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Research Article

CHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF EXTRACTS OFTHREE PLANTS USED IN TRADITIONAL MEDICINE IN BENIN: *TECTONA GRANDIS, UVARIA CHAMEAE AND JUSTICIA SECUNDA*

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ABSTRACT

Objective: The present study was devoted to the chemical analysis of three plants (Tectona grandis, Uvaria chameae and Justicia secunda) usually used by traditional healers in Benin, for their curative properties in the treatment of certain diseases.

Methods: After characterization of large chemical groups present in ethanolic extract of these plants, the total polyphenols were measured by the Folin method, the content of condensed tannins, total flavonoids, anthocyanins and anthocyanidins was evaluated by spectrophotometry.

Results: The results show the presence of several secondary metabolites such as saponins, alkaloids, tannin, mucilages, anthraquinones, leucoanthocyanins, anthocyanins and triterpene in varying proportions in the three plants. The contents of condensed tannins, of anthocyanidins, total polyphenols and total flavonoids are respectively higher in the leaves (13.409 mg/g, 1.529 mg/g, 3.479 mg/g and 24.640 mg/g) than the bark of T.g (8.612 mg/g, 0 mg/g, 2.694 mg/g and 12.410 mg/g). As against, leaves of U.care richer in condensed tannins, flavonoids and total polyphenols (44.290 mg/g, 9.135 mg/g, 4.779 mg/g) than J.s (0 mg/g, 2.011 mg/g and 1.478 mg/g) unlike anthocyanidins. Of three plants studied, U.c was revealed the richest in polyphenolic compounds (condensed tannins, total polyphenols) and J.s was least rich. This content of polyphenolic compounds explain the antiradical activities observed; an CI50 of 0.700 mg/mL for U.c, 14.340 mg/mL for leaves of T.g, 24.790 mg/mL for bark of T.g and 46.500 mg/mL for leaves of J.s.

Conclusion: This study contributes to increase those plants's phytochemical knowledge and allows having a better understanding of their pharmacological properties

Keywords: Polyphenols, Antiradical activities, Solid phase micro extraction, Tectona grandis, Uvaria chameae, Justicia secunda.

INTRODUCTION

Medicinal plants play an important role in the armamentarium of humanity. According to the World Health Organization, approximately 65-80% of the world population, due to poverty and lack of access to modern medicine of developing countries, use the medicinal plants for their needs in care of primary health [1]. With the new extraction techniques, identification and characterization of organic molecules, more than 25-50% of prescribed drugs today for active bioactive molecules of medicinal plants [2,3]. Africa in general and Benin in particular, the exploration of the active principles of plants for therapeutic use is experiencing a renewed interest in recent years. Nevertheless, the green legacy is still a huge reservoir of compounds with potential pharmacological and phytochemical properties waiting to be discovered. Therefore, the use of natural plant resources is a necessary and important concern through participation in the search for new drug molecules. The number of plants scientifically little known but widely used in traditional medicine in Benin for their healing properties in the treatment of several pathologies includes, among others Justicia secunda (JS) (Acanthaceae), Tectona grandis (TG) (Verbenaceae) and Uvaria chameae (UC) (Annonaceae).

JS is a perennial herb to 90 cm tall, purple stems and silver leaves which have flowers in terminal spikes with chalices and courolles red. As for TG, he is a tree up to 50 m tall with large elliptical leaves and downy below. Finally, UC is a highly fragrant shrub up to 3 m height often bushy with leaves alternate, oblong, acuminate and stellate hairs on the young shoots. The flowers are solitary, axillary, and greenish-yellow with nearly circular petals. Its petals are dull ochreous and brown fruits are competing at the top of a common stalk [4]. In the literature, very few studies are devoted to the determination of the chemical composition, quantification of polyphenolic compounds and measuring the radical scavenging activity of the extracts of these three plants. Hence, this work aims to contribute to the scientific knowledge of these plants through their chemical decryption and evaluation of the antiradical activity of their extracts (Photos 1 and 2).

