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PHYTOCHEMICAL SCREENING, QUANTITATIVE
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ACTIVITY**

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Original Article

STUDY OF CRUDE EXTRACTS FROM *CASSIA SIEBERIANA* ROOT BARK AND *KHAYA GRANDIFOLIOLA* TRUNK BARK: PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS AND RADICAL SCAVENGING ACTIVITYLANCINE TRAORE^{1,2}, YVES-ALAIN BEKRO^{1*}, JEAN-LUC PIRAT², JANAT AKHANOVNA MAMYRBEVA-BEKRO¹

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ABSTRACT

Objective: *Cassia sieberiana* and *Khaya grandifoliola* are two plants commonly used in traditional medicine in Côte d'Ivoire. Photochemical screening of crude extract obtained from *C. sieberiana* root bark and *K. grandifoliola* trunk bark revealed the presence of alkaloids, sterols, terpenes, polyphenols, flavonoids, coumarins, tannins, reducing sugars, glycosides, carbohydrates, cardiac glycosides and saponins.

Methods: Quantitative analysis was screened in *C. sieberiana* root bark and *K. grandifoliola* trunk bark.

Results: The results respectively showed high concentrations of total phenols (225.57±7.57 and 186.75±12.76 µgGAE/mg), total flavonoids (64.70±5.25 and 117.88±8, 68 µgQE/mg) and total tannins (170.60±5.85 and 39.96±1, 58 µgTAE/mg). The antioxidant activity of the glycosides extracts CS1, KG1 and their corresponding aglycones CS2, KG2 of these plants has been studied by scavenging free radicals by DPPH and that, compared with L-ascorbic acid (vitamin C, IC₅₀ = 0.07 µg/ml). IC₅₀ values of CS1 (2.69 µg/ml), KG1 (3.16 µg/ml) and CS2 (1.30 µg/ml), KG2 (0.726 µg/ml) showed that the aglycones are clearly more effective than the glycosides.

Conclusion: Qualitative analysis of *Cassia sieberiana* root bark and *Khaya grandifoliola* trunk bark showed a presence of a variety of secondary metabolites in these plants, when the quantitative analysis concludes that they contain phenolic compounds, flavonoids and tannins to varied contents.

Keywords: *Cassia sieberiana*, *Khaya grandifoliola*, Glycoside, Aglycone, Phytochemical screening, Quantitative analysis, DPPH, IC₅₀, Côte d'Ivoire.

INTRODUCTION

The last decades were marked by the particular interest in the development of medicinal plants as a source of natural bioactive substances. Consequently, many studies increasingly examine the therapeutic effects of antioxidants from natural sources [1]. However, this source seems inexhaustible since a small portion of the 250000 known plant species was investigated from phytochemical and pharmacological point of view; and that each species may contain several thousand different phytoconstituents [2]. Polyphenols are a family of secondary metabolites widespread in vegetables. Their beneficial effects on health, widely described in epidemiological studies are mainly based on their antioxidant activity. Indeed, they can scavenge free radicals, inhibit the enzymes responsible for the production of said radicals and some are even metal ion chelators [3]. Therefore, they arouse interest especially for the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4]. *Cassia sieberiana* DC. (Cesalpiniaceae) and *Khaya grandifoliola* C. DC. (Meliaceae) are two plants widely used in traditional medicine in West Africa. Their pharmacological properties have been demonstrated in many studies [5-15]. The objective of the present investigation was firstly, to conduct the phytochemical screening and the quantitative analysis of important phytochemical constituents like polyphenols, flavonoids, tannins from *C. sieberiana* root bark and *K. grandifoliola* trunk bark; and secondly to compare DPPH free radical scavenging activity of glycosides and corresponding aglycones extracted of the aforesaid organs.

MATERIALS AND METHODS**Plants materials**

Cassia sieberiana roots bark and *Khaya grandifoliola* trunk bark were harvested from Vavoua (west-central of Côte d'Ivoire) and authenticated at the Centre National de Floristique (CNF) at Felix Houphouët-Boigny University (Cocody-Abidjan/Côte d'Ivoire) and

the samples were deposited in our laboratory for future reference. The fresh barks were dried under shade for 30 days. Then they were ground into powder and store in the glass jar at room temperature. The powders obtained were then sampled and subjected to aqueous extractions and organic medium to give the crude extracts for analysis.

