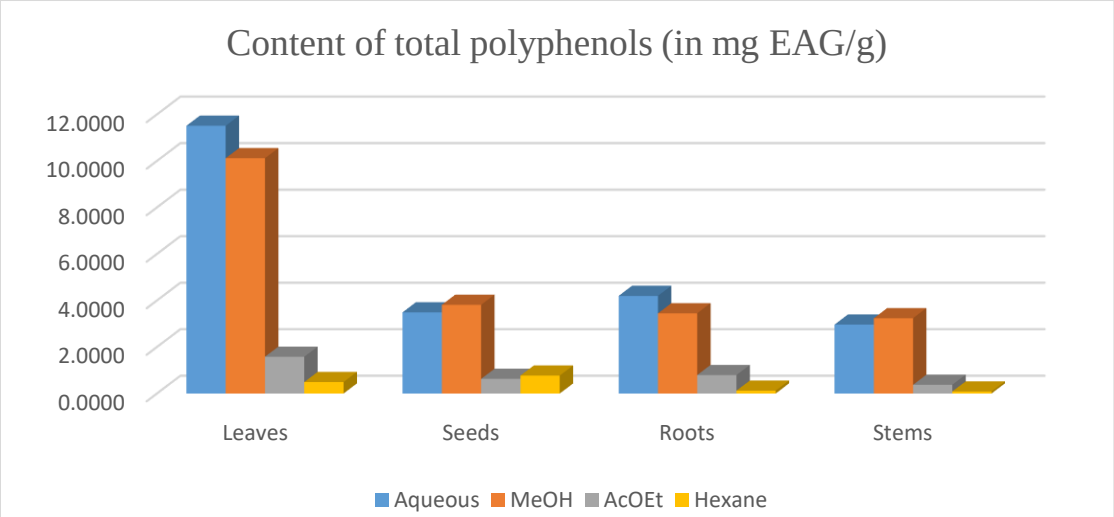

Fig. 3. Histogram of the content of total polyphenols in crude extracts of the different organs etudied of *C. occidentalis*

These results (Fig. 3) show that the leaves and roots are richer in polyphenols wich contents ranging from 0.4941 ± 0.0633 mg EAG/g to 11.5114 ± 0.0791 mg EAG/g for the leaves and 0.1235 ± 0.0220 mg EAG/g to 4.1968 ± 0.0481 mg EAG/g for roots.

For the other organs studied, namely he seeds and the stems, the examination of the results shows that the seeds have a higher polyphenol content compared to the stems.

However, the polyphenol content is dependent on the solvent. Indeed, of all the studied organs of *cassia occidentalis,* it is noted that the méthanolic and aqueous extracts present the best polyphenol contents. This can be explained by the fact that polyphenols are polar compounds therefore extractable by polar solvents [12]. This justifies the choice by some researchers of the use of hydro-alcoholic mixture for extraction of polyphenols [13].

It is also noted that the hexane extract for all organs have a low content of polyphénols. Nevertheless, the hexane extract of the seeds is richer in polyphenols than some ethyl acetate extracts. This may be due to the presence of fixed oil in the seeds which are extracted with hexane.

Moreover, the total polyphenol content obtained in our study on the leaves are higher than those obtained by Rajarajeswari and *al.* [14] wich show a content of 92.45 ± 1.26 µg/mg in the ethanolic extract of the leaves. This difference may be related to the extraction process or the method used for the assay.

The same study by Gaye and *al.* on *Carica papaya* [15], and using the same method as ours shows a higher total polyphenol content with a maximum value of 72.56 ± 3.16 mg EAG/g.

3.2 Total Flavonoid Content

The following calibration curve of Quercetin made it possible to determine the total content of flavonoids in the different extracts studied. This content is listed in the Table 2 and illustrated in Fig. 4.

