

Full Length Research Paper

Chemical composition and *in vitro* investigation of biological activities of *Hemizygia bracteosa* (Benth.) Briq leaves

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Hemizygia bracteosa (Benth.) Briq (Lamiaceae) is used in the treatment of mental disease, memory loss, HIV, malaria and had antimicrobial and hypoglycemic properties. To the authors' knowledge, no scientific study has been done on these properties except antimicrobial and hypoglycemic properties. The phytochemical investigation focused on the quantification of phenolic compounds, flavonoids, tannins, anthocyanins and reducing sugars. Gas chromatography-mass spectrometry (GC-MS) analysis was performed on derivatized and non-derivatized extracts for the determination of chemical composition. Phytochemical composition evaluation shows phenolics content between 9.3 and 24.8 mg eq GA/g, a flavonoids content of 9 to 10.5 mg eq Q/g, an anthocyanins content of 0.0005 to 0.0025 mg eq C3G/g, a reducing sugar content of 126.1 to 123.7 mg eq G/kg, and undetected tannin content. GC-MS results revealed the presence of sugars, alcohols, acids, sesquiterpenes, amino acids, ester, lactone, phenolic. It also revealed that acetylcholinesterase inhibition was between 16.9 and 36.5%; xanthine oxidase inhibition between 3.8 and 60.2%; α -amylase inhibition between 6.1 and 8.7%, anti-inflammatory activity between 4.7 and 8.1%, anti-cancer activity between 24 and 96.4% for MCF7 ; 33.1 and 96.8% for HCT116 ; antioxidant activity between 2.1 and 13.2%. The results of acetylcholinesterase inhibition of ethyl acetate and methanolic extracts may justify the use of the plant in the treatment of mental illnesses and memory loss. But *H. bracteosa* should be used with caution because of its very high anti-cancer activity that could induce cytotoxicity.

Key words: *Hemizygia bracteosa*, chemical composition, antioxidant, acetylcholinesterase, inflammation, xanthine oxidase, α -amylase, cancer.

INTRODUCTION

Hemizygia bracteosa (*Orthosiphon bracteatus*) (Benth. Baker] (Lamiaceae) is a herbaceous plant native to tropical region and is abundant in West Africa. This plant

is widely used in folklore to treat mental illness, memory loss, to prevent and treat HIV infection, AIDS symptoms and as a stimulant for dance (Mujovo, 2009; Stafford,

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2009). *H. bracteosa* is also used as an anti-mosquito (anti-*Plasmodium falciparum*) to treat malaria (Burkill, 1995). A previous work has also shown its antimicrobial and hypoglycemic properties (Konfo et al., 2014). The above various medicinal uses of *H. bracteosa* suggest that this plant could possess some biological activities including antioxidant, anti-cancer, anti-inflammatory, anti-diabetic, acetylcholinesterase inhibitory and xanthine oxidase properties. However, these biological activities of *H. bracteosa* have not been explored as suggested by the literature review. Therefore, this study aimed to study the chemical composition of *H. bracteosa* and to screen its biological activities including antioxidant, anti-cancer, anti-inflammatory, anti-diabetic, acetylcholinesterase inhibitory and xanthine oxidase properties.

MATERIALS AND METHODS

Chemicals and plant material

Fresh leaves of *H. bracteosa* were harvested in July 2014, in Savalou located in the center part of Benin. The plant was botanically identified by the National Herbarium of the University of Abomey Calavi (no. AA 6543/HNB) where voucher specimens are deposited. All chemicals and reagents used in this study were of analytical grade and obtained from Sigma-Aldrich-Fulka chemical company (Saint Quentin, France).

Preparation of crude extract

Sufficient plant material was harvested, rinsed with water and spread in the laboratory's air-conditioned drying room at 16°C until the plant material was dry. The dried plant was ground in a blender to obtain powder. The powder was extracted successively with solvents of increasing polarity including cyclohexane, dichloromethane, ethyl acetate and methanol. 100 g of the powder obtained are mixed with 1 L of cyclohexane. The mixture was left in continuous maceration with stirring. The extract was then filtered. The organic portion was evaporated to dryness with a rotary evaporator at 25°C. The residue was extracted again with 1 L of cyclohexane and the extraction procedure repeated until exhaustion grounds. The residue was successively extracted using 1 L of other solvents of increasing polarity namely dichloromethane, ethyl acetate and methanol. The extracts obtained were stored at -20°C for analysis.

Phytochemical studies

Quantification of phenolic content

Total phenolics content was determined by Folin-Ciocalteu method with modifications (Bekir et al., 2013). 20 µL of extract solution Total phenolics content was determined by Folin-Ciocalteu method with modifications (Bekir et al., 2013). 20 µL of extract solution (50 mg/L DMSO) were added to 100 µL of Folin-Ciocalteu reagent (0.2 N). After 5 min of incubation at ambient air away from the light, 80 µL of sodium carbonate solution (Na₂CO₃; 75 g/L) was added and incubated for 15 min at room temperature away from light. The absorbance was measured at 765 nm against a blank where Folin-Ciocalteu reagent was replaced by distilled water. Gallic acid (0 to 11.5 mg/L) was used as standard reference for calibration curve. All tests were carried out in triplicate. The concentration was obtained

from a calibration curve and expressed in mg equivalent of gallic acid per g of dry mass.