Chemicals

All chemicals used for analysis were purchased from Carlo-Erba or Sigma Aldrich and were analytical grade.

Preparation of plant extracts

Extraction of glycosides: 100 g of powdered root bark or trunk bark was added to 600 ml of distilled water and heated to 90 °C for 90 min. After cooling and filtration, the decoction is lyophilized for 24 hours. The extracts obtained were encoded CS1 and KG1 respectively for *Cassia sieberiana* and *Khaya grandifoliola*.

Extraction of aglycones: 100 g of powdered root bark or trunk bark was added to 600 ml of 2 N HCl and heated to reflux at 90 °C for 90 min. After cooling and filtration, the aglycones were extracted with ethyl acetate. The organic phase was dried over anhydrous MgSO₄, then concentrated in a rotary evaporator and dried again under vacuum. The total aglycones extracts were encoded CS2 and KG2, respectively for *Cassia sieberiana* and *Khaya grandifoliola*.

Phytochemical screening of crude extracts

The extract obtained was subjected to preliminary phytochemical screening.

Detection of alkaloids

Dragendorff's test: The extract was dissolved in 6 ml of ethanol and treated with 2 drops of Dragendorff's reagent. A precipitate or orange color indicated the presence of alkaloids [16].

Detection of sterols and terpenes

Liebermann-Büchard's test: In a test tube, an aliquot of extract was diluted in 1 ml of acetic anhydride. Then 0.5 ml of concentrated H₂SO₄ was poured slowly on the walls of the tube. The appearance of a purple color turning blue to green indicates a positive reaction [16].

Detection of polyphenols

FeCl₃ test: To 2 ml of aqueous extract were added a few drops of an aqueous solution of FeCl₃ 2% (w/v). A dark blue and dark green color indicated the presence of polyphenols [17].

Detection of flavonoids

Shinoda's test (Cyanidin reduction test): 5 to 7 drops of concentrated HCl and 10 to 15 mg of Zn or Mg shavings were added to 2 ml of aqueous extract. After 3 to 5 min, a red-orange coloration characterizes the flavonoids. To accelerate the reaction and color enhancement, the reaction mass was heated in a water bath for 2 to 3 min [16].

Concentred HCl test: To 1 ml of aqueous extract, was added 3 to 5 drops of a solution of concentrated HCl. A red color indicated a positive reaction [17].

NH₄OH test: To 1 ml of aqueous extract, was added 3-5 drops of concentrated ammonium hydroxide. The appearance of the yellow coloration when cold and orange or red when hot indicates the presence of flavones, flavanones and flavonols. The appearance of red coloration when cold indicates the presence of chalcones and aurones. The appearance of blue or violet coloration when cold highlights anthocyanins [17].

Oxalo-boric test: To 1 ml of aqueous extract, 3-5 drops of oxalic acid and boric acid are added. Light yellow coloration indicated the presence of 5-oxyflavone and 5-oxyflavonol [17].

Vanillin hydrochloride Test: To 1 ml of aqueous extract, was added 3-5 drops of a vanillin solution (1 g vanillin in 50 ml of concentrated HCl). A red strawberry color indicated the presence of catechin [17].

Detection of coumarins

Test on the lactone ring: In two test-tubes, 2 ml of aqueous extract were introduced. In one test tube, was added 0.5 ml of 10% NaOH (w/v), then the test-tubes were heated in a water bath until boiling. After cooling, 4 ml of water were added to each test tube. If the liquid is transparent or transparent (yellow) relative to the liquid from the test tube containing no alkali, the reaction is positive. Acidifying the transparent solution with a few drops of concentrated HCl, lead to the lost of yellow color to form a cloudy suspension or a precipitate [16].

Detection of tannins

Stiasny's test: aqueous plant extract (5 ml) was evaporated to dryness in a capsule. Stiasny's reagent (15 ml) was added to the residue. The mixture was kept in a water bath at 80 °C for 30 min and cooled. The precipitate observed in large flakes characterized the presence of catechic tannins. The solution was then filtered and saturated in sodium acetate. The addition of 3 drops of FeCl₃ to 2% (w/v) shows a deep blue-black color indicating the presence of gallic tannins [16].

