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Assessment of *in vivo* acute, subacute toxicity, *in vitro* anti-plasmodial activity, and UPLC-ESI-QToF-MS/MS prediction of *Annickia affinis* (Exell) Versteegh & Sosef (Annonaceae) roots extract

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Abstract

Background: *Annickia affinis* is a medicinal plant widely used in African traditional medicine to treat various diseases, including malaria. This study aimed to evaluate the toxicological profile, chemical composition, and antiplasmodial activity of the ethanolic (EtOH) root extract of *Annickia affinis*.

Methods: Acute (14-day) and subacute (28-day) toxicity studies were conducted in accordance with guidelines 423 and 407 of the Organisation for Economic Co-operation and Development. Chemical profiling was performed using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) and molecular networking analysis (GNPS). Antiplasmodial activity was evaluated *in vitro* against the chloroquine-resistant *Plasmodium falciparum* Dd2 strain.

Results: In the acute toxicity study, oral administration of the extract at doses of 2000 and 5000 mg/kg body weight caused no mortality or significant changes in clinical signs, body weight, or most biochemical and hematological parameters. Similarly, subacute administration at 200, 400, and 800 mg/kg for 28 days did not induce mortality. Mild increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in male rats, gamma-glutamyl transferase (γ-GT) levels in females, and urea levels in both sexes were observed, whereas serum creatinine and most hematological parameters remained unchanged. Histopathological examination of the liver, kidneys, and heart revealed no tissue damage. Chemical analysis identified several alkaloids, mainly protoberberine alkaloids, along with sesquiterpenes. The EtOH root extract exhibited strong antiplasmodial activity with an IC_{50} of 2.08 µg/mL against the *P. falciparum* Dd2 strain.

Conclusion: The EtOH root extract of *A. affinis* demonstrates a favorable safety profile and promising antiplasmodial activity, suggesting its potential as a source of bioactive compounds for antimalarial drug development

Keywords: Acute toxicity; *Annickia affinis*; Antiplasmodial; GNPS; Subacute toxicity; UPLC-MS/MS

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Background

The increasing global burden of malaria, reflected in rising incidence and mortality rates, as well as the emergence of resistance to all classes of available antimalarial drugs, has become a major public health concern. Malaria is a parasitic disease transmitted by the female mosquitoes of the genus *Anopheles* mosquito. In 2012, approximately 247 million malaria cases and more than 619,000 deaths were reported worldwide [1]. Children under five years of age account for 67% of malaria-related deaths globally, and more than 93% of these deaths occur in sub-Saharan Africa [2], including 12.8% recorded in Cameroon in 2017 [3]. Limited geographical and economic access to modern healthcare services, combined with insufficient healthcare resources, has resulted in more than 80% of the African population relying on traditional medicine for the treatment of malaria and other diseases [4].

Consequently, the scientific validation and valorization of traditional medicinal plants have become essential for the potential development of plant-derived therapeutic agents. In this context, the key principles guiding such research are safety, efficacy and quality [5]. The present study focuses on the roots of *Annickia affinis* (Exell) Versteegh & Sosef (Annonaceae), a plant widely used in traditional medicine in Cameroon. This species has traditionally been employed in the treatment of malaria, prostate cancer, and rheumatism [6, 7]. Despite its ethnomedicinal importance, there are limited reports on the biological activities of the ethanolic extract of its roots. Previous studies have shown that the aqueous extract of the stem bark *A. affinis* exhibited significant antiplasmodial activity against *Plasmodium falciparum* 3D7 strains, with an IC_{50} value of 1.49 µg/mL [8]. In addition, methanolic extracts of the leaves, stem bark and roots also demonstrated moderate antiplasmodial activity against chloroquine-sensitive *P. falciparum* 3D7 strains [9]. The cytotoxic and antioxidant activities of methanolic extracts from these plant parts have also been reported [10], while antimicrobial activities of methanolic extracts of stem bark extracts have previously been documented [11]. In antiplasmodial assays, chemosensitivity tests conducted on erythrocytes indicated that these extracts did not exhibit significant cytotoxic effects on red blood cells, suggesting that they may be safe upon systemic exposure. Furthermore, previous *in vivo* studies on other organs of *A. affinis* reported no major signs of toxicity in experimental animals, including acute toxicity studies of methanolic extracts of leaves, stem bark, and roots [11], as well as subacute toxicity studies of methanolic stem bark extracts [11]. However, the subacute toxicity of the ethanolic extracts of *A. affinis* roots was not evaluated.

Therefore, the present study aimed to investigate the *in vivo* acute and subacute toxicity, phytochemical composition, mass spectrometric dereplication of the secondary metabolites, and antiplasmodial activity of the ethanolic root extract of *A. affinis* against the chloroquine-resistant and artemisinin sensitive *Plasmodium falciparum* Dd2 strain.

Methods

Plant collection and identification

The roots of *A. affinis* used in this study were harvested in Mont Kala, a village in the Central Region of Cameroon, located in the commune of Mbankomo and the department of “Méfou-et-Akono”. The collected plant was identified by a taxonomist at the National Herbarium of Cameroon and recorded under the voucher specimen number 6420/HNC.

Extraction

The preparation of the ethanolic extract of the plant was performed according to the method described by Adesokan et al. [12]. The harvested roots were washed several times with distilled water to eliminate impurities that could affect the evaluation of biological activities. The plant material was then dried at room temperature out of direct sunlight to avoid decomposition of natural product contents. Thereafter, it was ground and yielded a mass of 1450 g of powder. This resulting powder was mixed with 7.2 L of ethanol 90% and the resulting mixture was stirred for 72 hours at room temperature (25°C); The mixture was filtered three times through cotton wool and once through 3 mm Whatman filter paper. Finally, the filtrate was evaporated at 60°C using a rotary evaporator (Heidolph LABOROTA 4000); The crude extract obtained was weighed (103.7 g) and stored in a refrigerator (−20ºC) prior to further uses.

Experimental animals

Healthy female and male Wistar rats, nulliparous, non-pregnant, and aged from six to nine weeks, which have not been subjected to previous experimental activities were used. Their weights were determined prior to feeding. The rats were acclimatized for two weeks. The experimental animals were housed in standard plastic cages and provided access to food and water *ad libitum* in a controlled environment maintained at a room temperature of 26 ± 2°C, relative humidity of 60–80%, and a standard 12-hour light/dark cycle. All experimental protocols were reviewed and approved by the Institutional Ethics Committee of the University of Douala (Approval N° 3170CEI-Udo/06/2022/T). Humane endpoints were strictly defined; animals exhibiting severe weight loss (> 20%), prolonged lethargy, or signs of severe distress would be humanely euthanized to prevent suffering.

Acute toxicity

A total number of twelve female Wistar rats were randomly selected and divided into four different treatment batches, each batch comprising three healthy animals that were subsequently fasted before dosing.

Batch 1, the neutral control group, were treated with 1 mL/100 $g_{body\ weight}$ of distilled water. Batch 2, the negative control group, were treated with 1 mL/100 $g_{body\ weight}$ of dimethylsulfoxide (DMSO)/olive oil (5/95, v/v). Batch 3 was treated with 1 mL/100 $g_{body\ weight}$ of ethanolic root extract at 2000 mg/$kg_{body\ weight}$ and batch 4 was treated with 1 mL/100 $g_{body\ weight}$ of ethanolic root extract at 5000 mg/$kg_{body\ weight}$.

Subacute toxicity

A total number of 30 Wistar rats were randomly selected and divided into five different treatments batches, each batch comprises of six healthy animals (three males and three females) each bearing a mark allowing them to be distinguished. The rats were housed under the following conditions: a room temperature of 22 ± 1°C, relative humidity 50 ± 5%, 12:12-h light/dark cycle.