Quantification of total flavonoids

Quantification of the flavonoids was carried out according to trichloride aluminum method (Bekir et al., 2013). 100 µL of extract solution (50 mg/L DMSO) was added to 100 µL of methanolic solution of aluminum trichloride (2%) and incubated for 15 min. The absorbance was read at 415 nm against a blank. All measurements are performed in triplicate. Quercetin is the reference used. Flavonoid content was calculated using the standard calibration curve and expressed in milligrams equivalent of quercetin per gram of dry mass (mg EQ/g).

Quantification of tanins

Tanins content was determined according to the vanillin method (Naczek et al., 2000). 100 µL of sulfuric vanillin solution (1%, 7M H₂SO₄) was mixed with 50 µL of the extract solution (50 mg/L of DMSO). The mixture was incubated for 15 min at 25°C in the absence of light. The absorbance was read at 500 nm against its blank. The reference used was catechine. All measurements were performed in triplicate. Tanin content was calculated using standard (catechin) calibration curve and expressed as milligrams equivalent of catechin per gram of dry mass (mg ECat/g).

Quantification of anthocyanins

The assay was carried out according to the differential pH method described by Bekir et al. (2013). Two reaction mixtures, one consisting of 100 µL of extract solution (50 mg/L DMSO) and 100 µL of buffer solution [pH = 1 (0.2 M KCl - HCl 0.2 M)] and the other consisting of 100 µL of extract solution (50 mg/L DMSO) and 100 µL of another buffer solution [pH = 4.5 (0.2 M acetic acid - 0.2 M sodium acetate)] were incubated for 15 min at ambient temperature and protected from light. Then, the absorbance of each mixture was measured at nm and 700 nm. All measurements were performed in triplicate. The anthocyanin content was calculated according to the equation:

$$An \text{ (mg/L)} = \frac{A * M * DF}{\epsilon * L} * 10^{-3}$$

A = sample absorbance = [(abs₅₁₀-abs₇₀₀)_{pH=1} - (abs₅₁₀-abs₇₀₀)_{pH=4.5}]; M = molar mass of cyanidin 3-glucoside = 449.2 g/mol; DF = sample dilution factor = 10 to 50; ε = molar absorption coefficient of the majority anthocyanin = 26900 L.mol⁻¹cm⁻¹; L = optical path = 0.52 cm; 10⁻³ = conversion factor from g to mg

Quantification of reducing sugars

100 µL of extract solution (50 mg/L DMSO) was mixed with 150 µL of 3,5-dinitrosalicylic acid (0.05 M), homogenized and then incubated in bain-marie at 100°C for 5 min. An addition of 750 µL of distilled water was followed by stirring. Finally, the absorbance was read at 540 nm against a blank and a negative control. Glucose was the reference used. All experiments were performed in triplicate. Reducing sugar content was calculated according to glucose calibration curve and expressed as milligram equivalent of glucose per gram of extract (mg eq G/g)

Preparation of extracts for GC-MS analysis

All extracts were analysed before and after derivatization. The

derivatisation was carried out according to the method adopted by Tzing and Ding (2010) with some modifications. 5 mg of each extract were solubilized in 1 mL of a tetrahydrofural solution. Then, 150 μL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), 1.5 μL of chlorotrimethylsilane were added to the mixture. The mixture was stirred, bubbled with nitrogen for 20 s and then incubated at 40°C for 15 min. Finally, 20 μL of the reaction mixture are taken for analysis by GC-MS.

GC-MS analysis

The analysis by GC-MS was carried out using a chromatograph Mark Varian 3400 Cx (Ullis, France) equipped with a fused silica capillary column DB-5MS (5% phenylmethylpolysiloxane, size 30 m x 0.25 mm, 0.25 mm film thickness) and a Varian Saturn GC-MS 4D mass spectrometer.

The chromatographic analysis conditions were: raising the temperature from 60 to 260°C with a first gradient of 5°C/min, 15 min to reach 260°C; a second gradient of 40°C/min to reach 360°C; the duration of the analysis was 30 min; the samples were solubilized in petroleum ether, the carrier gas is helium (99.999% purity) at a rate of 1 ml/min and the injector at 200°C. The conditions of analysis by mass spectrometry were: the emission current of the mass spectrometer was 10 μA , the voltage of the electron multiplier varies between 1400 and 1500V; the temperature of the trap was 150°C and that of the transfer line was 170°C, the mass digitization was 40-650 atomic mass unit.

Phenolics constituents were identified by comparing their retention times obtained on the apolar column DB-5MS with those of the C5-C24 alkanes of the literature, mass spectra obtained with those of the NIST 08 library (National Institute of Standards and Technology) and reported in published articles. The percentage composition of the extract was carried out according to the method of Normalization of GC peak areas, assuming that the mass response factors are the same for all compounds. The results are mean values of two extract injections, without taking into account correction factor. Only volatile compounds were identified.

