Heliyon 9 (2023) e21841

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Antihypertensive potential of the stem bark of *Canarium schweinfurthii* Engl. (Burseraceae) in wistar rats: UPLC-ESI-QToF-MS/MS-based prediction of antihypertensive phytochemicals

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ARTICLE INFO

Keywords:
Canarium schweinfurthii
Ethanolic extract
antihypertensive activity
Wistar rats
Sodium chloride
UPLC-MS/MS
GNPS
SIRIUS5

ABSTRACT

Ethnopharmacological relevance: Canarium schweinfurthii, also called ''Elemierd'Afrique'', is used in Cameroonian folk medicine (bark decoction) to treat patients suffering from hypertension.
Aim of the study: This study aimed at evaluating the antihypertensive activities of the stem bark of *Canarium schweinfurthii* and identifying potential compounds present in its extract that may support or oppose its ethnomedicinial use.
Materials and methods: Stem bark extract of *Canarium schweinfurthii* was prepared by maceration using 70 % ethanol followed by redissolution in methanol and hyphenated. Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) analysis for the detection and characterisation of secondary metabolites. Antihypertensive effects were assessed in Wistar rats after induction of hypertension with sodium chloride (NaCl) 18 % at a dose of 0.01mL/$g_{body\ weight}$ once a day for four weeks.Hemodynamic parameters were measured weekly by anon-invasive method using the CODA system.
Results: The ethanolic bark extract of *C. schweinfurthii* significantly inhibited the increase of blood pressure with a maximum of 23.18 % (systolic pressure, $p < 0.0001$), 24.77 % (diastolic pressure, $p < 0.001$) and 22.95 % (mean pressure, $p < 0.0001$) at a dose of 200 mg/$kg_{body\ weight}$ at the 4th

Abbreviations: UPLC-MS/MS, QToF, Quadrupole Time of Flight; ESI, electrospray ionization; SBP, systolic blood pressure; DBP, diastolic blood pressure; FA, formic acid; SEM, Standard Error on the Mean; W, week; C S, *Canarium schweinfurthii*; NaCl, sodium chloride; MeCN, acetonitrile; RBC, red blood cell; MCV, mean corpuscular volume; Ht, hematocrit level; Hb, Haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin content; WBC, white blood cell; LYM, lymphocyte count; PLT, platelet count; NHC, National Herbarium of Cameroun.

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https://doi.org/10.1016/j.heliyon.2023.e21841
Received 8 August 2023; Received in revised form 28 October 2023; Accepted 30 October 2023
Available online 4 November 2023

week, compared to agroup of Wistar rats that received only NaCl (negative control). Similarly, the extract significantly inhibited the increase in heart rate by 18.84 % ($p < 0.001$) at 200 $mg/kg_{body\ weight}$ at week four. Hematological parameters did not differ significantly between the extract-treated and control groups. The UPLC-MS/MS spectrometric analysis provided evidence for the presence of several C30 terpenoids containing three or five oxygen atoms and exhibiting pentacyclic triterpenoid structures, as well as C29 terpenoids and related compounds containing nitrogen in addition to oxygen, using spectral matching, and *in silico* molecular formula and structure prediction. Additionally, two features were annotated with high-confidence as lignans, structurally closely related to hinokinin and dehydrocubebin through MS/MS-based *in silico* structure prediction using CSI: Finger ID in SIRIUS5. The lignans have been previously reported from stem bark of plants belonging to the Burseraceae family. *Conclusion*: The ethanolic stem bark extract of *C. schweinfurthii* demonstrated antihypertensive properties on the tested Wistar rats. These results support the ethnopharmacological use of *C. schweinfurthii* concoctions for the treatment of hypertension and suggest a protective effect against salt damage, hypothetically by the up regulation of antioxidative enzymes and/or lipids, mitigatings membrane peroxidation.

1. Introduction

High blood pressure or hypertension, defined as a permanent elevation of systemic blood pressure, is a major risk factor of heart disease and stroke and remains a central public health concern worldwide. Hypertension is usually diagnosed in adults when the systolic blood pressure (SBP) at resting state is $\geq$ 140 mmHg and/or the diastolic blood pressure (DBP) $\geq$ 90 mmHg [1]. Worldwide, 31.1 % of people are hypertensive and 75 % of hypertensive people residein low-to middle-income countries [2]. Approximately 16.2 % of the population, or 74.7 million individuals in sub-Saharan Africa are estimated to have hypertension [3] and high blood pressure is endemic in over 30 % of the Cameroonian population [4]. The current treatment for hypertension is essentially based on the use of synthetic drugs, but because of the prolonged duration of treatment and the associated high financial costs as well as scepticism towards synthetic drugs in the African context, old and new solutions are favourably oriented towards the use of medicinal plants [5].

Ancient wisdom encoded in natural products has inspired approximately 60 % of the pharmaceutical drugs available in pharmacies currently [6]. Despite the vast potential, only a fraction of plants i.e. roughly 6–7 %, have undergone thorough biological evaluation, and merely 15 % have been subjected to phytochemical analysis [7]. Nevertheless, in our modern era, two-thirds of the global population still relies on the healing properties of plant-based remedies [6]. These statistics highlight both the untapped possibilities and the enduring significance of harnessing the therapeutic potential found in the plant kingdom. It is also known that many phytochemicals exhibit important antihypertensive effects. For example, alkaloids are reported to be diuretic and to reduce oxidative stress, lower blood pressure by affecting salt and water transport in the renal tubules, as well as through the inhibition of lipid peroxidation and microsomal protein oxidation. Coumarins contribute to blood fluidity, facilitating blood circulation and lower end arterial pressure via their venotonic, vasoprotective and vasodilator properties. Polyphenols and flavonoids exhibit antioxidant properties and contribute to the regulation of stress responses [8–10].

To contribute to the wide endeavour of investigating the utility of ethnopharmaceutical treatments, we chose to investigate the stem bark of *Canarium schweinfurthii* Engl., also known under the vernacular names "hehe" (Bassa), "mbeu" (Bamileke) and "abel" (Béti). *C. schweinfurthii* was chosen due to the wide-spread use of decoctions from its stem bark in traditional medicine to treat hypertension [11,12] and the paucity of reports on *C. schweinfurthii* antihypertensive activities. However, results fromphytochemical analyses, as well as anticancer, antihelminthic, antimalarial, nephroprotective, analgesic, antidiabetic, antibacterial and antioxidant assays of *C. schweinfurthii* extracts have been contributed to the scientific literature by various authors [12]. Furthermore, our recent work on the acute, subacute and subchronic toxicity of the same extract have shown encouraging results [13,14].

2. Material and methods

2.1. Plant materials

The stem bark of *Canarium schweinfurthii* Engl. used in this study was collected from Bamendjou village, Hauts-Plateaux Subregion, West region, Cameroon (latitude: 5023′23″ North; longitude 10019′48″ East; altitude above sea level: 1604 m) and identified by a botanistat the National Herbarium of Cameroon. A specimen of the plant was registered under the voucher number 40804/HNC.

2.2. Extraction

The plant material was dried and ground before a sample of 3.5 kg of the resulted powder of *C.schweinfurthii*stem bark was macerated in ethanol (7 L, 70 %) for 72 h at 25 °C. After filtration of the mixtureusing cotton and filter paper Wattman No 1, the filtrate was concentrated under reduced pressure (40 °C) using a rotary evaporator. The starting material was re-extracted two times and the resulting extract material was combined for further analysis.