Batch 1, the neutral control group was treated with 1 mL/100 $g_{body\ weight}$ of distilled water. Batch 2, the negative control group, was treated with DMSO/olive oil (5/95, v/v) at 1 mL/$g_{body\ weight}$. Batch 3 was treated with 1 mL/100 $g_{body\ weight}$ of root extract at 200 mg/$kg_{body\ weight}$. Batch 4 was treated with 1 mL/100 $g_{body\ weight}$ of extract at 400

mg/kg$_{body\ weight}$, and batch 5 was treated with 1 mL/100 g$_{body\ weight}$ of extract at 800 mg/kg$_{body\ weight}$. The choice of doses was made based on studies of acute toxicity and data from analogous substances in the literature.

Phytochemical screening

Detailed phytochemical screening was performed on the ethanolic extract of *A. affinis* roots using standard methods, as reported in the literature [13-16]. Other specific phytochemical tests were also utilized, based on either precipitation reaction via the generation of insoluble complexes, or on colorimetry reaction through the formation of colored soluble chemical species. The colored reactions were carried out in test tubes in the presence of the positive controls. The following tests were carried out: Dragendorff test (alkaloids), Tannins test (gallic tannins), Liebermann Burchard test (steroids and terpenoids), Shinoda test (flavonoids), Cardiotonic glycosides test (cardiotonic glycosides), Borntrager test (anthraquinones), Foam Index test (saponins), $FeCl_3$ test (polyphenols), Potash test (coumarin) and Reducing Sugars test.

UPLC-HR-ESI-MS/MS analysis of A. affinis ethanolic extract

The Supplementary Material (Text S1) contains specific UPLC-MS/MS acquisition parameters, such as gradient settings, MS fragmentation conditions, and data processing procedures utilizing MZmine3, GNPS, and SIRIUS5. Data processing, molecular networking, compound annotation, and visualization were executed using established MZmine3 [17], GNPS [18, 19], SIRIUS5/CANOPUS [20, 21], and Cytoscape [22] workflows.

Acute toxicity

Acute toxicity experiments were conducted according to guideline 423 of the Organisation for Economic Co-operation and Development (OECD) protocol [23] at the Pharmacology and Toxicology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of our University. Nine-week-old female Wistar rats were fasted over the night prior the experiment from 8 p.m. to 8 a.m. Two (2) batches of three (3) randomized rats received the ethanolic extracts of the root of *A. affinis*, at doses of 2000 and 5000 mg/kg, respectively. The control batch (three rats) received distilled water and the negative control group, treated with 5% DMSO + 95% olive oil at 1 mL/100 g$_{body\ weight}$. Once treated, the animals were observed for 2 hours after the administration of the extract. They were then fed and observed after 4 h, 8 h and then 14 days during which the symptoms of intoxication (stool appearance, noise sensitivity, grouping, locomotion, motility, coat modification, reaction to noise, grooming, trembling, as well as deaths) were noted. The dead rats in each batch were counted for the determination of the median Lethal Dose (LD_{50}). The plant extract and vehicle control were administered via oral gavage at a standard constant volume of 10 mL/kg body weight. Animals were randomly assigned to their respective treatment batches. Furthermore, the investigator performing the histopathological and biochemical assessments was blinded to the treatment groups.

Subacute toxicity

Subacute toxicity was studied as per OECD Guideline 407 with slight modifications [24] at the Pharmacology and Toxicology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of our University. Nine-week-old adult, Wistar albino, male and female rats were divided into three experimental batches of six animals each, three males and three females. They were fasted the night before the experiment from 8 p.m. to 8 a.m. The subacute toxicity tests were carried out on the three batches of six randomized rats which received the ethanolic extracts of the root of *A. affinis*, at different doses. Because no mortality or severe toxicity was observed at 2000 mg/kg in the acute study, doses of 200, 400, and 800 mg/kg (representing 1/10th, 1/5th, and less than 1/2 of the acute limit dose, respectively) were selected to systematically evaluate dose-dependent subacute effects. The control batches (six rats) received distilled water (neutral control to to establish an absolute physiological baseline) and the negative control group (account for any systemic background effects caused by the solvent), treated with 5% DMSO + 95% olive oil at 10 mL/kg. The administrations continued for 28 days. After 28 days, all the animals were sacrificed by an anesthesia (ether) after an overnight fast (8 h) [25]. Briefly, the rats were put to fast for 16 hours but had free access to the water. They were then anesthetized with ether by inhalation, after decapitation the blood was collected in tubes with or without anticoagulant (ethylene diamine tetra Acetate), for hematological and biochemical studies. Later, the animals were opened on the ventral side for organ collection. The organs that were removed, including liver, kidneys, heart, lung, and spleen, were rinsed with 0.9% saline solution (physiological solution), then observed *in situ* and weighed. During the experimental periods, animals were visually monitored twice daily for any clinical signs of toxicity or behavioral alterations for acute and sub-acute toxicity. The biochemical parameters measured were serum urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), the blood count and histology of organ sections. The following serum parameters: aspartate transaminase (AST), alanine aminotransferase (ALT), creatinine, uric acid (UA), were measured by enzymatic methods. These assays were carried out at the Animal Physiology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of our University. The histological procedure was carried out by the method described by Biswas et al. in 2010 with some modifications [26]. It comprises multiple stages including fixing, trimming, dehydration, inclusion, cutting, coloring, assembly and observation. This assay was carried out at the Animal Physiology Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. The effects of the extract on the hematological parameters of the animals were assessed using a "Mindray BC-2800" hematology analyser from the hematology laboratory of the Central Hospital of Yaoundé. This machine works by drawing blood from an Ethylenediaminetetra-acetic acid (EDTA) tube and performs red blood cell and platelet counts, hemoglobin levels, hematocrit, and erythrocyte constants. A differential lysis of the erythrocytes is then performed, and the different leukocyte populations are counted using specific enzymatic activity. The various parameters are plotted on a graph and printed [27].

In vitro antiplasmodial activity: Plasmodium falciparum culture and growth inhibition assay

Plasmodium falciparum Dd2 (multidrug resistant/artemisinin-sensitive) strain was obtained from the Biodefense and Emerging Infections (BEI) Research Resources (Manassas, VA) and maintained 5% CO_2 at 37°C using a modified Trager and Jensen method [28]. Parasites were grown in fresh human O^+ red blood cells at 3% (v/v) hematocrit in RPMI 1640 culture medium containing GlutaMax and $NaHCO_3$ (Gibco, UK), supplemented with 25 mM *4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid* (HEPES) (Gibco, Drewton, UK), hypoxanthine1X (Gibco, Waltham, MA, USA), 20 µg/mL gentamicin (Gibco, China), and 0.5%

Albumax II (Gibco, Waltham, MA, USA). When needed, *Plasmodium falciparum* Dd2 parasites were synchronized at the ring stage with a sorbitol (5%) treatment and further cultivated for one complete cycle (48 h) prior to the drug activity assays. EtOH extract of *A. affinis* roots dissolved in dimethylsulfoxide (DMSO) at 0.2% were diluted in RPMI 1640 and mixed with the parasite cultures (1.5% hematocrit and 1% parasitaemia) in 96-well plates to achieve final drug concentration of 10 µg. The final concentration of DMSO per 100 µL culture per well was 0.2%. Following a 72 h incubation at 37°C, with EtOH extract of *A. affinis* roots or drug controls (artemisinin and chloroquine at 1 µM respectively and DMSO at 0.1%), parasite growth was assessed using a SYBR green I-based DNA quantification assay. Briefly, 80 µL of parasitized erythrocytes were transferred to a dark plate and 40 µL of an SYBR green I-containing lysis buffer (3×) was added to each well. Following 30 min incubation in the dark, fluorescence was measured using a Fluoroskan Ascent multi-well plate reader with excitation and emission wavelengths at 485 and 538 nm, respectively [29]. The experiments were performed in triplicate and each one was repeated at least once. The concentrations at which 50% inhibition of growth (IC_{50} values) was obtained and were determined using GraphPad Prism 8.0, by plotting the logarithmic sample concentration on the x-axis against the percentage of inhibition on the y-axis.

Statistical analysis

Data were expressed as a mean ± standard error of mean (SEM). Statistical analyses were performed using Graphpad Instat software version 8.0.1. The analysis of the relative organ weights and biochemical parameters were performed using one-way ANOVA followed by TUKEY post-test. Two-way ANOVA with repeated measures followed by DUNNETT post-test was used to process data on hemodynamic parameters and body weight. Prior to ANOVA, the assumptions of normality and homoscedasticity were verified using the Shapiro-Wilk test and Levene's test, respectively.