Biological screening

All extracts were tested at a concentration of 50 mg/L of DMSO 5% in the wells.

Antioxidant activity assay

The antioxidant scavenging activity of extracts was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Bekir et al. (2013). 20 μL of extract solution were mixed with 180 μL DPPH methanolic solution (0.2 mM). The resulting mixture was incubated at 25°C for 25 min in the dark. Upon incubation, the absorbance of the mixture was measured at 524 nm against a blank. Negative control where the extract solution was replaced by solubilization solvent and its blank were also tested. Ascorbic acid was used as positive control. All tests were carried out in triplicate. The free radical-scavenging activity of each extract solution was expressed as percentage of inhibition and was calculated using the formula:

$$\text{Inhibition (\%)} = 100 - (A_{(\text{Blank})} - A_{(\text{sample})})/A_{(\text{Blank})}$$

Acetylcholinesterase inhibition assay

The evaluation of acetylcholinesterase inhibition of extracts was carried out according to the Ellman's colorimetric method as

previously reported by Bekir et al. (2013) with modifications. 50 μL of phosphate buffer A [(Na₂HPO₄, 12H₂O) 0.1M pH = 8], 25 μL of extract solution (50 mg/L and 5% DMSO in the well), 125 μL solution of dithiobisnitrobenzoate (3 mM) dissolved in buffer C [(Na₂HPO₄, 12H₂O) 0.1 M pH = 7], and 25 μL of the acetylcholinesterase (2.8 U/mL) dissolved in buffer B [(Na₂HPO₄, 12H₂O), (0.02M; pH = 7)] were added to the 96-well microplate. The mixture was incubated for 15 min at 25°C followed by addition of 25 μL of acetylthiocholine iodide. The mixture was again incubated for 10 min. Then, the absorbance was read at 412 nm against a blank. The same procedure was done with a negative control (0% acetylcholinesterase inhibition activity) where the sample was replaced by solubilization solvent and a blank, as well as the positive control which is galanthamine. The experiments were done in triplicate. Anticholinesterase inhibitory activity was expressed as percent of inhibition calculated as:

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

Xanthine oxidase inhibition assay

The anti-xanthine oxidase inhibition activity was realized according to method described by Lin et al. (2000) with many modifications. The mixture consisted of 60 μL of phosphate buffer [(Na₂HPO₄) (70 mM, pH=7.5)], 50 μL of extract solution (50 mg/L and 5% DMSO in the well) and 30 μL of xanthine oxidase (0.1 U/mL) was performed in wells of a 96-well and incubated at 25°C in a spectrophotometer for 15 min. This is followed by addition of 60 μL of xanthine (150 μM) and 5 min of incubation in the spectrophotometer, after which the absorbance was read at 295 nm against a blank. A negative control where extract solution is replaced by solubilization solvent and its blank were also tested (0% xanthineoxidase inhibition activity). Allopurinol was used as positive control. The assay was done in triplicate. The percent of xanthine oxidase inhibition was calculated using the following equation:

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

α -Amylase inhibition assay

The assay was carried out following Jyothi et al. (2011) method with some modifications. 50 μL of extract solution (50 mg/L and 5% DMSO in the well) and 50 μL of α -amylase (0.02 U/mL) was incubated at 25°C for 15 min. 100 μL of starch (1%) was added to the mixture followed by a further incubation for 3 min at 25°C. Addition of 100 μL of DNS (3,5-dinitrosalicylic acid) reagent (12 mL of distilled water, 12 g of sodium potassium tartrate dissolved in 8 mL of sodium hydroxide (2 M); 0.4379 g of 3,5-dinitrosalicylic acid dissolved in 20 mL of distilled water) was done to stop the reaction. This mixture was again incubated for 10 min at 100°C. Finally, the reaction mixture was diluted by adding 1 mL of phosphate buffer [NaH₂PO₄: 2H₂O (pH = 6.9)] containing sodium chloride 6.7 mM) and the absorbance was read at 540 nm against a blank. Blank was performed by replacing enzyme by buffer. A negative control sample was performed in the same manner by replacing the sample by solvent (0% α -amylase inhibition activity). Acarbose was used as positive control. α -Amylase inhibition activity was expressed as percentage inhibition and calculated using the formula:

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

5-Lipo-oxygenase inhibition assay

Evaluation of the inhibition activity of 5-lipoxygenase is carried out according to the method described by Bekir et al. (2013). A reaction

Table 1. Extraction yield and phytochemical composition of *Hemizygia bracteosa*.