METHODS

Plant material

The plant material used consists of the leaves and bark of the three plants (TG, UC and JS) collected in Benin. After collection and identification by botanists at the National Herbarium of Benin, the plant material was dried at laboratory temperature between 26°C and 30°C to constant mass and then pulverized using an electric grinder (Brand Retsch, type SM 100). The extraction was performed by soaking 50 g of powder in 500 mL of ethanol for 72 hr, and the supernatant was vacuum filtered through a Buchner funnel. The filtrate thus obtained was evaporated to dryness, weighed and stored at 6°C until analysis [5].

Phytochemical screening

The phytochemical screening is based on the differential reactions for major groups of chemical compounds in plants (color and

Photo 1: *Justicia secunda* (Acanthaceae)

Photo 2: *Uvaria chameae* (Annonaceae)

precipitation). Thus, sterols and terpenes research was realized by the reaction of Liebermann. The characterization of the compounds belonging to the group of polyphenols was realized by reacting ferric chloride. Flavonoids have been shown by the reaction cyanidin. The compounds belonging to the group of tannins has been demonstrated by the Stiasny reaction. The free or combined quinone compounds were revealed by reacting Borntraeger. Saponins identication is based on the property those aqueous solutions containing saponins after foaming the agitation. The alkaloids search was made using dragendorff reagent (reactive iodobismuthate potassium) and Bouchardat reagent [6,7].

Determination of phenolic compounds

Anthocyanidins

Two grams of each herb powder are introduced into 160 mL of hydrochloric acid solution (2 mol/L) and boiled for 30 minutes. After cooling and filtration, a liquid-liquid extraction was carried out with butanol (3 mL × 20 mL). The butanolic phase was used to determine spectrophotometrically the anthocyanidin content by scanning the wavelength from 480 nm to 600 nm. The highest absorbance was used against a blank consisting of the pure butanol to quantitate anthocyanidins [8,9]. Anthocyanidin content was calculated by the formula:

$$T_{antho} = \alpha.A_{ext}.M.d.V/(\varepsilon.m)$$

T_{antho}: Anthocyanidin content expressed as mg/g (cyanidols equivalent)
α: Correction factor = 6;
A: Absorbance at the wavelength of maximum absorption;
d: Dilution factor;
ε: Molar absorption coefficient of leukocyanidol (34700);
m: Mass of dry matter of plant material hydrolyzed;
M: Molecular weight of **leukocyanidol** (306 g/mol);
V: Volume of n-butanol solution;

Anthocyanins

A volume of 500 µL of ethanolic extract of each plant are mixed with 500 µL of ethanol solution acidified with pure hydrochloric acid at 0.1% and 10 mL of HCl at 2% in distilled water. The absorbance of 5 mL of this mixture is read in the presence of 2 mL of sodium bisulfite solution ($Na_2S_2O_5$, 150 g/L) in a spectrophotometer at 520 nm against a blank containing 2 mL of distilled water instead bisulfite [10,11]. The total content of anthocyanin expressed in mg per gram of extract standard is given by the following formula:

$$T_{anthocyanins} = (OD_A - OD_B).\ P/C_{ext}$$

OD_A: Optical density of the control solution (without bisulfite)
OD_B: Optical density of the mixture
P: The line slope obtained starting from the standard (p=875 for malvidin-3-glucoside)
C_{ext}: Concentration of the extract

Total flavonoids, condensed tannins and polyphenols content

The method of aluminum trichloride was used to quantify the total flavonoids [12]; condensed tannins were assayed by vanillin sulfuric method [10] and the total polyphenols were measured by the Folin method [13].

Antiradical activity

The antioxidant activity of each extract was assessed by measuring free-radical scavenging activity via the decoloration of a methanol solution of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Konan et al. and Duan et al. [14,15] as follows: 2 mL of methanol solution of each tested material at various concentrations (2-50 µg/mL) were added to a 2 mL solution of DPPH (100 µM) in methanol, and the reaction mixture was shaken vigorously. After incubation at room temperature for 30 minutes, the absorbance (A) of DPPH was determined with a spectrophotometer at 515 nm, and the radical scavenging activity of each sample was expressed as percentage inhibition:

$$\% \text{ inhibition} = ([A_{control} - A_{sample}]/A_{control}) \times 100$$

IC_{50} (sample concentration required for 50% inhibition) was obtained by linear regression analysis of the dose-response curve, plotted as the % inhibition and concentration (mg/L). Gallic acid, which is the better well-known natural antioxidant, was used as a positive control.