Results and Discussion

The yield of the ethanolic extract of *Annickia affinis* roots at the different stages was 72.5% after grinding and 7.5% after maceration. Extraction by maceration of the *Annickia affinis* roots was carried out in ethanol 90%, which is a polar protic solvent chosen in this study for its low toxicity. The extraction yield was found to be 7.15% which is very close to the methanolic extraction yield of *A. affinis* roots, i.e. 7.62% [9].

Table 1 shows the classes of secondary metabolites present in *A. affinis* roots. The results of the phytochemical screening of the ethanolic extract of *A. affinis* roots were positive for alkaloids, terpenoids, coumarins and reducing sugars, while saponins, flavonoids, steroids, phenols, anthraquinones, anthocyanins and tannins were not detected.

The different colorimetric phytochemical screening assays suggested the presence of different classes of secondary metabolites in the ethanolic extract of *A. affinis* roots, including alkaloids, triterpenoids, coumarins, reducing sugars. These results are similar to the studies conducted by Ali et al. in 2023 which showed the presence of several groups of compounds in the methanolic extracts of *A. affinis* roots including alkaloids and coumarins [9] and those conducted by Erhunse et al., in 2023 which showed the presence of alkaloids, flavonoids, phenolics, quinones, coumarins in the aqueous extracts of *A. affinis* stem bark [8]. The 2010 study by Odoh et al., in Nigeria also reported the presence of alkaloids, reducing sugars, flavonoids, tannins, saponins and glycosides in ethanolic extracts of *A. affinis* roots [30]. This difference i.e. the scarcity of flavonoids, saponins and tannins in our extract, may be related to the type of soil and climate at the site where the plant was harvested.

Figure 1 shows the molecular network output (visualized in Cytoscape [22]) from GNPS [19], as well as a summary of the CANOPUS [21] natural product compound classification results. Most of the detected features were classified as alkaloids by CANOPUS, and in agreement with published reports of *A. affinis* natural products [31], spectral matches were obtained to protoberberine alkaloids such as jatrorrhizine, dihydroberberine, palmatin and tetrahydropalmatine. Based on the MS/MS spectral matching results, it is not possible to conclusively identify which exact ion feature represents e.g, jatrorrhizine, dihydroberberine (or e.g. columbamine, another isomer previously reported from *A. affinis* [31], as they are structurally very similar and show very similar MS/MS spectra. Accordingly, several isomeric features with very similar fragmentation spectra, precursor *m/z* of 352.15, but differing in retention time and appearing as different chromatographic features, were matched to palmatin, probably representing palmatin and isomers such as pseudopalmatin, which differ in the position of the methoxy groups or double bonds. A spectral match was observed for N-methylurabaine, which was associated several closely related features, probably representing similar bisaporphine alkaloids. Aporhine alkaloids have previously been reported from *A. affinis* [31], and bisaporphines have previously been isolated from the leaves of *A. kummeriae* before [32].

Many features in the cluster shared by bisaporphines and protoberberine were not successfully matched to any known alkaloids by spectral matching, and their *m/z* values did not correspond to alkaloids previously reported from *Annickia*. Therefore, they may represent new, as yet unknown alkaloids. The presence of several terpenoids was detected, some of which were matched to costunolide, a-isabolol, cootkatone and b-caryophyllene oxide using GNPS, and similar sesquiterpenoids have been reported from this genus before [31]. In addition, a feature was matched to and spectral matching led to the putative identification of the carotenoid derivative (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-6,10,14,18-tetraene-1,2,3,22,23-pentol and other alkaloids such as (E)-9-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylnon-8-en-1-one, piperanine, piperine, magnocuranine, feruloyltyramine and 2-aminoanthraquinone, and the flavonoids tetramethylscutellarein and sinensetin.

The LC-MS/MS analysis of the ethanolic root extract of *A. affinis* led to the putative identification of abundant protoberberine and bisaporphine alkaloids such as jatrorrhizine, dihydroberberine and palmatin, previously reported from this source [31], in addition to sesquiterpenoids, and less abundant signals for piperine and other related alkaloids. These results are consistent with the results of the phytochemical screening which showed the presence of alkaloids. The molecular networking analysis also revealed the presence of possible new alkaloids, not previously isolated from *Annickia*, warranting future preparative efforts to access these relatively minor components.

After oral administration of a single dose of the ethanolic extract of *A. affinis* roots, no abnormal variation in physiological parameters was observed in different batches of rats compared with those in control batch which received distilled water (Table 2). Therefore, the ethanolic extract of *A. affinis* roots was considered

as non-toxic at doses of 2000 and 5000 mg/kg, indicating that the lethal dose 50 (LD50) was greater than 5000 mg/kg. Batch 2, the negative control group, was treated with DMSO/olive oil (5/95, v/v) at 1 mL/kg.

The acute toxicity study of the ethanolic extract of the *A. affinis* roots on female Wistar rats showed that the extract administered orally did not cause any mortality at the limit doses of 2000 mg/kg and 5000 mg/kg body weight. Therefore, the $LD_{50,}$ if it exists, would be higher than 5000 mg/kg. This confirms the study by Ali et al. in 2023 on the acute toxicity at 2000 mg/kg and 5000 mg/kg not only of the methanolic extract of the *A. affinis* roots, but also on those of the leaves, and stem bark which showed no mortality in rats during the study [9].

As shown in the Figure 2, an increase in the weight of the rats was observed every two days over the 14 days of observation, irrespective of the solution administered. At doses of 2000 and 5000 mg/kg, ethanolic extract of A. affinis roots resulted in an increase in the body weight of the animals over the 14 days of observation. However, in the 5000 mg/kg group, there was a significant decrease in body weight gain compared with the normal control that received distilled water ($p < 0.05$) and also compared with the normal control that received the 5% DMSO + 95% olive oil mixture ($p < 0.05$, $p < 0.01$) throughout the experiment. In the 2000 mg/kg group, there was a non significant ($p > 0.05$) decrease in body weight gain (Figure 2).

The results showed no significant difference in the relative weight of organs ($p > 0.05$) such as heart, lungs, kidneys and spleen in the batches receiving the extract at doses of 2000 and 5000 mg/kg compared to the control batches. On the other hand, there was a significant increase in liver weight in batches 2-3 ($p < 0.05$) and batch 4 ($p < 0.01$) compared to the normal control batch that received distilled water (Figure 3).

No variation in behavioural parameters was observed in any of the rats (male and female), either in the test or control batches (Table 3).

In the subacute toxicity study, an ethanolic extract of *A. affinis* roots administered orally at repeated doses of 200, 400 and 800 mg/kg did not cause any deaths. The extract would have a toxicity index of 5 on the Hodge and Sterner scale of chemical toxicity, depending on the LD_{50} and route of administration [33]. However, no signs of toxicity were observed during the first few hours after administration of the ethanolic extract, including decreased sensitivity to stimuli (pain and noise), decreased mobility, or softening of the feces.

In the female rat's groups, there was a significant decrease in body-weight gain ($p < 0.05$) in the batch receiving the extract at 200, 400 and 800 mg/kg compared to those receiving distilled water or the DMSO + olive oil from day 4 to day 16. From day 20 to day 28, a significant increase ($p < 0.001$) in body-weight gain was observed in the groups receiving the 5% DMSO + 95% olive oil mixture and the extract at 800 mg/kg. The same increase was observed on days 24 and 28 in the groups receiving the extract at 200 and 400 mg/kg compared with the control receiving distilled water. A significant decrease in weight gain ($p < 0.05$) was observed throughout the experiment in the test batches compared with the control receiving the 5% DMSO + 95% olive oil mixture, except on days 20 and 28 when an increase in weight gain was observed (Figure 4A). In male rat groups, the group receiving the DMSO + olive oil mixture and the extract at doses of 200 and 400 mg/kg throughout the experimental period had a significant increase in body-weight gain. A decrease in weight gain was observed from day 8 to day 12. From day 16 to day 28, there was a significant increase in body-weight gain compared to the control with distilled water. Compared with the group receiving the 5% DMSO + 95% olive oil mixture, the results showed an increase in body-weight gain throughout the experimental period in the groups receiving the plant extract at 200 and 400 mg/kg, and a significant decrease in this gain in the group receiving the extract at 800 mg/kg during the 28 days of treatment (Figure 4B).