Extracts	Yields (%)	Phenolics (mg eq GA/g).	Flavonoids (mg eq Q/g)	Tanins (mg eq Cat/g)	Anthocyanins mg eq C3G/g	Reducing Sugar (mg eq G/kg)
Cyclohexane	0.92	9.3 ± 0.3	nd	nd	0.0015 ± 0,0	nd
Dichloromethane	0.55	17.4 ± 0.4	nd	nd	0.0025 ± 0,0	nd
Ethyl acetate	2.72	24.4 ± 0.5	9.0 ± 0.1	nd	0.0005 ± 0,0	126.1 ± 0.6
Methanol	1.00	24.8 ± 1.2	10.5 ± 0.2	nd	nd	127.3 ± 0.3

nd = Not detected.

mixture composed of 150 µL of sodium phosphate buffer [(Na₂HPO₄, 2H₂O), (KH₂PO₄), (NaCl), (pH = 7.4)], 20 µL of extract solution (50 mg/L and 5% DMSO in the well), 60 µL of linoleic acid (3.5 mM), 20 µL of enzyme, was incubated at 25°C for 10 min against a blank. The absorbance was measured at 234 nm. A negative control where the extract solution is replaced by the solvent solubilizing the extract, and a blank was also tested (0% 5-lipo-oxygenase inhibition activity). Nordihydroguaiaretic acid was used as positive control. The tests are carried out in triplicate. The inhibition activity of 5-lipoxygenase expressed as percentage inhibition is calculated according to the formula:

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

Cytotoxicity evaluation

Anti-cancer activity of the extracts was evaluated on human breast cancer cells (MCF-7) and human colon cancer cells (HCT116) according to the method described by Natarajan et al. (2011) with modifications. 100 µL of cells suspension was deposited in the wells of the 96-well plate at a rate of 3.104 cell/well. 100 µL of culture medium was added to have a sufficient volume for growth. The plate was incubated at 37°C for 24 h. The culture medium was gently removed taking care not to detach the adherent cells. 100 µL of culture medium containing the extract were added to 100 µL of culture medium in order to have 50 mg/L for concentration of extract in the wells. The contact with the cells was carried out at 37°C for 48 h. After removal of culture medium and PBS washing, 50 µL of MTT solution (1 mg/mL in PSB) were added to each well and then the plate was incubated for 40 min at 37°C. The MTT was gently removed and then 50 µL of DMSO were added to each well for solubilization of the formazan crystals formed.

Homogenization within the spectrophotometer and reading of the optical density at 605 nm completed the test. The experiments were repeated three times and the results were compared with cells grown only in the presence of culture medium. Controls were carried out in parallel with anti-cancer drug, Tamoxifen.

Statistical analysis

All data were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at P<0.05. Correlations were carried out using the correlation and regression in the EXCEL program.

RESULTS AND DISCUSSION

Extraction yield

The yield of each extract is shown in Table 1. Ethyl

acetate extract had the highest yield which was 2.72% followed by methanol (1.00%) and cyclohexane extracts (0.92%). The lowest yield was obtained with dichloromethane extract (0.55%). To the best of the authors knowledge, there is no data on the yields of the various extracts.

Chemical composition

Phenolic compounds content

Table 1 shows phenolic compounds contents of *H. bracteosa* extracts. The lowest content, 9.3 ± 0.3 mg equivalent gallic acid/g, was obtained with cyclohexane extract. The highest content, 24.8 ± 1.2 mg equivalent gallic acid/g, was obtained with methanol extract. These results showed that ethyl acetate and methanol extracts have more phenolic content than non polar extract, suggesting that the compounds are polar. This low content could influence the biological activities of the plant because polyphenols are revealed to be responsible for several activities. There is lack of information on phenolics content of genus, *Hemizygia*. However, a comparison with some Lamiaceae (Khomdram and Singh, 2011) showed that, *H. bracteosa* contains fewer phenolics compounds than *Elsholzia blanda* (46.28 ± 0.543), *Elsholzia stachyodes* (32.07 ± 0.566), *Hyptis suaveolens* (32.07 ± 0.363), *Ocimum americanum* (26.15 ± 0.808) and *Ocimum basilicum* (39.31 ± 0.439). This difference could be explain by the difference between the organs, extraction technique, extraction solvents and nature of the plants.

Flavonoids content

Flavonoids were quantified only in ethyl acetate extract and in methanol extract with respective values of 9 ± 0.1 mg eq Q/g and 10.50 ± 0.2 mg eq Q/g dry mass (Table 1). Information on flavonoids content of *H. bracteosa* plant or on species of the genus *Hemizygia* is scarce. Nevertheless these results can be compared with those of other species in the family. Thus, the value of the methanol extract is lower than those obtained with ethanolic extracts (80%) of the aerial parts of the eight

Lamiaceae species studied by Khomdram and Singh (2011). This difference could be explained by the difference of extraction method, plant organ, edaphic, climatic and environmental conditions.

Tanin content

Tanin content was not detected. However, comparison with eight species of Lamiaceae showed that they contain them (Khomdram and Singh 2011). This difference can be explained by difference of extraction technique, plant organs, and climatic and environmental conditions.

