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Jean Emmanuel Mbosso Teinkela
Professor, Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Iselin Fipa Pieumeu
Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Jean Baptiste Hzounda Fokou
Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Thierry Fokou Nzodjou
Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Jules Clément Assob Nguedia
Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Edwige Laure Nguemfo
Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

Corresponding Author:
Professor, Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box. 2701 Douala, Cameroon

In vivo subacute toxicity and antimicrobial properties of *Annickia affinis* (Exell) Versteegh & Sosef stem bark

Jean Emmanuel Mbosso Teinkela, Iselin Fipa Pieumeu, Jean Baptiste Hzounda Fokou, Thierry Fokou Nzodjou, Jules Clément Assob Nguedia and Edwige Laure Nguemfo

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Abstract
Annickia affinis is a medicinal plant of the Annonaceaes family widely used in traditional medicine for the management of typhoid, diarrhoea and urinary tract infections, hence our interest in this plant. Therefore, our work focused on the study of the subacute toxicity and the antimicrobial activities of the methanolic extract of the *Annickia affinis* (Exell) Versteegh & Sosef stem bark.
The *in vivo* subacute toxicity test was performed according to the modified Organisation for Economic Co-operation and Development (OECD) guidelines 407 with slight modification over a period of 28 days, with 4 batches of 6 rats (3 male and 3 female albino Wistar rats). The antimicrobial potential was evaluated on three bacterial strains (*Escherichia coli*, *Salmonella typhi* and *Neisseria gonorrhoea*) and one fungal strain (*Candida albicans*) using the micro-dilution broth method for the determination of MICs and MFC and MBCs.
Phytochemical screening of different extracts revealed the presence of anthocyanins, alkaloids, saponins, triterpenoids, coumarins and anthraquinones. Regarding the toxicity testing, No deaths were recorded at the different doses and, physiological, biochemical and histological parameters analyses revealed a non-significant statistical difference. The methanolic extract of *A. affinis* stem bark exhibited the best antibacterial activity on *S. tiphy* and *N. gonorrhoea* strains (MIC 39 µg/ml), weak antibacterial activity on *E. coli* (MIC = 2500µg/ml) and not antifungal activity was observed on *Candida albicans* (MIC > 10000 µg/ml). According to the BMC/MIC ratios, our extract is bactericidal on *E. coli*, *S. typhi*, and *N. gonorrhoea*.
This study highlights the antibacterial effect of the methanolic extract of *Annikia affinis* on *S. typhi* and *N. gonorrhoea* strengthening his folkloric usage in management of typhoid and bacteril infection of the smooth muscle of acceptable safety.

Keywords: Chhani, consumption, fuel-wood, households, Lanchaan

Introduction
Infectious diseases are considered as a major threat to human health due to the unavailability of vaccines or limited chemotherapy. It accounts for about half of all deaths in tropical countries. Infectious diseases, ranked 5th in 1981, became the 3rd leading cause of death in 1992, with an increase of 58% [1]. The WHO estimated the number of cases due to infectious diseases at 582 million in 2015, including 351,000 related deaths [2]. Most current antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects and their excessive use on a large scale has led to an increase in clinical resistance of previously susceptible microorganisms and the appearance of infrequent infections [3]. Thus, bacterial resistance to antibiotics and the risks of toxicity related to the massive use of drugs and synthetic antibiotics, have led researchers to find other natural sources such as plants, thus providing new effective and low-toxic drugs.
The African continent because of its great biodiversity is full of plants, a very high number of which are used as herbs, natural foods, for therapeutic purposes. In addition, many natural substances have been identified and many of them are used in traditional medicine for prophylaxis and treatment of diseases. In order to contribute to the improvement of knowledge about medical plants, and to find an alternative to the problems of resistance of

microorganisms, we were interested in *A. affinis*, a plant from the Annonaceae family, that is also called *Enantia affinis* Exell (1926). The vernacular names are African yellow wood, and yellow wood (in Englis), moambe Jaune (in French).

The choice of this plant was motivated on the one hand by its use in traditional medicine in the treatment of typhoid, diarrhea, urinary tract infections and to treat lesions and ulcers [36], on the other hand, a literature review revealead no study on the antibacterial and antifungal activities of *A. affinis.* However, recent study have been carried out on phytochemical screening, acute toxicity, antioxidant activity [5] and also cytotoxic and antiplasmodial activities [6, 7] of different parts, of *A. affinis*. Therefore this study aimed at evaluating the subacute toxicity and antimicrobial activities of *A. affinis* stem bark methanolic extract.