The results showed no significant difference ($p > 0.05$) in the relative weight of its organs except for the left kidney, where a significant increase in its relative weight was observed in the batches that received the extract at the respective doses of 200 ($p < 0.05$), 400 ($p < 0.01$) and 800 mg/kg ($p < 0.01$) in male rat groups (Figure 5A). In male rat groups, no significant difference ($p > 0.05$) in relative organ weights was noted except for the liver in animals given the plant extract at 800 mg/kg where a significant increase ($p < 0.01$) in relative weight was observed compared to the normal control batch (Figure 5B).

The average body weight of rats in all lots increased during the observation period. Comparison of the initial values on day zero with the final values on day 28 of the test and control batches using the two-way ANOVA test showed no significant difference ($p > 0.05$). These results support the hypothesis of a probable safety of the extract administered to the rat batches, since otherwise a decrease in the body mass of the different batches would have been observed due to the disruption of the normal metabolism of these animals [34]. The body weight gain of males in all test batches is higher than that of female rats test batches with an average gain of 54.25 g in male rats and 43.62 g in female rats between day 0 and day 28. These results are similar to those of Etame et al. and Tankeu et al., where the weight gain of males and females generally increased regardless of the selected batch, and the weight gain of male rats was greater than that of female rats [35, 36].

Biochemical markers liver (ALT, AST and gamma-glutamyl transferase (gamma-GT)) and of kidney (urea and creatinine) function to assess the subacute toxicity are shown in Figure 6. For ALT, no significant difference ($p > 0.05$) was observed except in the female group receiving the extract at the dose of 800 mg/kg where an increase in ALT activity was observed compared to the control group receiving distilled water (Figure 6A, $p < 0.001$) in female rats. For male rats, we observed an increase in ALT activity in the batches receiving the extract at 400 and 800 mg/kg compared to the control group receiving distilled water ($p = 0.002$) and control group receiving DMSO (Figure 6B, $p = 0.05$). In Figure 6C, no significant difference ($p > 0.05$) in AST activity in the female batches receiving the different doses of the extract compared with the female control batches, whereas, in male groups, a significant increase of its activity ($p < 0.05$) was observed in the batches receiving the extract at 200 and 800 mg/kg and a decrease in the batch receiving the extract at 400 mg/kg compared to the batch receiving DMSO and the control batch with distilled water (Figure 6D). Compared to the normal female control batch receiving distilled water, there was a significant increase in gamma-GT activity in the female batches receiving the DMSO ($p < 0.05$) and the extract at 400 mg/kg (Figure 6E, $p < 0.001$) whereas, no significant ($p > 0.05$) variation in gamma-GT activity was observed between the male batches receiving the plant extract compared with the control batches (Figure 6F). Concerning the concentration of urea, there was a significant decrease of this concentration in the female batches receiving the plant extract at the different doses compared with the control receiving distilled water ($p < 0.001$) and the control batch receiving the DMSO (Figure 6G). In male rats, the data showed a decrease in urea levels in the batches receiving the DMSO and the extract at 200 and 800 mg/kg compared with the control receiving distilled water

(Figure 6H). There were no significant differences in serum creatinine levels between female rats (Figure 6I). The data showed an increase in creatinine concentrations in the male batches receiving DMSO and the extract at 200 and 800 mg/kg, compared with the control receiving distilled water and a decrease in the batch receiving the extract at 400 mg/kg (Figure 6J).

The enzyme ALT is more specific for liver injury, but AST is slightly more sensitive [37]. Subacute treatment of rats at different doses resulted in an increase in serum ALT in female rats given the 800 mg/kg extract ($p < 0.05$). In males, a significant increase ($p < 0.05$) in ALT was observed in batches receiving the extract at 400 and 800 mg/kg compared to control batches. In their study, Tankeu observed a decrease in ALT levels in all rats tested. This difference may be explained by the fact that males are more sensitive to the effect of repeated doses of methanolic extract of *A. affinis* [36]. With regard to AST, no significant difference ($p < 0.05$) was observed in the batches of female rats receiving the different doses of the extract compared with the control batches. With regard to AST in male rats, a significant increase ($p > 0.05$) in its activity was observed in the batches receiving the extract at the doses of 200 and 800 mg/kg, and a decrease in the batch receiving the extract at 400 mg/kg compared to the batch receiving solution of DMSO (5% in 95% olive oil) and the control batch with distilled water. These results are supported by those of Etame et al. who also observed a decrease in the serum AST levels in male rats in all batches [35]. Biologically, the dose-dependent increases in ALT and AST, despite normal macroscopic organ architecture, indicate the initiation of mild, early hepatic stress or subclinical hepatocellular leakage, underscoring the liver's function in the extract's detoxification. The isolated urea assay is used to study protein metabolism, while the combined urea-creatinine assay is used to assess renal function [38]. While creatinine levels remained stable, the dose-dependent increase in urea observed in both sexes suggests an increased renal filtration burden or altered nitrogenous waste metabolism, highlighting the need for caution during prolonged use. In the present study, we observed a decrease in serum urea and creatinine levels independent of dose and gender. Serum urea and creatinine are considered the main markers of nephrotoxicity, although serum urea is often considered a more reliable predictor of renal function than serum creatinine [39].

In female groups, a significant increase of the blood platelet rate in the batches receiving the extract at different doses was observed compared to the batch receiving the DMSO (Table 4). This increase was of the order of 55.57% ($p < 0.001$), 36.22% ($p < 0.001$) and 18.04% ($p < 0.01$) in the batches receiving the *A. affinis* extract at the different doses of 200, 400 and 800 mg/kg respectively, no significant difference ($p > 0.05$) was observed for the other parameters. In male groups, no significant difference ($p > 0.05$) was observed for these different parameters except for the blood platelet rate with a significant increase in the batches receiving the extract at the different doses compared to the batch receiving the DMSO. This increase was of the order of 54.25% ($p < 0.001$), and 27.29% ($p < 0.001$) in the batches having received the extract of *A. affinis* at the respective doses of 200 and 400 mg/kg (Table 4). The observed thrombocytosis (elevated platelet counts) may reflect a secondary, compensatory physiological response to mild systemic inflammation induced by the extract, though it did not culminate in observable overt pathology. Although the increase in platelet counts reached statistical significance, the values generally remained within the broader physiological reference range for Wistar rats, indicating a sub-clinical stress response rather than a severe pathological thrombotic risk.

Figure 7 shows the effects of ethanolic extract of *A. affinis* roots on the structure of the liver, kidney, and heart in females after 28 days of treatment. Both in female (Figure 7A) and male (Figure 7B) groups, the batches treated with the plant extract at 200, 400 and 800 mg/kg show a normal architecture of the liver (hepatic parenchyma with a centro-lobular vein and distinct hepatocytes), kidney (normal parenchyma with a distinct glomerulus and urinary space) and heart (distinct muscle fibres and nuclei) as observed in the batches receiving distilled water or DMSO. No evidence of structural alteration changes was observed in the different batches.

Histological analysis of the major organs involved in the detoxification mechanisms showed no damage to the liver, kidneys or heart. Vascular congestion is usually caused by back pressure in the vein leading to the build-up of blood cells. When a vein becomes congested, fluid flows into the parenchyma of the affected organ, causing interstitial oedema [40]. All these results support the hypothesis that the ethanolic extract of *A. affinis* roots, administered in repeated doses for 28 days, shows a safety profile; however, the mild elevations in liver enzymes and urea indicate that prolonged systemic exposure at high doses requires caution. A semi-quantitative histological assessment revealed negligible scoring (Grade 0-1 on a 4-point scale) for hepatocyte vacuolation, vascular congestion, and interstitial edema across all treated groups compared to controls.

