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Original Research Article

Phytochemical Profil, Hypoglycemic and Antihyperglycemic of Two Medicinals Plants of Cameroon, *Anthocleista vogelii* (Loganiaceae) and *Hallea stipulosa* Leroy (Rubuaceae)

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Abstract

Anthocleista vogelii (Loganiaceae) is a plant used in traditional medicine in the Cameroon, Ghana, Togo, Nigeria and Guinea for the treatment diabetes. *Hallea stipulosa* (Rubuaceae) is a plant used in traditional medicine for the treatment of several illnesses such as hernia, metrorrhagia and dysmenorrhea, colic, psychosomatic disorder and diabetes. This study aims to determine the level of toxicity following the protocol of OECD guideline 423 after phytochemical analysis by using screening, High performance thin layer chromatography (HPTLC) and quantitative analysis of the extract's total polyphenol contents (TPC) and total flavonoids contents (TFTs)were done using folin-ciocalteu and aluminiun trichloride test respectively. The evaluation of the hypoglycaemic activity and a hypoglycaemic activity of the aqueous extracts of the bark of *Haleas stipulosa* and *Anthocleista vogelii*. Combined at the concentrations 250mg / kg and 500mg / kg each at the respective proportions 50/50; 30/70; 70/30 using as a method the measurement of blood sugar every 30min for 3 hours. We obtained a yield of 11.65 for the extract of *Hallea stipulosa* and 10.2 for the extract of *Anthocleista vogelii*. The analysis of the phytochemical screening revealed the presence of tannins, flavonoids, antocyanins and terpenoids, alkanoids and phenols. The *Haleas stipulosa* and *Anthocleista vogelii* fractions have a relatively high TPC of 95,78 ± 0,02 (mg EAG/g de ES), 169,66 ± 0,03 (mg EAG/g de ES), respectively and the TFTs of 62,93 ± 0,06 (mg EQ/g d'ES), 73,45 ± 0,05 (mg EQ/g d'ES) respectively. Acute toxicity tests in female rats at doses of 2000mg / kg and 5000mg / kg revealed no behavioral disturbances and no death. The administration of the combination of the aqueous extracts of the bark of the trunks of *Hallea stipulosa* at the concentrations of 250 mg / kg BW and 500 mg / kg BW produced a significant hypoglycaemic effect ($P < 0.05$) in rats compared to the batches of rats positive controls. These results suggest that the combination of the aqueous extracts of the bark of the trunks of *Haléas stipulosa* and *Anthocleista vogelii* exhibits hypoglycemic and antihyperglycemic activity at certaines concentrations.

Keywords: *Hallea stipulosa*, *Anthocleista vogelii*, HPTLC, diabetes, hypoglycemia, anti-hyperglycemia.

INTRODUCTION

Diabetes is a metabolic disease characterized by the presence of chronic hyperglycemia that occurs when the pancreas does not produce enough insulin, or when the body does not properly use the insulin it produces, or a combination of both [1]. According to Goldenberg and *Coll.* in 2013, diabetes can be defined as a fasting plasma glucose (<8 hours of fasting) greater than 1.26 g/l or 7mMol/l [2]. It is a long-term carrier of severe and disabling micro and macrovascular complications affecting certain target organs such as the heart, central and peripheral nervous system, kidneys, eyes and feet [3], and is therefore a major public health problem due to its significant and growing prevalence on the one hand, and its socio-economic impact on the other [4]. Globally, the number of diabetic patients has increased dramatically in recent years, with 356 million registered. This compares to 463 million according to the International Diabetes Federation [5, 6]. It is also estimated that this figure will reach 578 million in 2030, and then 143%. Cameroon in particular is no exception

with 615,000 people affected, i.e. a prevalence of 6% among adults over 20 years of age [7].

This upsurge can be explained by several factors, on the one hand by the improvement in screening and deaths in care; on the other hand, the modification of diagnostic criteria and the ageing of the population; but above all by the increase in risk factors for diabetes such as: overweight and obesity [8]. Although its management is based on 4 levels, namely: oral antidiabetic drugs (ODAs), a healthy and balanced diet, sports and psychological activity. According to Nissan and Wolski, these ODAs have several significant disadvantages including high costs on the one hand and on the other hand, harmful effects (hypersensitivity, drug resistance, kidney failure and many others) [5, 9, 10]. Hypoglycemic agents remain an important area of investigation with bioactive compounds from readily available traditional medicinal plants offering great potential to discover new anti-diabetic molecules [11, 12], which would limit the use of these ADOs. Our work is part of the study of the hypoglycemic and antihyperglycemic activity of the extract from the trunk bark of *Hallea stapulosa* and *Anthocleista vogelii*, which are the plants widespread throughout intertropical Africa. They are used in combination in traditional recipes as an anti-diabetic, anti-inflammatory, and antioxidant agent [11]. In view of their functional properties, the combination of these extracts from the trunk bark of *Hallea stapulosa* and *Anthocleista vogelii* in defined proportions could increase their degree of efficacy. Therefore, the general objective of this work is to study the hypoglycemic and antihyperglycemic activity of the trunk bark extract of *Hallea stapulosa* and *Anthocleista vogelii*.

I. MATERIALS AND METHODS

I.1. Materials

I.1.1 Plant material

The bark of *A. voguelii* was harvested on November 28, 2020 in Bonepoupa, a locality in the Littoral Region of Cameroon (GPS coordinates: Latitude 4^0 02’ N, Longitude 10^0 01’ E). The plant was identified in the National Herbarium of Cameroon compared to sample number 20729/SFR. CAM. De A.J.M. LEUWENBERG No. 7670.

The bark of *H. stipulosa* was collected on 30 October 2020 in Ntonde in the region of the Cameroon (GPS coordinates: Latitude 9^0 50’ N, Longitude 4^0 13’ E) coast has been identified to comparison with the reference sample number 049319 previously collected.

