



**PHYTOCHEMICAL APPROACH AND RESEARCH INTO THE ANTIOXIDANT  
ACTIVITY OF THE ROOTS AND STEMS OF CASSIA SIEBERIANA DC. : A  
MEDICINAL PLANT FROM COTE D'IVOIRE**

N'Guessan Patrick Audrey<sup>1\*</sup>, Mouho Guéi Didier Roselin<sup>2</sup>, Ouattara Logopho Hyacinthe<sup>2</sup>, Koné Songui Issouf<sup>3</sup>,  
Zon Doumadé<sup>2</sup> and Kablan Ahmont Landry Claude<sup>2</sup>

<sup>1</sup>Laboratoire De Chimie Bio-Organique Et De Substances Naturelles, Ufr-Sfa, Université Nangui Abrogoua, Abidjan, Côte D'Ivoire.

<sup>2</sup>UPR De Chimie Organique, Département De Mathématiques-Physique-Chimie, Ufr Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte D'Ivoire.

<sup>3</sup>Département De Biologie-Animale, Ufr Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte D'Ivoire.



\*Corresponding Author: N'Guessan Patrick Audrey

Laboratoire De Chimie Bio-Organique Et De Substances Naturelles, Ufr-Sfa, Université Nangui Abrogoua, Abidjan, Côte D'Ivoire.

Article Received on 28/08/2024

Article Revised on 27/09/2024

Article Accepted on 07/10/2024

**ABSTRACT**

This work involved phytochemical investigations and research into the antioxidant activity of *Cassia sieberiana* root and stem extracts. This study aims to justify the traditional use of *C. sieberiana* in the traditional treatment of certain diseases linked to oxidative stress. To achieve this, hydroethanolic crude extracts obtained by maceration and selective extracts obtained by liquid-liquid contact were prepared. The selective extracts were used for phytochemical identification and antioxidant activity research by TLC, while the hydroethanolic crude extracts were used for tube identification tests, UPLC-MS analysis, phytophenol determination and research into their antioxidant potential. Phytochemical sorting of specialized metabolites using color tube reactions and TLC revealed the presence of polyphenols, flavonoids, tannins, phenolic acids, sterols, terpenes and saponins in both plant organs. UPLC-MS analysis detected a flavonoid in the roots. The assays showed that total phenolics, total flavonoids and total condensed tannins were more concentrated in the roots than in the stems. The presence of these specialized metabolites could justify the use of these two organs in the traditional treatment of numerous pathologies, mainly those linked to oxidative stress.

**KEYWORDS:** Oxidative stress, phytochemical sorting, UPLC-MS, flavonoids.

**INTRODUCTION**

Plants have always been and still are the basis of almost all medicines, long before the development of synthetic drugs in the 19th century.<sup>[1,2]</sup> It is estimated that around half of all bioactive molecules in clinical use come from plants.<sup>[3]</sup> Thus, plants are an important natural source for the treatment of many diseases.<sup>[4]</sup> What's more, despite the fact that plants are regularly subjected to various stresses that can lead to withering and/or death<sup>[3]</sup>, they manage to survive, grow and multiply, while synthesizing anti-free radical substances for their defences. Plants are therefore natural sources of antioxidant substances designed to protect them against oxidative stress.<sup>[5]</sup> These defensive antioxidants are mainly derived from specialized metabolites: flavonoids, coumarins, tannins, alkaloids, saponins, sterols and terpenes. Indeed, specialized metabolites are endowed with numerous pharmacological properties, including

antioxidant properties.<sup>[6,7]</sup> However, the plant kingdom is not alone in suffering the deleterious effects of oxidative stress, as the human organism is not immune. Like all mammals, we produce free radicals on a daily basis. These free radicals are unstable, highly reactive compounds which, through successive reactions, damage numerous cellular components such as proteins, lipids and DNA.<sup>[8]</sup> They are the ultimate cause of numerous pathologies and are thought to be at the root of certain chronic diseases (cardiovascular, neurodegenerative and Alzheimer's diseases, cancer and diabetes) as well as ageing.<sup>[9,10]</sup> Antioxidant molecules of synthetic origin do exist for healthcare use, but they are currently being called into question because of the potential toxicological risks they may pose.<sup>[11,12]</sup> Thus, the search for new sources of antioxidants appears to be a necessity. With this in mind, we turned our attention to *Cassia sieberiana* following an ethnobotanical survey carried

out in the north of Côte d'Ivoire with several traditional practitioners. *Cassia sieberiana* is traditionally used to treat certain pathologies directly or indirectly linked to oxidative stress, such as sleeping sickness<sup>[13]</sup>, hemorrhoids<sup>[14]</sup>, sterility and dysmenorrhea.<sup>[13]</sup> The aim of this work is to scientifically confirm or refute the use of *Cassia sieberiana* roots and stems in the traditional treatment of diseases caused by oxidative stress.

## 1. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. Plant material

The plant material consists of stems and roots of *Cassia sieberiana*. The plant was selected on the basis of an ethnobotanical survey of tradithérapeutes practising in northern Côte d'Ivoire. It was harvested in February 2022 in the town of Korhogo (9° 27' 28" North, 5° 37' 46" West). The various plant organs were dried for 5 days in a room at room temperature, away from the sun. Finally, these dried organs were ground in a mortar and sieved to obtain fine powders, which were used to prepare the different extracts to be tested.