Anthocyanins content

Only dichloromethane extract contains a tiny amount of 0.0023 ± 0 mg eq cyanidine-3-glucoside/g of dry mass (Table 1). No data is available on the anthocyanin content of *H. bracteosa* in the literature or on the genus *Hemizygia*.

Reducing sugars content

The determination of reducing sugar content showed that only the ethyl acetate and methanol extracts contained respectively 126.1 ± 0.6 and 127.3 ± 0.3 mg eq glucose/kg (Table 1). Reducing sugars have not been quantified in the less polar extracts. This determination of the sugar content of *H. bracteosa* would be the first in the genus *Hemizygia*.

Compounds identified before and after derivatisation

GC-MS analysis before and after derivatization of *H. bracteosa* extracts identified sugars, alcohols, acids, sesquiterpene, amino acid, ester, lactone and a single phenolic compound called catechollactate (Table 2). Sesquiterpene (E) - β -farnesene was very abundant in the cyclohexane extract. It was the major compound of the essential oil of the aerial part of the plant (Kpoviessi et al., 2016). The only phenolic compound identified and its low abundance could account for the low phenolic content.

Biological activities

The antioxidant activity

The results of DPPH assay (Table 3) showed that *H. bracteosa* extracts were poor in antioxidant. The antioxidant activity of extracts was low. The best activity was obtained with methanol extract which presented $13.2 \pm 0.6\%$ of inhibition. Cyclohexane extract is the lowest in antioxidant ($2.1 \pm 0.2\%$ of inhibition). Methanol is the best

antioxidant extraction solvent. Ascorbic acid (5.3 mg/L) was used as the positive control. It presented $53.01 \pm 0.4\%$ of inhibition. This low activity could be explained by the poverty of phenolic compounds. Phenolic compounds could be responsible for this activity.

Antioxidant activity of *H. bracteosa* leaves methanolic extract was different from ethanolic extract of *Coriandrum sativum* plant which gave $0.21 \pm 0.1\%$ at 0.1 mg/ml (100 mg/L) (Maya and Sarada, 2014). *H. bracteosa* at 50 mg/L was rich in antioxidant with $13.2 \pm 0.6\%$ than *C. sativum*.

The acetylcholinesterase inhibition

These results (Table 3) show that the *H. bracteosa* extracts have moderate activity ranging from $16.9 \pm 0.7\%$ (dichloromethane extract) to $36.5 \pm 1.5\%$ (Ethyl acetate extract). The results could justify its use in the treatment of memory loss in pupils and the elderly. Percent of galanthamine (1.5 mg/L) inhibition which was used as positive control was 96.2 ± 1.8 mg/L. No data on acetylcholinesterase inhibition are available in the literature. Methanolic extract of *H. bracteosa* leaves would be more active than *Lavandula angustifolia* ethanolic extract which is a Lamiaceae, (Ferreira et al., 2006) because 0.5 mg/ml (500 mg/L) of *L. angustifolia*, is 10 times the concentration of *H. bracteosa* which gave $26.6 \pm 9.5\%$ inhibition. *H. bracteosa* at 50 mg/L was inhibited at $24.4 \pm 0.6\%$.

5-lipoxygenase inhibition


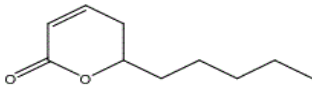
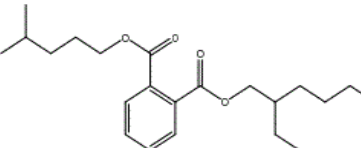
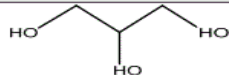
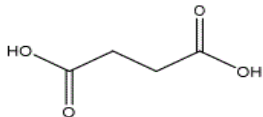
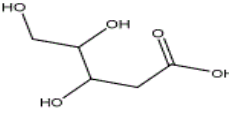
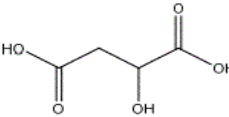
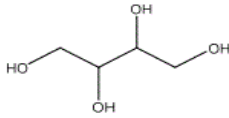
The evaluation of 5-lipoxygenase inhibition *H. bracteosa* extracts anti-inflammatory activity gave Results (Table 3) which show a very low activity. The lowest value, $4.7 \pm 0.2\%$, was obtained with methanolic extract. The highest value was $8.1 \pm 0.2\%$ inhibitions, it was obtained with ethyl acetate extract. The positive control used was nordihydroguaiaretic acid (NDGA) (4 mg/L) presenting percent of inhibition of $81.9 \pm 0.2\%$ of inhibition. The results of this study were the first in the literature. No comparison can be made with genus *Hemizygia*.

As compared to *Canthium parviflorum* studied by Sirigiri et al. (2014), *H. bracteosa* was less active. While *H. bracteosa* at 50 mg/L showed $4.7 \pm 0.2\%$ inhibition, *C. parviflorum* another Lamiaceae showed 59% inhibition at 25 μ g/mL (25 mg/L). *H. bracteosa* extract was more concentrated but had a lower percentage and *C. parviflorum* is less concentrated but with a higher percentage.