I.1.2 Animal Material

The experiments were carried out on a single animal species. These are rats (*Rattus norvegicus*) of the wistar strain, born and then raised in the animal facility of the Laboratory of Experimental Pharmacology of the Faculty of Medicine and Pharmaceutical Sciences (FMSP) of the University of Douala. For the assessment of hypoglycemic and antihyperglycemic activities, we used rats aged 8 to 12 weeks, physically healthy with a weight between 150 and 200 grams. We rejected diseased rats (tumors), immature rats used in other experimental studies with a weight of less than 150 g and pregnant female rats. food: the diet in this experiment is a composition of the different crushed products for a total of 58kg.

I.2. Methods

I.2.1. Phytochemical Screening

Screening is a set of qualitative techniques making it possible to implement evidence certain chemical compounds through the formation of insoluble complexes or reactions.

I.2.1.1 Identification of secondary metabolite classes

Phytochemical screening is the set of qualitative techniques used to determine the various chemical groups contained in a plant organ. Tube tests carried out on methanolic plant extract, to make a preliminary determination of the phytochemical classes contained in the plant. Their presence on the basis of the formation of insoluble complexes (precipitates) or the formation of coloured complexes was therefore assessed by the following tests.

The various groups of compounds such as alkaloids, flavonoids, sterols, polyterpenes, coumarins, saponins, phenols, tannins and anthocyanins contained in stem bark extracts will be highlighted according to the methods described.

Detection of alkaloids

Alkaloids are detected in residues using Dragendorff’s and Burchard’s precipitation reagents: 0.1 g of residue will be taken up in 6 ml of 60% ethanol, then divided into 2 test tubes. In the first tube, 2 drops of Dragendorff’s reagent are added. The appearance of an orange-red or reddish-brown precipitate indicates a positive test. Add 2 drops of Burchard’s reagent to the second tube. The appearance of a brown precipitate indicates a positive test.

Detection of flavonoids

Flavonoids will be detected in residues using the cyanidin reaction. Evaporate 2 ml of the extract and dilute the residue in 5 ml of 2-fold hydrochloric alcohol. Add 2 to 3 drop of Mg and a few drops of isopentanol. The appearance of an intense pink-orange or purplish coloration (red or red-orange with Zn) indicates a positive reaction.

Detection of sterols and triterpenes

Detection of sterols and triterpenes in residues using the Liebermann reaction. Dissolve an aliquot of hot residue in 1 ml acetic anhydride. Add 0.5 ml concentrated sulphuric acid. The appearance of a violet color that turns blue, blue and then green indicates a positive reaction for sterols and triterpenes respectively.

Detection of coumarins

To 5 ml of an alcoholic solution of the extract, add a few drops of 10% potassium hydroxide. Interpretation: The appearance of a blue to purple-yellow coloration indicates the presence of coumarins.

I.2.1.2 Qualitative analysis by High-Performance Thin-layer Chromatography (HPTLC)

Principle

Thin-layer chromatography (TLC) is a separation and analysis technique that identifies the constituents of homogeneous mixtures. It makes it possible to highlight the subfamily of secondary metabolites. This is a technique that allows the different compounds contained in a mixture to be analysed quickly, reliably and accurately.

Three steps were then used:

Deposit the migration sites.

The various spots were laid in horizontal strips. An initial concentration of 10 mg/mL of each extract was prepared in distilled water (10 mg in 1 mL, w/v). After filtration through fine 0.2 µm filters, 20 µL of solution of each extract was automatically deposited in strips by spraying liquid nitrogen onto HPTLC 60F254 plates (20 cm x 10 cm glass holder from Merck, Darmstadt, Germany). The sample deposition was carried out using a semi-automatic Linomat5 applicator (Camag® Muttenz, Switzerland) controlled by the vision CATS CORE SOFTWARE (CAMAG® HPTLC). In this study, quercetin, coumarin and tannic acid were used as positive controls for flavonoids, coumarins and tannins, respectively.

Migration

After drying the stains, migration was carried out (7 cm path) using a saturated CAMAG container for 30 minutes with a specific mobile phase as appropriate: ethyl acetate/formic acid/acetic acid/water (100:11:11:26; v/v/v/v) for flavonoid migration, toluene - ethyl acetate (20:4, v/v) for the migration of sterols and triterpenes, Ethyl acetate/methanol/water/chloroform (18:2, 4:2.4:6; v/v/v/v) for tannins, sterols and triterpenes. Ethyl acetate/diethylamide/toluene (70:10:20, v/v/v) for alkaloid migration and chloroform/ethyl acetate/methanol/water (6:18:2.4:4, v/v/v/v) for flavonoid migration. After development, the different plates were dried at 110 °C for 2 min on a heating plate (ThermoFischer®) and chromatographic fingerprints were observed at visible and UV wavelengths of 254 nm and 366 nm in a C In camera [13]

Disclosure of secondary metabolites

Neu's reagent and ferric chloride (5%) were used as developers for flavonoids and tannins respectively. The Liberman-Buchard reagent has been used for the revelation of sterols and triterpenes. In addition, sulfuric anisaldehyde has been used for the revelation of steroidal and triterpene saponins. Potassium hydroxide (KOH 2%) was used to highlight coumarins under 366 nm UV light [13].

I.2.2 Quantitative analysis by UV spectrometry dosage

I.2.2.1 Total polyphenol content's dosage (TPC)

The quantification of the TPC was performed using the Folin-Ciocalteu colorimetric method with slight modifications. First, a stock solution of 1 g/mL was prepared for each extract. 1 mL of stock solution was added to 1 mL of Folin Ciocalteu reagent and incubated for 10 min at 105 °C in an oven. Subsequently, 2 mL of sodium carbonate solution (7.5%) was added to the previous mixture to incubate for 30 min at room temperature. At the end of this time, absorbances were read using a 760 nm UV-visible spectrophotometer (model UV-1800 240V, UV spectrophotometer, Shimadzu Japan). A cascade dilution of the stock solution was used as a blank control. The CPR was then calculated using the gallic acid (GA) equation used as a reference: Y = 10.46X + 0.03 (r2 = 0.99).

$$TPC = (A - B) \times FD \times V/(a \times m)$$

I.2.2.2 Total flavonoid content (TFC)

The TFCs were estimated using the aluminum trichloride colorimetric method described above. Briefly, a stock solution of 1 g/mL was first prepared for each extract, and quercetin was used as a reference. Solutions of gallic acid and quercetin were first prepared at a concentration of 1 mg/ml in triplicate. A cascading dilution at 1/2; 1/4; 1/8; 1/16; 1/32 and 1/64 of the initial concentration were reached. The absorbance of each concentration was used to plot the calibration curve. In a hemolysis tube, 1 mL of stock solution was mixed with 1 mL of AlCl3 (2%) of solution. The absorbance of the reaction mixture was measured at 415 nm after a 10-minute incubation in the dark. A blank was prepared under the same conditions using methanol as the sample. The TFC was calculated from the equation of quercetin (Q): Y = 10.43X - 0.11 (r2=0.98) and applying the formula.