#### 1.1.2. Laboratory materials and equipment

Laboratory equipment includes: beakers, test tubes, pipettes, measuring cylinders, flat-bottomed flasks, Erlenmeyer flasks, funnels, oven, electronic balance, micropipettes, water bath, hot plate, spatula, filter paper, thermometer, refrigerator, gallows, vats with lids, sprayer, dryer, micropipettes, JENWAY 7315 spectrophotometer, fume hood, Waters ACQUITY UPLC-MS mass spectrometer.

#### 1.1.3. Reagents and chemicals

The chemicals used are of analytical quality. They include ethanol, hexane, dichloromethane, ethyl acetate, acetic acid, acetone, methanol, acetonitrile and formic acid. For thin-layer chromatography (TLC) tests, silica gel chromatoplates 60 F<sub>254</sub> on an aluminum support were used.<sup>[15]</sup> The developers and reagents used for TLC and tube reactions were Dragendorff's reagent, 2% FeCl<sub>3</sub>, Shinoda's reagent, 10% KOH, CH<sub>3</sub>CO<sub>3</sub>CCH<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub> and DPPH.

## 2.2. METHODS

### 2.2.1. Preparing study extracts

#### 2.2.1.1. Preparation of hydroethanol crude extracts

A 10g mass of each organ powder was macerated in 100 mL ethanol/water mixture (70mL / 30 mL) for 24h. Each maceration was performed 3 times. After filtration, the macerates obtained were combined, then placed in an oven at 50°C for 2h to remove the ethanol. The resulting extract was kept for 24h in a refrigerator at 4°C to precipitate lipophilic compounds. After decantation, hydroethanol extracts were obtained. One quantity was used for phytochemical screening in tubes, to assess antioxidant activity in tubes, to assay certain secondary metabolites and for analysis by ultra performance liquid chromatography (UPLC). The remainder was used to prepare selective extracts.<sup>[16]</sup>

#### 2.2.1.2. Preparation of selective extracts

A volume of 15 mL of each hydroethanol extract was exhausted by successive fractionations with 3×10 mL of hexane, dichloromethane and ethyl acetate. The various selective organic fractions were concentrated to dryness in an oven, weighed, then recovered with the respective extraction solvents and stored in a refrigerator at 4°C. The selective extracts were used for phytochemical screening and evaluation of antioxidant activity on TLC plates.<sup>[16]</sup>

### 2.2.2. Phytochemical studies

#### 2.2.2.1. Color reaction tests of hydroethanolic crude extracts

To carry out these tests, the analytical methods used are those described in the literature.<sup>[17-19]</sup> This involves bringing specific reagents and the hydroethanolic extracts tested into contact and observing the changes in state.

#### 2.2.2.2. Phytochemical screening on TLC plate

TLC screening of the selective extracts was carried out according to the methods described by.<sup>[20,21]</sup> Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank containing the migration solvents (developing agents). After development, chromatograms were visualized using visible light developers. Colorations appearing as spots are recorded, and frontal ratios (Rf) calculated. Secondary metabolites were identified using the following migration solvents.

- Hexane (C<sub>6</sub>H<sub>14</sub>) / ethyl acetate (AcOEt) : (5 : 0.375; v/v) for hexane extracts.

- Dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) / ethyl acetate (AcOEt) / acetic acid (CH<sub>3</sub>COOH): (1:4:1; v/v/v) for dichloromethane and ethyl acetate extracts.

#### 2.2.2.3. UPLC analysis of hydroethanolic extracts

UPLC-MS analysis was carried out using a Waters ACQUITY UPLC-MS instrument. It comprises a quadrupole detector, A mass spectrometer equipped with an Electrospray Ionisation Interface (ESI) and a diode array detector. To perform the analysis, a 5 mg mass of each crude hydroethanol extract of *Cassia sieberiana* was dissolved in 1 to 1.5 mL of methanol. The resulting suspension was filtered, and a sufficient quantity of the filtrate was introduced into A small vessel (vial) specially designed for the analysis, to determine the chromatographic profile of the extract and the mass spectra of the various associated compounds.

The binary solvent system used consisted of solvents (A; B) with A: H<sub>2</sub>O/acetonitrile 98/2 + 0.1% formic acid and B: acetonitrile 100%+0.1% formic acid. In order to identify the compounds obtained, the crude formulae of several secondary metabolites were given, based on information from the literature on *Cassia sieberiana* in particular and on plants in general. The appearance of

one or more peaks in green confirms the presence of the secondary metabolites recorded in the instrument, while the appearance of peaks in red invalidates their presence.

#### 2.2.2.4. Dosages

##### Assay of total phenols

The total phytophenol contents were determined using the Folin-Ciocalteu colorimetric method.<sup>[22]</sup> To 1 mL of each extract are added 1.5 mL of Na<sub>2</sub>CO<sub>3</sub> (17%, mv) and 0.5 mL of Folin-Ciocalteu reagent (0.5N). The whole is incubated at 37°C for 30 min; the absorbance is read at 760 nm against a blank without extract taken as a reference. The quantification of total polyphenols is carried out according to a linear calibration line ( $y = ax+b$ ) carried out by a standard extract of gallic acid at different concentrations (0 to 1000 µg/mL) under the same conditions. Than the sample. The results are expressed in milligrams of gallic acid equivalent per gram of dry matter of the powdered plant (mg EAG/g DM).