The xanthine oxidase inhibition

Analysis of xanthine oxidase inhibition results (Table 3), showed an activity between $3.8 \pm 0.1\%$ (dichloromethane

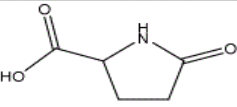
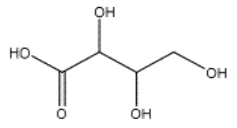
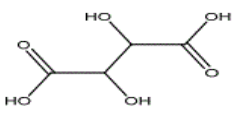
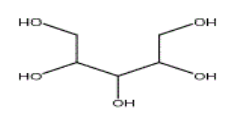
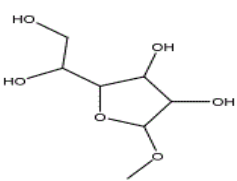
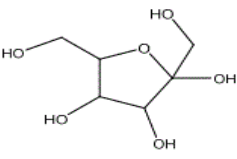
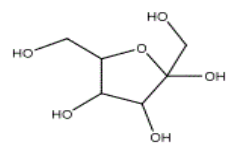
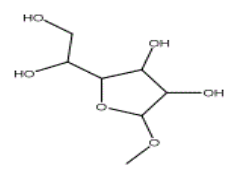
Table 2. Compounds identified before and after derivatisation.

N°	Tr (min)	compounds	structures	Presence in extracts of			
				Cyclo	DCM	AcE	MeO H
Before derivatisation							
1	13.23	(E)- β -Famesene		++++			
2	13.66	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-		++	+		
3	21.06	Phthalic acid 2-ethylhexyl isohexyl ester			++		
After derivatisation							
4	11.33	Glycerol				++	+
5	11.76	succinic acid				+	
6	12.33	3,4,5-trihydroxypentanoic acid				+	
7	13.34	2-hydroxysuccinic acid				+	+
8	13.42	Threitol				+	+

extract) and $60.2 \pm 0.9\%$ inhibition (methanolic extract). The xanthine oxidase inhibition of *H. bracteosa* is summarized in Table 3. The most polar extracts showed the best inhibitions. Allopurinol which was the positive control used, presented 1 mg/L inhibition of $52 \pm 1.0\%$. There is no data on this activity in the literature. A

comparison with Shivraj and Se (2014) work showed that leaves methanolic extract of *H. bracteosa* was more active than root methanolic extract of *Rapidx negundo*. Indeed, two extracts were tested at the same concentration of 50 mg/L but the percentage inhibition of *H. bracteosa* which is 60.2 ± 0.9 was greater than that of

Table 2. Contd.

9	13.71	5-oxoproline		+	
10	13.94	L-threonic acid		+	
11	14.54	L-tartaric acid		+	
12	15.20	Arabinitol		++++	+
13	15.78	glucofuranoside methyl		+	+
14	15.87	D-Fructofuranose		+	+
15	15.95	D-Fructofuranose isomere 1		+	+
16	16.15	glucofuranoside methyl isomere 1		+	

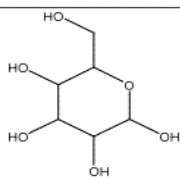
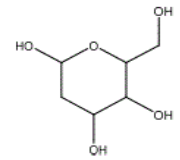
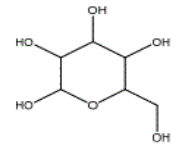
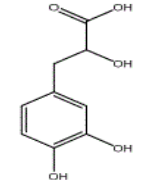
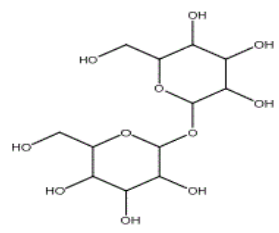
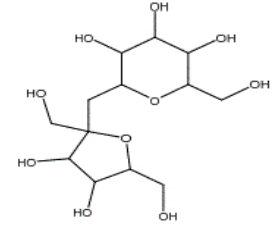
Vitex negundo which was $45.8 \pm 0.2\%$. This difference could be explained by the difference of organs, the technic, edaphic conditions, climatic conditions and geographical area. *H. bracteosa* may be indicated for the treatment of gout disease.

The α -amylase inhibition

The evaluation of anti α -amylase activity of *H. bracteosa*

extracts gave results shown in Table 3. The results show that all extracts exhibited α -amylase inhibition. Methanol extract showed the highest inhibition with $8.7 \pm 0.4\%$. The lower inhibition, $6.1 \pm 0.4\%$ was obtained with ethyl acetate extract. Cyclohexane and dichloromethane extracts showed no activity. This shows that the bioactive compounds responsible for this activity are present only in the polar extracts. Polar solvents are the best solvents extracting these bioactive compounds.

Table 2. Contd.

17	16.55	D-talopyranose		+	+
18	16.67	Deoxy-galactopyranose		+	+
19	17.01	D-Manopyranose		+	+
20	17.56	catéchollactate		+	+
21	20.52	D-Turanose		+	+
22	21.06	Sucrose		+	+

Acarbose (50 mg/L) is the positive control used which presented $56.4 \pm 2.6\%$ inhibition.

