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Antiproliferative, antimicrobial, antiplasmodial, and oral acute toxicity of *Ficus elastica* Roxb. Ex Hornem lianas

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Abstract

Background: *Ficus elastica* is a plant used in traditional medicine for the treatment of allergies and skin infections. This study aimed to investigate the antimicrobial, antiplasmodial, and antiproliferative activities of the crude extract and fraction of *F. elatica* as well as the study of the *in vivo* oral acute toxicity of the most active fraction.

Methods: The antimicrobial activity of the total extract and the different fractions was evaluated by the determination of the inhibition diameter using the agar well diffusion method on 3 Gram (+) bacteria, 4 Gram (-) bacteria, and 2 fungi, by the determination of MIC using the micro-dilution method only on the strains that showed sensitivity on the agar and by the determination of MMC on Muller Hinton agar. The evaluation of antimalarial activity was done on *Plasmodium falciparum* 3D7 cells by measuring the parasite lactate dehydrogenase (pLDH) activity using the mixture of Malstat and NBS/PES. Extracts were afterward evaluated for their antiproliferative effect on 2 human cell lines (human oligodendroglioma and breast cancer) and mouse melanoma using colorimetric MTT assay. The toxicity assessment of the most active fraction was performed *in vivo* according to the modified OECD 425 guidelines.

Results: Total extract and different fractions were not active on different strains because the MICs values are > 1024 µg/mL. Dichloromethane and hexane fractions were bactericidal with a ratio CMM/CMI of 2 and the total extract would be bacteriostatic. The antimalarial activity showed that the hexane fraction reduced the viability of *P. falciparum* 3D7 cells by 61.4% with an IC_{50} value of 26.41 µg/mL. As for the antiproliferative activity, the dichloromethane fraction significantly inhibited different cell lines with IC_{50} values between 13 and 16.5 µg/mL. The toxicity study conducted on the hexane fraction, being the most active fraction, resulted in no apparent signs of toxicity and no death at doses of 2000 mg/kg bw and 5000 mg/kg bw over a period of 14 days.

Conclusion: Methanolic crude extract of *F. elastica* lianas and its hexane as well as dichloromethane fractions possess low antibacterial activity. The dichloromethane fraction possesses antiproliferative activity while the hexane fraction possesses antiplasmodial activity and presented no signs of acute toxicity by the oral route. These results support the use of *F. elastica* in the treatment of several ailments such as skin infections.

Keywords: Acute oral toxicity; antimicrobial; antiplasmodial; antiproliferative; *Ficus elastica;* phytochemical screening.

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Background

Every year, approximately seven million people suffer from cancer, making this disease responsible for at least 12% of deaths worldwide [1]. On the other hand, antimicrobial resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 [2]. In addition, Malaria is one of the most death-leading diseases caused by a mosquitoes-borne parasite. Despite efforts to eradicate malaria, the disease is globally rather causing an increasing number of deaths. In fact, according to the World Health Organization (WHO) there is an increasing number of malaria cases i.e. from 227 million in 2019 to 241 million in 2020 in 85 malaria-endemic countries. This disease affects mostly children under 5 years and pregnant women [3].

The use of plants in therapeutics has existed throughout the world for thousands of years. Since 1990, it has made a significant appearance in many developed and developing countries [4]. Traditional medicine is not only the main mode of health care delivery worldwide, but also a complement to it, and an important and often underestimated part of health care [4]. In Africa, WHO statistics estimate that 80% of the population still uses traditional medicines rather than modern ones for primary health care [5]. This situation could be explained by the poverty of the population, the lack of infrastructure and of social and health personnel in modern medicine, and religious or superstitious considerations [6]. Cameroon's flora is rich in nearly 8000 species and its potential in medicinal plants indicates 600 to 800 species recognized and used in traditional and modern medicine [7]. The data on the safety and efficacy of traditional medicine are too insufficient in both quantitative and qualitative terms to justify advocating its adoption throughout the world [8]. Due to the emergence of drug resistance, there is a permanent need for the discovery of new treatments for malarial, microbial infections, and cancer. To provide a solution to this major problem, medicinal plants remain a good target to overcome this problem [9].