##### Assay of total flavonoids

This assay was carried out according to the method used by Arvouet-Grand *et al.*<sup>[23]</sup> 500 µL of 2% aluminum chloride (AlCl<sub>3</sub>) in methanol was added to an equal volume of extract. After 10 minutes of incubation, the reading was taken with a spectrophotometer at 415 nm. Quercetin (0-100 µg/L), used as a standard, allowed the establishment of the calibration curve. Three (3) tests were carried out for each extract and the result given is an average of the three readings. Results expressed in milligrams Quercetin Equivalent (EQ) per gram of dry matter (mg EQ/g DM).

##### Assay of total condensed tannins

The determination of condensed tannins in the different extracts was carried out according to the method described by Heimler *et al.*<sup>[24]</sup> For 400 µL of each sample or standard, 3 mL of a 4% methanolic vanillin solution and 1.5 mL of concentrated hydrochloric acid are added. The mixture is incubated for 15 min and the absorbance

is read at 500 nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin (0-300µg/ml), and are expressed in milligrams of catechin equivalent per g of dry matter (mg EC/g DM).

#### 2.2.3. Research of antioxidant activity

##### 2.2.3.1. Tube screening for DPPH

The method used to carry out this test is that proposed by Popovici *et al.*<sup>[25]</sup> In a volume of 0.5 mL of hydroethanolic extract solution from each organ are added 1.5 mL of purple-colored DPPH and the positive reaction results in the appearance of a yellow color in the medium after 15 min. 'incubation.

##### 2.2.3.2. Screening by TLC against DPPH of selective extracts

The screening of antioxidant power by TLC used is that developed by the method described by Takao *et al.*<sup>[26]</sup> A volume of 10 µL of each plant extract solution is placed on a chromatoplate (silica gel 60 F<sub>254</sub>, on aluminum support (Merck)), which is then placed in a chromatography tank saturated with migration solvent. After development, the chromatograms are dried then revealed using an ethanolic solution of DPPH (2 mg/mL). After 30 min of optimal time, the constituents of the extract presenting potential anti-radical activity are revealed in the form of pale yellow spots on a purple background.

The same migration solvents used during the phytochemical screening by TLC also made it possible to search for the antioxidant potential on TLC plates.

### 3. RESULTS AND DISCUSSION

#### 3.1. Extraction yields

The different extractions carried out made it possible to determine the extraction yields of the roots and stems of *Cassia sieberiana*. The different values obtained are recorded in table 1.

**Table 1: Yields of different *Cassia sieberiana* extracts.**

	Stems	Roots
Hydroethanolic extracts	22.02 %	17.46 %
Hexane extracts	2.11 %	1.67 %
Dichloromethane extracts	3.08 %	2.59 %
Ethyl acetate extracts	5.17 %	4.86 %

The extraction method used in this study is maceration with the ethanol-water solvent mixture (70 : 30; v/v). This method, reputed to extract a large number of organic compounds, was used to obtain hydroethanolic plant extracts. Yields of ethanolic extracts were 22.02% for stems and 17.46% for roots. These values are relatively high compared with those reported in the literature.<sup>[27,28]</sup> This may be due to the presence of water, which increases the permeability of plant tissues and favours the phenomenon of mass diffusion in the extraction stage.<sup>[29,30]</sup> The yields of the selective extracts

(hexanic, dichloromethane and ethyl acetate) vary from 1.67% for the hexanic root extract to 5.17% for the ethyl acetate stem extract. Hydroethanol yields are thus far higher than those of selective extracts. With identical solvents, stem yields are always higher than root yields. We also note that for both organs, taken separately, the yields of ethyl acetate extracts are higher than those of dichloromethane extracts, which are in turn higher than those of hexane extracts. This order of magnitude can be explained in part by the polarity of the solvents used. Indeed, the polarity of ethyl acetate (1.78) is higher than

that of dichloromethane (1.14), which in turn is higher than that of hexane (0.00).

### 3.2. Phytochemical screening in tube

#### 3.2.1. Detection of saponins (Foam index)

Saponins were detected in stem and root decoctions of *Cassia sieberiana* and moss index (Im) values were determined (Table 2).

**Table 2: Foam index detection.**

	Foam height (cm)									
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>	T <sub>10</sub>
<b>CST</b>	0.2	0.5	0.6	0.7	1.7	2.1	2.2	2.4	2.5	2.8
<b>CSR</b>	0.0	0.0	0.7	0.8	1.3	1.4	1.5	1.6	2.2	2.3

**CSR: Hydroethanolic root extract; CST: Hydroethanolic extract of the stems; T: Tube**

The height of moss is greater than 1 cm from tubes 5, then after calculating the moss index (Im) by the relationship  $Im = (1000/N^{\circ}Tube)$ , the presence of saponins in the roots and stems of *Cassia sieberiana* was confirmed by the value of their moss index (Im=200) which is well above 100.<sup>[18]</sup>

#### 3.2.2. Detection of polyphenols, flavonoids and coumarins

Tube detection of polyphenols, flavonoids and coumarins in hydroethanolic extracts of stems and roots of *Cassia sieberiana* was carried out and the results are illustrated in Table 3.