Material and Methods

Plant material

The stem bark of *A. affinis* was collected at Mount Kala, a village in Yaounde neighbourhood, Department of Méfou-et-Akono (Latitude 30 47' 31" North; longitude 110 24' 13" East). The voucher specimen was deposited at the National Herbarium of Cameroon under the number 6420/HNC.

Extraction

The preparation of the methanolic extracts was performed by maceration in 96° as described by Bidié *et al*. in 2008 [8]. Briefly, the harvested stem bark was washed several times with distilled water in order to eliminate any impurity that could affect the evaluation of biological activities. The plant material was then cutted in small pieces and dried at room temperature in a dry and shade place, the reafter, it was ground and yielded a mass of 1800 g of powder. The resulted powder was mixed with 8L ofmethanol 96° and the resulting mixture was stirred for 72 hours at room temperature (25 °C); the mixture was filtered three times through cotton wool and on 3 mm Wattman filter paper. Finally the filtrate was evaporated at 60 °C using a rotary evaporator (Heidolph LABOROTA 4000); the crude extract obtained was weighed (65 g) and stored in a refrigerator (4 °C) prior to further uses.

Animal material

A total number of 30 Wistar rats were randomly selected and divided into five (5) different treatments batches, each batch comprises of 6 healthy animals (3 males and 3 females).

Phytochemical screening of crude extract

Detailed phytochemical screening was performed on the methanolic extract of *A. affinis* stem bark using standard methods described by Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro *et al*. (2007), as reported in the literature [9-12]. Other specific phytochemical tests were also realized, all based on a precipitation reaction via the generation of insoluble complexes called precipitates, and on colorimetry through the formation of colored soluble chemical species. The color reactions were carried out in test tubes in the presence of the reference positive controls. The following tests were used: Drangendorff test (alkaloïds), Tannins (gallic tannins), Liebermann-Burchard test (steroïds and triterpenoids), Shinoda (flavonoïds), anthocyanins test (anthocyanins), Borntrager (anthraquinones), Foam Index test (saponins), $FeCl_3$ test (polyphenols), coumarins (Potash test) and Reducing Sugars test. All observations were recorded.

Subacute Toxicity

Subacute toxicity has been studied as per OECD Guideline 407 with slight modifications [13] at the Pharmacology and Toxicology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. Nine-week-old Wistar albino, male and female rats were divided into three experimental batches of six (6) 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 (3) batches of six (6) randomized rats which received the methanolic extract of *A. affinis* stem bark, at doses of 200, 400 and 800 mg/kg body weight, respectively. The control batch received distilled water. The continue administrations for 28 days with 6 days of administration out of seven per week. After 28 days, the organs removed: liver, heart and kidneys were rinsed with 0.9% saline solution (physiological solution), then observed *in situ* and weighed.

The biochemical parameters measured were serum urea, creatinine, aspartate aminotransferase (AST) alanine aminotransferase (ALT); Organ sections in histology.

Biochemical Analysis

The following serum parameters were measured by enzymatic methods: Aspartate transaminase (AST) by the optimized UV IFCC kinetic method, using the GSMitalia GOT-AST LR kit; alanine aminotransferase (ALT) by the optimized UV IFCC kinetic method, using the GSMitalia GPT-ALT LR kit; Creatinine (Creatine) by the Jaffé colorimetric method, using the CREATININE LR SGMitalia kit; Uric acid (UA) by the Urease-GLDH UV kinetic method, using the UREA UV SGMitalia kit; These assays were carried out at the Animal Physiology Laboratory Laboratories of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala.

Histology Examination

The histological procedure was carried out by the method described by Biswas *et al* in 2010 with some modifications [14]. The liver, heart and kidney from both the treated and control groups was processed with automatic tissue processor (STP 120) by tissue processing method as described by Galen and Gambino, 1975 [15]. Histology preparation was done in 4 µm tissue sections with a Microtome (Leica, RM 2145). These sections were then deparaffinated in xylene, dehydrated through a graded ethanol series, and stained with haematoxylin-eosine and cleared in xylene I and xylene II and these organs were preserved for microscopic examination. The slides prepared by this process were observed under microscope (Model Nikon Labophot. 223425 Japan) and photographed through Nikon labophot Advanced Research Microscope, Model 223425 Japan, with Sony Digital 12.1 MEGA PIXELS. This assay was carried out at the Animal Physiology Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1.