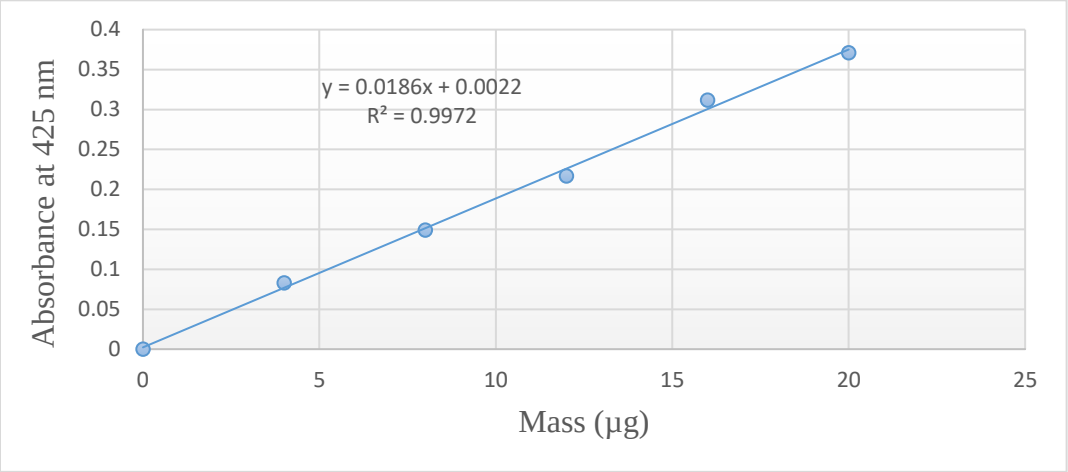

Fig. 4. Calibration curve of Quercetin (0.1 mg/mL)

Table 2. content of total flavonoids in crude extracts of the various organs studied of *Cassia occidentalis*

	Content of total Flavonoids (in mg EQ/g)			
	Aqueous extract	**Methanolic extract**	**Ethyl acetate extract**	**Hexane extract**
Leaves	2.0703 ± 0.0072	7.2115 ± 0.1728	2.2201 ± 0.0485	1.6645 ± 0.0215
Seeds	1.7931 ± 0.0395	2.6717 ± 0.0062	0.3335 ± 0.0027	0.0507 ± 0.0006
Stems	0.7584 ± 0.0072	1.9785 ± 0.0538	0.3795 ± 0.0012	0.1200 ± 0.0022
Roots	1.2674 ± 0.0215	3.4839 ± 0.0538	0.7458 ± 0.0043	0.0813 ± 0.0022

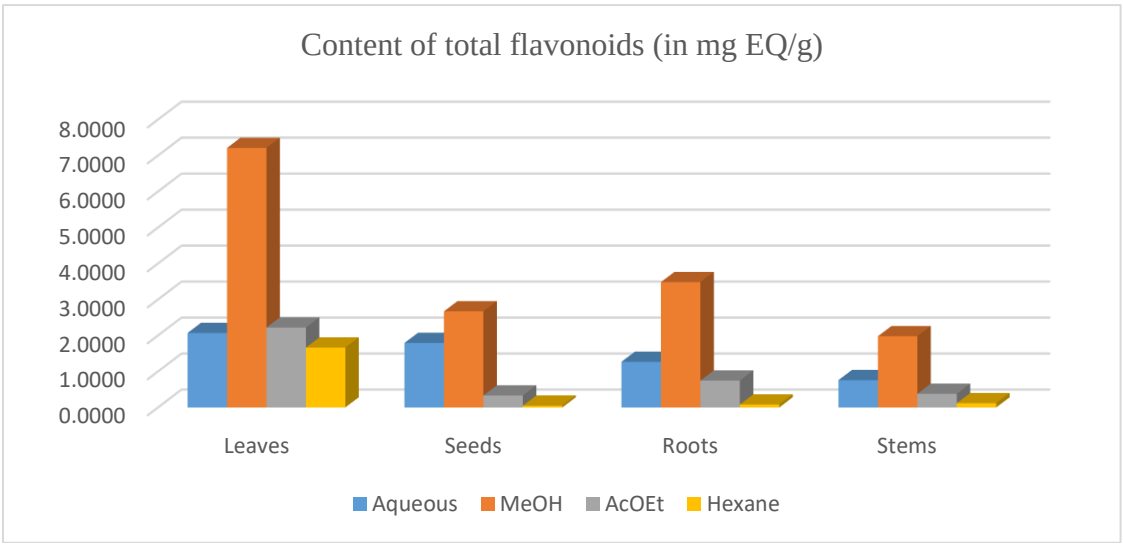

Fig. 5. Histogram of the content of total flavonoids in crude extracts of the different organs etudied of *C. occidentalis*

These results show that, like the dosage of polyphenols, the leaves and roots are still the richest organs in flavonoids among those studied in the plant. Indeed, the total flavonoid contents range from 1.6645 ± 0.0215 mg EQ/g to 7.2215 ± 0.1728 mg EQ/g for the leaves and 0.0813 ± 0.0022 mg EQ/g at 3.4839 ± 0.0538 mg EQ/g for roots Fig. 5.

The content of total flavonoids is higher in the leaves and this in all the extracts. This unequal disribution of flavonoids could be explained by

the fact that the leaves are more exposed to the sun and to parasites than the other organs of the plant. Indeed, flavonoids ensure the protection of plant tissues against the harmful effects of solar radiation [16].