2.3. UPLC-HR-ESI-MS/MS analysis of Canariumschweinfurthii ethanolic extract

Chromatographic separation of the ethanolic extract was carried out on a Dionex UPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA). A Fortis C18 column (2.1 × 100 mm, 3 μm; Fortis Technologies Ltd, Cheshire, UK) maintained at 40 °C was connected to a guard column (5 mm × 2.1 mm, 3 μm) with the same stationary phase and experiments were conducted at a flow rate of 0.300 mL/min. The mobile phase was a mixture of water and acetonitrile (MeCN), each adjusted with 0.1 % formic acid (FA). A multistep gradient condition with constant ramping between segments was executed as follows: 0–5 min (H_2O–MeCN–FA, 95:5:0.1, *v/v/v*), 10–15 min (H_2O–MeCN–FA, 85:15:0.1, *v/v/v*), 20–25 min (H_2O–MeCN–FA, 75:25:0.1, *v/v/v*), 30–35 min (H_2O–MeCN–FA, 65:35:0.1, *v/v/v*), 40–45 min (H_2O–MeCN–FA, 45:55:0.1, *v/v/v*). The auto-sampler vial compartment was kept at 40 °C throughout the analysis and a sample volume of 10 μL was injected, with the sample having been prepared at a final concentration of 1 mg/mL in methanol. The chromatograph was hyphenated to a Bruker Compact Quadrupole Time of Flight (QToF, Bruker, Bremen, Germany) high-resolution tandem mass spectrometer (HR-MS/MS) and data were recorded using an electrospray ionization (ESI) probe, operated in positive mode. The ESI conditions were set with an end plate offset of 500 V, a nebulizer pressure of 3.0 bar, a dry gas flow rate of 9.0 L/min, and a dry temperature of 220 °C. The capillary voltage was set to 4.5 kV. Nitrogen was used as both nebulizer and dry gas. A data-dependent acquisition method including the recording of fragmentation spectra (product ion scan) of the 10 most intense precursors per scan was used, with collision energy of 40 eV to induce dissociation. The mass range for the acquisitions was set to 50–2000 *m/z*. Raw data was converted to mzXML format and processed in MZmine3, followed by a feature-based molecular networking workflow and spectral matching using the GNPS online tool (gnps.ucsd.edu), as well as*in silico* structure and compound class predictions using CSI:FingerID [15] and CANOPUS [16], respectively, within the SIRIUS5 suite [17] (detailed description of parameters in S.1).

2.4. Antihypertensive activity

2.4.1. Reagents

Mobile phase reagents were procured from Merck Millipore (Johannesburg, South Africa) and formic acid was obtained from Sigma-Aldrich (Johannesburg, South Africa), NaCl (Camphardis, Cameroon) and captopril (Denk Pharma, Germany).

2.4.2. Experimental animals

Wistar female rats aged 10–12 weeks, weighing between 150 and 200 g were used to assess the antihypertensive activity of the samples. The animals were housed in a colony cage in the animal house of the University of Douala, Cameroon, under normal conditions: 12 h of light/dark cycleat 23–25 °C. They were fed with a standard commercial diet and tap water *ad libitum*.

2.4.3. Study of antihypertensive effect

The animals first underwent a five day acclimazation period, which consisted of placing them in CODA restraining cages for three to four sessions of 10–15 min each. The antihypertensive effect was evaluated in rats by the model of induction of hypertension by sodium chloride (NaCl) 18 % at the daily dose of 10 mL/$kg_{body\ weight}$ for four weeks. After NaCl-induced hypertension, animals were screened to exclude those with blood pressure over 140/90 mmHg. Thereafter, 25 female rats were randomly divided into fivegroups of five rats each. The first group (normotensive group or neutral control) received distilled water (10 mL/$kg_{body\ weight}$). The second group (hypertensive group or negative control) received NaCl (18 %) solution. The third and fourth groups (essays groups) received NaCl solution concomitantly with extract of *C. schweinfurthii* at the doses of 200 mg/$kg_{body\ weight}$ and 400 mg/$kg_{body\ weight}$, respectively [18]. The animals received the various solutions orally every day for 4 weeks, in repeated daily doses. Animals on NaCl overload were given to drink a 1 % NaCl solution, while the neutral control animals received tap water. The choice of these two doses of 200 and 400 mg/$kg_{body\ weight}$ in this study was due to the fact that study of the *in vivo* subchronic toxicity of ethanolic extract from the stem bark of *C. schweinfurthii* in wistar rats at doses of 200, 400 and 800 mg/$kg_{body\ weight}$ showed onset of liver toxicity at the highest dose [14]. The fifth group (positive control group) received NaCl solution concomitantly with captopril (20 mg/kg), an anithypertensive reference drug. All products were dissolved in distilled water and administered daily by oral routeto the rats for fourweeks. Blood pressure and heart rate measurements were performed twice a week by a non-invasive method using the CODA system (Kent Scientific Co., USA).

2.4.4. Blood sampling

After the final treatment, the test animals were euthanized by decapitation. Blood samples were collected in dry tubes, and centrifuged at 3000 rpm for 15 min. The resulting serums were stored at −20 °C. The Na^+, K^+, Ca^{2+} ions analysis assay was performed using the method described by Odie et al., 2011 [19].

Na^+: This method involves an exchange of electrons between an electrode and an electroactive substance in solution. The plate consists of two selective electrodes, each containing methylmonensine (a sodium ionophore), a reference layer (silver chloride) and a silver layer and silver chloride covering a polyester support. After applying a drop of sample of the rat to be determined on one side of the plate and a drop of reference liquid on the other side, a migration of the two liquids towards the center of the paper bridge was observed. A stable junction of liquids was formed, connecting the reference electrode to the indicator electrode of the sample and the results were expressed in mmol/L.

K^+: The potassium ions were assayed using the enzymatic method with the ready-to-use BIOLABO reagent. The potassium ion was measured by a coupled kinetic system using pyruvate kinase which is potassium dependent. The generated pyruvate was converted and NADH was converted to NAD^+ and H^+. The decrease in absorbance measured at 380 nm was proportional to the concentration of

potassium in the serum. The results were expressed in mmol/L.

Ca^{2+}: Arsénazo III (AIII) is a metallic pigment dye [20] was used for the determination of calcium in biological samples [21]. The method is based on the variation in absorption at the 650 nm wavelength of the complex formed by the specific bond of Arsenazo III with calcium in an acidic medium (pH = 6.5). The intensity of the chromophore formed was proportional to the total calcium concentration of the sample [22]. The samples, a volume of 150 μL of serum were placed in the cups and were placed in order in the tanks at the level of the PLC (Biosystem A 15). Calcium results were collected after programming PLC system parameters and serum calcium concentrations expressed in mg/dL.

2.5. Exploration of results and statistical analysis

Data were recorded, and then expressed as Mean ± Standard Error on the Mean (SEM). Statistical analysis was done using GraphpadInstat version 8.0.1. The analysis of the blood pressure was performed with "one-way" ANOVA followed by the TUKEY post-test. The "two-way withrepeated measures" ANOVA test followed by the DUNNETT post-test was used for the treatment of data on hemodynamic parameters. The differences were considered significant for $p < 0.05$ (moderate), $p < 0.01$ (good) and $p < 0.001$ (strong).

3. Results

3.1. Extraction efficiency

The yield of the ethanolic extract of *C. schweinfurthii* stem bark at the different stages was 60.5 % after drying; 97.22 % after grinding and 2.29 % after maceration (Table 1).