Antimicrobial activity

The evaluation of the antimicrobial activity was carried out after the sterility control of the methanolic extract of *A.*

affinis stem bark. In order to determine the parameters of inhibition: "The minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC)". Following the Principle Based on the microdilution method, adopted by the Société Française de Microbiologie (SFM) and updated in their report with the EUCAST in 2019 (European Committee on Antimicrobial Susceptibility Testing). This method consists in observing the color change of the colored indicator (phenol red) which passes either from red to yellow due to the release in the medium of acid metabolites (acetic acid), or from red to purple when the products resulting from the metabolism of the bacteria during their growth are basic. In the absence of growth, there is no change in coloration [16]. For the demonstration of antimicrobial activity, the extract was tested on several bacterial strains (*Escherichia coli*, *Salmonella typhi* and *Neisseria gonorrhoea*) and a fungal strain (*Candida albicans*). This assay was carried out at the Bacteriology Laboratory of the District Hospital of the Cité des Palmiers.

Data Analysis and Statistical Parameters

Excel software was used to record the data of rat weight tracking, organ masses and biochemical marker concentration. Both disk GraphPad Prism 8.0.1. Software allowed us to plot the curves and perform statistical analysis by one-way ANOVA and two-way ANOVA methods through the parametric test of DUNNETT with a significance level of 5%. Probability values (p) less than 0.05 indicated a significant difference between the samples compared.

Results

Phytochemical Screening

Phytochemical screening (Table 1) showed that methanolic extract of *A. affinis* stem bark contains alkaloids, saponins, triterpenoids, coumarins, anthocyanins and anthraquinones, while flavonoids, phenols, sterols and tannins are absent.

Table 1: Phytochemical screening of methanolic extract of *A. affinis* stem bark

Types of tests	Phytochemicals	Results
Dragendorff	Alkaloids	+
Foam index	Saponins	+
Shinoda	Flavonoids	-
Libermann-buchard	Triterpenoids	+
Libermann-buchard	Steroids	-
Ferric Chloride ($FeCl_3$)	Phenols	-
Borntrager	Anthraquinones	+
Anthocyanins	Anthocyanins	+
Coumarins	Coumarins	+
Stiasny	Tannins	-

(+) = presence; (-) = absence

Subacute toxicity

Clinical parameters of rats

The animals were observed after the first 30 minutes, then at 1 h, 2 h, 4 h, 8 h, and then every 24 h for 28 days, and the results were the same for all the rats tested (male and female) in our research (Table 2).

Table 2: Observation of clinical parameters of rats of subacute toxicity

Parameters observed	Batch 1	Batch 2	Batch 3	Batch 4
Number of rats	6	6	6	6
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; Batch 1, Batch 2 and Batch 4: tests batches that received the *A. affinis* extract at the doses of 200, 400 and 800 mg/kg of body weight respectively; Batch 4: normal control group that received 1 ml/kg of distilled water body weight.

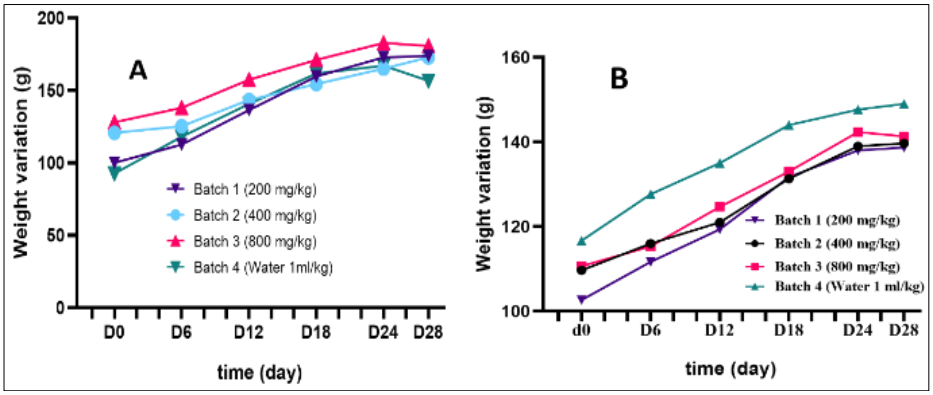


Fig 1: Evolution of the animal weight of male (A) and female (B) during the tests,

Evolution of the weight growth of male rats

The weight monitoring along the 28 days is summarised in Figure 1. From that figure, it come neither out that there were no weight loses nor gain in both sex (Figure 1 A and B) during the tests.

Effect of repeated doses of methanolic extract of *A. affinis* stem bark on animals organs Globally, the variation in organ weight in both sex was not significant when comparing each treated animal with the correct control batch, with $p > 0.05$ (Figure 2). However, the weight of the lung seems to be affected by the concentration of the extract as a decrease in the lung relative weight is visually observed in both sexes. However, these differences were not statistically different from the control.