**Table 3: Detection of polyphenols, flavonoids and coumarins.**

	Polyphenols	Flavonoids	Coumarins
<b>CST</b>	+	+	+
<b>CSR</b>	+	+	-

**CSR: Hydroethanolic root extract; CST: Hydroethanolic extract of the stems**

Phytochemical tube screening revealed the presence of polyphenols and flavonoids in the roots and stems of *Cassia sieberiana*. The positive test for polyphenols in the plant's stems and roots could be explained in part by the presence of flavonoids, which were also detected in this study in both organs, as well as coumarins, which were only detected in the stems.

### 3.3. Phytochemical screening by TLC

The presence of sterols and terpenes was highlighted in the hexane extracts with visible sulfuric vanillin. As for the alkaloids, they were searched for in the dichloromethane extracts with Dragendorff's reagent in the visible light. Finally, the ethyl acetate extracts were used to search for tannins and phenolic acids with iron III chloride (FeCl<sub>3</sub>). The compounds detected are presented in Table 4.

**Table 4: Phytochemicals identified in selective extracts from the roots and stems of *Cassia sieberiana*.**

Extracts		Possible phytochemicals [Rf] molecular spot coloring
<b>E<sub>1</sub></b>	<b>R</b>	<b>Terpenes</b> : [0.86] Vi <sup>a*</sup> ; [0.50] Vi <sup>a*</sup> ; [0.20] Vi <sup>a*</sup> ; [0.08] Vi <sup>a*</sup> <b>Sterols</b> : [0.90] B <sup>a*</sup> ; [0.13] B <sup>a*</sup>
	<b>T</b>	<b>Terpenes</b> : [0.86] Vi <sup>a*</sup> ; [0.79] Vi <sup>a*</sup> ; [0.62] Vi <sup>a*</sup> ; [0.50] Vi <sup>a*</sup> ; [0.30] Vi <sup>a*</sup> ; [0.23] Vi <sup>a*</sup> ; [0.20] Vi <sup>a*</sup> ; [0.10] Vi <sup>a*</sup> ; [0.08] Vi <sup>a*</sup> <b>Sterols</b> : [0.90] B <sup>a*</sup> ; [0.13] B <sup>a*</sup>
<b>E<sub>2</sub></b>	<b>R</b>	<b>Alkaloids</b> : NI
	<b>T</b>	<b>Alkaloids</b> : NI
<b>E<sub>3</sub></b>	<b>R</b>	<b>Phenolic acids</b> : [0.27] V <sup>c*</sup> <b>Tannins</b> : [0.19] G <sup>c*</sup> ; [0.13] G <sup>c*</sup>
	<b>T</b>	<b>Phenolic acids</b> : [0.92] V <sup>c*</sup> ; [0.27] V <sup>c*</sup> <b>Tannins</b> : [0.80] G <sup>c*</sup> ; [0.20] G <sup>c*</sup> ; [0.06] G <sup>c*</sup>

**A: Sulfuric vanillin; B: Dragendorff; C: FeCl<sub>3</sub>; Vi: Purple; B: Blue; V: Green; G: Gray; \*: Visible; NI: Not identified; E<sub>1</sub>: Hexanic extracts; E<sub>2</sub>: Dichloromethane extracts; E<sub>3</sub>: Ethyl acetate extracts; R: Roots; T: Stems**

Sterols were revealed with sulphuric vanillin in visible blue<sup>[31]</sup> and terpenes as purple spots<sup>[32]</sup> in the roots and stems of *Cassia sieberiana*. Tannins and phenolic acids were found in the roots and stems of *C. sieberiana*. The presence of tannins was marked by a grey coloration,

while phenolic compounds appeared green.<sup>[31]</sup> Qualitative tube and TLC analysis of *C. sieberiana* stem and root extracts revealed the presence of polyphenols (flavonoids, tannins and phenolic acids), saponins, sterols and terpenes. However, only the stems contained coumarins, and no alkaloids were detected in either plant organ. These results are clearly in line with those obtained by several authors. Indeed, Sam *et al.*<sup>[33]</sup> showed the co-presence of saponins, sterols, tannins and triterpenes in the roots of the same plant species. Traore

*et al.*<sup>[34]</sup> also showed that roots contain polyphenols, flavonoids, tannins, saponins and sterols. They also noted a total absence of alkaloids. In the work of Awomukwu *et al.*<sup>[35]</sup>, chemical analysis of aqueous crude extracts of *C. sieberiana* stems and roots revealed the presence of tannins, flavonoids, saponins, phenol compounds and, above all, alkaloids. The presence of alkaloids in stems and roots is at odds with the results obtained in this study. This difference could be explained by the difference in harvesting site and period, as the studies carried out by Awomukwu and colleagues were carried out on the Nigerian species.

The presence of these bioactive secondary metabolites seems to justify the use of *C. sieberiana* in the traditional

treatment of toothache<sup>[36]</sup>, headaches, malaria<sup>[14]</sup>, skin diseases<sup>[33]</sup> and venereal diseases.<sup>[13]</sup> In fact, polyphenols (flavonoids, coumarins, tannins and phenolic acids), saponins, sterols and terpenes contain numerous pharmacological properties. These include antibacterial, antiviral<sup>[6]</sup>, antiparasitic<sup>[38,39]</sup>, antimicrobial<sup>[40]</sup>, analgesic<sup>[41]</sup>, anti-inflammatory<sup>[42]</sup> and haemostatic<sup>[43]</sup> properties.