Determination of volatile compounds present in the plant powder: Solid phase micro extraction (SPME) coupled with gas chromatography-mass spectrometry (GC/MS)

The solid phase extraction has taken over the years an important role in the preparation of samples. This extraction method can easily perform extractions of extractable compounds difficult by organic solvents and were, therefore, analyzed after a simple precipitation [16,17]. It is used in combination with gas chromatography to identify and measure volatile substances in many fields such as biology, oenology, cosmetology, study key species of plants [18] etc.

Hence, the volatile compounds of our plant samples were identified by SPME coupled with GC/MS. experiments by SPME were performed using 75 mm fiber divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bornem, Belgium). The extraction and desorption were carried out automatically by an automatic sampler (Gerste IMPs). For the preparation of the Samples, 1 g of each powder plant incubated at 30°C for 2 minutes with agitation 250 rev/minutes speed. To calculate the concentrations of the components, the fiber was exposed to the head space for 60 minutes at 35°C.

Analyzes of extracts by GC/MS were performed with an Agilent 6890 clinical proteomics group coupled to a 5973 quadrupole mass

spectrometer MSD (Agilent technologies, Diegem, Belgium), equipped with a Varian CP-Q Pora BOND, column capillary (length 25 m, diameter: 0.320 mm, film thickness: 5 µm). The injector was set at 250°C, the transfer line of MSD 250°C, the carrier gas is helium (1 mL/min); desorption was made by SPME in split mode with the injector 4 cooled injection inlets-programmable temperature vaporizing (Gerstel) lasted 120 s and at 70 eV ionization. The oven temperature was programmed from 60 to 350°C [18]. The identification of volatile compounds was done by comparing their retention indices with those of reference compounds in the literature and confirmed by comparison of their mass spectra (GC-MS) with those of reference compounds [19].

RESULTS AND DISCUSSION

Phytochemical screening

Various secondary metabolites have been identified in plants studied by a series of color or precipitation reactions more or less specific to each class of active ingredients as shown in Table 1. From this table, we can retain that: The leaves of UC contain abundant alkaloids, saponosides, flavonoids, catechin tannins as well as gallic. However, triterpenoids and anthocyanins are present in small amounts while reducing compounds, anthraquinones (free and combined), coumarins, mucilage, and leukoanthocyanins are absent.

The leaves of TG contain a high proportion of saponosides, free anthraquinones, gallic tannins, catechic tannins, flavonoids, triterpenoids and mucilages. However, alkaloids, reducing compounds and combined anthraquinones (O-hétérosides) are present in small quantities. Reverse against, the combined anthraquinones (C-hétérosides and O-hétérosides with reduced genins), coumarins, anthocyanins and leukoanthocyanins are absent. These results are consistent with those of Mohammad [20] with regard to the presence of tannins, saponosides, free anthraquinones, alkaloids and flavonoids. Contrary to leaves, in the bark of TG there are plenty of flavonoids, tannins catéchin and free anthraquinones, but a low presence of alkaloids and combined anthraquinones O-glycosides. As against, saponosides, triterpenoids and gallic tannins present in the leaves are absent in the bark. The two parts of the plant are lacking the C-hétérosides, O-hétérosides, coumarins, mucilage, reducing compounds and leukoanthocyanins.

Leaves of JS contain abundant mucilages, saponosides, anthraquinones (O-heterosides with reduced genins, C-hétérosides) triterpenoids and alkaloids. But, reducing compounds, free anthraquinones and leuco-anthocyanins are present in small quantities. However, the catechin tannins, gallic tannins, anthocyanins, flavonoids, anthraquinones combined (O-hétérosides) and coumarins are absent.