3.2. UPLC-MS/MS analysis of C. schweinfurthii ethanolic extract

Metabolite profiling of ethanolic extract of *C. schweinfurthii* stem bark was performed through reversed-phase liquid chromatography tandem mass spectrometry in positive ESI ionization mode. The raw data was converted to mzXML format and preprocessed using MZmine3 (ver.3.4.14) [23] followed by analysis through feature-based molecular networking [24,25] using the spectral matching online utility GNPS, in addition to structural and compound class predicitons using SIRIUS5 [15–17,26]. The molecular networking output from GNPS was visualized in Cytoscape (ver.3.9.1) [27] and enriched with the compound class predictions calculated with SIRIUS5 (ver.5.6.3), the molecular structures of potential spectral matches and *in silico* structural predictions from SIRIUS5 (Fig. 1 **A**). The compound class predictions are summarised in Fig. 1 **B**, which depicts a comparison of the number of features belonging to each particular predicted compound class. Spectral matching through GNPS led to the putative annotations of various terpenoids including sumaresinolic acid, oleanolic acid (previously reported from *C. schweinfurthii*) and glycyrrhetinic acid, while *in silico* structure prediction additionally provided indications for the presence of prostaglandins and steroids. Importantly, for terpenoids the structures annotated based on spectral matching are often only of limited reliability since the fragmentation spectra of terpenoids can be highly similar due to their structural similarity and expanisve isomerism. Therefore, these annotations should be interpreted as an indication of the presence of the broader compound class (summary of annotation parameters given in S.2). Triterpenoids of similar structures to those predicted here, have been previously isolated from *C. schweinfurthii* resin [28] and shown moderate inhibitory activity against the human leukemia cell line HL-60 (TB). Spectral matching through GNPS furthermore provided an indication for the presence of deoxybenzoine, while CSI: Finger ID led to high confidence *in silico* structure predictions indicating the presence of lignans, possibly corresponding to hinokinin and dihydrocubebin. Hinokinin was found to be a major constituent in the bark extract of *Bursera simaruba* (family Burseraceae) [29]. This compound has been shown to exhibit anti-inflammatory, anti-trypanosomal, anti-microbial, anti-viral and cytotoxic effects [30] and we are not aware of reports of this natural product from *C. schweinfurthii*. Dihydrocubebin has to our knowledge not been reported from *C. schweinfurthii*, nor the family Burseraceae, however it has previously been isolated from the leaves of *Piper gunineense* Schum. (Piperaceae) [31], the fruit of *Piper cubeba* [32], as well as the stem bark of *Ixora cibdela* Craib (Rubiaceae) [33]. This compound has been shown to exhibit antihelmintic activity [32]. Several putative alkaloids have also been detected, with the CSI:FingerID predictions for two features suggesting the presence of peptide related compounds including leupeptin and an aminolipid and a spectral match suggesting the presence of a parbenate related alkaloid. In addition, three putative alkaloid features were detected which may show structural similarities to prostaglandins.

Table 1
Extraction yield of the ethanolic extract of *C. schweinfurthii* stem bark.

Step	Initial mass (g)	Final mass (g)	Yield (%)
Drying	11900	7200	60.5
Grinding	7200	7000	97.22
Maceration	3500	80	2.29

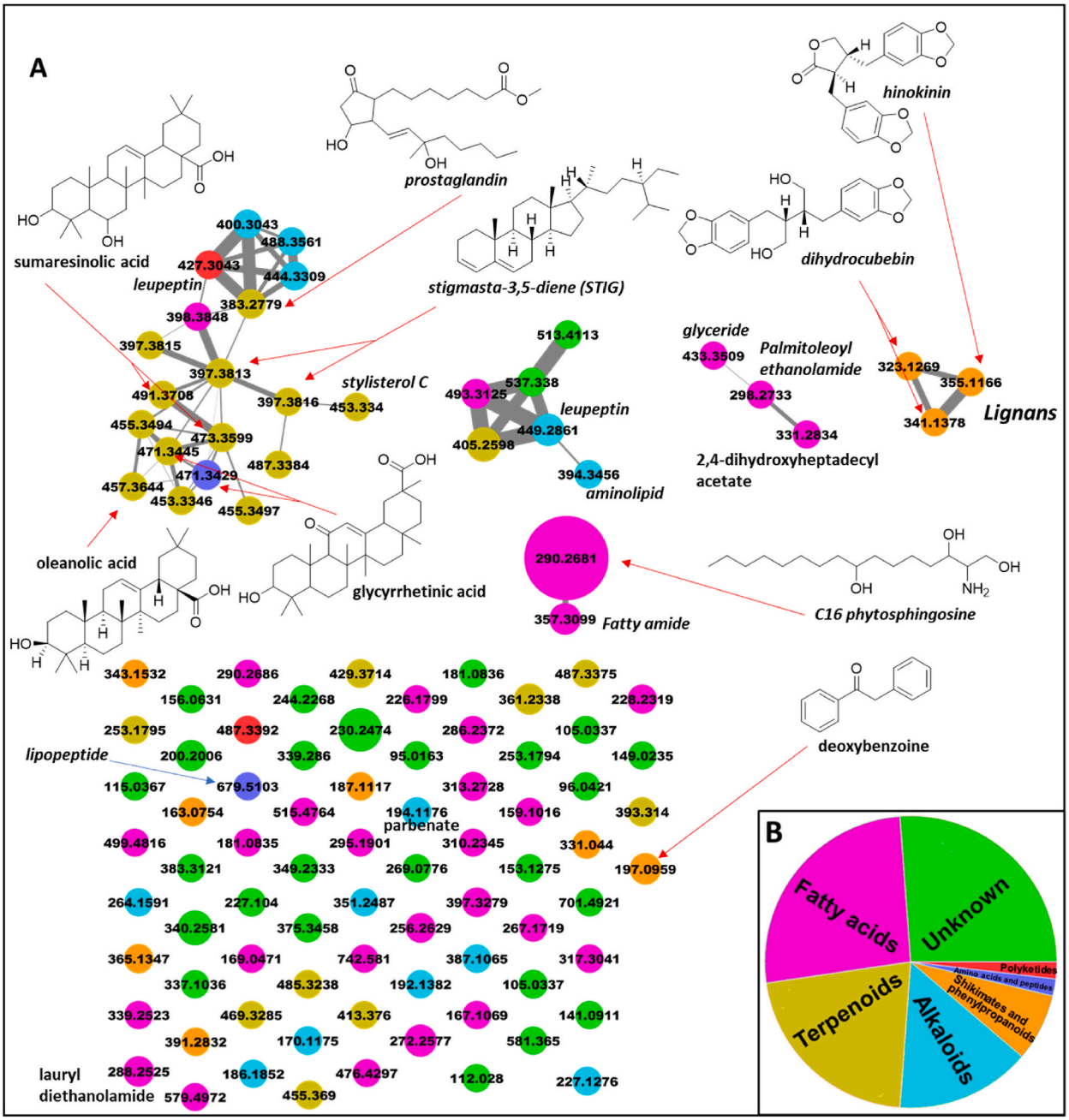

Fig. 1. Annotated molecular network (**A**) and *in silico* compound class predictions (**B**). Molecular structures indicate putative annotations with the names of those derived from spectral matches printed in normal font and of *in silico* structure predictions in *italics*. Compound class predictions are indicated by node color, with violet denoting fatty acids, yellow terpenoids, turquoise alkaloids, orange shikimates and phenylpropanoids, blue amino acids and peptides and red polyketides. A comparison of the number of features predicted through SIRIUS5 to belong to each compound class, is shown in **B**. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Antihypertensive activity

3.3.1. Effect of the ethanolic extract of Canarium schweinfurthii on systolic blood pressure

Co-administration of ethanol extract of *C. schweinfurthii* at doses of 200 and 400 mg/kg and NaCl solution (Fig. 2) to Wistar rats, significantly prevented the increase in systolic blood pressure (SBP) from the 3rd week of treatment ($p < 0.0001$; $p < 0.0001$) compared to animals given only NaCl. This inhibition was maximal at week four with values of 117 and 116 mmHg at doses of 200 mg/kg (22.51 %) and 400 mg/kg (23.18 %), respectively, compared to the value obtained in hypertensive controls which was 151 mmHg.