The content of total flavonoids is sovent-dependent. Indeed, of all the crude extracts studied, the methanolic extracts are the richest in flavonoids.

The aqueous extracts have very low contents compared to the contents of the methanolic extracts. This may be related to the fact that the flavonoids contained in ours amples are poorly soluble in water, which is a very polar solvent.

Our results are better than those obtained by Vats and Kamal [17] who reveal a maximum flavonoid content of 1.95 mg/g of dry matter in the leaves and a minimum of 1.24 mg/g in the stems.

3.3 Antioxidant Capacity and IC_{50}

The equation of the following TROLOX calibration line made it possible to determine the antioxidant capacity of each extract, that is to say the mass of antioxidant in the extract, equivalent to TROLOX, capable of reacting with the DPPH radical. These capabilities are summarized in Table 3 and illustated in Fig. 6.

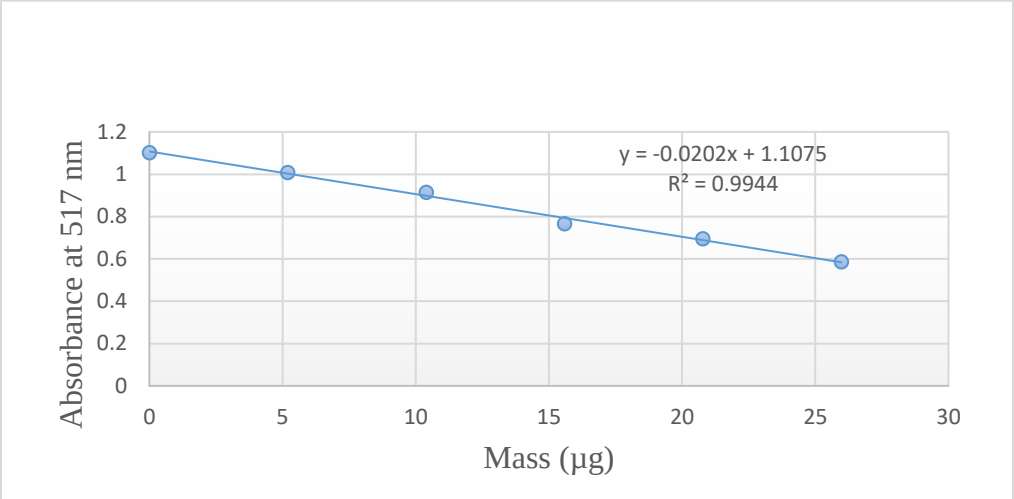

Fig. 6. Calibration curve of TROLOX ((519 µmol/L)

Table 3. antioxidant capacity and IC_{50} of crude extracts of the various studied organs of *Cassia occidentalis*

Organs	Extracts	Total antioxidant capacity (mg ETr/g)	CI_{50} (mg/mL)
	Aqueous	5.1980 ± 0.4950	N/D
Leaves	Methanolic	14.1502 ± 0.3781	0.2409 ± 0.0006
	Ethyl Acetate	2.1823 ± 0.0869	N/D
	Hexane	1.3923 ± 0.0124	N/D
	Aqueous	1.2364 ± 0.4946	N/D
Seeds	Methanolic	4.4967 ± 0.3781	N/D
	Ethyl acetate	0.8540 ± 0.0429	N/D
	Hexane	0.1176 ± 0.0248	N/D
	Aqueous	0.5569 ± 0.0371	N/D
Stems	Methanolic	4.5895 ± 0.1288	N/D
	Ethyl acetate	0.7116 ± 0.0124	N/D
	Hexane	N/D	N/D
	Aqueous	1.0932 ± 0.1891	N/D
Roots	Methanolic	7.7042 ± 0.0619	0.1415 ± 0.0005
	Ethyl acetate	2.0689 ± 0.0189	N/D
	Hexane	0.0722 ± 0.0189	N/D