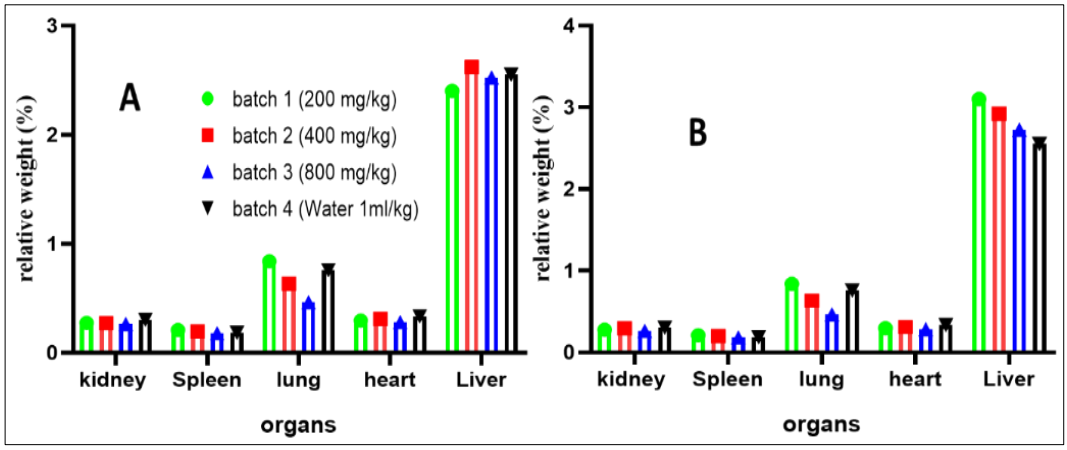


Fig 2: Effects of methanolic extract of *A. affinis* stem bark on the relative weight of some organs of male (A) and female (B) rats.

Determination of some biochemical markers of renal and hepatic function

Biochemical markers of hepatic (ALT and AST) and renal (urea and creatinine) function and were monitored in the blood of the tested to assess the subacute toxicity of our sample.

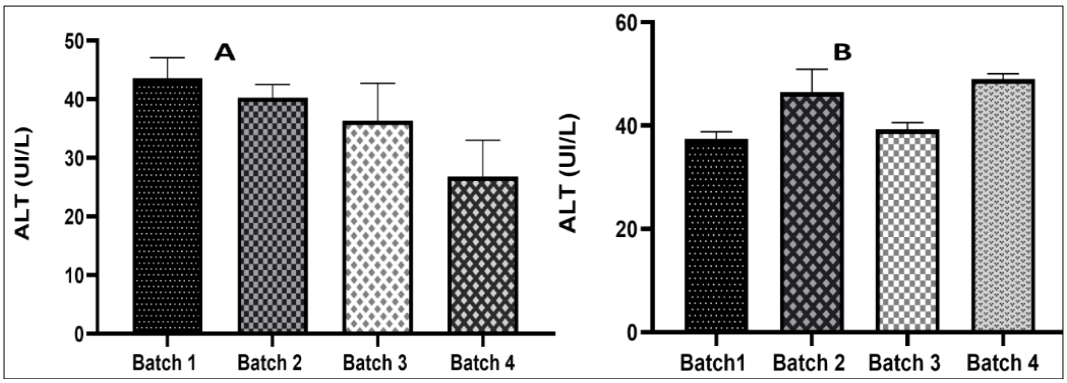
Effect of repeated doses of methanolic extract of *A. affinis* stem bark on serum transaminase concentration

Effect of repeated doses on serum ALT concentration rats

Figure 3 presents the values of hepatic Alanine transferase level. From that figure, the following observation can be drawn.

There is a significant increase in the level of ALT in males (Figure 3A) when the extract is administered at doses of 200 and 400 mg/kg body weight in comparison with the negative control using a paired test. While there were no statistical differences between the treatment at the dose of 800 mg/kg body weight in comparison with the negative control. In contrast, there is no statistical difference when analysing global the ALT level in test groups compared to the negative control. Showing globally that there is no difference between the ALT between treated and untreated groups.

In females, the response is belt-shaped with the maximum decrease observed at the doses of 200 and 800 mg/kg body weight. At these two later doses, the decreases were significantly different from the control and the dose of 400 mg/kg body weight. While no significant difference was observed between the treatment at the dose of 400 mg/kg body weight and negative control.