The qualitative phytochemical study made it possible to highlight the metabolites secondary present in the extracts of *H. Stipulosa* and *A. vogelii*. It was carried out using standard Bruneton protocols.

The general principle is based on the formation of insoluble and colored complexes, using respectively precipitation and coloring reactions, due to the formation of a conjugation or an instauration in a molecule. The intensity of the coloring or the degree of turbidity will thus be proportional to the quantity of complexes formed.

I.2.3 Estimated LD50

The acute toxicity test was carried out according to guideline 423 of the Organization for Economic Co-operation and Development (OECD). A

test limits doses of 2000 and 5000 mg/Kg of body weight of the animal.

I.2.3.1 Administration of the active substance

Randomly chosen (susceptible) female rats between 8 and 12 weeks of age were deprived of food for 12 h before the test but water was provided to them ad libitum. After fasting, the rats were weighed and the test substance was administered orally using an orogastric tube according to the following distribution:

- ***Change in body mass***

The rats were weighed 8 times respectively in order to evaluate the weight variation: D0; J2; J4; J6; J8; J10; J12; J14.

I.2.4 Evaluation of hypoglycemic activity

This study made it possible to evaluate the regulatory capacity of aqueous extracts of the trunk bark of *Hallea Stipulosa* Leroy and *Anthocleista vogelii* in the body with regard to carbohydrate overload. For this, 36 normal rats were divided into 9 groups of 04 rats and fasted for 12 hours. The distribution of batches for evaluation of the hypoglycemic activity of the aqueous extracts of the trunk barks of *Hallea Stipulosa* Leroy and *Anthocleista vogelii* combined is identical as follows:

Batch 1: Negative control:
- Distilled water (10ml/Kg)
Batch 2: Postive control:
- Glucose (2g/Kg)
Batch 3: Reference:
- Glibenclamide (10mg/Kg)
Batch 4: 250mg/kg of PC
- 50% +50%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 125mg/kg +AV=125mg/kg
Batch 5: 250mg/kg of PC
- 30%+70%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 75mg/kg + AV = 125mg/kg
Batch 6: 250mg/kg of PC
- 70%+30= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 125mg/kg + AV = 75mg/kg
Batch 7: 500mg/kg of PC
- 50% +50%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 250mg/kg +AV=250mg/kg
Batch 8: 500mg/kg of PC
- 30%+70%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 150mg/kg + AV = 350mg/kg
Batch 9: 500mg/kg of PC
- 70%+30= *Hallea Stipulosa* (HS) + *Anthocleista vogelii*
HS = 350mg/kg + AV = 150mg/kg

After a light puncture of the distal tip of the tail using a lancing device, blood was collected for blood glucose determination; Baseline blood glucose was determined after 12 hours of fasting before administration of the different products. Just after baseline blood glucose, glucose was administered except to the negative control groups by gavage 30 min before administering the extracts. Subsequently, blood sugar levels were successively determined at times 30 min, 60 min, 90 min, 120 min and 180 min using a glucometer and code-free brand strips.

I.2.5 Evaluation of antihyperglycemic activity

The distribution of batches for evaluation of the hypoglycemic activity of the aqueous extracts of the trunk barks of *Hallea Stipulosa* Leroy and *Anthocleista vogelii* combined is identical as follows:

Batch 1: Negative control:
- Distilled water (10ml/Kg)
Batch 2: Postive control:
- Glucose (2g/Kg)
Batch 3: Reference:
- Glibenclamide (10mg/Kg)
Batch 4: 250mg/kg of PC
- 50% +50%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 125mg/kg +AV=125mg/kg
Batch 5: 250mg/kg of PC
- 30%+70%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 75mg/kg + AV = 125mg/kg
Batch 6: 250mg/kg of PC
- 70%+30= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 125mg/kg + AV = 75mg/kg
Batch 7: 500mg/kg of PC
- 50% +50%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 250mg/kg +AV=250mg/kg
Batch 8: 500mg/kg of PC
- 30%+70%= *Hallea Stipulosa* (HS) + *Anthocleista vogelii* (AV)
HS = 150mg/kg + AV = 350mg/kg
Batch 9: 500mg/kg of PC
- 70%+30= *Hallea Stipulosa* (HS) + *Anthocleista vogelii*
HS = 350mg/kg + AV = 150mg/kg

After a light puncture of the distal tip of the tail using a lancing device, blood was collected for blood glucose determination; Baseline blood glucose was determined after 12 hours of fasting before administration of the different products. Just after baseline blood glucose, the extracts were administered by gavage 30 min before glucose administration except for the negative control groups. Subsequently, blood sugar levels were successively determined at times 30 min, 60 min, 90 min, 120 min and 180 min using a glucometer and SDcode-free brand strips.

II. RESULTS

II.1 Qualitative Phytochemical

The reactions were positive to alkaloids, antocyanes, anthraquinones, polyphenols, steroids, saponosides, flavonoids, tannins in the aqueous extracts of *Hallea Stipulosa* and *Anthocleista vogelii*. Reactions to coumarins were negative in aqueous extracts of *Hallea Stipulosa* and *Anthocleista vogelii* (Table I).