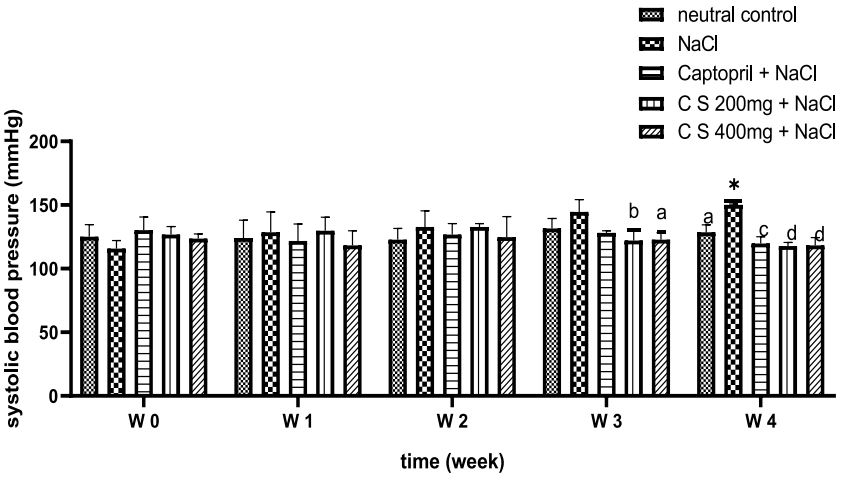

Fig. 2. Effect of different doses of *C. schweinfurthii* extract on systolic blood pressure during four weeks. Each bar represents the mean value ± S.E.M; n = 5; $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; $^{d}p < 0.0001$ significant difference when compared to hypertensive group; *$p < 0.05$ significant difference when compared to normotensive group. W0-4: week 0–4; C S: ethanolic extract of *C. schweinfurthii*stem bark.

Similarly, concomitant administration of the reference product, captopril, and NaCl solution also significantly inhibited the increase in systolic blood pressure from the 3rd to the 4th treatment compared to the hupertensive rats ($p < 0.001$). The maximum of inhibition was 20.16 % (four weeks).

3.3.2. *Effect of the ethanolic extract of Canarium schweinfurthii on diastolic blood pressure*

As presented in Fig. 3, the diastolic blood pressure was significantly elevated from the 3rd week of treatment in hypertensive rats compared to the normotensive control ($p < 0.05$). This elevation amounted to 109 mmHg in hypertensive, compared to 88 mmHg in normotensive rats at the fourth week with the maximum increase of 23.86 %. Concomitant administration of ethanolic extract of *C. schweinfurthii* at doses of 200 and 400 $mg/kg_{body\ weight}$ and NaCl solution, significantly inhibited the increase in diastolic blood pressure (PAD) from the 3rd week of treatment ($p < 0.01$; $p < 0.05$). This inhibition was maximal at week four with values of 82 mmHg (200 mg/kg) and 86 mmHg (400 mg/kg) compared to the value of the hypertensive rats which was 109 mmHg. The percentage of inhibition was 24.77 % and 21.10 %, respectively. Captopril also significantly inhibited the increase in diastolic blood pressure from the 3rd to the 4th week of treatment compared to the hypertensives groups with values of 86 mmHg versus 109 mmHg for the negative control ($p < 0.05$). The percentage of inhibition was 21.10 %.

3.3.3. *Effect of the ethanolic extract of Canarium schweinfurthii on mean blood pressure*

Oral administration of NaCl solution alone for four weeks elicited an increase of the *mean blood pressure* (PBM) compared to the normotensive rats (Fig. 4). This significant elevation of 23.23 % was observed at the 4th week of evaluation ($p < 0.05$). Concomitant administration of sample extract and NaCl solution significantly mitigated the increase in mean blood pressure from the 3rd week with rates of diminution of 22.95 % (94 mmHg) and 21.31 % (96 mmHg) at doses of 200 and 400 $mg/kg_{body\ weight}$ of the sample extract at the 4th week of treatment ($p < 0.0001$; $p < 0.001$). The maximum of inhibition was obseved at the 4th weekat a dose of 200 mg/kg compared to the hypertensive group.Administration of captopril + NaCl significantly inhibited the increase in mean blood pressure of

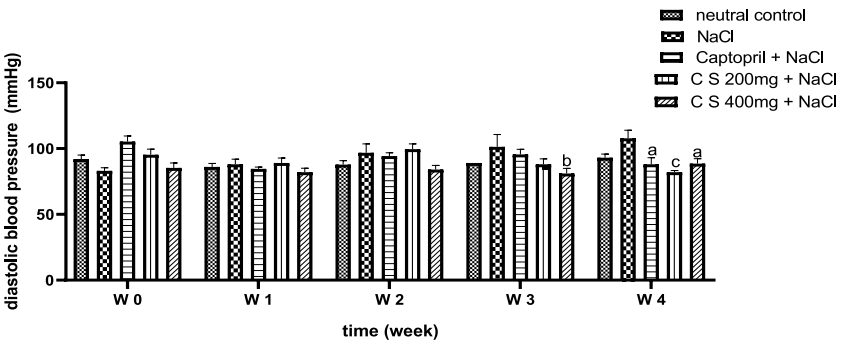

Fig. 3. Effect of different doses of *C. schweinfurthii* diastolic blood pressure during four weeks. Each bar represent the mean value ± S.E.M; n = 5; $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$significant difference when compared to hypertensive group. W0-4: week 0–4; C S: ethanolic extract of *C. schweinfurthii* stem bark.

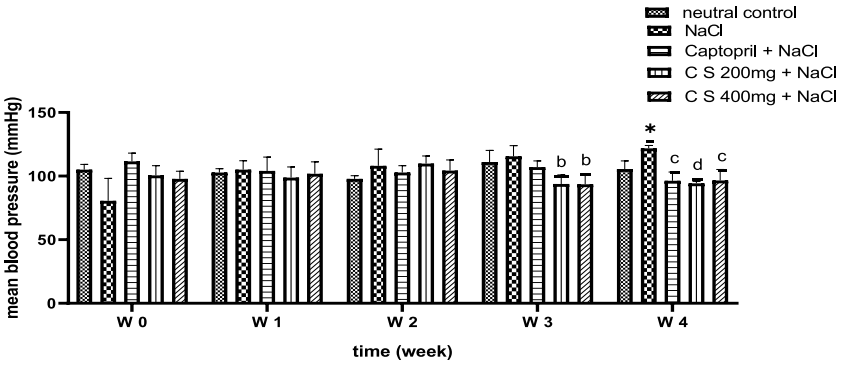

Fig. 4. Effect of different doses of *C. schweinfurthii* mean blood pressure during four weeks. Each bar represents the mean value ± S.E.M; n = 5; $^{b}p < 0.01$; $^{c}p < 0.001$; $^{d}p < 0.0001$ significant difference when compared to hypertensive group. *p < 0.05 significant difference when compared to normotensive group. W0-4: week 0–4; C S: ethanolic extract of *C. schweinfurthii* stem bark.

the 4th week of treatment (21.31 %) compared to the hypertensive group ($p < 0.001$). The mean blood pressure at week four of treatment was 122 mmHg in the hypertensive group and 96 mmHg in the animals that received captopril.

3.3.4. Effect of the ethanolic extract of Canarium schweinfurthii on heart rate

After four weeks of treatment, the heart rate of rats receiving NaCl solution only, increased significantly when compared to rats which received distilled water ($p < 0.001$, 22.67 %) (Fig. 5). The increase was maximal (22.67 %) at the 4th week and reached a value of 368 bpm in the hypertensive rats compared to 300 bpm for the normotensive rats. Concomitant administration of *C. schweinfurthii* sample extract and NaCl significantly inhibited the increase in heart rate from 3rd to 4th week of treatment compared to the hypertensive untreated group ($p < 0.001$; $p < 0.001$). The heart rate was measured at 295 bpm (200 mg/kg) and 306 bpm (400 mg/kg), respectively, at week four of treatment. The percentages of reduction were 19.84 % (200 mg/kg) and 16.85 % (400 mg/kg) for plant extract and 21.20 % for captopril, the reference drug.

3.3.5. Effect of the ethanolic extract of Canarium schweinfurthii on serum calcium

The levels of calcium measured in the blood of test animals after euthanization are shown in Fig. 6. No significant difference was found between the hypertensive and normotensive groups, treated respectively, with NaCl solution or distilled water ($p > 0.05$). However, after concomitant administration of NaCl and *C. schweinfurthii* extract, only the dose of 200 mg/kg significantly decreased the serum calcium when compared to the animals that received NaCl only ($p < 0.05$).