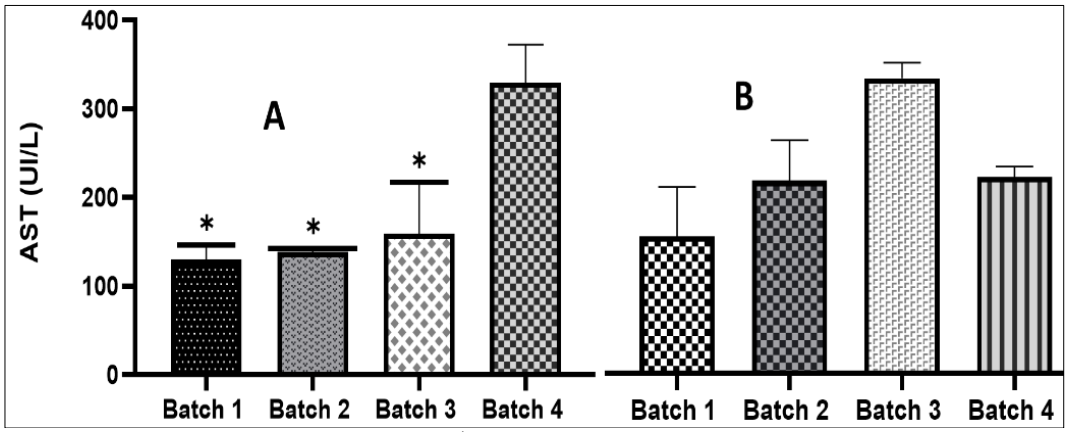


Each histogram represents the mean ± Standard Error on the Mean (SEM); n = 6; Batch 1, Batch 2 and Batch 4: tests batches that received the *A. affinis* extract at the doses of 200, 400 and 800 mg/kg of body weight respectively; Batch 4: normal control group that received 1 ml/kg of distilled water body weight.

Fig 3: Comparison of serum ALT concentration in male (A) and female (B)

Effect of repeated doses of *A. affinis* stem bark extract on serum AST concentration in male (A) and female (B) rats: The result of the record of the AST monitoring is summarised in the Figure 4. The results from that Figure 4 highlight that, in male, there were a reduction in the level of AST in the treated male rat in comparison to the control and more interesting these reductions was significant in comparison to the control group (figure 4A). In contrast, in

female (figure 4 B) there were not statistical difference between the level of the AST at doses 200 and 400 mg/kg body weight in comparison with the negative control that receive distilled water. While, at the dose of 800 mg/kg body weight, there were a significant increase in the level of these enzyme.

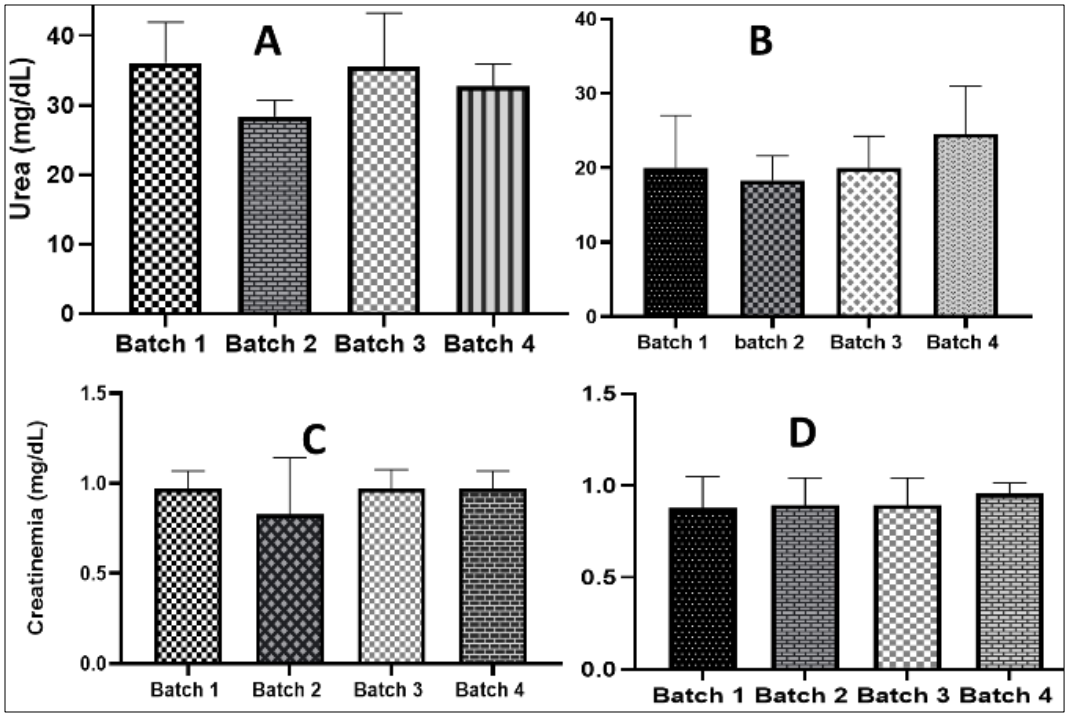


Each point represents the mean ± SEM; n = 6; $^{*}p < 0.05$: significant differences from batch 4. Batch 1, Batch 2 and Batch 4: tests batches that received the *A. affinis* extract at the doses of 200, 400 and 800 mg/kg of body weight respectively; Batch 4: normal control group that received 1 ml/kg of distilled water body weight.