Table I: Qualitative composition of the extracts in some metabolites

Secondary metabolites	**Coloring or precipitation**	***Hallea Stipulosa* aqueous extract**	***Anthocleista vogelii* aqueous extract**
Alkaloids	Yellowish white precipitate	+	+
Antocyanin	Purplish blue color	+	+
Anthraquinone	More or less red coloring	+	+
Coumarins	Violet fluorescence	-	-
Polyphenols	Blackish coloring	+	+
Steroids	Brownish red ring	+	+
Saponosides	Presence of foam	+	+
Flavonoids	Red/orange coloring	+	+
Gallic tannins (FeCL3)	Blackish blue	+	+
Catechic tannins HCL	Soluble red precipitate	+	+

II.2 Qualitative analysis: Metabolites identified by HPTLC

II.2.1 Detection of sterols and triterpenes

Figure 1 shows the chromatographic profile of sterols and triterpenes of the study extracts. Analysis of this figure shows that the Liberman-Buchard reagent has highlighted sterols and triterpenes that appear as purple, blue, pink, gray, green, red, and yellow spots on the various chromatographic plates. The extract of the bark of *H. stipulosa* has 7 flourescent bands and *A. vogelii* has 5 bands. These bands represent the identification compounds present in each extract when observed under UV light at 366nm wavelength.

Figure 1 HPTLC sterol and triterpene detection chromatogram
***Elution Solvent**: hexane/acétate d'éthyle (20: 4, v/v)*
***Revealing**: Liebermann Buchard*
Absorbance wavelength: 366nm

II.2.2 Flavonoid detection

Figure 2 shows the chromatographic fingerprint of the flavonoids in the extracts. The chromatogram revealed blue, green, yellow, yellow, orange, greenish and fluorescent dots in the extract of the bark of *H. stipulosa* and *A. vogelii*. Flavonoids are known to interact with Nue's reagent to generate brilliantly coloured complexes that glow under UV light at a wavelength of 366nm. Yellow spots and greenish spots indicate the presence of flavonols. In addition, the presence of flavonones and aurones has been indicated by the presence of green spots. It is noticeable that all the extracts contain flavonoids. The extract of the bark of *H. stipulosa* has 11-12 bands and *A. vogelii* has 9 bands. Green spots were observed on this samples indicating the presence of aurones and flavones in these extracts.

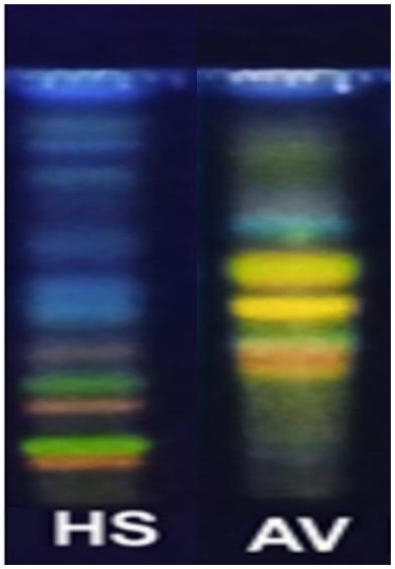

Figure 2: HPTLC flavonoid detection chromatogram
Elution Solvent: *Acétate d'éthyle/acide formique/acide acétique/eau (100 :11 :11 :26, v/v/v/v)*
Revealing: *Réactif de Neu*
Absorbance wavelength*: 366nm*

II.2.3 Alkaloids detection

Figure 3 shows the chromatographic fingerprint of the alkaloids present in the extracts. The identification of alkaloids in the extracts was carried out in the different samples of the study, the spraying of the plates with the Dragendorff reagent revealed the alkaloids that appear orange under visible light. According to the chromatogram, we can see that the concentration of alkaloids is very low. No blemishes were observed on the extracts. These spots appear at low intensities under UV light with a wavelength of 366nm.

Figure 2 HPTLC alkaloid detection chromatogram
Elution Solvent*: Ethyl Acetate/Diethylamide/Toluene (70:10:20, v/v/v)*
Developer*: Dragendorff*
Absorbance wavelength*: 366nm*

II.2.4 Tannin detection

Figure 4 shows the chromatographic fingerprints of the tannins in the extracts. Vaporization with a 2% iron (III) chloride solution revealed some spots under 366nm wavelength UV light. The AI, AV and HS extracts showed low-intensity patches. No blemishes were observed on the extracts.

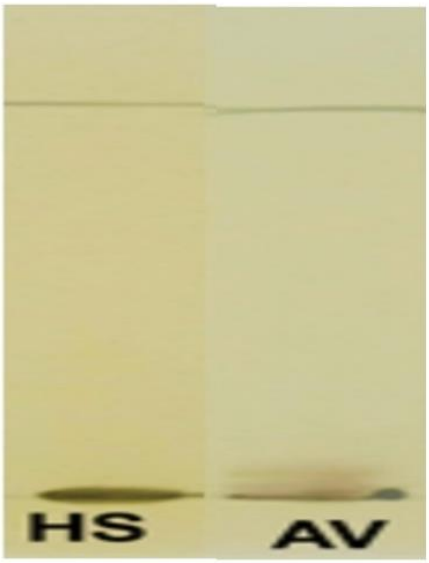

Figure 4: HPTLC tanins detection chromatography
***Elution Solvent**: Chloroform/Ethyl Acetate/Methanol/Water (6:18:2.4:2.4, v/v/v/v)*
***Revealing**: Iron Trichloride (2%)*
***Absorbance wavelength:** 366nm*

II.3 Results of the determination of total phenolic compounds (TPCs) and total flavonoids **(TFTs)**

Table XI shows the determination of total phenolic compounds (TPCs) and total flavonoids (TFTs) in the different samples. The values are expressed in milligrams of gallic acid equivalent per gram of extract and milligrams of quercetin equivalent per gram of extract respectively. The extracts of *H. stipulosa* and *A. vogelii* which have a TPC of 95.78 ± 0.02 and 220.93 ± 0.03 mg EAG/g respectively.

In addition, the TFTs of *H. stipulosa* and *A. vogelii* are relatively high of, 62.93 ± 0.06 and 73.45 ± 0.05 mg EQ/g respectively.