The leaves exhibited low activity, while the whole plant was hypoglycemic in Wistar rats (Chabi et al., 2015). Although, origin of the two plants is the same city, the difference in activity can be explained mainly by the difference in the organs used (leaves in the current study and whole plant in the study carried out by Chabi et al. (2015), and also by the type of extract (aqueous extract in the study of Chabi et al. (2015) and extract with cyclohexane, dichloromethane, ethyl acetate and methanol in the current investigation) and the mode of

evaluation of the activity (*in vivo* in Chabi et al. (2015) and *in vitro* in the current study).

H. bracteosa was less active than the methanolic extracts of *Phlomis bruguieri*, *Phlomis rigida*, *Stachys byzantina* and *Scutellaria toumefortii* which presented at 15 mg/mL (1500 mg/mL), an inhibition percentage respectively, of 49.7 ± 5.7 ; 94.8 ± 3.3 ; 86.1 ± 2.8 and 52.1 ± 0.1 (Hedieh et al., 2014). It takes 30 times the concentration of *H. bracteosa* to have these high percentages.

On the other hand, it would be less active than other Lamiaceae whose methanolic extracts showed $60.24 \pm$

Table 2. Contd.

23	21.70				+
		D-turanose			

+ = Little presence; ++ = average presence; +++ = abundant presence; ++++ = very abundant presence.

Table 3. *Hemizygia bracteosa* extracts activities.

Extracts and standards	Antioxydant activity (%)	Acetylcholinesterase inhibition (%)	Anti inflammatory activity (%)	Xanthine oxydase inhibition (%)	α -Amylase inhibition (%)
Cyclohexane	2.1 \pm 0.2	27.2 \pm 1.0	6.3 \pm 0.2	29.3 \pm 0.3	na
Dichlorométhane	4.6 \pm 0.3	16.9 \pm 0.7	7.8 \pm 0.2	25.8 \pm 1.1	na
Acétate d'éthyle	6.4 \pm 0.3	36.5 \pm 1.5	8.1 \pm 0.2	59.0 \pm 1.1	6.1 \pm 0.4
Méthanol	13.2 \pm 0.6	24.4 \pm 0.6	4.7 \pm 0.2	56.4 \pm 2.0	8.7 \pm 0.4
ascorbic acid (5.3 mg/L)	53.01 \pm 0.4	-	-	-	-
Gаланthamine (1.5 mg/L)	-	96.2 \pm 1.8	-	-	-
Nordihydroguaiaretic acid NDGA (4 mg/L)	-	-	81.9 \pm 0.2	-	-
Allopurinol (1 mg/L)	-	-	-	52 \pm 1.0	-
Acarbose (50 mg/L)	-	-	-	-	56.4 \pm 2.6

Table 4. *Hemizygia bracteosa* leaves extracts anti-cancer activities: MCF7 and HCT116 cell line inhibition.

Extracts and standard	MCF7 inhibition (%)	HCT116 inhibition (%)
Cyclohexane	46.3 \pm 3.2	78.9 \pm 2.3
Dichloromethane	96.4 \pm 2.4	96.8 \pm 0.7
Ethyl acetate	24 \pm 5.6	33.1 \pm 3.9
Méthanol	29.5 \pm 10.4	44.4 \pm 6.6
Tamoxifen (IC ₅₀)	2.70 \pm 0.2	2.68 \pm 0.1

0.69% for *Mangifera indica*, 47.30 \pm 1.27% for *Azadirachta indica*, 47.12 \pm 0.40% for *Phyllanthus amarus* (Dineshkumar et al., 2010). These differences can be explained by concentration, geographical location, edaphic conditions, climatic conditions and extraction technique.

The anti-cancer activity

Table 4 shows the results of the anti-cancer activity. The table reveals that all extracts had an activity on cancer

cells. Ethyl acetate extract was the least active on MCF7 (24 \pm 5.6%) and HCT116 (33.1 \pm 3.9%) cells. Dichloromethane extract was most active on both MCF7 (96.4 \pm 2.4%) and HCT116 (96.8 \pm 0.7%) lines. No data on anti-cancer on MCF7 and HCT116 lines exist in the literature. The apolar extracts were more active than polar extracts. This can be explained by the affinity of the compounds responsible of this activity with apolar solvents. As the extracts strongly inhibit the growth of cancer cells, they will therefore be toxic at this concentration for normal cells. This cytotoxicity is corroborated by the study carried out by Chabi (2015)

which highlights the toxicity of the plant at certain doses on normal cells of liver and kidneys of Wistar rats. *H. bracteosa* should therefore be taken with caution. Further study is needed to identify and isolate the compounds responsible for the manufacture of medicines. What was involved in the search for anti-cancer drugs was weaking havoc in the populations today.