### 3.4. UPLC analysis of hydroethanolic extracts from *Cassia sieberiana* roots and stems

UPLC-MS analysis of hydroethanolic extracts of *C. sieberiana* was carried out in order to characterize certain molecules in these extracts. This analysis highlighted the five major compounds in each extract (Table 5).

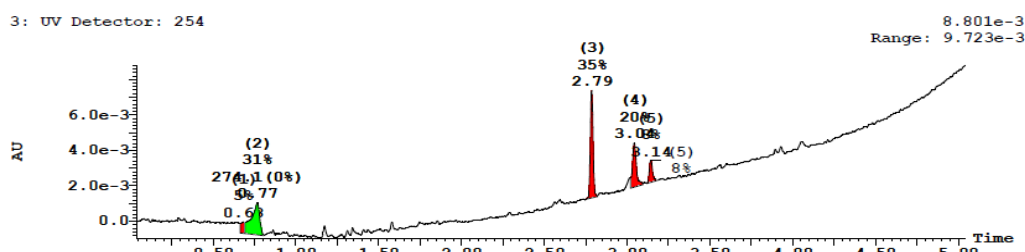
**Table 5: Identification of the majority compounds in the roots and stems of *Cassia sieberiana*.**

	CSR					CST				
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Tr (mn)	0.68	0.77	2.79	3.04	3.14	0.77	1.17	2.79	3.04	3.14
P (%)	5.13	31.41	34.90	20.37	8.20	16.67	5.86	57.94	13.78	5.74
Compound Formula	NI	Flavonoid	NI	NI	NI	NI	NI	NI	NI	NI
M (g/mol)	NI	C <sub>15</sub> H <sub>14</sub> O <sub>5</sub>	NI	NI	NI	NI	NI	NI	NI	NI
	NI	274.08	NI	NI	NI	NI	NI	NI	NI	NI

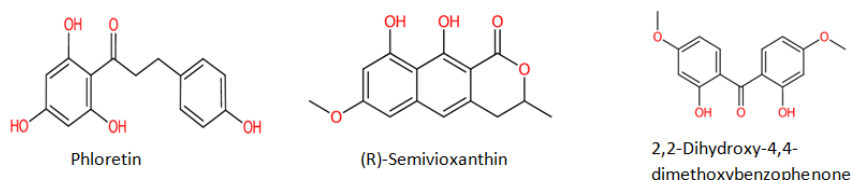
**Tr:** retention time of compounds; **P (%):** percentage of presence of compounds; **CSR:** Hydroethanolic root extract; **CST:** Hydroethanolic extract of the stems

Each compound was analyzed both by comparison with the chromatographic profile of the standard molecules used, and by analysis of the mass spectra of each

compound. Thus, A flavonoid (peak 2) with A molecular mass of 274.08 and formula C<sub>15</sub>H<sub>14</sub>O<sub>5</sub>, shown in green in Figure 1, was identified only in the hydroethanol extract of *Cassia sieberiana* roots. Possible structures of this flavonoid are shown in figure 2.



**Figure 1: Chromatographic profile of the hydroethanolic extract of *Cassia sieberiana* roots.**



**Figure 2: Possible structures of flavonoid with chemical formula C<sub>15</sub>H<sub>14</sub>O<sub>5</sub>.**

### 3.5. Determination of total phytochemicals

Quantitative analysis was carried out on hydroethanol extracts using spectrophotometry. It assessed the phenol,

total flavonoid and condensed tannin content of *Cassia sieberiana* roots and stems (Table 6).

**Table 6: Contents of some phytochemicals in the roots and stems of *Cassia sieberiana*.**

Compounds dosed	Roots	Stems
Total phenols (mg EAG/g DM)	1912.13±64.08	1721.18±63.65
Total flavonoids (mg EQ/g DM)	10.18±0.94	7.65±0.56
Condensed tannins (mg EC/g DM)	556.99±23.68	438.72±27.06

Total phenolics, total flavonoids and total condensed tannins in the hydroethanol extract of *Cassia sieberiana* roots were found to be higher than in the hydroethanol extract of the stems. These higher values for total phenolics in the roots compared to the stems could be explained by the fact that the roots of this plant concentrate more flavonoids and tannins. It should also be noted that polyphenols are organic phytochemicals that are widely present in the plant kingdom and contain multiple pharmacological properties, which could once again justify the use of these two organs in traditional medicine.<sup>[40, 44]</sup>

### 3.6. Antioxidant activity

In-tube antioxidant activity was tested by adding DPPH solution to hydroethanolic extracts of *Cassia sieberiana* stems and roots, and a yellow coloration was observed after a few minutes incubation. This suggests that both extracts may have antioxidant activity. This assertion was confirmed by TLC by monitoring the reduction of the DPPH radical, which was accompanied by its change from violet to yellow. The frontal ratios (Rf) of these spots were calculated and compared with those of the phytochemical screening (Table 7).