Table II: Total phenolic content (TPC) and total flavonoids contents (TFC) results

Samples	TPC (mg EAG/g de ES)	TFT (mg EQ/g d'ES)
H. stipulosa	95.78 ± 0.02	62.93 ± 0.06
A. vogelii.EAV	169.66 ± 0.03	73.45 ± 0.05

***EQ**: quercetin equivalent, **EAG:** gallic acid equivalent, **ES:** dry matter*

II.4 Acute oral toxicity: observation of physiological parameters

The results obtained for the observation of physiological parameters after administration of the combined extract of the trunk bark of H. stipulosa and A. vogelii are shown in Table 2.

Table III: Results of the physiological parameters observed on rats of the different batches

Observed parameters	Sample batch (Distilled water 10 ml/kg)	Lot 1 (extract combined with 2000 mg/kg)	Lot 2 (extract combined with 5000 mg/kg)
Mobility	N	N	N
Aggressiveness	A	A	A
Stool condition	N	N	N
Sensitivity to pain	N	N	N
Vomit	A	A	A
Vocalization	N	N	N
Erection Pilot	A	A	A
Tail Condition	N	N	N
Vigilance	N	N	N
Death toll	00	00	00

A= Absent; N = Normal; 0 = zero.

The results obtained after 14 days of observation showed that of all the parameters observed, no anomalies were found in all batches.

II.4.1 Effect of Trunk Extracts of Hallea stipulosa and Anthocleista vogelii on Weight Growth

Rats were followed for 14 days after acute treatment. During this period, weight growth was recorded to assess the percentage change in weight over time. At the end of the experiment, the animals were necropsied and then the masses of their organs were measured.

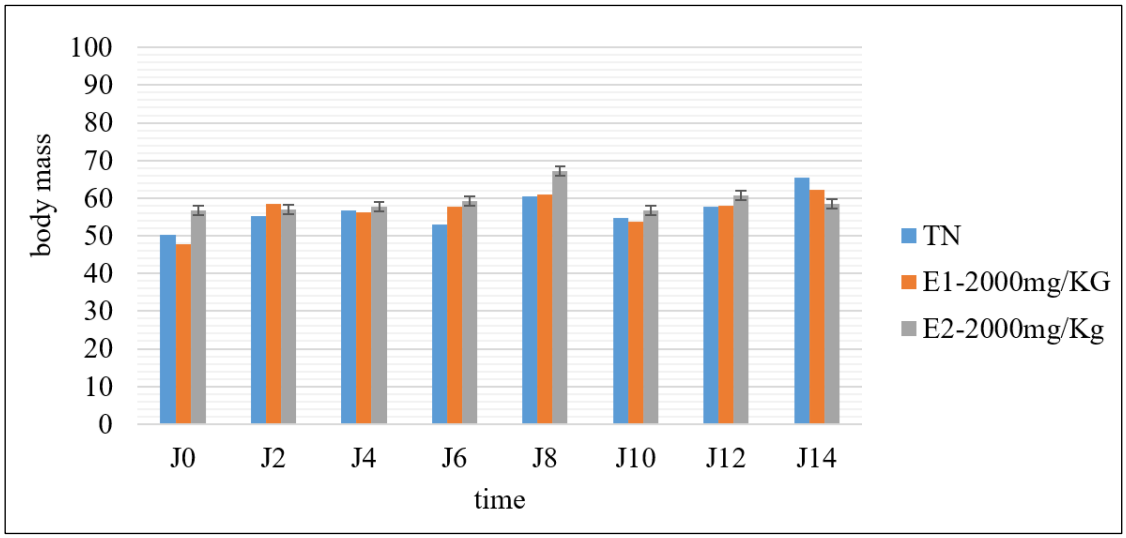

Figure 5: Change in mass as a function of time during the evolution of toxicity

TN: negative control; E1: aqueous extract from the bark of the trunk of Hallea stipulosa Leroy; E2: aqueous extract from the bark of the trunk of Anthocleista vogelii.

The *figure 5* shows the evolution of rats' body weights as a function of time. This figure shows a non-significant change ($p>0.05$) in the body weight of rats at the beginning and end of the experiment. Combined trunk extracts of *H. stipulosa* and *A. vogelii* significantly increased and did not decrease ($p>0.05$) body weight in rats, respectively.

II.4.2 Effect of Trunk Extracts of Hallea stipulosa and Anthocleista vogelii on Relative Organ Weights After Acute Oral Toxicity

He relative weight of the organs is shown in the figure below:

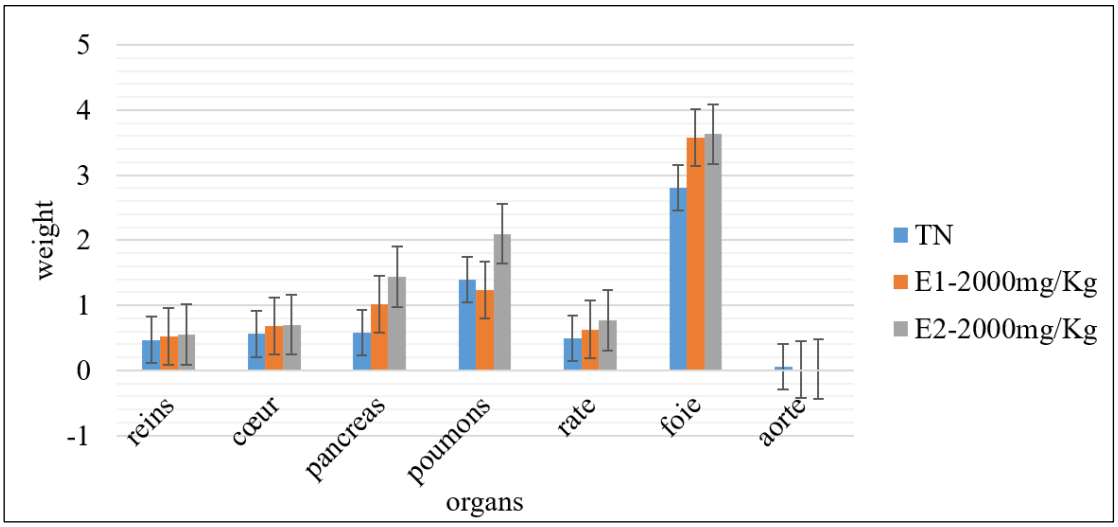

Figure 6: Organ's relative weight after acute oral toxicity test

TN: negative control; E1: aqueous extract from the bark of the trunk of Hallea stipulosa Leroy; E2: aqueous extract from the bark of the trunk of Anthocleista vogelii,

Figure 6 shows a non-significant increase ($p<0.05$) in the relative weight of the organs compared to the negative control group.