Fig 4: Comparison of serum AST concentration in male rats from test versus control batches

Effect of repeated doses of methanolic extract of *A. affinis* stem barkon urea concentration in male and female rats

Figure 5 shows the level of urea and creatine of the control batch along with the treated batches. From that figure, it can be observed that for male and female, there is no difference between treated group and the controls that just received distilled water in both urea and creatinine level.

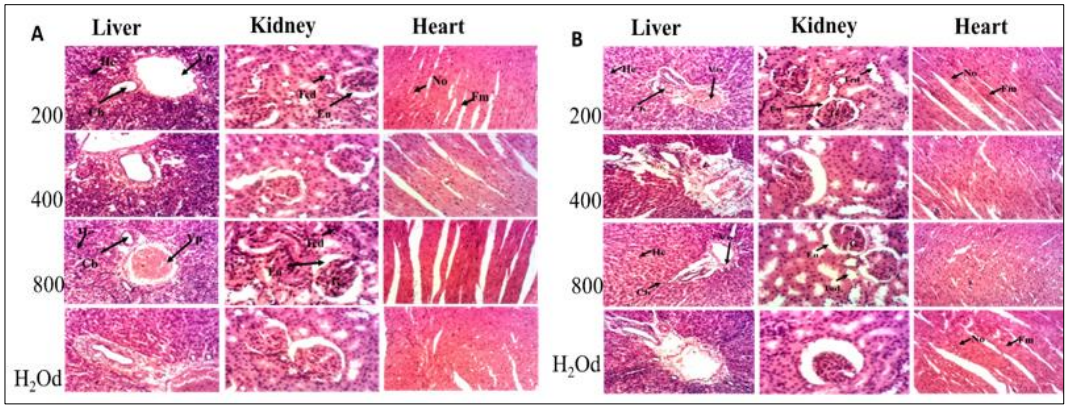


Each point represents the mean ± SEM; n = 6; Batch 1, Batch 2 and Batch 4: tests batches that received the *A. affinis* extract at the doses of 200, 400 and 800 mg/kg of body weight respectively; Batch 4: normal control group that received 1 ml/kg of distilled water body weight.

Fig 5: Comparison of serum urea concentration (male (A) and female (B)) and creatinine (male (C) and female (D)) rats.

Histological sections of livers, kidneys and hearts of test rats

The sections showed 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 fibers and nuclei). No signs of tissue toxicity were observed on both male and female (Figure 6).



Liver: Vp = hepatic portal vein, He = hepatocyte, Cb = bile duct; Kidney: Eu = urinary space; Tcd = distal convoluted tubule; Heart: No = cardiac muscle fiber nucleus, Fm = cardiac muscle fiber. 200, 400, 800, H_2Od: Batches that received distilled water; 5% DMSO + extract of *A. affinis* stem bark at the doses of 200, 400 and 800 mg/kg and distilled water, respectively.

Fig 6: Effects of methanolic extract of *Annickia affinis* stem bark on liver (X100), heart (X100), and kidney (X200) in male (A) and female (B) rats for the different samples compared to the control batch.

Antimicrobial activities

Antibacterial activity

The MICs of the methanolic extract of *A. affinis* stem bark on *E. coli*, *Salmonella typhi* and *N. gonorrhoea* strains are presented in Table 3.

From the Table 3, the extract showed MIC values of 2500; 39 and 39 respectively for *E. coli*, *Salmonella typhi* and *N. gonorrhoea* strains. The MBCs were 2500; 39 and 78 µg/ml respectively for these strains.

Table 3: Antibacterial activity of the methanolic extract of *A. affinis* stem bark

Sample	*N. gonorheae*			*E. coli*			*S. typhi*		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
Extract	39	78	2	2500	2500	1	39	39	1
Ceftriaxone	0.004	0.008	2	0.012	0.082	1.38	0.05	/	/

/. Not determined because the sample was not soluble or not active by the disc diffusion test. MIC: minimum inhibitory concentration (µg/mL); MBC: minimum bactericidal concentration (µg/mL). Ceftriaxone: Reference antibiotic.