Ficus elastica Roxb. ex Hornem is a plant of Moraceae family. Its leaves are used in traditional medicine for the treatment of skin diseases such as allergies and microbial infections, and as a diuretic. Previous studies conducted on the leaves by Preeti *et al.* [10] in 2015 had shown that they have antimicrobial and cytotoxic activity; In 2012, Mbosso *et al.* [11] had shown that the bark of aerial roots had antimicrobial and antiproliferative activities; studies done by Mbosso *et al.* [1] in 2016 on the wood of the aerial roots had also shown that they have antiproliferative activities, we should mention here that no study has been conducted on *F. elatica* lianas to the best of our knowledge. The present study, therefore, focuses on the evaluation of the antimicrobial, antiplasmodial, and antiproliferative activities of the crude extract and fraction of *F. elatica* as well as the study of the acute toxicity of the most active fraction.

Methods

Plant material

The lianas of *F. elastica* used in this study were harvested in the Centre region of Cameroon, more precisely at "*Mélen*", in the district of "*Yaoundé*" III. The identification was done at the National Herbarium of Cameroon where it was identical to a specimen deposited under voucher number 65646/HNC.

Bacterial and fungal

Growth inhibition tests were carried out with three Gram-negative bacteria (*Salmonella enteric typhimurium* ST2850, *Salmonella enterica munchen* NR4311, and *Escherichia coli* NR32771), three Gram-positive bacteria (*Staphylococcus epidermidis* NRS848, *Staphylococcus aureus* VCU006, and *Staphylococcus saprophyticus*), and two fungal isolates (*Candida albicans* and *Candida kruzei*). All strains were kindly supplied by the Microbiology Laboratory of the University of Buea and are reference strains for bacteria and clinical specimens for fungal isolates. Samples were stored at -80°C until use.

Malaria parasite

In this study, a cell line of the malaria parasite was used, namely the *Plasmodium falciparum* strain 3D7, which was provided by the Biomedical Research Center of the University of Rhodes in South Africa.

Cancer cell lines

To demonstrate the antiproliferative activity, three cancer cell lines were used namely Hs683 (human oligodendroglioma, primary brain cancer), MCF7 (human breast carcinoma), and murine B16F10 (mouse melanoma). The culture medium was an RPMI 1640 medium adjusted with phenol red at 25 mM hepes (Biowittacker, Verviers, Belgium), 10% Beaf Fetal Serum (GIBCO-Lige Technology, Merelbecke, Belgium), 2% L-glutamine at 200 mM, 2% penicillin-streptomycin at 10.000 units/mL of penicillin, 10.000 µg/mL of streptomycin (GIBCO-Life Technology, Verviers, Belgium) and 0.2% gentamicin at 50 mg/mL (GIBCO-Life Technology, Verviers, Belgium). All cell lines have been purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA).

Animals

The animal material consisted of 9 nulliparous, non-pregnant, 7-week-old female Wistar rats from the Faculty of Sciences of the University of Yaoundé I, weighing between 96 and 155 grams of body weight. They were placed in ventilated plastic cages containing wood shavings bedding which were changed daily. The rats were acclimatized to the conditions of the animal house (12 hours of light and 12 hours of darkness) for 7 days prior to treatment and then divided into 3 homogeneous batches of 3.

Extraction and fractionation

Plant material (i.e. *F. elastica* lianas) was cut into small pieces, air-dried, and ground into a powder. Then, 1.2 kg of the powder was macerated at room temperature with 95% methanol. After evaporation under reduced pressure using a rotary evaporator at a speed of 60 rpm at 40°C, 56 g of methanol extract (denoted MESCf) from which 5 g was removed for the subsequent activity tests. Next, 200 mL of water was added to the remaining 51 g of methanolic dry extract in order to carry out successive liquid-liquid extractions using a separating funnel and with different solvents of increasing polarity ranging from hexane, dichloromethane, and ethyl acetate. We obtained 14 g (yield of 27.45%) of the hexane fraction (HFSCf), 10 g (yield of 19.6%) of the dichloromethane fraction (DFSCf), 9 g (yield of 17.65%) of the ethyl acetate fraction (EFSCf), and 12 g (yield of 23.52%) of the aqueous fraction

(AqFSCf) were obtained.

Phytochemical screenings

Detailed phytochemical screening was performed on the five fractions using standard methods, as reported in the literature [12-15]. 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 positive controls. The following tests were performed: Drangendorff test (alkaloids), Tannins (gallic tannins), Libermann-Buchard test (triterpenoids), Shinoda (flavonoids), Borntrager (anthraquinones), Foam index test (saponins) and $FeCl_3$ test (polyphenols). All observations were recorded.