A. affinis roots were subjected to an antimalarial test on multi-Drug-Resistant *Plasmodium falciparum* Dd2 strain. The dose-response curve fitting for the crude extract demonstrated high reliability ($R^2 = 0.98$), with the extract exhibited an IC_{50} of 2.08 µg/mL (95% CI: 1.85 – 2.34 µg/mL). For comparison, the positive controls chloroquine and artemisinin yielded IC_{50} values of 0.2 µg/mL (95% CI: 0.18 – 0.23) and 0.01 µg/mL (95% CI: 0.009 – 0.012), respectively (Table 5). This value is much higher than the IC_{50} obtained for chloroquine, to which the strain is resistant. This suggests that a strong resistance signal has been identified, with the Dd2 *P. falciparum* strain showing a more than 10-fold increase in IC_{50} to the crude extract tested compared to the values for chloroquine.

The *in vitro* evaluation of the antiplasmodial activity of the *A. affinis* root extract on a Dd2 strain of *Plasmodium falciparum* expresses a percentage of inhibition of 67.59%. This percentage is lower than that of the methanolic extract of the roots on the 3D7 strain of *Plasmodium falciparum* which was about 83.6% [9]. The difference in activity would be related to the fact that these extracts are of different polarities. Furthermore, *Plasmodium falciparum* strain 3D7 (chloroquine and artemisinin sensitive) was not a multidrug resistant strain compared to Dd2 strain (chloroquine resistant and artemisinin sensitive) used in these studies. On the other hand, the *A. affinis* root extract showed an IC_{50} of 2.08 µg/mL which was much lower than that of the methanolic root extract on *Plasmodium falciparum* 3D7 strains (19.7 µg/mL) [9]. These results are also close to those of Erhunse et al., in 2023 on *Plasmodium falciparum* 3D7 strains, who reported that the aqueous extracts of *Annickia affinis* stem bark have potent antiplasmodial activity with an IC_{50} value of 1.49 µg/ml [8]. According to Singh et al., 2015 efficacy score for *in vitro* antiplasmodial activity, the plants were classified for their antiplasmodial potential as highly active ($IC_{50} < 5$ µg/mL), promisingly active (IC_{50} 5.1 – 10 µg/mL), good activity (IC_{50} 10.1 – 20 µg/mL), moderate activity (IC_{50} 20.1 – 40 µg/mL), Marginal potency (IC_{50} 40.1 – 70 µg/mL), and poor or inactive ($IC_{50} > 70.1$ µg/mL); we can say that the ethanolic extract of *A. affinis*

roots have high activity [41] and is more active than of the methanolic extract of the roots. Phytochemical screening of the ethanolic extract of *A. affinis* roots revealed that they are rich in alkaloids, and sesquiterpenes, suggesting potential antimalarial activities for these classes of secondary metabolites. For example, according to Wright et al., in 2000, protoberberine alkaloids such as berberrine, jatrorrhizine, columbamine and canadine possess potent antiplasmodial activity against *Plasmodium falciparum* with IC_{50} values of 0.97, 3.16, 1.93, 1.93 and 4.14 mM respectively [42]. These results are similar to studies reported by Muganza et al., in 2012 on aqueous extracts of *Enantia chlorantha* stem bark against a chloroquine and pyrimethamine resistant strain of *Plasmodium falciparum,* which showed an IC_{50} of 7.77 µg/mL [43]. Vennestrom and Klayman reported in 1988 that palmitin and jatrorrhizin isolated from *E. chlorantha* had an antimalarial activity against clones D-6 (clones obtained in Sierra Leone and resistant to mefloquine) and W-2 (clones obtained in Indonesia and resistant to chloroquine, pyrimethamine, sulfadoxine and quinine) with IC_{50} values of 0.46 and 4.75 µM, respectively [44]. However, the ethanolic extract of *A. affinis* roots showed reduced antiplasmodial activity compared to the reference molecules used, chloroquine (IC_{50} of 384.60 nM) and artemisinin (IC_{50} of 22.47 nM). It is important to note that protoberberine alkaloids are very effective against *Plasmodium* species, but they can also be toxic to mammalian cells in a dose-dependent way. This alkaloid load may help to explain the mild liver stress and high urea levels seen at the highest test doses.

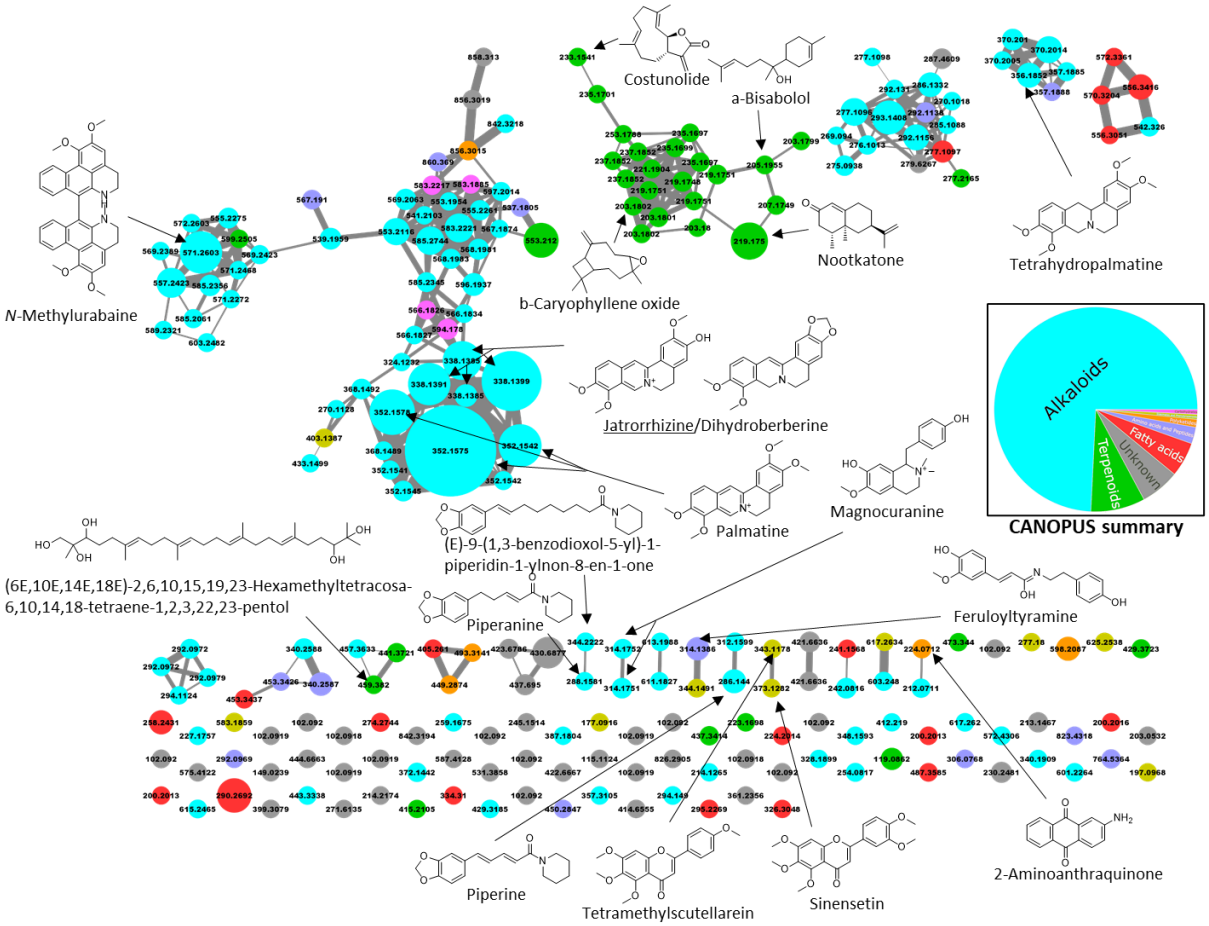

Figure 1. Annotated molecular network of *A. affinis* ethanolic roots extract.