Antifungal activity

No inhibition was observed at up to 10mg/ml on *Candida albicans* (Table 4)

Table 4: Antifungal activity of the methanolic extract of *A. affinis* stem bark

Sample	*Candida albicans*		
	MIC	**MFC**	**MFC/MIC**
Extract	> 10000	/	/
Fluconazole	> 2000	/	/

/. Not determined because the sample was not soluble or not active by the disc diffusion test. MIC: minimum inhibitory concentration (µg/mL); MFC: minimum fungicidal concentration (µg/mL). Fluconazole: Reference antifungal.

Discussion

Extraction by maceration of *Annickia affinis* stem bark done in methanol, this solvent was chosen on the one hand for its low boiling temperature of around 64.7°C, a temperature which minimizes the risk of damage to secondary metabolites during concentration of the macerate and on the other for its ability to dissolve a high proportion of polar and non-polar compounds [17]. The drying yield was around 58%, while the grinding yield was 62%. After maceration and evaporation, we obtained an extraction yield of 3%. These results are very different from those obtained by Madeli *et al.* 2015 [18], who obtained a yield of 16.56% after aqueous extraction and 20.84% after hydroalcoholic extraction of the stem bark of *A. chloranta*, a plant belonging to the same genus as *A. affinis*. This difference in extraction yield might be due to the impact of the place and time of harvesting, climatic conditions, and the nature of the extraction solvent, but above all, to the fact that these two plants belong to two different species. On the other hand, Mbosso *et al.* 2023, obtained a yield of 4.23% from methanolic extract of *A. affinis* stem bark. This yield is very close to that of this study, as it involves the same extraction solvent, the same plant and the same harvesting period [5].

The results of phytochemical screening showed the presence of alkaloids, saponins, triterpenoids, coumarins, anthraquinones and the absence of phenols, anthocyanins and tannins. These results are in straight line with that of Mbosso *et al.* 2023, who reported that the methanolic extracts of *A. affinis* stem bark, contain alkaloids, saponins, triterpenoids, anthocyanins and anthraquinones while flavonoids, phenols, coumarins and tannins were not detected [5]. These results are similar to those of Adesokan *et al.* 2008, and Adesokan and Akanji 2010, who showed the presence of several groups of compounds in aqueous and ethanolic extracts of a plant of the same genus *Enantia chlorantha* stem bark, such as alkaloids, saponins and terpenes [19, 20]. Other studies shown the presence of other classes of metabolites, such as the study by Gill and Akinwunmi 1986, which showed that stem bark contained lignans and tannins in addition to saponins and alkaloids on *E. chlorantha* [21]. The difference between these results (the absence of flavonoids and tannins in our extracts) may be related to the difference in extraction solvent: aqueous extracts are more polar than methanol extracts, and could therefore contain more tannins, for example.

In the subacute toxicity study, methanolic extract of *A. affinis* stem bark administered orally at repeated doses of 200, 400 and 800 mg/kg body weight did not cause any death throughout the study. The extract can be classify as toxicity index equivalent to 5, according to the Hodge and Sterner toxicity scale for a chemical substance based on LD_{50} and route of administration [22]. However, no signs of toxicity were observed during the first few hours after administration of the methanolic extract, including reduced sensitivity to stimuli (pain and noise), decreased mobility or softening of the faeces.

The average body weight of rats in all batches increased over the observation period. These results support the hypothesis that the extract administered to the rat batches is probably harmless, since if this had not been the case, a drop in the body weights of the different batches would have been observed due to the disruption of the normal metabolism of the test animals [23]. The body weight gain of males in all test batches was greater than that of female test batches, with an average gain of 59 g in male rats and 32.33 g in female rats between day zero and day 28. These results are close to those of Tankeu *et al.* 2020, and Fokou *et al.* 2023, who found that weight gain in males and females generally increased regardless of the batch chosen, and that weight gain was greater in male rats than in female rats [24,25].

Subacute treatment of rats at different doses resulted in a decrease in serum ALT levels in female rats in all test batches compared with the control batch, whereas in males we observed a non-significant increase in serum levels in all test batches compared with the control batch. Tankeu *et al.* 2020, observed a decrease in ALT levels in all female and male rats tested. This difference may be explained by the fact that males are more sensitive to the effect of repeated doses of *A. affinis* methanolic extract [24]. With regard to AST, we noted a decrease in AST levels in all rats of the male batches tested in comparison with the control batch, whereas in female rats we observed a decrease in the 200 mg/kg batch and an increase in the 400 and 800 mg/kg batches. These results are consistent with those of Etame *et al.* 2017, who also reported a decrease in serum AST levels in male rats in all batches and an increase in serum levels in female rats in several batches [26]. Urea determination can be used to explore protein metabolism, while combined urea-creatinine determination can be used to assess renal function [27]. Urea concentration increases during renal failure or protein catabolism. In conjunction with nephropathy, changes in creatinine levels provide a convenient means of monitoring glomerular filtration rate and assessing renal function. In this study, we observed a dose- and gender-independent decrease in serum urea and creatinine levels. These results are very similar to those of Manda *et al.* 2017, who also noted a decrease in serum urea and creatinine levels in all rats tested compared with the control lot [28].