N/D: Not/Determined

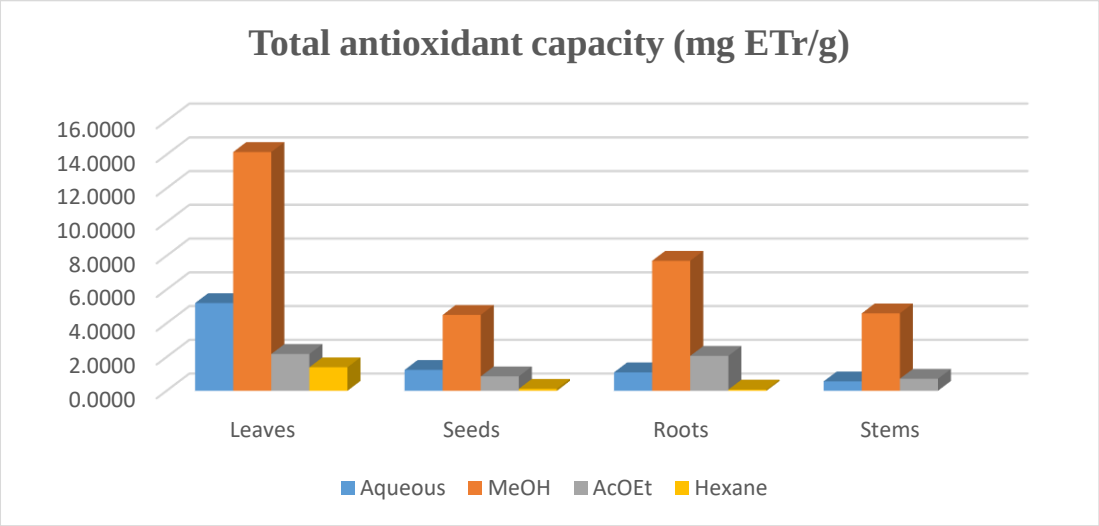

Fig. 7. histogram of antioxidant capacity versus DPPH radical. crude extracts of various studied organs of Cassia occidentalis

Examination of these results shows that leaves and roots have the highest total antioxidant capacities with values ranging from 1.3923 ± 0.0124 mg ETr/g to 14.1502 ± 0.3781 mg ETr/g for leaves and 0.0722 mg ETr/g ± 0.0189 mg ETr/g to 7.7042 mg ETr/g ± 0.0619 mg ETr/g for roots.

This result can be explained by the fact that these organs have a such high rates of polyphenols and flavonoids, which are metabolites known to have strong antioxidant power [4].

The stems and the seeds have almost equal total antioxidant capacities, the maximum values of which are 4.4967 ± 0.3781 mg ETr/g for the seeds and 4.5895 ± 0.1288 mg ETr/g.

It is also noted that the amount of antioxidant is a function of the solvent. Indeed, of all the extracts of the different organs studied, the methanolic extracts present the highest total antioxidant capacities followed by the aqueous extracts. Hexane extracts have very low antioxidant capacities.

By comparing the total antioxidant capacities of the methanolic extracts of the different organs, we note that the methanolic extract of the leaves has the highest antioxidant capacity as illustrated in fig. 7.

These results are well correlated with the polyphenol contents of the extracts of the different organs. Therefore, the extracts richest in polyphenols show a higher antioxidant capacity as reported in other studies [18].

The IC_{50}s are determined to conclude on the antioxidant power of the extracts. The results show that only the methanolic extracts of the leaves and roots have IC_{50}s with values of 0.2409 ± 0.0006 mg/mL for the leaves and 0.1415 ± 0.0005 mg/mL for roots. The IC_{50}s of the other extracts are not determined (N/D) because of the low antioxidant content.

The IC_{50} values (50% inhibitory concentration) obtained (Table 3) allows us to say that the methanolic extract of the roots has a higher antioxidant power than that of the leaves despite the fact that the methanolic extract of the leaves contains a higher quantity in antioxidants. This may be due to the fact that the molecules responsible for this antioxidant activity, although less numerous in the methanolic extract of the roots, are more effective.

4. CONCLUSION

In this work, we carried out the dosage of total polyphenols and flavonoids and the study of the antioxidant activity of different extracts of organs of *Cassia occidentalis*, a plant widely used in traditional Senegalese medicine to fight against several infections.

The results of this study show that leaves and roots are the richest organs in polyphenols and flavonoids. However, these contents are low compared to other studies on other plants.

These same organs also have the highest total antioxidant capacities, especially the level of methanolic extracts. Only the IC_{50}s of the methanolic extracts of the leaves and roots could be determined. On the other hand, The IC_{50} of the methanolic extract of the roots is lower than that of the methanolic extract of the leaves.

This lets us say that of all the extracts of the different organs studied, only the methanolic extracts of the leaves and roots have antioxidant activity. This activity is better on the methanolic extract of the roots despite the fact that the methanolic extract of the leaves is richer in polyphenols and flavonoids. These results justify the use largely of the leaves and roots of C. occidentalis in traditional Senegalese medicine.

It would therefore be important for a sequel to confirm this antioxidant activity noted in some of our extracts using other evaluation methods such as ABTS and CUPRAC.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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