Node sizes increase with signal intensity, node colors indicate natural product classification according to CANOPUS (peak area-based summary shown in the inlet; alkaloids = turquoise, dark green = terpenoids, gray = unknown, red = fatty acids, blue = amino acids and peptides, orange = polyketides, olive = shikimates and phenylpropanoids, purple = carbohydrates). Molecular structures indicate spectral matches found through GNPS.

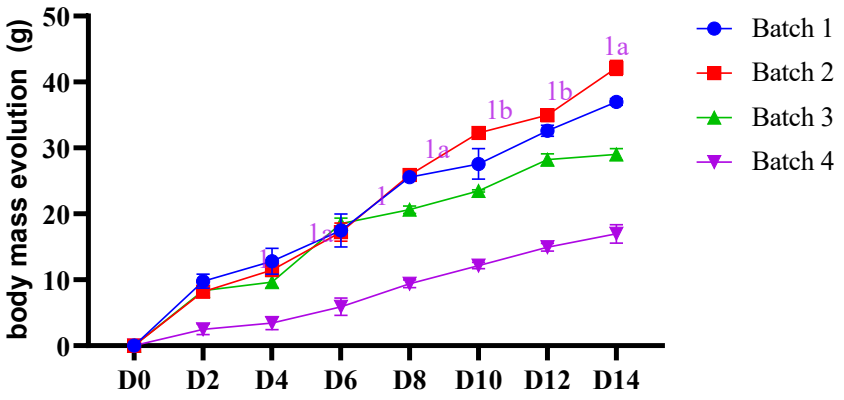

Figure 2. Evolution of the weight growth of rats (3 rats per batch) during acute toxicity.
Each point represents the mean ± Standard Error on the Mean (SEM); Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3 and Batch 4: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 2000 and 5000 mg/kg, respectively; [1]p < 0.05: significant differences from batch 1, [a]p < 0,05, [b]p < 0,01: significant differences from Batch 2.

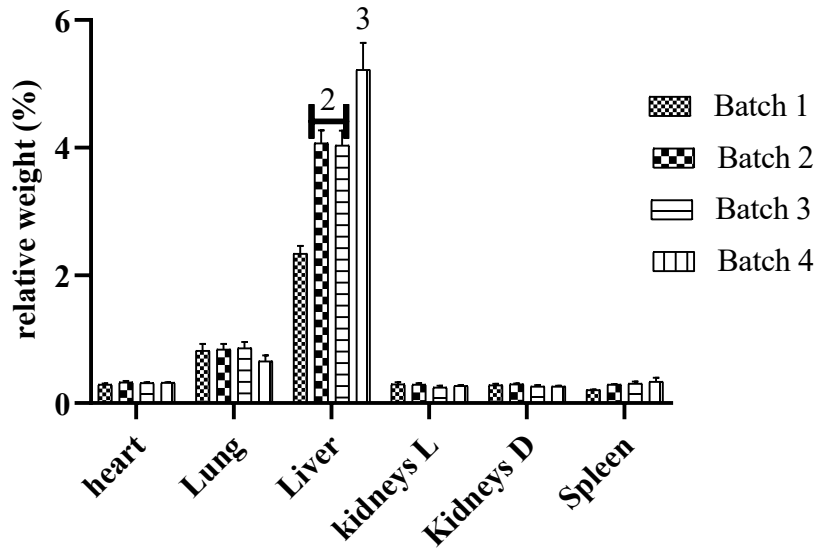

Figure 3. Effects of ethanolic extract of *A. affinis* roots on the relative weight of some organs of rats (3 rats per batch).
Each bar represents the mean ± SEM; Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3 and Batch 4: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 2000 and 5000 mg/kg, respectively; [2]p < 0.05, [3]p < 0.01: significant differences from batch 1.

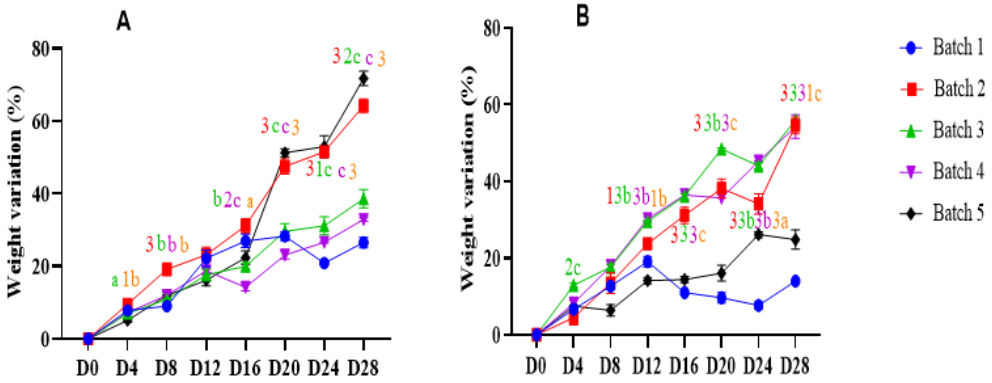

Figure 4. Effects of ethanolic extract of *A. affinis* roots on weight gain in female (3 rats per batch) (A) and in male (3 rats per batch) (B).

Each point represents the mean ± SEM; Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3, Batch 4 and Batch 5: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 200, 400 and 800 mg/kg, respectively. [1]p < 0,05, [2]p < 0,01, [3]p < 0,001: significant differences from batch 1, [a]p < 0,05, [b]p < 0,01, [c]p < 0,001: significant differences from Batch 2.

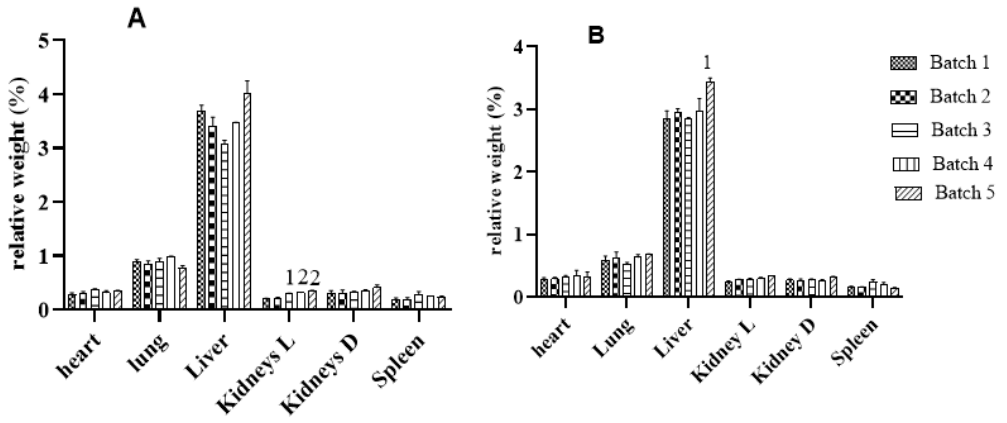

Figure 5. Effects of repeated doses of ethanolic extract of *A. affinis* roots on the relative weight of some organs of female rats (A) and male (B) with 3 rats per batch

Each bar represents the mean ± SEM; Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3, Batch 4 and Batch 5: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 200, 400 and 800 mg/kg, respectively. [1]p < 0,05, [2]p < 0,01: significant differences from batch 1.