II.4.3 Determination of biochemical parameters at the end of the acute toxicity study

Although from a behavioural and physical point of view the animals showed no signs of poisoning as well as from the observation of the organs, the dosage of some biochemical parameters was evaluated in order to verify its various observations and the results obtained which made it possible to obtain the graphs below:

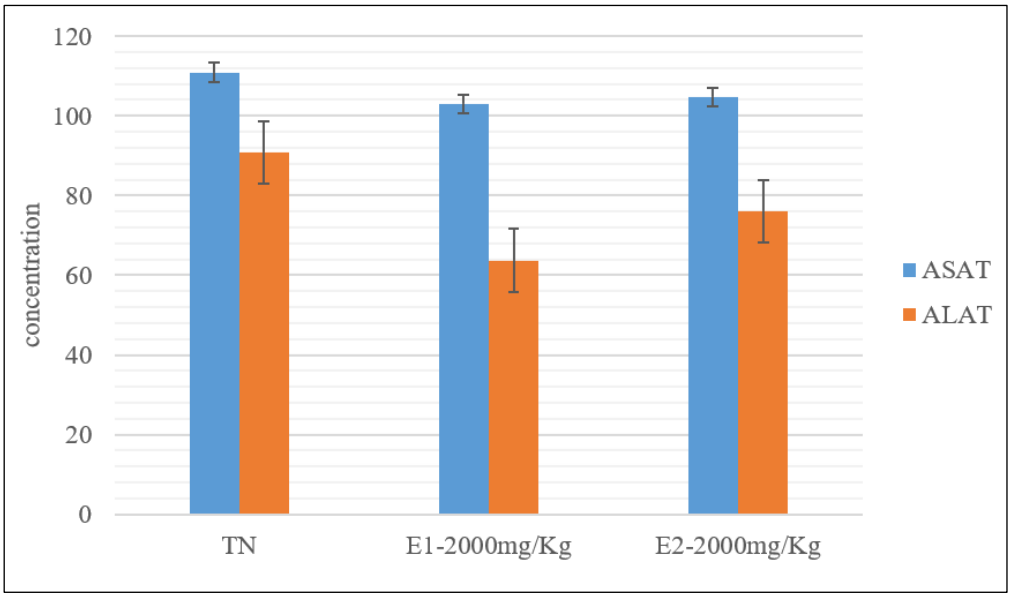

Figure 3 Serum transaminase activity of female rats after toxicity study

The figure shows that, compared to controls, there were no significant differences between the AST and ALT values of the test and control rats, as well as between the creatinine and protein.

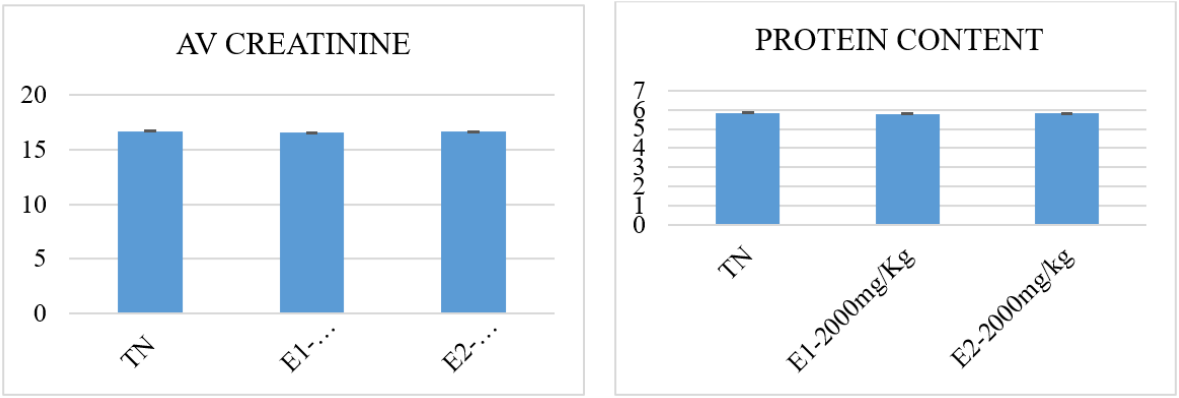

Figure 4Creatinine and serum protein levels of female rats after toxicity studies

TN: negative control; E1: aqueous extract from the bark of the trunk of Hallea stipulosa Leroy; E2: aqueous extract from the bark of the trunk of Anthocleista vogelii.

Changes in total protein and creatinine were not significant compared to the negative control rats.

II.5 Evaluation of the antihyperglycemic activity of combined aqueemic extracts from the trunk bark of *Hallea stipulosa* and *Anthocleista vogelii*:

The following figure indicates the effect of combined aqueous extracts of trunk barks of *Hallea Stipulosa* Leroy and Anthocleista vogelii on hyperglycemia induced by glucose overload in rats.