Conclusion

This study showed that *H. bracteosa* leaves have low concentration of phenolic compounds, contain bio-active substances which are sugars, alcohols, acids, phenolic compounds, sesquiterpenes, amino acid, ester and lactone. The leaves have low antioxidant activity. It also showed a moderate xanthine oxidase inhibition, acetylcholinesterase inhibition and a strong anti-cancer activity. It is cytotoxic, therefore, its use must be with caution. This work is a preliminary assessment study that deserves further investigations to identify the bioactive substances through several fractionation of extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Bekir J, Mars M, Souchard JP, Bouajila J (2013). Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegrate (*Punica granatum*) leaves. *Food Chem. Toxicol.* 55:470-475.
- Burkill HM (1995). The useful plants of west tropical Africa Families J-L, vol 3, Royal Botanic Gardens, Kew. P 638.
- Chabi WN, Konfo CTR, Adjagba M, Moussedikou L, Ahoussi-Dahouenon E, Laleye A, Gbaguidi C, Soumanou MM (2015). Evaluation of the toxicity of *Hemizygia bracteosa* (Benth) Plant used in traditional medicine for treatment of diabetes Mellitus in Benin, *Am. J. Biomed. Sci.* 3:40-44.
- Dineshkumar B, Mitra A, Manjunatha M (2010). A comparative study of alpha amylase inhibitory activities of common anti-diabetic plants at Kharagpur 1 Bloc. *Ind. J. Green Pharm.* pp. 115-121.
- Ferreira A, Proença C, Serralheiro MLM, Araujo MEM (2006). The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *J. Ethnopharmacol.* 108:31-37.
- Hedieh S, Marjan N, Gholamreza A, Parisa S, Ahmad RG, Mahdieh KM, Soodabeh S (2014). α -amylase inhibitory activity of some traditionally used medicinal species of Labiatea. *J. Diabetes Metab. Disord.* pp. 1-5.
- Jyothi KSN, Hemalathr P, Calla S (2011). Evaluation of alpha amylase inhibitory potential of three medicinally important traditional wild food plants of india. *Ind. J Green Pharm.* pp. 95-99.
- Khomdram SD, Singh PK (2011). Polyphenolic compounds and free radical scavenging activity in eight Lamiaceae herbs for Manipur. *Not. Sci. Biol.* 3(2):108-113.
- Konfo CTR, Chabi WN, Agbadjizo J, Dahouenon-Ahoussi E, Soumanou MM, Sohounhloué DCK (2014). Influence de la feuille de *Hemizygia bracteosa* (Benth) sur la qualité de la bière du sorgho « tchakpalo » produite au Bénin/Influence of *Hemizygia bracteosa* (Benth) sheet on the quality of sorghum beer « tchakpalo » produced in Benin. *Int. J. Innov. Appl. Stud.* pp. 453-463.
- Kpoviessi S, Agbani P, Gbaguidi F, Gbénou J, Sinsin B, Accrombessi G, Moudachirou M, Quetin-Leclercq (2016). Seasonal variations of volatile constituents of *Hemizygia bracteosa* (Benth). *Briq. parts from Benin. C. R. Chim.* 19:890-894.
- Lin JK, Chen PC, Ho CT, Lin-Schiao SY (2000). Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by thea flavin-3,3'-digallate, (-)-epigallocatechin-3-gallate and propyl gallate. *J. Agric. Food Chem.* 48:2736-2743.
- Maya M, Sarada S (2014). In vitro screening for anti-cholinesterase and anti oxidant activity of methanolic extract of Ayurvedic medicinal plants used for cognitive disorders. *PLOS ONE* 9:1-7.
- Mujovo SF (2009). Antimicrobial activity of compound s isolated from *Lippia Javanica* (Burn. F.) Spreng and *Hoslundia opposita* against *Mycobacterium tuberculosis* and HIV- Reverse transcriptase. These 125p.
- Nacz M, Amarowicz R, Pink D, Shahidi F (2000). Insoluble condensed tannins of anola/rapeseed. *J. Agri. Food Chem.* 48:1785-1762.
- Natarajan N, Thamaraiselvan R, Lingaiah H, Srinivasan P, Balasubramanian M, Peryaasamy BM (2011). Effect of flavonone hesperidin on the apoptosis of human mammary carcinoma cell line MCF-7. *Biomed. Pev. Nutr.* 1:207-215.
- Shivraj HN, Se WP (2014). Total phenolics, antioxidant and xanthine oxidase inhibitory activity of three colored onions (*Allium cepa* L.), *Front. Life Sci.* 7:2-3, 224-228.
- Sirigiri CK, Kokkanti M, Challa SR (2014). In vitro pharmacological investigations of using leaf Callus extracts of *Canthium parviflorum* Lam. *Br. Biotechnol. J.* 4(3):325-338.
- Stafford GI (2009). Southern African plants used to treat central nervous system related Disorders, Ph.D, thesis. P 105.
- Tzing SH, Ding WH (2010). Determination of melamine and cyanuric acid in powdered milk using injection-port derivatization and gas chromatography tandem mass spectrometry with furan chemical ionization. *J. Chromatogr. A.* 1217:6267-6273.