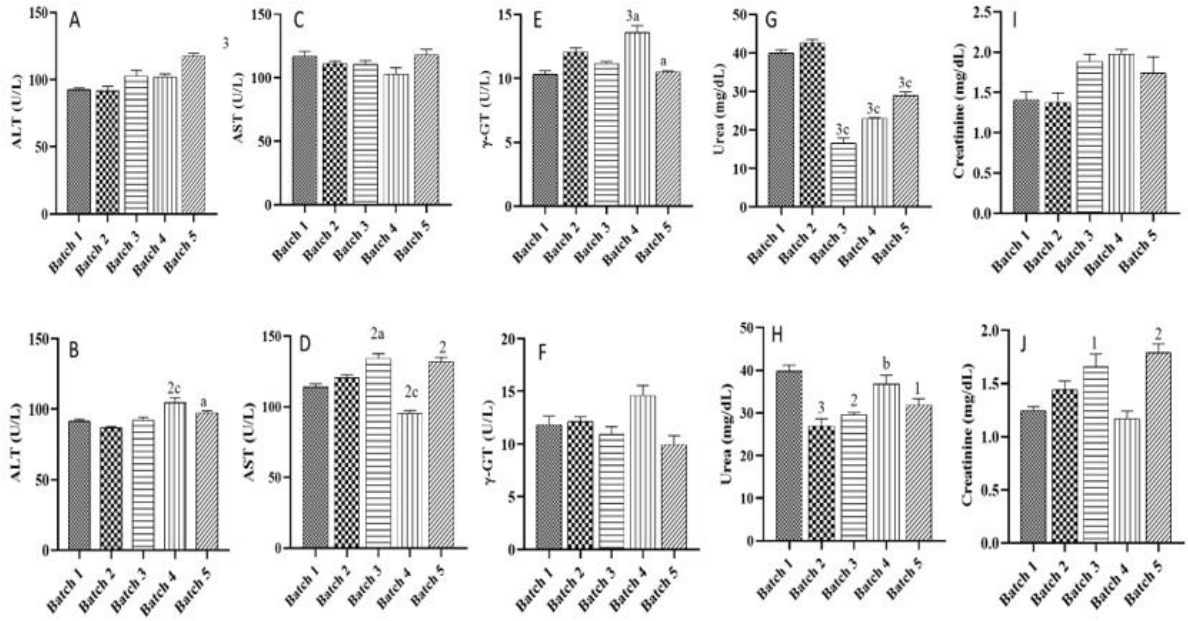

Figure 6. Variation of serum ALT concentration in female (A) and male (B) rats, serum AST concentration in female (C) and male (D) rats, serum gamma-GT concentration in female (E) and male (F) rats, serum urea concentration in female (G) and male (H) rats and serum creatinine concentration in female (I) and male (J) rats.

Each bar represents the mean ± SEM. Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3, Batch 4 and Batch 5: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 200, 400 and 800 mg/kg, respectively. Fig 6A, [3]p < 0.001: significant differences from batch 1. [1]p < 0.05, [2]p < 0.01, [3]p < 0.001: significant differences from batch 1; [a]p < 0.05, [b]p < 0.01, [c]p < 0.001: significant differences from Batch 2. Dunnett's test was used for statistical analysis.

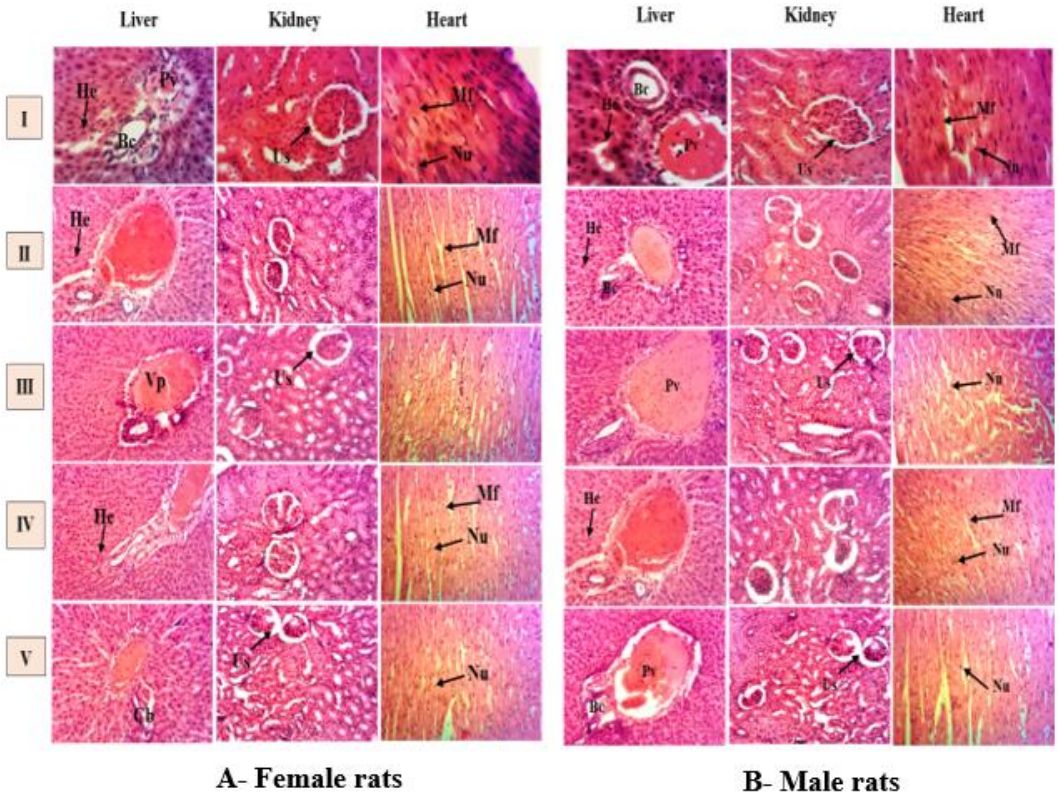

Figure 7. Effects of ethanolic extract of *Annickia affinis* roots on liver (100X), kidney (200X) and heart (100X) structure in female rats (A) and male (B).

Batches receiving distilled water (I); 5% DMSO + 95% olive oil mixture (II); extract of *A. affinis* roots mixed with DMSO + olive oil at 200 (III), 400 (IV) and 800 mg/kg body-weight (V). *Pv = Portal vein, He = Hepatocyte, Bc = Bilary canaliculi, Ha = Hepatic artery; Kidney: G = Glomerulus, Us = Urinary space, Heart, Nu = Nucleus, Mf = Muscle fiber; Images were captured at a total magnification of 100x for liver/heart and 200x for kidney tissues*

Table 1. Phytochemical constituents of ethanolic extract of *Annickia affinis* roots

Types of tests	Phytochemicals	Results
Dragendorff	Alkaloids	+
Foam index	Saponins	-
Shinoda	Flavonoids	-
Liebermann-Burchard	Terpenoids	+
Liebermann-Burchard	Steroids	-
Ferric Chloride ($FeCl_3$)	Phenols	-
Borntrager	Anthraquinones	-
Anthocyanins	Anthocyanins	-
Coumarins	Coumarins	+
Stiasny	Tannins	-
Fehling	Reducing sugars	+

(+) = presence ; (-) = absence

Table 2. Observation of physiological parameters of rats of acute toxicity (n = 3 rats/group)

Variables	Distilled water at 1 mL/100 g	5% DMSO in 95% olive oil at 1 mL/kg	Extract of *A. affinis*	
			2000 mg/kg	5000 mg/kg
Number of rats	3	3	3	3
Mortality	0	0	0	0
Coat modification	A	A	A	A
Impaired gait	A	A	A	A
Posture and reaction to manipulation	N	N	N	N
Excessive agitation	A	A	A	A
Trembling	A	A	A	A
Convulsions	A	A	A	A
Stool appearance	N	N	N	N
Reaction to sound	N	N	N	N
Intense thirst	A	A	A	A
Vomiting	A	A	A	A
Salivation	A	A	A	A
Alteration of the process	A	A	A	A

A= Absent; N = Normal

Table 3. Observation of physiological parameters of rats in subacute toxicity (n = 6 rats/group)

Variables	Distilled water at 1 mL/100 g	5% DMSO in 95% olive oil at 1 mL/kg	Extract of *A. affinis*		
			200 mg/kg	400 mg/kg	800 mg/kg
Number of rats	6	6	6	6	6
Mortality	0	0	0	0	0
Coat modification	A	A	A	A	A
Impaired gait	A	A	A	A	A
Posture and reaction to manipulation	N	N	N	N	N
Excessive agitation	A	A	A	A	A
Trembling	A	A	A	A	A
Convulsions	A	A	A	A	A
Stool appearance	N	N	N	N	N
Reaction to sound	N	N	N	N	N
Intense thirst	A	A	A	A	A
Vomiting	A	A	A	A	A
Salivation	A	A	A	A	A
Alteration of the process	A	A	A	A	A

A = Absent; N = Normal; Batch 1: normal control that received distilled water; Batch 2: normal control batch that received the 5% DMSO + 95% olive oil mixture; Batch 3, Batch 4 and Batch 5: tests batches that received the extract of A. affinis roots mixed with DMSO + olive oil at the doses of 200, 400 and 800 mg/kg, respectively.