3.3.6. Effect of the ethanolic extract of Canarium schweinfurthii on sodium levels

The effect of different doses of *C. schweinfurthii* extract, NaCl, and control solution on the test animals is shown in Fig. 7. It appears that the natremia of the animal cohort that received only NaCl solution, is significantly elevated relative to the neutral control ($p <$

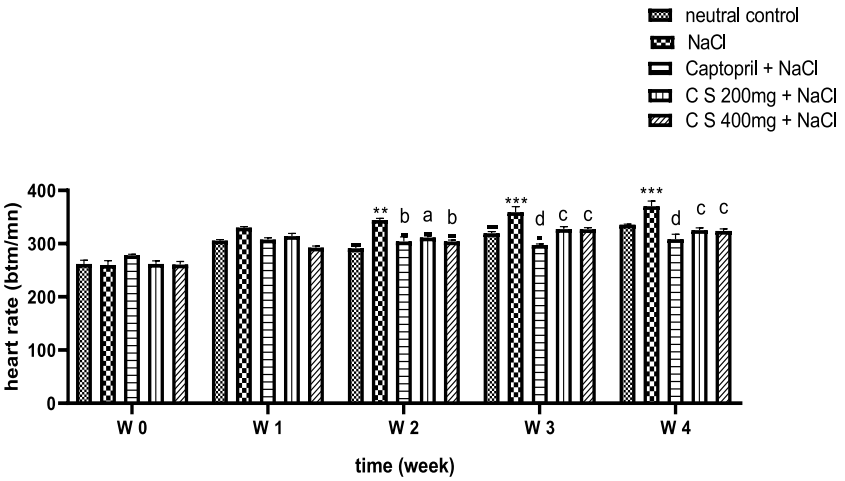

Fig. 5. Effect of different doses of *C. schweinfurthii*heart rate during four weeks. Each bar represent the mean value ± S.E.M; n = 5; $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ $^{d}p < 0.0001$; significant difference when compared to hypertensive group. **p < 0.01; ***p < 0.001 significant difference when compared to normotensive group. W0-4: week 0–4; C S: ethanolic extract of *C. schweinfurthii* stem bark.

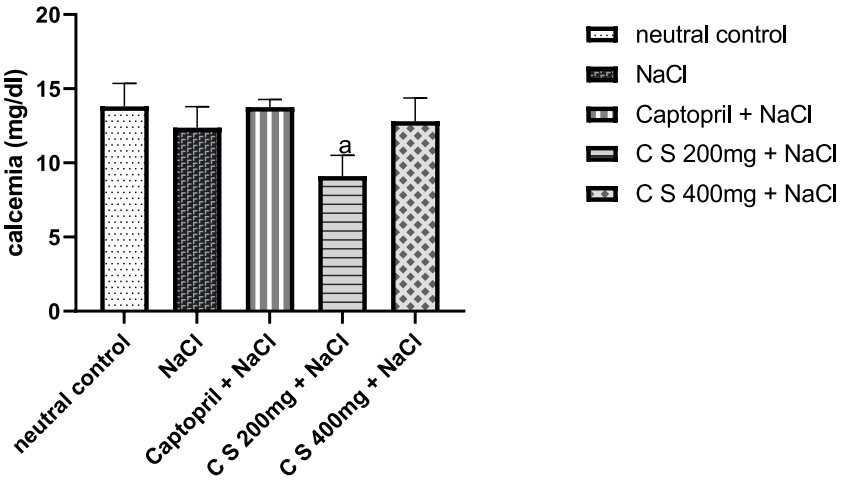

Fig. 6. Effect of different doses of *C. schweinfurthii* on calcium levels during four weeks. Each bar represents the mean value ± S.E.M; n = 5; $^{a}P <$ 0.05 significant difference when compared to hypertensive group; C S: *C. schweinfurthi*; C S: ethanolic extract of *C. schweinfurthii*stem bark.

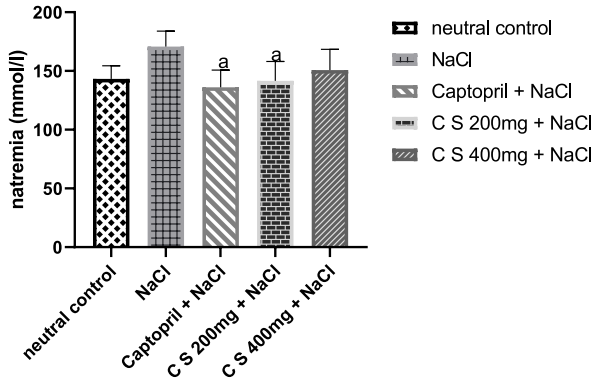

Fig. 7. Effect of different doses of *C. schweinfurthii* on sodium levels pressure during four weeks. Each bar represents the mean value ± S.E.M; n = 5; $^{a}P < 0.05$ significant difference when compared to hypertensive group; C S: ethanolic extract of *Canarium schweinfurthii*stem bark.

0.05). The concomitant administration of the ethanolic extract of *C. schweinfurthii* stem bark at 200 mg/$kg_{body\ weight}$ and NaCl correlated with a significant decrease in natremia in comparison to the neutral controlbatch ($p < 0.05$), whereas in the batch treated with the extract at 400 mg/kg, no such significant distinction to the control batch was observed.

Finally, the concomitant administration of captopril and NaCl led to a significant decrease of natremia compared to the neutral control cohort ($p < 0.05$).

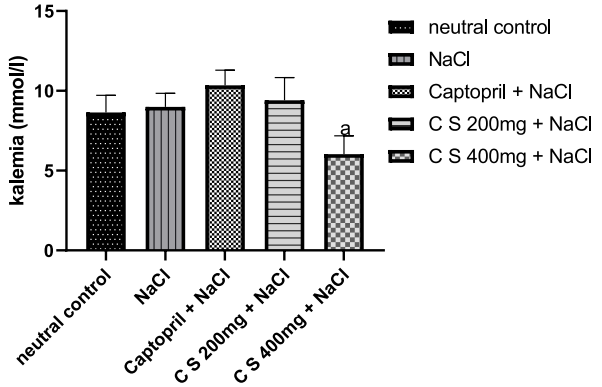

Fig. 8. Effect of different doses of *C. schweinfurthii*on potassium levels during four weeks.

3.3.7. Effect of the ethanolic extract of Canarium schweinfurthii on kalemia

The effects of administration of the sample extract as well as of control solutions on kalemia in test animals is summarised in Fig. 8. The kalemia of the group of animals treated only with NaCl solution, did not significantly differ from the control group.

Concomitant administration of the ethanolic extract of *C. schweinfurthii* stem bark and NaCl resulted in a significant decrease in kalemia in the 400 mg/$kg_{body\ weight}$ batch in comparison to the hypertensive group ($p < 0.05$) and a non-significant effect when 200 mg/$kg_{body\ weight}$ were administered, relative to the hypertensive group.

Treatment with captopril and NaCl resulted in a non-significant increase in serum potassium in comparison to the hypertensive group ($p > 0.05$).

Each bar represents the mean value ± S.E.M; n = 5; $^{a}P < 0.05$ significant difference when compared to hypertensive group; C S: ethanolic extract of *Canarium schweinfurthiistem* bark.

3.3.8. Effect of the ethanolic extract of Canarium schweinfurthii on blood count

The effect of repeated administration of *C. schweinfurthii* extracton on the complete blood count of experimental animals is shown in Table 2. No change occurred in red blood cell (RBC) levels between rats that received the plant extract and control rats. Also, there was no significant change in mean corpuscular volume (MCV) and hematocrit level (Ht)between these twogroups. Haemoglobin (Hb) concentration (g/dL), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin content (MCH) did not vary significantly between the treatment and control groups. Similarly, the white blood cell (WBC) count per liter of blood, the lymphocyte count (LYM) and platelet count (PLT) did not vary significantly between the animals tested compared to controls.

4. Discussion

The extraction by maceration of *Canarium schweinfurthii* stem bark was carried out in 70 % ethanol, which is a protic polar solvent chosen in this study for its relatively low toxicity. The extraction yield was 2.9 % as compared to dry material. This result is in accordance with reports by Mbosso et al., in 2022 on the same type of extract from *C. schweinfurthii* [13].