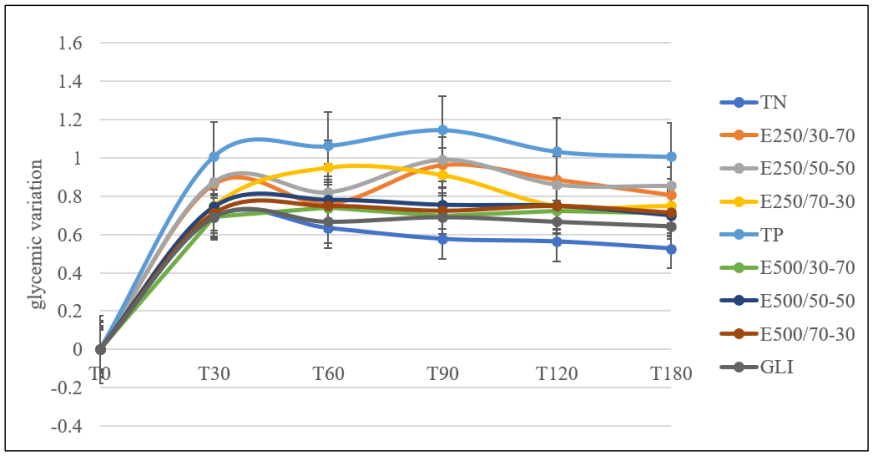

Figure 5 The antihyperglycemic activity of combined aqueous extracts from the trunk barks of *Hallea stipulosa* and *Anthocleista vogelii*

TN: negative control; TP: positive control; GLI: glibenclamide; E500/50-50: extract combined with concentration 500 mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 250mg/kg +AV=250mg/kg; E500/70-30: extract combined at concentration 500 mg/70% +30%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 350mg/kg +AV=150mg/kg; E250/50-50: extract combined at the concentration 250 mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 125mg/kg +AV=125mg/kg; E250/70-30: extract combined at concentration 250mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 125mg/kg +AV=75mg/kg; E250/30-70: combined extract at the concentration 250mg/30% +70%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 75mg/kg +AV=125mg/kg.

In general, a hyperglycemic peak was observed in all batches treated with glucose at T60 and T90 min compared to the TN batch treated with distilled water only, which had the lowest of all groups of rats. Blood glucose levels in the TP group increased significantly ($p<0.05$) compared to the glucose and extract treated batches. On the other hand, at T60, T90 and T120 min the batches treated with combined aqueous extracts of the trunk bark of *H. stipulosa* and *A. vogelii* at doses of 250 mg/kg BW and 500 mg/kg BW showed hypoglycemic activity with blood glucose levels that varied significantly ($p<0.05$) from 80.66 mg/dl to 85.66 mg/dL respectively; 79 mg/dL to 89.33mg/dL; 86.66 mg/dL to 88.66 mg/dL and 81 mg/dL to 87.66 mg/dL compared to the positive control group (122.33±0.12 mg/dL).

II.6 Evaluation of the hypoglycemic activity of combined aqueous extracts from the trunk barks of *Hallea stipulosa* and *Anthocleista vogelii*

Figure 10 shows the hypoglycemic activity of the combined aqueemic extracts of the trunk bark of *H. stipulosa* and *A. vogelii.*

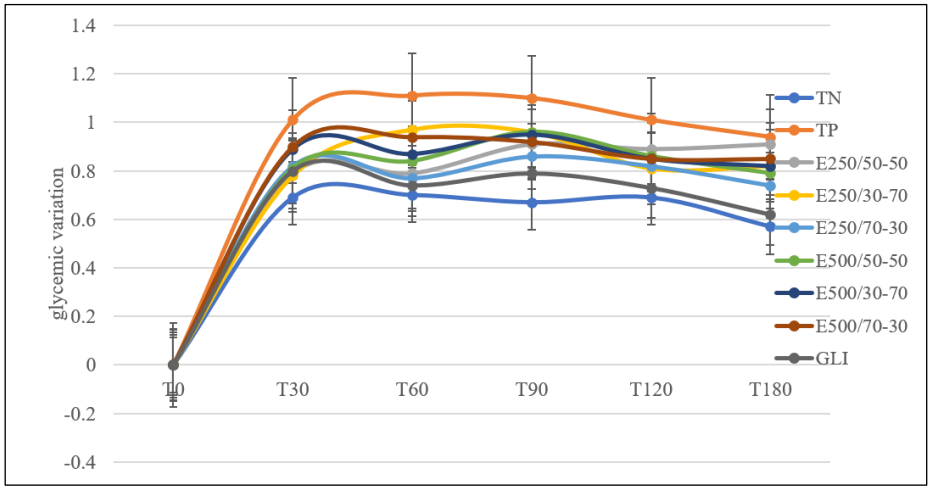

Figure 6 The hypoglycemic activity of combined aqueous extracts from the trunk barks of Hallea stipulosa and Anthocleista vogelii

TN: negative control; TP: positive control; GLI: glibenclamide; E500/50-50: extract combined with concentration 500 mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 250mg/kg +AV=250mg/kg;E500/70-30: extract combined at concentration 500 mg/70% +30%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 350mg/kg +AV=150mg/kg;E250/50-50: extract combined at the concentration 250 mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 125mg/kg +AV=125mg/kg;E250/70-30: extract combined at concentration 250mg/50% +50%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 125mg/kg +AV=75mg/kg;E250/30-70: combined extract at the concentration 250mg/30% +70%= Hallea stipulosa (HS) + Anthocleista vogelii(AV) HS = 75mg/kg +AV=125mg/kg.

The reference molecule at a concentration of 10 mg/kg BW resulted in a significant hypoglycemic effect (P<0.001) after administration up to time T180 compared to the blood glucose levels observed in controls in the positive control (PT) batch. Combined aqueous extracts of the trunk bark of *H. stipulosa* and *A. vogelii* at concentrations of 250 mg/kg BW and 500 mg/kg BW caused the significant hypoglycemic effect (P<0.05) in rats compared to positive controls. Administration of the aqueous extract combined with the concentration of 500 mg/kg BW, the combined aqueous extract caused a return to baseline blood glucose at times T120 and T180 min. The results showed that the aqueous extract, at a concentration of 500 mg/kg BW, led to a decrease in hyperglycemia from 1.23 g/L to 0.98 g/L or 20.49% compared to 1.24 g/L to 0.72 g/L or 41.93% for the reference molecule.