Detection of reducing compounds

Fehling's test: In a test tube containing 5 ml of aqueous extract, were added Fehling's reagent (5 ml) the formation of a brick red precipitate after 2-3 min heating in a water bath at 70 °C indicated a positive reaction [16].

Tollens's test: In a test tube containing 5 ml of the same extract, Tollens's reagent (5 ml) was added, then ammonium hydroxide was dripped until the disappearance of the precipitate. The formation of a silver mirror confirmed the presence of reducing compounds [16].

Detection of carbohydrates and glycosides

Benedict's test: To 0.5 ml of extract, Benedict's reagent (0.5 ml) was added. The mixture was heated in a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugars [18].

Detection of glycosides

Keller-Killiani test: To 2 mg of plant sample extract dissolved in a few ml of glacial acetic acid were added respectively a drop of (5%, w/v) FeCl₃ and a drop of concentrated H₂SO₄. The presence of glycosides is confirmed if at the junction of two liquid phases, a reddish brown color develops and the upper liquid phase becomes bluish green [18].

Borntraeger's test: To the filtrate hydrolysate (2 ml), was added of chloroform (3 ml). After separation, the chloroform phase is treated with an (10%, v/v) ammonia solution. The formation of a colored precipitate confirmed the presence of glycosides [18].

Detection of saponins

Test for foam value (FV): 1g of powdered root bark or trunk bark was transferred to the conical flask containing 100 ml of distilled water. It was maintained at moderate boiling for 30 min. After cooling and filtration, the volume was adjusted to 100 ml with distilled water. The stock solution was poured into 10 test tubes (of same internal diameter) in successive portion of 1, 2, 3, up to 10 ml and the liquid in each test tube was to 10 ml with distilled water. Each tube was vigorously stirred in a horizontal position for 15 seconds. After 15 min of rest in an upright position, the height of the persistent foam is noted. If it is less than 1 cm in the tube, the desired foam value is less than 100. If it is of 1 cm in one of the tube, the dilution of the drug in this tube serves to calculate the foam value. If 1 cm of foam is measured in the fourth tube containing 4 ml of decoction at 1% (0.4 g of drug), FV is then 10/0.04 = 250 [17].

Test for characterization of saponins: In a test tube were introduced 5 ml of HCl (0.1N) and in another 5 ml of NaOH (0.1 N). In each tube, 2 to 3 drops of plant extract were added and the mixture was stirred vigorously. If a stable foam equal in volume and height form in each tube, then one is in the presence of triterpenic saponins. If the tube containing the alkaline solution forms a very important volume foam which is more stable compared to the tube containing the acidic solution, one is in the presence of steroidal saponins [17].

Quantitative analysis

Quantification of total phenols

The assay of total phenols was carried out according to the method described by Michel *et al.*, [20]. 20 µl of plant extract (10 mg/ml) are mixed with 100 µl of Folin-Ciocalteu reagent and 1.58 ml of distilled water. After 10 min, 300. µl of Na₂CO₃ (20%, w/v) are added to the mixture. The mixture is left for 20 min at room temperature and the absorbance is read using a UV-VIS spectrophotometer (HP 8453) at 765 nm against a blank without plant extract. A calibration curve is drawn in parallel in the same operating conditions using gallic acid (31.25-500 µg/ml). The total phenols are expressed in micrograms gallic acid equivalent/mg extract (µgGAE/mg).

Quantification of total flavonoids

The determination of total flavonoids was performed according to the method described by Basma *et al.*, [21]. 1 ml of plant extract (1 mg/ml) was mixed with 0.5 ml of EtOH, 100 µl of AlCl₃ (10%, w/v) 100 µl of CH₃COOK (1M) and 0.8 mL of distilled water. The whole is incubated at room temperature for 30 min. The absorbances were measured at 520 nm using a UV-VIS spectrophotometer (HP 8453) nm against a blank without plant extract. A calibration curve is drawn in parallel in the same operating conditions using quercetin (50-400 µg/ml). The total flavonoids are expressed in micrograms quercetin equivalent/mg extract (µgQE/mg).

Quantification of total tannins

The determination of total tannins was performed according to the method described by Palici *et al.*, [22] with some modifications. 2 ml of extract (0.4 mg/ml) were mixed with 1 ml of tungstophosphoric acid and 17 ml of NaCO₃ (50%, w/v). After 3 min, the absorbance at 750 nm was read using a UV-VIS spectrophotometer (HP 8453). A calibration curve was drawn in parallel in the same operating conditions using tannic acid (1-5 mg/ml). The total tannins are expressed in micrograms of tannic acid equivalent/mg of extract (µgTAE/mg).