Subsequent UPLC-MS/MS analysis allowed to putatively identify triterpenoids, lignans, fatty acids and alkaloids in the ethanolic extract of *C. schweinfurthii* stem bark. These classes of compounds have previously been reported from phytochemical screening of the genus *Canarium* with isolations however limited to fatty acids, terpenoids, coumarins, flavonoids and simple phenolic compounds [34].

Administration of NaCl solution for four weeks to male Wistar rats led to significant increasesof systolic, diastolic and mean blood pressure compared to the neutral controls ($p < 0.05$). The maximal increases were 20.80 %, 23.86 % and 23.23 % for systolic, diastolic and mean blood pressure, respectively, at week four of treatment. Van et al., in 2006 reported that excessive NaCl intake can lead to a slow and gradual progressive increase in blood pressure [35]. Although the mechanisms by which NaCl influences blood pressure remainnot fully understood, the prevailing hypothesis suggests that the increase in plasma sodium concentration leads to a shift of fluid from the intracellular to the extracellular space. This stimulates thirst and increases the secretion of vasopressin or antidiuretic hormone [36]. The binding of this hormone to V1a receptors results in vasoconstriction of vascular smooth muscle cells, and/or its binding with V2 receptors, to increase the permeability of the collecting canal to water and thus leading to an increase in blood pressure [37].

Co-administration of NaCl and ethanolic *C. schweinfurthii* stem bark extract significantly inhibited systolic, diastolic and mean blood pressure increases ($p < 0.0001$; $p < 0.01$; $p < 0.0001$). These inhibition effects were already maximal at a dose of 200 mg/kg at the 4th week of treatment and could be explained by the neutralization of salt by the extract. This antihypertensive activity of *C. schweinfurthii* might be due to its effect on the mechanism of induction of the increase in blood pressure by NaCl. The presence of alkaloids in the extract could decrease blood pressure by affecting the transport of salt and water in the renal tubules by increasing the excretion of Na^{+} as suggested by Ratianarijaonaas part ofthe study of the diuretic action of SAMAH01 [38]. Moreover, a significant decrease (dose of 200 mg/kg) in the level of Na^{+} was observed. Alkaloids could also be exhibit diuretic and antioxidant effects by mitigating lipid peroxidation and oxidation of microsomal proteins. The presence lignans could be compatible with antioxidant properties and may contribute to the regulation of stress responses [9,10]. Similarly, results obtained in rats that received captopril and NaCl showed a significant decrease systolic, diastolic and mean blood pressure compared to negative control ($p < 0.001$; $p < 0.05$; $p < 0.001$).

Administration of NaCl in rats resulted in a significant increase of heart rate compared to neutral controls ($p < 0.001$). This increase may be due to a sympathetic mechanism [39]. Indeed, excessive levels of intracellular Na^{+} increase the reactivity of smooth vascular muscle to sympathetic stimulation. Sympathetic preganglion neurons located in the intermediolateral horn of the spinal cord, and responsible for the degree of constriction of vessels will send cholinergic (short) projections to sympathetic ganglia localized on each side of the spinal cord and to the adrenal medulla gland. The sympathetic lymph nodes will in turn send noradrenergic postganglion projections (long) on their target organs. Noradrenaline released by sympathetic projections will act via the activation of β1 adrenergic receptors on the heart by an increase in heart rate (positive chronotropic effect) [40]. In addition, a significant decrease in heart rate was observed in rats which received in addition to NaCl, the ethanolic extract of *C. schweinfurthii* stem bark or captopril ($p < 0.001$), with maximum inhibitions of 19.84 % (dose 200 mg/kg) and 21.20 %, respectively. The extract could have exerted its activityby inhibiting the action of the sympathetic nervous system. These results are similar to those reported by Bella et al., in 2012, showing that the aqueous extract of *Tetrapleura tetraptera* significantly inhibited heart rate increase in the NaCl induction model of hypertension [41]. As for captopril, it inhibits the production of angiotensin II, thus preventing the increase of peripheral resistance, by inhibiting vasoconstriction of blood vessels which causes the decrease in heart rate [42].

Table 2
Means of haematological parameters of the different batches of rats.

	Distilled water	NaCl	CS200 mg/kg + NaCl	CS400 mg/kg + NaCl	Captopril + NaCl
Red (10^6/L)	4.05 ± 1	5.26 ± 0	4.63 ± 0	4.43 ± 0	3.95 ± 0
Leukocytes (10^3/L)	5.63 ± 0	5.23 ± 0	4.50 ± 0	5.00 ± 1	4.40 ± 1
Haemoglobin (g/dL)	10.3 ± 2	11.2 ± 1	9.9 ± 1	9.98 ± 1	7.9 ± 0
Haematocrit (%)	0.46 ± 0	0.69 ± 0	0.53 ± 0	0.57 ± 0	0.47 ± 0
VGM (FL)	59.8 ± 9	68.3 ± 9	66.3 ± 9	63.0 ± 9	44.5 ± 6
TCMH (pg)	38.0 ± 5	39.3 ± 6	37.12 ± 5	37.13 ± 5	39.5 ± 2
CCMH (G/dL)	42,3 ± 1	40.5 ± 5	42.5 ± 3	42.5 ± 3	39.5 ± 1
Platelets (10^2/mL)	1.43 ± 2	1.5 ± 3	1.51 ± 0	1.49 ± 3	1.50 ± 2
Lym + (%)	506.7 ± 9	547.5 ± 9	497.62 ± 9	1770.0 ± 9	540.0 ± 9

Each valueis expressed as mean ± S.E.M; n = 5; C S: ethanolic extract of *C. schweinfurthii* stem bark.

Ions play an important role in regulating blood pressure [43]. All movements of sodium in the body are necessarily related to water: when we ingest sodium, we retain water and when we eliminate sodium, we lose water. Thus, an increase in salt consumption causes an increase in the volume of blood circulating in the arteries and therefore an increase in blood pressure. In our study, the significant increase in natremia in rats in the batch that received only NaCl compared to other batches therefore justifies the hypertension of these rats related to the hypertensive effect of NaCl which was used as an inducer of hypertension. On the other hand, the significant decrease in natremia in the batch that received NaCl and the extract at 200 mg/kg concomitantly for four weeks could reflect the fact that, at this dose, the extract mayhave a diuretic effect and therefore would have facilitated sodium elimination. It is the same for the batch that received NaCl and captopril concomitantly, which, by inhibiting the converting enzyme, facilitates the elimination of sodium. The similar natremia in the batch that received the distilled water and the batch that received NaCl solution and the extract at 400 mg/kg concomitantly, would reflect that at this dose, the extract would assist in maintaining normal natremia.

A mechanism then allows sodium to leave the cell, but it requires the presence of potassium to replace it through an ingenious system of cellular pumps. The adequate distribution of sodium and potassium in the body is one of the conditions of cellular life, especially for nervous and muscular function. Sodium and potassium form an essential tandem for the proper regulation of blood pressure. The significant decrease in serum potassium in the batch that received concomitant NaCl and the extract at 400 mg/kg would have an effect on the maintenance of sodium/potassium balance [43].

Calcium, in addition to its role on bone mass, would also have a favorable role on the relaxation of the artery wall (excess dietary salt would cause urinary calcium loss) which would explain the significant decrease in serum calcium in the batch that simultaneously received NaCl and extract at 200 mg/kg rats.

Blood is one of the most important systems in determining the index and physiological and pathological status in humans as well as animals. According to Adeneye et al., in 2006 and Hazra et al., in 2008, some therapeutic substances may have an action on hematopoietic differentiation cells [44,45]. Complete blood counts in treated rats showed no significant change in the blood parameters studied, thus reflecting the absence of effect on hematopoietic stimulation. These results are similar to those obtained by Bedou in 2019 on the evaluation of the activity inhibitor of *Bauhinia thonningii* (Fabaceae) fruits on two glycosidases and diabetes treatment trial in Wistar rats where they also found no significant variations on haematological parameters [46].