Table 1: Secondary metabolites identified in samples

Major chemical groups	UC	TG		JS
	Leaves	Bark	Leaves	Leaves
Alkaloids	+	+/-	+/-	+
Saponosides	+	-	+	+
Flavonoids	+	+	+	-
Gallic tannins	+	-	+	-
Catechic tannins	+	+	+	-
Mucilage	-	-	+	+
Reducing compound	-	-	+/-	+/-
Free anthraquinones	-	+	+	+/-
Combined anthra-quinones				
O-heterosides	-	+/-	+/-	-
O-heterosides with reduced genins	-	-	-	+
C-heterosides	-	-	-	+
Coumarins	-	-	-	-
Triterpenoids	+/-	-	+	+
Anthocyanin	+/-	+	-	-
Leuco-anthocyanin	-	-	-	+/-

+: Presence, +/-: Track, -: Absent, UC: Uvaria chameae, TG: Tectona grandis, JS: Justicia secunda

Phenolic compounds present in plants studied

Total flavonoids and condensed tannins content

The total flavonoids contents of our samples range from 2.01 mg/g to 24.64 mg/g. Leaves of TG present the highest content of flavonoids, whereas the leaves of JS have the lowest content. At the same plant (TG) there is a higher concentration of flavonoids in the leaves than in the stem bark. The total flavonoids content of UC Bark (9.14 mg/g) is between the values obtained by Donatus et al. in 2009 (5.70 mg/g) [21] and that obtained by Omajali et al. in 2011 (10.37 mg/g) [22] for the same plant (Fig. 1).

Moreover, the content of condensed tannins in our samples ranged from 0 mg/g to 44.29 mg/g. We noted that condensed tannins are more concentrated in the leaves than in the bark as the flavonoids contents recorded on the two bodies of TG These results (44.29 mg/g for UC) are close to those obtained by Donatus et al. (40 mg/g) on the same organ collected in Nigeria in 2009 (Fig. 2).

Anthocyanins and anthocyanidins content

The contents of anthocyanins and anthocyanidinsin our samples vary respectively from 0 mg/L to 0.394 mg/g and 0 mg/L to 1.529 mg/g.

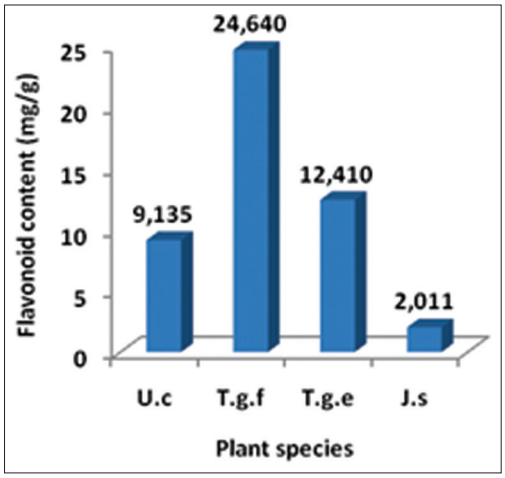

Fig. 1: Flavonoids contents of different plant species. *UC*: Leaves of *Uvaria chameae*, *TG* f: Leaves of *Tectona grandis*, *TG e*: Bark of *Tectona grandis*, *JS*: Leaves of *Justicia secunda*

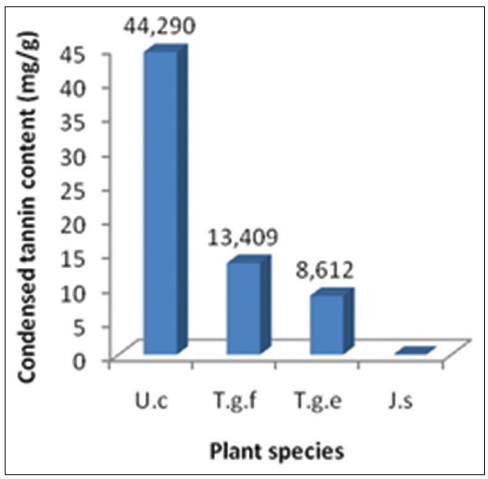

Fig. 2: Condensed tannins contents of different plant species. *UC*: Leaves of *Uvaria chameae*, *TG* f: Leaves of *Tectona grandis*, *TG e*: Bark of *Tectona grandis*, *JS*: Leaves of *Justicia secunda*

Bark of TG have the highest anthocyanin content, while the leaves of TG have the highest content of anthocyanidins (Fig. 3).