DPPH radical scavenging activity

The scavenging potential of extracts on DPPH was evaluated using the method described by Basma et al., [21] with some modifications. 1 ml of each extract at different concentrations (12.5-200µg/ml in MeOH) was added to 1 ml of a DPPH solution (0.002%, w/v) and incubated in dark for 30 min. At the same time a solution with no extract and containing vitamin C (L-ascorbic acid) taken as standard, was subjected to the same operating conditions. The reduction of DPPH was measured at 520 nm (HP 8453 UV-VIS spectrophotometer). Scavenging effect was calculated as: %I= (A₀-A₁) × 100/A₀; A₀: absorbance of the control; A₁: absorbance of the extract.

The efficient concentration of plant extract to which 50% of the DPPH have been reduced (IC₅₀) was determined graphically by a curve of standardization of the extract concentration according to the scavenging activity.

RESULTS AND DISCUSSION

Phytochemical screening of plant extracts

The results of the preliminary phytochemical analysis *Cassia sieberiana* root bark and *Khaya grandifoliola* trunk bark are summarized in table 1. Different types of secondary metabolites such as alkaloids, sterols/terpenes, polyphenols, flavonoids, flavones, flavanones, flavonols, [17] and flavanones and dihydroflavonols [19], 5-oxyflavone and/or 5-oxyflavonol [17] catechic tannins, glycosides, carbohydrates, cardiac glycosides and reducing sugars are present in CS1 and KG1.

We note however, the presence of gallic tannins in CS1 and their absence in KG1. Furthermore, it is noticed that the steroid and triterpenic type saponins are respectively present in CS1 and KG1. Moreover, while CS1 and KG1 contain sugars, CS2 and KG2 do not. Indeed, in these extracts aglycones were separated from glycone by acid hydrolysis. These phyto compounds are known to have a curative activity against several pathogens and therefore can be proposed for the treatment of various diseases [23].

Assay of total phenols, flavonoids and tannins

The calibration curves of gallic acid ((y = 0,644x±0.184 and R² = 0.999), quercetin (0,044x±0.021 and R²) and tannic acid (0,091x±0.002 and R² = 0.999) allowed us to estimate respectively

the levels of total phenols, total flavonoids and total tannins in the extracts of studied plants. Results are reported in the table 2. The values are the average of three repetitions plus or minus the standard deviation. These are high.