Table 4. Mean ± SEM of hematological parameters of different female rats for the different samples compared to the control batch (n = 6 rats/group)

Parameters	Mixture (5% DMSO in 95% olive oil)	Extract of *A. affinis*, mg/kg body weight		
	1 mL/kg	200 mg/kg	400 mg/kg	800 mg/kg
Female rats				
RBC (10^3/µL)	7.08 ± 0.19	7.95 ± 0.42	7.51 ± 0.43	8.11 ± 0.28
Hb (g/dL)	13.83 ± 0.83	13.60 ± 0.26	14.40 ± 0.40	13.10 ± 0.55
HCT (%)	44.64 ± 2.00	43.03 ± 0.84	40.72 ± 1.17	40.74 ± 1.23
WBC (106/µL)	6.40 ± 1.20	8.12 ± 0.21	7.24 ± 1.06	7.96 ± 0.57
MCV (fL)	61.33 ± 2.40	55.33 ± 1.45	58.67 ± 2.40	60.67 ± 1.20
LYM (103/µL)	6.90 ± 0.14	6.42 ± 1.15	5.85 ± 0.89	7.77 ± 0.59
GRA (103/µL)	1.16 ± 0.28	0.89 ± 0.19	0.88 ± 0.10	0.70 ± 0.14
MCHD (g/dL)	32.90 ± 0.78	32.50 ± 0.87	32.70 ± 0.12	32.37 ± 0.44
MCMC (g/dL)	18.43 ± 0.59	18.23 ± 0.23	17.73 ± 0.64	18.03 ± 0.24
PLT (103/µL)	341.00 ± 7.51	515.50 ± 7.22^c	464.50 ± 3.78^c	402.50 ± 13.57^b
VPM (fL)	7.00 ± 0.12	6.93 ± 0.26	6.83 ± 0.12	6.87 ± 0.9
Male rats				
RBC (10^3/µL)	7.86 ± 0.14	7.25 ± 0.31	7.57 ± 0.26	7.21 ± 0.47
Hb (g/dL)	15.00 ± 0.50	13.33 ± 0.41	13.70 ± 0.12	13.00 ± 0.75
HCT (%)	46.03 ± 0.93	41.02 ± 2.07	42.79 ± 1.29	42.86 ± 1.66
WBC (106/µL)	8.87 ± 1.57	7.92 ± 0.65	8.68 ± 0.87	7.74 ± 1.09
MCV (fL)	60.00 ± 0.58	55.00 ± 0.60	60.67 ± 2.60	61.67 ± 1.76
LYM (103/µL)	7.04 ± 1.27	5.37 ± 1.31	6.74 ± 0.87	5.44 ± 0.80
GRA (103/µL)	1.06 ± 0.09	1.28 ± 0.16	0.66 ± 0.20	1.02 ± 0.24
MCHD (g/dL)	32.23 ± 0.52	31.00 ± 0.85	32.90 ± 0.75	31.83 ± 0.24
MCMC (g/dL)	18.67 ± 0.57	18.23 ± 0.33	18.90 ± 0.36	18.53 ± 0.07
PLT (103/µL)	354.30 ± 8.67	546.50 ± 7.79^3	451.00 ± 8.08^3	368.00 ± 14.43
VPM (fL)	7.20 ± 0.06	6.93 ± 0.18	7.00 ± 0.58	6.70 ± 0.15

Measurements were taken for each test, with 3 rats per batch. Each value represents the mean ± SEM. RBCs = Red blood cells, Hb = Hemoglobin, HCT = Hematocrit, WBC = White blood, MCV = mean globular volume, LYM = lymphocyte, GRA = granulocytes, MCHD = mean corpuscular hemoglobin content, MCHC = mean corpuscular hemoglobin concentration, PLT = Platelets. $^b p < 0.01$, $^c p < 0.001$: significant differences with the control.

Table 5. Sensitivity of Dd2 *P. falciparum* strain to crude extract of *Annickia affinis* roots and known antimalarial drugs

Samples	IC_{50} (nM)	IC_{50} (µg/mL)
Chloroquine	384.6	0.2
Artemisinine	22.47	0.01
Annickia affinis roots	/	2.08

[a] Values are from one representative experiment

Conclusion

Hyphenated liquid chromatography-tandem mass spectrometry analysis indicated the presence of protoberberine, bisaporphine and other related alkaloids, as well as sesquiterpenoids in the ethanolic root extract of *A. affinis*. No evidence of toxicity to Wistar rats was observed throughout the study, suggesting that the ethanolic root extract of *A. affinis* is non-toxic. This extract represents a potentially good candidate for the formulation of improved traditional medicines. Accordingly, the ethanolic extract of *A. affinis* roots reduced the viability of *Plasmodium falciparum* Dd2 cells with an IC_{50} value of 2.08 µg/mL, with improved activity compared to the methanolic extract. This activity is likely to be directly related to its alkaloid content based on previously reported data. Furthermore, the antiplasmodial activity of the test extract was found to be weak compared to the positive controls on the laboratory strains. It would be interesting to test these extracts and their fractions on field isolates and purify alkaloids, especially potentially new ones, to test the isolated compound material for antiplasmodial activity.

Additional file

The GNPS supporting results associated with this article can be found at:

https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=e44def99dc3f4641b3086a9b1360e2be.

Abbreviations

ALT: Alanine aminotransferase
ANOVA: Analysis of Variance
AST: Aspartate aminotransferase
BEI: Biodefense and Emerging Infections
CPC: Centre Pasteur du Cameroun
DMSO: Dimethylsulfoxide
DNA: Deoxyribonucleic acid
EDTA: Ethylenediaminetetra-acetic acid
ESI: Electrospray Ionization
EtOH: Ethanolic / Ethanol
FA: Formic acid
$FeCl_3$: Ferric chloride
γ-GT / GGT: Gamma-glutamyl transferase
GNPS: Global Natural Product Social Molecular Networking
GRA: Granulocytes
Hb: Hemoglobin
HCT: Hematocrit
HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HNC: National Herbarium of Cameroon (Herbarium National du Cameroun)
HR-MS/MS: High-resolution tandem mass spectrometry
IC_{50}: Half-maximal inhibitory concentration
LC-MS: Liquid Chromatography-Mass Spectrometry
LD_{50}: Median Lethal Dose
LYM: Lymphocytes
m/z: Mass-to-charge ratio
MCHC: Mean corpuscular hemoglobin concentration
MCV: Mean corpuscular volume / Mean globular volume
OECD: Organisation for Economic Co-operation and Development
PLT: Platelets
RBC: Red blood cells
RP-UPLC: Reversed-Phase Ultra-Performance Liquid Chromatography
RPMI: Roswell Park Memorial Institute (culture medium)
RT: Retention time
SEM: Standard error of the mean
UA: Uric acid
ULB: Université Libre de Bruxelles
UPLC: Ultra-Performance Liquid Chromatography
VPM: Mean platelet volume (Volume plaquettaire moyen)
WBC: White blood cells

Authors' Contribution

Conceptualization, JEMT and CEEM; Methodology, JEMT, JANK and GMMEL, SVF; Software, PBEK; Validation, JEMT, JANK, GMMEL, CEEM; Formal Analysis, JEMT, ETM, L.A. and NME; Investigation, ETM, NME and LA; Resources, TFN and JEMT; Data Curation, PBEK, TFN and CEEM; Writing – Original Draft Preparation, JEMT and CEEM; Writing – Review & Editing, all authors.; Visualization, JEMT and SVF; Supervision, JEMT, G.M. GMMEL and CEEM. All authors have reviewed the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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