**Table 7: DPPH radical scavenger phytochemicals present in selective extracts of *Cassia sieberiana*.**

Extracts		Possible phytochemicals [Rf] molecular spot coloring
E <sub>1</sub>	R	Terpenes: [0.86] J <sup>d*</sup> ; [0.50] J <sup>d*</sup> ; [0.20] J <sup>d*</sup> ; [0.08] J <sup>d*</sup> Sterols: [0.90] J <sup>d*</sup> ; [0.13] J <sup>d*</sup> NI: [0.95] J <sup>d*</sup> ; [0.70] J <sup>d*</sup>
	T	Terpenes: [0.86] J <sup>d*</sup> ; [0.79] J <sup>d*</sup> ; [0.62] J <sup>d*</sup> ; [0.50] J <sup>d*</sup> ; [0.30] J <sup>d*</sup> ; [0.23] J <sup>d*</sup> ; [0.20] J <sup>d*</sup> ; [0.10] J <sup>d*</sup> ; [0.08] J <sup>d*</sup> Sterols: [0.90] J <sup>d*</sup> ; [0.13] J <sup>d*</sup> NI: [0.95] J <sup>d*</sup> ; [0.70] J <sup>d*</sup> ; [0.42] J <sup>d*</sup>
E <sub>2</sub>	R	NI: [0.94] J <sup>d*</sup> ; [0.04] J <sup>d*</sup>
	T	NI: [0.65] J <sup>d*</sup> ; [0.55] J <sup>d*</sup> ; [0.46] J <sup>d*</sup> ; [0.34] J <sup>d*</sup> ; [0.26] J <sup>d*</sup> ; [0.15] J <sup>d*</sup> ; [0.09] J <sup>d*</sup> ; [0.04] J <sup>d*</sup>
E <sub>3</sub>	R	Phenolic acids: [0.27] J <sup>d*</sup> Tannins: [0.19] J <sup>d*</sup> ; [0.13] J <sup>d*</sup> NI: [0.50] J <sup>d*</sup> ; [0.45] J <sup>d*</sup> ; [0.39] J <sup>d*</sup> ; [0.34] J <sup>d*</sup> ; [0.25] J <sup>d*</sup>
	T	Phenolic acids: [0.92] J <sup>d*</sup> ; [0.27] J <sup>d*</sup> Tannins: [0.80] J <sup>d*</sup> ; [0.06] J <sup>d*</sup> NI: [0.82] J <sup>d*</sup> ; [0.65] J <sup>d*</sup> ; [0.59] J <sup>d*</sup> ; [0.49] J <sup>d*</sup> ; [0.40] J <sup>d*</sup> ; [0.35] J <sup>d*</sup> ; [0.20] J <sup>d*</sup>

**d : DPPH; J: Yellow; \*: Visible; NI: Not identified; E<sub>1</sub>: Hexanic extracts; E<sub>2</sub>: Dichloromethane extracts; E<sub>3</sub>: Ethyl acetate extracts; R: Roots; T: Stems**

Antioxidant activity was assessed in tubes and by TLC. Both methods are based on the same principle. They involve the anti-free radical activity of plant extracts in inhibiting the DPPH radical. In the presence of free radical scavengers (H donors), the violet DPPH (H acceptor) is reduced to the yellow 1, 1-diphenyl-2-picrylhydrazine (DPPH-H).<sup>[26]</sup> This study is of the utmost importance, as oxidative stress is not only the main initial cause of many diseases<sup>[45]</sup>, but also the factor that potentiates the onset of multi-factorial diseases.<sup>[10]</sup> Antioxidant activity in tubes showed that both hydroethanol extracts from roots and stems have antioxidant activity. This observed activity is due, among other things, to the presence of polyphenols (flavonoids, coumarins) and saponins, which were detected by tube reactions. As for antioxidant activity by TLC, the various selective extracts (hexanic, dichloromethane and ethyl acetate) were found to exert antioxidant activity. The compounds responsible for this activity include sterols, terpenes, phenolic acids and tannins.

In short, after assessing antioxidant activity in tubes and by TLC, we can say that the antioxidant activity of *Cassia sieberiana* root and stem extracts could be

explained in part by the presence of phenolic substances such as phenolic acids, tannins, coumarins and flavonoids, as well as saponins, sterols and terpenes detected within them. Indeed, numerous studies have already demonstrated the antioxidant properties of these compounds.<sup>[6, 16, 40, 44]</sup>

Ultimately, the presence of these various secondary metabolites and the antioxidant activity exerted by the roots and stems of *Cassia sieberiana* could justify the frequent use of these two organs in the traditional treatment of numerous pathologies such as toothache<sup>[36]</sup>, headache, malaria<sup>[14]</sup>, skin diseases<sup>[33]</sup> and venereal diseases<sup>[13]</sup> on the one hand, and on the other, be used to treat or prevent diseases linked to oxidative stress.

### 4. CONCLUSION

The main aim of this work was to confirm or refute the use of *Cassia sieberiana* roots and stems in the traditional treatment of oxidative stress-related diseases through phytochemical and biological investigations.

Phytochemical investigations included phytochemical screening, UPLC-MS analysis and assays of selected phenolic compounds. The results of this phytochemical study highlighted the presence of polyphenols, flavonoids, tannins, phenolic acids, sterols, terpenes and

saponins in *Cassia sieberiana* roots and stems. However, only the stems contained coumarins, with an absence of alkaloids in both organs. UPLC-MS analysis showed the presence of a flavonoid with the gross formula  $C_{15}H_{14}O_5$  in the roots. Assays showed that total phenolic compounds, total flavonoids and total condensed tannins were more concentrated in *C. sieberiana* roots than in stems.