DISCUSSION

Phytochemical studies show that aqueous extracts from the trunk barks of *H. stipulosa* and *A. vogelii* are rich in metabolites: flavonoids, tannins, anthocyanins, saponins, terpenoids, alkaloids and phenols. The presence of these secondary metabolites is also reported by several authors such as [15]. These results suggest that these extracts have the power to inhibit α-amylase activity. Several studies have shown that polyphenols (tannins, flavonoids and anthocyanins), terpenoids and glycosides are α-amylase inhibitors and could be an alternative to the treatment of type 2 diabetes by reducing postprandial hyperglycemia [16].

The application of HPTLC provides real-time analysis for screening tools. This method confirms the presence of triterpenes and biomarker lipids such as ursolic acid, retinol, and ergosterol identified by the red spots, cholesterol by the blue-green spots, and stigmasterol which appears as an orange band. With the help of Leibermann buchard's reagent as revelatory agent, sterols and terpenes were identified in all extracts. This corroborates with studies conducted on these same plants or phytochemical screening tests were positive for these metabolites [14, 15]. Sterols and triterpenes are commonly known for their antioxidant and anti-inflammatory effects [14, 15].

Also, flavonoids are a diverse group of phytonutrients found mainly in plants, known for their antioxidant properties [18]. They have been identified by visualizing the different extracts on the chromatographic plate using Neu's reagent as revelator. The results show that all extracts exhibited flavonoids and the various subfamilies such as flavones and flavonols were present as yellow bands in the bark extracts, while anthocyanins appeared in red-purple in all extracts. All extracts presented the isoflavones in the form of a band in blue or pink colors, or yellow or orange colours [18]. In short, the evidence of flavonoids in our different samples corroborates the studies conducted on the same plants [14, 15].

Quantitative analysis of the total polyphenol contents and total flavonoids contents were done using the folin ciocalteu reagent and aluminiun trichloride test respectively. The values are expressed in milligrams of gallic acid equivalent per gram of extract and milligrams of quercetin equivalent per gram of extract respectively. The extracts of *H. stipulosa* and *A. vogelii* which have a TPC of 95.78 ± 0.02 and 220.93 ± 0.03 mg EAG/g respectively. The TFTs of *H. stipulosa* and *A. vogelii* are relatively high of, 62.93 ± 0.06 and 73.45 ± 0.05 mg EQ/g respectively. These results corroborates with those of [24, 25] who studied the quantitative and qualitatives analysis of these plants.

According to the 2008 OECD protocol, a substance is considered not to be acutely toxic if it does not cause death or specific signs of poisoning two weeks after it has been administered at a single dose of 2000 mg/kg bw. After administration of such a dose of aqueous extracts from the trunk barks of *H. stipulosa* and *A. vogelii*, no deaths were recorded, the values of AST, ALT, total protein and creatinine were not increased, the values of AST, ALT, Total Protein and creatinine were not significantly different compared to the control group which indicates the condition of the heart, liver, muscles and kidneys was not significantly degraded and no particular signs of poisoning on either the animals or their organs were noted. Non-insulin treatment of diabetes uses sulfonamide hypoglycemic agents, biguanides and alpha-glucosidase inhibitors. The effect of sulfonamides, to which glibenclamides belongs, is well known [17].

Glibenclamide exerts a hypoglycemic effect, in agreement with the results of Rydberg and *coll.* (1994) [26]. The binding of glibenclamide to its membrane receptor promotes the entry of glucose into the cell, thus resulting in the accumulation of glucose in the blood, which explains the observed reduction in hyperglycemia. Glibenclamide induces a significant glucose-lowering effect 1 hour after administration in glucose-overloaded rats. However, the observed value remains higher than the normal blood glucose value after 4 hours of experimentation. These observations support the results of some authors (Luzi and Pozzi, 1997) [27] who have demonstrated that the plasma half-life of glibenclamide is between 5 and 6 hours after acute administration.

The aqueous extract combined with concentrations of 250 mg/kg BW and 500 mg/Kg BW caused a significant hypoglycemic effect. The extract, in high concentrations, is said to have sustained hypoglycemic activity. These results corroborate those obtained by Ateufack and coll., 2006 [28] who showed in their study that the administration of the ethanolic extract of *Anthocleista* roots at doses ranging from 125, 250 and 500 mg/kg BW leads to a significant decrease in alloxane-induced hyperglycemia. In view of these results, it could be stated that the hypoglycemic activity

of the extracts is related to the choice of extraction solvent and the plant organ used.

Therefore, at a concentration of 250 mg/kg BW, the combined aqueous extract of the bark of *Anthocleista vogelii* and *H. stipulosa* would not have a significant hpoglycemic effect in normoglycemic rats.

However, at 500 mg/kg BW, the combined aqueous extract caused a return to baseline blood glucose levels in rats with hyperglycemia at T120 and T180 min. These results suggest that at high doses, bark extract leads to a normalization of induced hyperglycemia. Thus, the reduction in hyperglycemia observed in rats treated with the high concentration of the extract could be explained by a stimulation of insulin secretion by the pancreas [28] and/or, probably by an increase in peripheral glucose utilization in the presence of the extract [29].

CONCLUSION

At the end of our study, which consisted in studying the hypoglycemic and antihyperglycemic activity of the aqueous extract of the trunk bark of *Hallea stipulosa* Leroy. and *Anthocleista vogelii*, it appears that the phytochemical screening of these aqueous extracts from the trunk bark of *Hallea stipulosa* and *Anthocleista vogelii* revealed the presence of secondary metabolites with anti-diabetic properties, in particular tannins, flavonoids, saponins, anthocyanins, alkaloids, phenols. Trunk bark extracts of *Hallea stipulosa* and *Anthocleista vogelii* showed no evidence of toxicity at 2000 mg/kg BW. Combined aqueous extracts of the trunk bark of *Hallea stipulosa* and *Anthocleista vogelii* exert antihyperglycaemic and hypoglycemic activity at concentrations of 250 mg/kg BW and 500 mg/kg BW. Thus, we can say that the combined extracts studied optimized their antihyperglycemic and hypoglycemic activities through the important phenolic compounds due to their combination.

III.1 Additional Information

III.1.1 Funding

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III.1.2. Disclosure statemen

No potential conflict of interest was reported by the authors

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