Total polyphenols content

Leaves of UC have the highest content of total polyphenols. However, the value obtained in Benin (4.780 mg/g) is less than that found in Nigeria by Donatus et al. (100 mg/g), but greater than that reported by Omajali et al. (0.470 mg/g) (Fig. 4).

Antiradical activity of various plant extracts studied

Drawing the graph expressing the rate of radical scavenging DPPH according to the concentration of extract was used to determine concentrations of ethanolic extracts to trap 50% of DPPH radicals (CI_{50}). We note that these concentrations vary from one plant to another and within the same plant; they vary from one organ to another (Table 2).

The concentration of the extract to 50% trapping of free radicals is lower with UC. This plant, which had already proved richest in polyphenols, therefore has the highest antiradical activity.

Drawing the graph of the total polyphenols content based on scavenging activity for the most active extract compared with less active shows a good correlation between antiradical activity and polyphenol content with a determination coefficient of $R^2 = 0.950$. The antiradical activity of these extracts would be strongly related to their phenolic content as evidenced by several studies in the literature on plant extracts [8,14,15,23-25] (Fig. 5).

Identification of aromatic compounds present in the powder of the plants studied

The analysis of Table 3 revealed that 96% of the compounds in the powder of UC leaves were identified of which only 21.9% are aromatic.

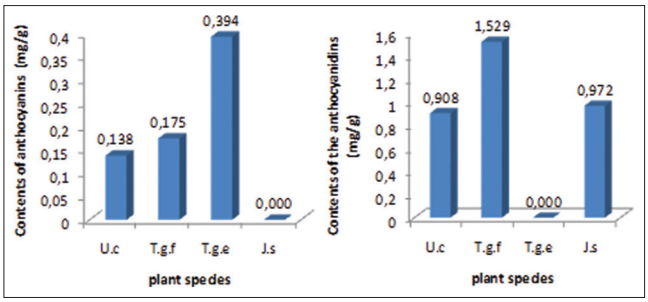

Fig. 3: Anthocyanins content. *UC*: Leaves of *Uvaria chameae*, *TG* f: Leaves of *Tectona grandis*, *TG e*: Bark of Tectona grandis, *JS*: Leaves of *Justicia secunda*

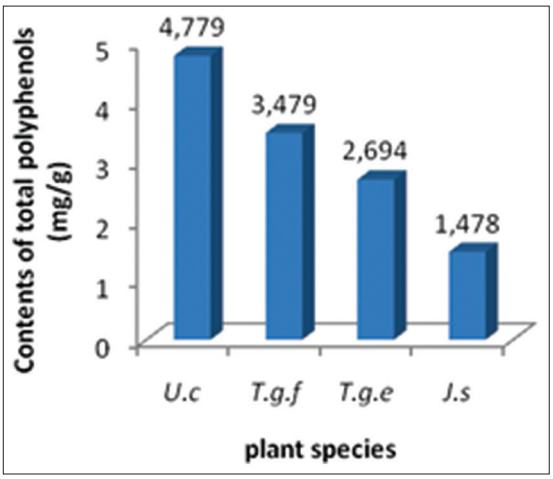

Fig. 4: Total polyphenols content

The aromatics compounds identified in the powder of UC leaves are essentially E-caryophyllene.

It appears from the analysis of Table 4, 93.200% of the compounds contained in the leaves powder of TG have been identified against 97.710% in the bark of the same plant. The same compounds have been identified in both organs of the plant with the exception of 2-methylbutan-1-ol, α-pinene and p-cymene, which are present only in a sheet while hexanal, γ-muurolene and β-selinene are only present in the bark. The compounds common to both bodies of TG are more abundant in the bark than in the leaves except toluene, α-terpinene and limonene.

As we can note trough the results presented in Table 5, it is clear that 97.600% of the compounds contained in the powder of JS leaves were identified of which only 40.200% are volatile. The aromatics compounds present in the leaves of JS are essentially the ethyl acetate, α-copaene, E-caryophyllene, and allo-aromadendrene.

CONCLUSION

The present work was devoted to the identification of major chemical groups, the determination of phenolic compounds, followed by the evaluation of the antiradical activity and volatile compounds of three plants of Beninese pharmacopoeia to justify their use in the fight against various pathologies.