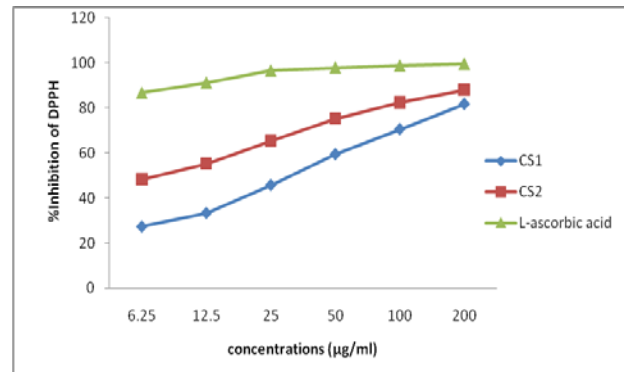


Fig. 1: Cassia sieberiana scavenging activity

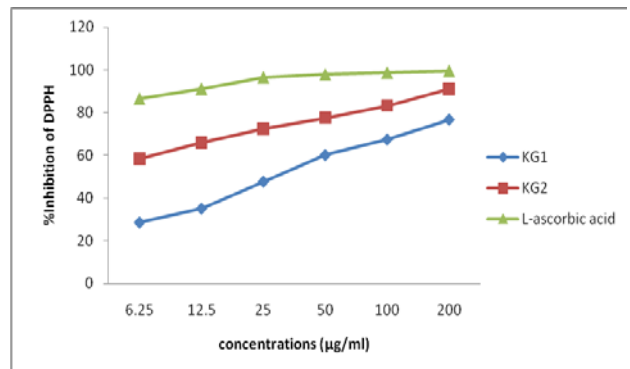


Fig. 2: Khaya grandifoliola scavenging activity

Table 1: Screening of phytocomponents in CS1, KG1, CS2, KG2

Secondary metabolites	Test/reagent	Results			
		CS1	KG1	CS2	KG2
Alcaloids	Dragendorff	+	+		
Sterols/terpenes	Liebermann-Bürchard	+	+		
Polyphenols	FeCl ₃	+	+		
Flavonoids	ZnShinoda Mg	++Flava/Dihy	++Flava/Dihy		
	HCl _{conc}	+	+		
	NH ₄ OH	+(yellow)*	+(red)*		
	Oxalo-boric	+	+		
	Chlorhydric vanillin	OxyflOxyflv+	OxyflOxyflv+		
Coumarins	NaOH	Catechin+	Catechin+		
Tanins	Catechic	+	+		
	StiasnyGallic	+	-		
Reducing sugars	Fehling	+	+	-	-
	Tollens	+	+	-	-
	Benedict	+	+	-	-
Carbohydrates/glycosides	Keller-Killiani	+	+	-	-
Cardiac glycosidesglycosides	Börntraeger	+	+	-	-
Saponins	FV	130	400		
	SteroidicTriterpenic	+-	++		

(+)= presence; (-)= absence, Flava/Dihy= Flavanones/Dihydroflavonols; Oxyfl= 5-Oxyflavone; Oxyflv= 5-Oxyflavonol; *= presence of flavones, flavonones and flavonols.

Table 2: Total phenols, flavonoids and tannins contents of CS1 and KG1

	Total phenols (µgGAE/mg)	Total flavonoids (µgQE/mg)	Total tannins (µgTAE/mg)
CS1	225,57±7,57	64,70±5,25	170,60±5,85
KG1	186,75±12,76	117,88±8,68	39,96±1,58

It has been reported that polyphenols have many biological and pharmacological properties, including antibacterial [24], antioxidant and anti-inflammatory [25]. Recent studies have shown that among the phenolic compounds, flavonoids and tannins help fight against certain disorders in the body caused by oxidative stress, thanks to their antioxidant potential [26].

Scavenging activity of crude extracts

The relatively stable radical DPPH is widely used to test the ability of a compound to act as a free radical scavenger or hydrogen acceptor and therefore assess its antioxidant activity [27]. The fig. 1 and 2 show that CS1 (IC₅₀ = 2.69 µg/ml), KG1 (IC₅₀ = 3.16 µg/ml), CS2 (IC₅₀ = 1.30 µg/ml), KG2 (IC₅₀ = 0.726 µg/ml) signed a modest scavenging profile compared with vitamin C (IC₅₀ = 0,07µg/ml). In besides, the aglycones CS2 and KG2 are more active than their respective glycosides CS1 and KG1. Like that, the glycone inhibit certain properties of plants. This result does not contradict other authors who have shown that aglycones have greater antioxidant power than their equivalents glycosylated [28]. Also, this type of correlation in the same plants had he been observed and reported by Bekro *et al.*, [6] and Traore *et al.*, [7] comparing *in vitro*, respectively, the anti-inflammatory activity CS1, KG1, CS2, KG2 and their antibacterial potential. Thus, it seems clear that there is also a direct link between antioxidant and pharmacological activities. Furthermore, a study showed a correlative link between antioxidant and anti-inflammatory activities [29].

CONCLUSION

Plant organs are useful for the treatment of several human pathologies. These are undoubtedly potential sources of drugs. Qualitative analysis of *Cassia sieberiana* root bark and *Khaya grandifoliola* trunk bark showed a presence of a variety of secondary metabolites in these plants, when the quantitative analysis concludes that they contain phenolic compounds, flavonoids and tannins to varied contents. The scavenging activity survey made notice with regard to the vitamin C, each plant extracts signs a modest scavenging activity that depends on its content in polyphenolic compounds. However, it is noted that aglycones CS2 and KG2 have a scavenging power more important than their equivalent glycosides CS1 and KG1. This activity supports the use of *Cassia sieberiana* and *Khaya grandifoliola* in traditional medicine against some diseases related to oxidative stress.

A chromatographic analysis of CS2 and KG2 is underway to isolate and characterize certain molecules responsible for the pharmacological properties of these plants.

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CONFLICT OF INTERESTS

Declared None

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