5. Conclusion

The tested extract of *C. schweinfurthii* stem bark significantly inhibited the elevation of blood pressure, the increase in heart rate, and allowed to conclude that the ethanolic extract of the stem bark of *C. schweinfurthii* exhibits antihypertensive activity. This effect might be related to its antioxidant potential and supports the traditional use of the stem bark of *C. schweinfurthii* to treat hypertension and suggest a protective effect against salt damage. The UPLC-HR-ESI-MS/MS analysis of the ethanolic extract of *C. schweinfurthii* stem bark allowed the putative annotation of triterpenoids related to oleanolic acid, steroids and prostaglandin related structures, as well as lignan polyphenols closely related or identical to hinokinin an dihydricubebin, compounds previously isolated from the stem barks of Burseraceae and other plants but not previously reported from *C. schweinfurthii*. The interesting activity observed for this extract suggests that it could be included in the formulation of improved traditional medicines and further studies are warranted to isolate the active components and understand the mechanisms of activity underlying the antihypertensive activity here reported for *C. schweinfurthii* ethanolic stem bark extract.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Jean Emmanuel Mbosso Teinkela: Conceptualization, Supervision, Writing – review & editing. **Edwige Laure Nguemfo:** Methodology, Validation, Writing – review & editing. **Thierry Fokou Nzodjou:** Data curation, Investigation, Writing – original draft. **Calvin Bogning Zangueu:** Data curation, Investigation. **Jarmo-Charles Kalinski:** Formal analysis, Software, Writing – review &

editing. **Bienvenu Tsakem:** Data curation, Writing – review & editing. **Jules Clement Assob Nguedia:** Conceptualization, Resources, Validation. **Xavier Siwe Noundou:** Software, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Dr Xavier Siwe Noundou is grateful for a South Africa National Research Foundation (NRF) Competitive Support for Unrated Researchers (CSUR) grant Number: SRUG2203291031. This work was materially supported by the Department of Pharmaceutical Sciences of the University of Douala and the laboratory of biology and annal organisms of the Faculty of Sciences of the same University. We also express our gratitude to the Department of Pharmaceutical Sciences, School of Pharmacy, Sefako Makgatho Health Sciences University and the Department of Biochemistry and Microbiology, Faculty of Science, Rhodes University for the UPLC-ESI-QToF-MS/MS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21841.

References

[1] J. Chalmers, S. MacMahon, G. Mancia, J. Whitworth, L. Beilin, L. Hansson, B. Neal, A. Rodgers, C. Ni Mhurchu, T. Clark, 1999 world health organization-international society of hypertension guidelines for the management of hypertension. Guidelines sub-committee of the world health organization, Clin. Exp. Hypertens. 21 (1999) 1009–1060, https://doi.org/10.3109/10641969909061028.

[2] K.T. Mills, J.D. Bundy, T.N. Kelly, J.E. Reed, P.M. Kearney, K. Reynolds, J. Chen, J. He, Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries, Circulation 134 (2016) 441–450, https://doi.org/10.1161/CIRCULATIONAHA.115.018912.

[3] O.S. Ogah, B.L. Rayner, Recent advances in hypertension in sub-Saharan Africa, Heart 99 (2013) 1390–1397, https://doi.org/10.1136/heartjnl-2012-303227.

[4] J.P. Nkeck, P.E.W. Yone, J.R. Nkeck, C. Ekono, N.M.E. Komo, A.E. Ze, Etiologies de la dyspnée aiguë dans le service de pneumologie de l'hôpital Jamot de Yaoundé, Health Sci. Dis. 19 (2018) 53–57.

[5] Oms Afrique, Rapport du secrétariat (2013) 1–3.

[6] R. Verpoorte, Pharmacognosy in the new millennium: leadfinding and biotechnology, J. Pharm. Pharmacol. 52 (2000) 253–262, https://doi.org/10.1211/0022357001773931.

[7] G.M. Cragg, D.J. Newman, Natural products:acontinuing source of noveldrug leads, Biochim. Biophys. Acta 1830 (2013) 3670–3695, https://doi.org/10.1016/j.bbagen.2013.02.008.

[8] F. Sénejoux, Etudes phytochimique et pharmacologique de *Ziziphora clinopodioides* (Lamiaceae), *Nitraria sibirica* (Nitrariaceae) et *Echinops integrifolius* (Asteraceae), plantes anti-hypertensives de la Pharmacopée ouïghoure, Doctoral dissertation, Université de Franche-Comté, 2011.

[9] C.T. Quenum, H. Ahissou, P. Gouthon, A. Laleye, Etude de l'activité antihypertensive d'une association de plantes (*Schrankialeptocarpa, Garcinia kola* et *Ocimum americanum*) chez le rat Wistar, Int. J. Biol. Chem. Sci. 8 (2014) 2685–2695, https://doi.org/10.4314/ijbcs.v8i6.28.

[10] J. Yinyang, M.E. Mpondo, M. Tchatat, R.C. Ndjib, O.P.B. Mvogo, S.D. Dibong, Les plantes à alcaloïdes utilisées par les populations de la ville de Douala (Cameroun), J. Appl. Biosci. 78 (2014) 6600–6619, https://doi.org/10.4314/jab.v78i1.7.

[11] J.B.L. Okullo, F. Omujal, C. Bigirimana, P. Isubikalu, M. Malinga, E. Bizuru, et al., Ethno-medicinal uses of selected indigenous fruit trees from the lake victoria basin districts in Uganda, J. Med. Plants Studies 2 (2014) 78–88.

[12] O.T. Tcheghebe, A.J. Seukep, F.N. Tatong, A review on traditional uses, phytochemical composition and pharmacological profile of *Canarium schweinfurthii* Eng, Nat. Sci. 14 (2016) 17–22, https://doi.org/10.7537/marsnsj141116.03.

[13] T.J.E. Mbosso, N.T. Fokou, E.L. Nguemfo, N.J.C. Assob, N.X. Siwe, Screening phytochimique et étude de la toxicité aigüe et subaigüe de l'extrait ethenolique des écorces du tronc de *Canariums chweinfurthii* Engl. (Burseraceae) chez le rat, J. Appl. Biosci. 174 (2022) 18043–18055, https://doi.org/10.35759/JABs.174.2.

[14] N.T. Fokou, N.J.C. Assob, J. Ngoupayo, B. Ngameni, T.J.E. Mbosso, Quantitative screening and study of the *in vivo* subchronic toxicity of ethanolic extract from the stem bark of *Canarium schweinfurthii* Engl. (Burseraceae) in wistar rats, Saudi J. Med. Pharm. Sci. 92 (2023) 74–93, https://doi.org/10.36348/sjmps.2023.v09i02.005.

[15] K. Dührkop, H. Shen, M. Meusel, J. Rousu, S. Böcker, Searching molecular structure data bases with tandem mass spectrausing CSI:FingerID, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 12580–12585, https://doi.org/10.1073/pnas.1509788112.

[16] K. Dührkop, L.-F. Nothias, M. Fleischauer, R. Reher, M. Ludwig, A.M. Hoffmann, et al., Systematic classification of unknown metabolite susing high-resolution fragmentation mass spectra, Nat. Biotechnol. 39 (2020) 462–471, https://doi.org/10.1101/2020.04.17.046672.

[17] S. Böcker, C.M. Letzel, Z. Lipták, A. Pervukhin, SIRIUS: decomposing isotope patterns for metabolite identification, Bioinformatics 25 (2009) 218–224, https://doi.org/10.1093/bioinformatics/btn603.

[18] A. Kuinze, Effet antihypertenseur de l'extrait aqueux des feuilles de *Azelia bipindensis Harms*. (Fabaceae) chez le rat, Master dissertation, University of Douala, Douala, 2018.

[19] L. Odie, L.F. Cunff, C. Naudat, Réactivité physico-chimique d'un couple émulsion de bitume/granulats : suivi du pH et dosage des éléments alcalins (Na, Ca, K et Mg) par spectrophotométrie d'absorption atomique à partir d'une solution d'extraction : projet de méthode d'essai n°77, in: Techniques et méthodes des laboratoires des ponts et chaussées, Méthode d'essai PME77, Institut français des sciences et technologies des transports, de l'aménagement et des réseaux, 2011.