Histological analysis of some of the main organs involved in detoxification mechanisms revealed no damage to the liver, kidneys or heart. The cross-sections showed normal architecture of the liver (hepatic parenchyma with a centrolobular vein and distinct hepatocytes), kidney (normal parenchyma with a distinct glomerulus and urinary space) and heart (distinct muscle fibers and nuclei). No signs of tissue toxicity were observed. Vascular congestion usually results from back pressure in the vein, leading to accumulation of blood cells. When a vein becomes congested, fluid flows into the parenchyma of the affected organ, resulting in interstitial oedema [29]. All these results support the hypothesis that methanolic extract of *A. affinis* stem bark administered in repeated doses over 28 days shows no signs of toxicity.

The MICs of 2500, 39 and 39 µg/ml for *E. coli*, *S. tiphy and N. gonorrhoea* respectively. The best activity was observed on *S. tiphy* and *N. gonorrhoea*, with MICs of 39 mg/ml. This value is higher than that of the reference (ceftriaxone), i.e. MIC = 50 µg/ml. According to Kuete *et al.* 2023 [30], methanolic extract of *A. affinis* stem bark has excellent activity on *S. tiphy* and *N. gonorrhoea* strains and a weak activity on *E. coli*. On the other hand, according to the Tabougia *et al.* 2017 [31], the extract of *A. affinis* stem bark shows strong activity against *S. tiphy* and *N. gonorrhoea*, and moderate activity against *E. coli*. This could justify its use in traditional medicine for the treatment of typhoid fever, diarrhoea and urinary tract infections. To the best of our knowledge, we did not find a report on the antibacterial activity of the methanolic stem bar extract of *A. affinis*. However, these results contrast with those of Atata *et al.* 2003, and Adesokan *et al.* 2008, who showed that the ethanolic and aqueous extracts of the stem bark of *A. chloranta*, a plant belonging to the same genus as *A. affinis*, possessed weak antimicrobial activity against *Salmonella typhi* and *E. coli*, with MIC values of 1000 µg/ml [19, 23]. This difference in MICs could be due, on the one hand, to the secondary metabolite obtained after extraction, as the solvent used in our study extracts most of the polar and apolar compounds, whereas the solvents used in the study by Atata *et al.*, extracted mainly polar compounds. On the other hand, the area and period of harvesting could influence the phytochemical composition of the extract. Another study, by Nyegue *et al.* 2008, on the essential oil of *Enantia chloranta*, a plant belonging to the same genus as *A. affinis*, showed that the extract was highly active against *E. coli*, with a MIC of 125 µg/ml. This germ is responsible for urinary tract infections. This result differs from our own, as the methanolic extract of *A. affinis* has a low activity on *E. coli* with a MIC of 2500 µg/ml [32].

Determination of the MIC of the methanolic extract of *A. affinis* stem bark on the fungal strain showed that the extract are not active on *Candida albicans* because the MICs values are > 10000 µg/ml. This result corroborates that of Atata *et al.* 2003, who showed that the ethanolic and aqueous extracts of *A. chloranta* stem bark were not active on *Candida albicans* with an MIC greater than 50000 µg/ml [23]. However, these results contradict those of Nyegue *et al.* 2008, which showed that *E. chloranta* essential oil have moderate activity against *Candida albicans* with an MIC of 500 µg/ml [29]. This difference may be due to the nature of the extract.

According to the Marmonier scale in 2010, we can say that our extract is bactericidal against *E. coli*, *S. typhi*, and *N. gonorrhoea* [33]. Also, the presence in our extract of triterpenoids and alkaloids, which are classes of secondary metabolites known for their antibacterial properties, could account for the antibacterial activities observed [34, 35].

Conclusion

The results of the present studies provide clear evidence that repeated-dose administration of the methanolic extract of *A. affinis* stem bark did not lead to any disturbance of the biochemical metabolism of the rats. No signs of toxicity

were observed throughout the study, and no deaths were reported. The methanolic extract of *A. affinis* stem bark possesses excellent activity on *S. tiphy* and *N. gonorrhoea* and no antifungal activity on *Candida albicans*. Based on all these observations and results, we can conclude that *A. affinis* stem bark has very interesting antibacterial activity against *S. typhi* and *N. gonorrhoea*, and is virtually harmless for medium-term oral use.

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Conflicts of interest
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