Minimum inhibitory concentration (MIC)

MICs were determined by broth microdilution technique using 96-well plates as previously described [16]. Culture media (nutrient broth) were supplemented with 0.005% phenol red endpoint indicator. The wells were filled with 50 µL of broth, and 100 µL of extract or fraction were added in triplicate to the first column previously prepared in DMSO to make a final concentration of 100 mg/mL. Successive dilutions were done by transferring 50 µL of the mixture from the first well to the eleventh well. An aliquot (50 µL) was discarded from the eleventh well. The twelfth well served as control since no sample (extract) was added to it. Finally, 50 µL of a standardized inoculum at 10^6 CFU/mL was added in each test well for Gram-negative bacteria and 10^5 CFU/mL for Gram-positive and yeasts. The final concentration of the extracts used to evaluate the antimicrobial activity ranged from 50 to 0.0975 mg/mL. Tests were incubated aerobically at 37 ± 1°C for 24 and 48 hours for bacteria and *Candida* species respectively. The endpoint was revealed by a color change of the indicator from red to pink or to yellow by comparing the test wells to control wells (media, diluted extract, and distilled water). The MIC was considered as the lowest concentration of sample that could prevent visible growth of microorganisms (no change of the indicator). The results were recorded as the mean of MICs from 3 independent experiments.

Minimum microbicidal concentration (MMC)

MMC value, defined as the lowest concentration of sample that can cause the death of approximately 99.99% of inoculum was further investigated. An aliquot (10 µL) from each microwell obtained from MIC determination and exhibiting no visible growth was inoculated and incubated at 37°C for 24 h, on fresh drug-free Mueller Hinton agar (for bacteria cultures) or Sabouraud agar (for yeast) plates, respectively. A microbicidal effect was reported for each sample resulting in plates displaying no growth [17]. All experiments were carried out in triplicate.

Antiplasmodial activity

The activity against *Plasmodium falciparum* chloroquine-sensitive 3D7 strain was assessed following the procedure reported by Makler & Hinrichs [18] in 1993 and Mbosso *et al*. [19] in 2018. Absorbances from parasite lactate dehydrogenase activity were measured at 620 nm for cultures incubated for 48 h at different concentrations of the *S. campanulata* methanolic extract and fractions in 96-well plates to assess parasite viability. Chloroquine was used as the reference drug, 10% DMSO as solvent, L-lactic acid, Acetyl Pyridine Adenine Dinucleotide (APAD), Nitro Blue Tetrazolium (NBT), Phenawine Etho Sulphate (PES), triton X-100 and trizma were used as reagents. Experiments were performed in triplicate.

Antiproliferative activity

The MTT assay is a colorimetric method for assessing the viability of cells in a sample. By extension, it allows the determination of cell death induced by a compound. The half maximal inhibitory concentration values (IC_{50}) of all fractions were determined *in vitro* after 3 days of culture using the MTT 3-[4,5]-dimethylthiazol-2yl-diphenyltetrazolium bromide assay (Sigma, Ostende, Belgium), as previously described in a panel of three cancer cell lines [20]. The optical densities of the plates were measured at 570 nm by visible spectrophotometry using a 680XR Bio-Rad plate reader. Only the dichloromethane fraction was tested as we encountered solubility issues with total extract and the other fractions (TEFEl, HFFEl, and AFFEl) which could not be dissolved at a solvent level preserving cell integrity (i.e. < 1% DMSO). Experiments were performed in triplicate. The results obtained were first recorded in an Excel file and then translated into a graph of the percentage of living cells as a function of the concentration of the product tested. The concentration of product at which 50% of the cell growth is inhibited, i.e. the IC_{50} at a given time for a given cell density, can finally be determined using a regression curve.

Acute toxicity

The assessment of acute oral toxicity was determined according to the modified OECD guidelines 423 at a fixed dose [21]. Female Wistar strain laboratory rats aged 8 to 12 weeks were randomly selected and fasted 12 hours before the test by receiving *ad libitum* water. After this fasting, the rats were weighed (D0) and the test substance was administered to them orally using an orogastric tube according to the following distribution: the control group (3 rats) received distilled water at 10 mL/kg body weight; 3 rats (group 1) received the aqueous extract at 2 g/kg bw; and 3 rats (group 2) received the aqueous extract at 5 g/kg bw (Figure 1). After the substance was administered, the animals were observed individually at least once during the first 30 minutes and regularly during the first 24 hours after administration, with particular attention during the first 4 hours. They were observed for 14 days following the administration of the substance. The observations focused on changes in the skin, body hair, somatomotor activity, and behavior. Particular attention was paid to various manifestations such as tremors, convulsions, diarrhea, lethargy, sleep, and coma. The rats underwent weighing during a study period respectively each day: from D0 (the day of administration) to D13 (the last day of observation) to assess the weight change.