[20] V.C.K. Chiu, D. Haynes, The pH dependence and the binding equilibria of the Calcium Indicator — arsenazo III, Membr. Biochem. 3 (1980) 169–183, https://doi.org/10.3109/09687688009063884.

[21] H.M. Brown, B. Rydqvist, Arsenazo III-Ca^{2+}: effect of pH, ionic strength, and arsenazo III concentration on equilibrium binding evaluated with Ca^{2+} ion-sensitive electrodes and absorbance measurements, Biophys. J. 36 (1981) 117–137, https://doi.org/10.1016/S0006-3495(81)84720-0.

[22] V. Michaylova, P. Ilkova, Photometric determination of micro amounts of calcium witharsenazo III, Anal. Chim. Acta 53 (1971) 194–198, https://doi.org/10.1016/S0003-2670(01)80088-X.

[23] R. Schmid, S. Heuckeroth, A. Korf, A. Smirnov, O. Myers, S. Thomas, S.T. Dyrlund, et al., Integrative analysis of multimodal mass spectrometry data in MZmine 3, Nat. Biotechnol. 41 (2023) 447–449, https://doi.org/10.1038/s41587-023-01690-2.
[24] M. Wang, J.J. Carver, V. Phelan, V.V. Phelan, M.L. Sanchez, N. Garg, Y. Peng, et al., Sharing and community curation of mass spectrometry data with global natural products social molecular networking, Nat. Biotechnol. 34 (2016) 828–837, https://doi.org/10.1038/nbt.3597.
[25] L.F. Nothias, D. Petras, R. Schmid, K. Dührkop, J. Rainer, A. Sarvepalli, et al., Feature-based molecular networking in the GNPS analysis environment, Nat. Methods 17 (2020) 905–908, https://doi.org/10.1038/s41592-020-0933-6.
[26] K. Dührkop, M. Fleischauer, M. Ludwig, A.A. Aksenov, V.A. Melnik, M. Meusel, et al., Sirius 4: turning tandem mass spectra into metabolite structure information, Nat. Methods 16 (2019) 299–302, https://doi.org/10.1038/s41592-019-0344-8.
[27] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, et al., Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (2003) 2498–2504, https://doi.org/10.1101/gr.1239303.
[28] T. Maffo, M. Takou, G.B. Tabekoueng, M.K. Langat, J.C. Vardamides, P. Wafo, et al., Triterpenoid derivatives from *Canarium schweinfurthii* Engl. (Burseraceae), J. Pharmacogn. Phytochem. 8 (2019) 3064–3067.
[29] M. Maldini, P. Montoro, S. Piacente, C. Pizza, Phenolic compounds from *Bursera simaruba* Sarg. bark: phytochemical investigation and quantitative analysis by tandem mass spectrometry, Phytochemistry 70 (2009) 641–649, https://doi.org/10.1016/j.phytochem.2009.02.009.
[30] M.C. Marcotullio, A. Pelosi, M.H. Curini, An emerging bioactive lignan, Molecules 19 (2014) 14862–14878, https://doi.org/10.3390/molecules190914862.
[31] D. Dwuma-Badu, J.S. Ayim, T.T. Dabra, Constituents of West African medicinal plants. IX. dihydrocubebin, a new lignan from *Piper guineense*, Lloydia 38 (1975) 343–345.
[32] C.M. Souza de Paula, A. Féboli, A. Carlos de Laurentiz, F. Rosemeire da Silva, A.H. Prizantelli de Oliveira, M.L. Andrade de Silva, et al., *In vitro* anthelmintic activity of the crude hydroalcoholic extract of *Piper cubeba* fruits and isolated natural products against gastrointestinal nematodes in sheep, Vet. Parasitol. 275 (2019), 108932, https://doi.org/10.1016/j.vetpar.2019.108932.
[33] R. Buathong, F. Schindler, J. Schinnerl, K. Valant-Vetschera, M. Bacher, A. Potthast, et al., Uncommon fatty acids, Iridoids and other secondary metabolites from the medicinal plant species *Ixora cibdela* Craib (Rubiaceae), Phytochem. Lett. 33 (2019) 77–80, https://doi.org/10.1016/j.phytol.2019.07.011.
[34] R. Mogana, C. Wiart, L. Canarium, A phytochemical and pharmacological review, J. Pharm. Res. 4 (2011) 2482–2489.
[35] L.A.J. Van Mierlo, L.R. Arends, M.T. Streppel, M.P.A. Zeegers, F.J. Kok, D.E. Grobbee, J.M. Geleijnse, Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials, J. Hum. Hypertens. 20 (2006) 571–580, https://doi.org/10.1038/sj.jhh.1002038.
[36] H.E. Wardener, F.J. He, G.A. MacGregor, Plasma sodium and hypertension, Kidney Int. 66 (2004) 2454–2466, https://doi.org/10.1111/j.1523-1755.2004.66018.x.
[37] J. Perucca, Rein, vasopressine et pression artérielle : importance de la concentration de l'urine et du rythmenycthéméral d'excrétion d'eau et de sodium, Doctoral dissertation, Université Pierre et Marie Curie, 2008. Paris vol. I, https://tel.archives-ouvertes.fr/tel-00812627. (Accessed 1 July 2023).
[38] F. Ratianarijaona, Etude de l'activité diuretique de l'extrait RTF Chez le cochon d'Inde, Master dissertation, University of Antananarivo, Antananarivo, 2015. http://www.biblio.univ-antananarivo.mg/pdfs/ratianarijaonaFenitra_SN_MAST_17.pdf. (Accessed 18 February 2023).
[39] R.C. Christie, W.B. Michael, Nitric oxide opposes glucose-induced hypertension by suppressing sympathetic activity, Hypertension 41 (2003) 274–278, https://doi.org/10.1161/01.hyp.0000049620.70909.2e.
[40] C. Brouillard, Mécanismes centraux sous-tendant les altérations cardiorespiratoires induites par un stress chronique, Neurosciences [q-bio.NC], Doctoral dissertation, Université Pierre et Marie Curie, Français, Paris VI, 2015.
[41] N.M.T. Bella, L.T.E. Ngo, O.B.F. Aboubakar, D.E. Tsala, T. Dimo, Aqueous extract of *Tetrapleura tetraplera* (Mimosaceae) prevents hypertension, dyslipidemia and oxidative stress in high salt-sucrose induced hypertensive rats, Pharmacologia 3 (2012) 397–405, https://doi.org/10.5567/pharmacologia.2012.397.405.
[42] P. Lechat, Pharmacologie, Service de pharmacologie clinique, Faculté de Médecine Pierre et Marie-curie, Paris, 2006. https://www.paperpile.com/s/heliyon-citation-style/. (Accessed 30 August 2023).
[43] M.P. Blaustein, Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis, Am. J. Physiol. 232 (1977) 165–173, https://doi.org/10.1152/ajpcell.1977.232.5.C165.
[44] A. Adeneye, A. Benebo, E. Agbaje, Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on alcohol-induced hepatotoxicity in rats, West Afr. J. Pharmacol. Drug Res. 22 & 23 (2006) 42–50, https://doi.org/10.4314/wajpdr.v22i1.14767.
[45] B. Hazra, S. Biswas, N. Mandal, Antioxidant and free radical scavenging activity of *Spondias pinnata*, BMC Complement. Altern. Med. 8 (2008) 63, https://doi.org/10.1186/1472-6882-8-63.
[46] K.D. Bédou, Evaluation de l'activite inhibitrice des fruits de *Bauhinia thonningii* (Fabaceae) sur deux glycosidases et essai de traitement du diabete chez le rat Wistar, Doctoral dissertation, Université Félix Houphouët-Boigny, Abidjan, 2019. https://theses.hal.science/tel-02537203/document. (Accessed 15 January 2023).