Biological investigations consisted in investigating antioxidant activity using DPPH by qualitative methods in tubes and by TLC. It was found that these organs contain an antioxidant profile. These results could justify the use of these two organs in the traditional treatment of numerous pathologies, mainly those linked to oxidative stress.

In the future, it would be wise to study the toxicity of these organs in order to prove whether or not the plant is harmless to the human organism, with a view to contributing to the manufacture of a phytomedicine with antioxidant properties.

## 5. REFERENCES

- Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Indian J Pharmacol*, 2000; 32: 81–118.
- Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. *J Agric Food Chem*; 2002; 50(19): 5294–99.
- Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE. Climate Change Effects on Plant Disease: Genomes to Ecosystems. *The Annual Review of Phytopathology*, 2006; 44: 489-509.
- Mpiana PT, Balanganayi Ek, Kanangila AB, Kolonda EM, Ngbolua KN, Tshibangu DST, Atibu EK, Lumbu JB. Anti-sickle cell activity and thermodegradation of anthocyanins extracted from *sterculia quinqueloba* and *ficus capensis*. *Int. J. Biol. Chem. Sci*; 2009; 3(3): 551-560.
- Sarr SO, Fall AD, Gueye R, Diop A, Diatta K, Diop N, Ndiaye B, Diop YM. Study of the antioxidant activity of *Vitex doniana* (Verbenaceae) leaf extracts. *Int. J. Biol. Chem. Sci*; 2015; 9(3): 1263-1269.
- Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. 2nd edition, Tec et Doc., Lavoisier, Paris, 1993; 915.
- Rolland Y. News on lipids in cosmetics: Natural plant antioxidants. *Oilseeds and fats, Crops and Lipids*, 2004; 11 (6): 419 – 424.
- Milane H. Quercetin and its derivatives: molecules with a peroxidative or therapeutic nature. Doctoral thesis. Louis Pasteur University Strasbourg I., 2004; 155.
- Pastre COJ. Interest in antioxidant supplementation in the diet of domestic carnivores. Doctoral thesis, Paul-Sabatier University, Toulouse, 2005; 116.
- Sergeant C, Hamon C, Simonoff M, Constans J, Conri C, Peuchant C, Delmas-Beauvieux MC., Clerc C, Pellegrin JL, Leng B, Pellegrin I, Fleury H. *Oxidative Stress in Cancer, AIDS and neurodegenerative diseases*. Editors : L. Montagnier, R. Olivier, C. Pasquier, Marcel Dekker. Inc. New York- Basel-Hong Kong, 1998; 409-427.
- Brieger K et al. Reactive oxygen species: from health to disease. *Swiss Medical Weekly*, 2012; 142, w13659.
- Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. *Journal of Food Composition and Analysis*, 2006; 19: 531–537.
- Schmelzer GH., Gurib-Fakim A., Arroo R., Bosch C.H. *et al.* *Plant Resources of Tropical Africa 11(1) – Medicinal Plants 1*. Backhuys Publishers, Wageningen, Netherlands. ISBN 978-90-5782-204-9: 2008.
- Mshana NR. Traditional medicine and pharmacopoeia: contribution to the revision of ethnobotanical and floristic studies in Ghana. Organization of AfricanUnity/Scientific, Technical & Research Commission, 2000.
- Merck E. Revealers for thin-layer and paper chromatography. In: Darmstadt ed; 1980; 153.
- Ouattara LH, Kabran MGR, Kadja AB, Tano BM, Mamyrbekova-Bekro JA, Bekro YA. Phytochemical study and antioxidant activity of plant extracts from Côte d'Ivoire used in the traditional treatment of hemorrhoids. *International Journal of Innovation and Applied Studies*, 2016; 15(4): 881-893.
- Ladyguina E, Safronitch L, Otriachenkova V, Bolandina I, Grinkevitch N, Sorokina A, Glizin VI, Molodojnikova LM, Mitin YS, Samilina IA, Ermakova VA. Chemical analysis of medicinal plants. Moska Edition; Vischaya Chkola (translated from Russian), 1983; 46-347.
- Dohou N, Yamni K, Tahrouch S, Idrissi HLM, Badoc A, Gmira N. Phytochemical screening of an endemic Ibero-Moroccan *Thymelaea lithroides*. *Bulletin of the Bordeaux Pharmacy Society*, 2003; 142: 61-78.
- Békro YA, Békro MJA, Boua BB, Tra Bi FH, Ehile EE. Ethnobotanical study and phytochemical screening of *Caesalpinia benthamiana* (Baill.) Herend. and zarucchi (Caesalpinaceae). *Sciences and Nature*, 2007; 4: 217-225.
- Dawson R, Elliott D, Elliott W, Jones K. *Biochemist's Dictionary*. Mir Edition, Moscow, 1991.
- Mamyrbékova-Békro JA, Konan MK, Békro YA, Djié Bi MG, Zomi Bi TJ, Mambo V, Boua BB. Phytocompounds of the extracts of four medicinal plants of Côte d'Ivoire and assessment of their potential antioxidant by thin layer chromatography. *European Journal of Scientific Research*, 2008; 24(2): 219-228.
- Singleton VL, Ortofer R, Lamuela-Raventos RM. Analysis of total phenol and other oxidation substrates and antioxidants by means of folin-