UC is the richest plant in phenolic compounds and presented the most interesting antiradical activity**.** The TG polyphenol content varies from one organ to another, and there is a high content at the supposed leaves most exposed to solar radiation. One could, therefore, conclude that

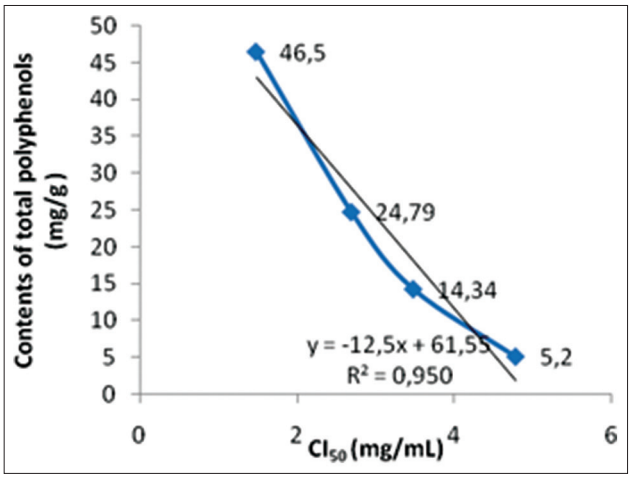

Fig. 5: Total polyphenol content based scavenging activity

Table 2: Antiradical activity of ethanolic extract of the plants studied

	UC	TG		JS
	Leaves	Leaves	Bark	Leaves
CI_{50} (mg/ml)	5.20	14.34	24.79	46.50

UC: Uvaria chameae, TG: Tectona grandis, JS: Justicia secunda

Table 3: Volatile compounds identified in powder of UC leaves

RT[a] (min)	KI[b] (cal)	KI (L)	Compounds identified	Area %
1.830	517	606	Ethyl acetate	57.500
21.930	1368	1374	α-copaene	1.400
23.690	1410	1417	E-caryophyllene	23.800
25.350	1452	1458	Allo-aromadendrene	15.000

[a]RT (min) : Retention time, [b]KI: Index Kovacs, UC: Uvaria chameae

Table 4: Volatile compounds identified in the powder of TG leaves and bark

RT[a] (mn)	KI[b] (cal)	KI (L)	Compounds identified	TG l	TG
1.840	519	606	Ethyl acetate	12.500	18.500
2.480	733		2-methylbutan-1-ol	1.400	
2.760	765	767	Toluene	2.600	1.850
3.070	801	801	Hexanal		1.890
5.440	930	932	α-pinene	1.200	
7.750	1014	1014	α-terpinene	1.200	0.330
8.010	1021	1020	p-cymene	2.300	
8.130	1025	1024	Limonene	1.200	0.560
21.910	1368	1374	α-Copaene	3.600	4.310
23.680	1410	1411	E-caryophyllene	31.900	33.390
24.380	1428	1432	α-E-bergamotene	2.100	2.820
25.040	1444	1452	α-humulene	5.400	7.240
25.350	1452	1449	Spirolepechinene	22.900	27.230
26.010	1469	1478	γ-muurolene		1.160
26.370	1478	1479	ar-curcumene	4.900	10.310
26.750	1487	1489	β-selinene		4.770

[a]RT (min): Retention time, [b]KI: Index Kovacs, TG: Tectona grandis

Table 5: Volatile compounds identified in the powder of JS

RT[a] (min)	KI[b] (cal)	KI (L)	Compounds identified	Area %
1.830	517	606	Ethyl acetate	57.500
21.930	1368	1374	α-Copaene	1.400
23.690	1410	1417	E-Caryophyllene	23.800
25.350	1452	1458	allo-Aromadendrene	15.000

[a]RT (min): Retention time, [b]KI: Index Kovacs, JS: Justicia secunda

the solar radiation stimulates the growth of phenolic compounds in the aerial parts of plants.

Furthermore, this work has shown a variety of secondary metabolites and aromatic compounds in plants studied and revealed their high polyphenol potential, natural antioxidants products, to considerable interest in the pharmacological field. This work thus makes a significant contribution to the phytochemical knowledge of the three plants investigated and allows to better understanding the pharmacodynamic properties of extracts explaining their use in traditional medicines.

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