- ciocalteu reagent. Packed L (ed) Methods in enzymology Orlando Academic Press, 1999; 152-178.
23. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of a propolis extract and identification of the main constituents, Journal of Pharmacy of Belgium, 1994; 49: 462-468.
  24. Heimler D, Vignolini P, Giulia DM, Francesco VF, Rmani A. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chemistry, 2006; 99: 464-469.
  25. Popovici C, Saykova I, Tylkowsk B. Evaluation of the antioxidant activity of phenolic compounds by reactivity with the free radical DPPH. Revue de Genie Industriel, 2009; 4: 25-39.
  26. Takao T, Kitatami F, Watanabe N, Yagi A, Sakata K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shell fish. Bioscience, Biotechnology and Biochemistry, 1994; 58: 1780-1783.
  27. Ouattara LH. Phytochemical and biological study of seven medicinal plants used in traditional therapy for hemorrhoids in Côte d'Ivoire. Single doctoral thesis, Nangui ABROGOUA University, 2017; 154.
  28. Kabran GRM. Chemical and cytotoxic study of ten medicinal plants from Ivory Coast, used in the traditional treatment of breast cancer. Single Doctoral thesis, Nangui ABROGOUA University, 2014; 265.
  29. Moure A, Cruz M, Franco D, Manuel D, Sineiro J, Dominguez H, Nunez M, Carlos P. Natural antioxidants from residual sources. Food Chemistry, 2000; 72(2): 145-171.
  30. Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Soumaya B, Hajlaoui H, Abdelly C. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT - Food Science and Technology, 2010; 43(4): 632-639.
  31. Lagnika L. Phytochemical Study and Antimalarial Activity of Natural Substances from Beninese Plants. Doctoral thesis, Louis Pasteur University of Strasbourg (France)/University of Abomey-Calavi, Benin, 2005; 268.
  32. Benkiki N. Phytochemical study of Algerian medicinal plants: *Ruta Montana*, *Matricaria pubescens* and *Hypericum perforatum*. State Doctorate thesis, El-Hadj Lakhdar Batna University (Algeria), 2006; 198.
  33. Sam GH, Mensah MLK, Nyakoa-Ofori N. Pharmacognostic studies and standardization of *Cassia sieberiana* roots. Pharmacogn. J., 2011; 3(21): 12-17.
  34. Traore A, Ouedraogo S, Belemlilga MB, Kabore A, Guissou IP., Phytochemical analysis and ovidal activity of *Cassia sieberiana*, *Guiera senegalensis* and *Excoecaria grahamii* extracts. African J. Pharm. Pharmacol, 2017; 11(44): 554-560.
  35. Awomukwu DA, Nyananyo BL, Ikpeama AI, Adieze CU. Comparative chemical constituents of some *Cassia* species and their pharmacognostic importance in South Eastern Nigeria. Sci. J. Chem; 2015; 3(3): 40-49.
  36. Burkill HM. The useful plants of West tropical Africa, Volume 3, Royal Botanic Gardens, Kew, UK; 1985.
  37. Sannomiya M, Fonseca V, Da silva M, Rocha L, Dos Santos L, Hiruma-Lima C, Britoc A, Vilegas W. Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. Journal of Ethnopharmacology, 2005; 97: 1-6.
  38. De Barros M, Lemos M, Maistro E, Leite M, Soosa J, Bastos J. Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. Journal of Ethnopharmacology, 2008; 120: 372-377.
  39. Tripoli E, Guardia M, Giammanco S, Di Majo D, Giammanco M. Review Citrus flavonoids: Molecular structure, biological activity and nutritional properties. Food chemistry, 2007; 104: 466 - 479.
  40. Hitara T, Fujii M, Akita K, Yanaka N, Ogawa K, Kuroyanagi M, Hongo D. Identification and physiological evaluation of the components from Citrus fruits as potential drugs for anti-corpulence and anticancer. Bioorganic & Medicinal Chemistry, 2009; 17: 25-28.
  41. Melagraki G, Afantitis A, Igglessi-Markopoulou O, Detsi A, Koufaki M, Kontogiorgis C, Hadjipavlou, Litina D. Synthesis and evaluation of the antioxidant and anti-inflammatory activity of Novel coumarin - 3 - aminoamides and their  $\alpha$ -lipoic acid adducts. European Journal of Medicinal Chemistry doi: 10.1016/j.ejmech.2008.12.087, 2009.
  42. Sutradhar R, Rahman A, Ahmad M, Bachar S. Bioactive flavones of *Sida cordifolia*. Phytochemistry Letters, 2008; 1: 179 -182.
  43. De Bruyne T *et al.* Condensed vegetable tannins: Biodiversity in structure and biological activities. Biochemical Systematics and Ecology, 1999; 27: 445-459.
  44. Okamura H, Mimura A, Yakou Y, Niwano M, Takahara Y. Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. Phytochemistry, 1993; 33: 557-561.
  45. Mates JM, Sanchez-Jimenez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol; 2000; 32: 157-170.