Ethical considerations

Ethical clearance and research authorizations were obtained for the performance of our work at our various study sites. We certify that all the total extract and different fractions obtained in this work were used only for phytochemical screening, biological activities evaluation, and acute oral toxicity evaluation. Microsoft Word 2013 was used for data entry, and Microsoft Excel 2013 for making tables and diagrams. GraphPad Prism program version 5.02 was used for data analysis.

Results

Phytochemical composition

Table 1 shows the secondary metabolites contained in *F. elastica* lianas after phytochemical screenings. The detection of these compounds was based on precipitation, turbidity, color change, or UV lamp tests. These results confirmed that *F. elastica* lianas contain several classes of secondary metabolites such as alkaloids present in the total extract, hexane fraction, and dichloromethane fraction; tannins present in total extract and dichloromethane fraction; triterpenoids and saponins present in total extract; flavonoids and anthraquinones are not present in these samples. The acetate fraction of *F. elastica* lianas could not be screened because of the low yield obtained during fractionation.

Antimicrobial activity

Following the strains that exhibited sensitivity, the minimum inhibitory concentrations, the minimum microbicidal concentrations, and the ratio MMC/MIC of the total extract and of the various fractions of *F. elastica* concerned at a concentration of 50 mg/mL were carried out by microdilution and the results are recorded in Table 2.

Table 2 shows that the total extracts and the hexane and dichloromethane fractions displayed different MICs on the different strains for which they were tested. The total extract has a MIC value of 12.5 on *S. aureus* against 3.13 mg/mL on *S. epidermidis*. The hexane and dichloromethane fractions exhibited MIC values of 3.13 and 6.25 mg/mL on *S. aureus*, respectively: and a MIC value of 6.25 mg/mL on *S. epidermidis*. The total extract has a CMM of 50 mg/mL on *S. aureus* and *S. epidermidis* with respective CMM/CMI ratios of 4 and 16; the dichloromethane fraction has a CMM of 12.5 mg/mL and a CMM/CMI ratio of 2 on the 2 strains tested and the hexane fraction has a CMM of 6.25 mg/mL with a ratio of 2 on *S. aureus* against an MMC of 25 mg/mL on *S. epidemidis*.

Antiplasmodial activity

The viability percentage of *Plasmodium falciparum* 3D7cells and the standard deviation obtained for each sample is reported in Figure 2. Only the hexane fraction (HFFEl) significantly reduced the viability of *Plasmodium falciparum* 3D7 by 38.46% at a concentration of 25 µg/mL. The other fractions did not reduce viability and showed percentages of viable cells of 107.27% for aqueous fraction (AFFEl); 97.18% for ethyl acetate fraction (EFFEl); 100% for total extract (TEFE1); 92.81% for dichloromethane fraction (DFFEl). Subsequently, the IC_{50} of hexane fraction (HFSCf), was determined by graphical regression method on dose-response curves at a fixed concentration of parasite (25 µg/mL). Promising antiplasmodial activities were obtained with IC_{50} of 26.41 µg/mL (Figure 3). For comparison purposes, chloroquine (an antimalarial drug) was used as a standard with IC_{50} of 0,015 µM (Figure 4).

Antiproliferative activity

The total extract and the different fractions (hexane, dichloromethane, ethyl acetate, and aqueous) of the *F. elastica* lianas were submitted to the antiproliferative tests but only the fraction in dichloromethane could be tested since the other fractions were insoluble. The IC_{50} values obtained after spectrophotometric plotting are given in Table 3.

According to Table 3, the dichloromethane fraction exhibits antiproliferative activity with an IC_{50} of 16.16 ± 5.5,13 ± 3, and13.5 ± 1.8 µg/mL for Hs683, B16F10, and MCF7 lines, respectively.

Acute toxicity

The evaluation of the acute oral toxicity was made according to the modified OECD guidelines 423 on the hexane fraction because the latter showed more activity compared to the other fractions and to the total extract (2 activities, antibacterial and antiplasmodial). The LD_{50} is the concentration of substance in mg/kg causing the death of 50% of a given animal population under precise experimental conditions. After oral administration of a single dose of the hexane fraction, abnormal variation of the physiological parameters listed in Table 4 was not observed during 14 days of the assay for batches 2 and 3 of rats compared to the rat control group (batch 1) and therefore was considered as non-toxic at doses of 2000 and 5000 mg/kg. Consequently, the LD_{50} can be considered as greater than 5000 mg/kg.

Discussion

Phytochemical study

Extraction

Methanol extraction of *F. elastica* lianas was carried out by double maceration. This polar solvent was chosen for our study on one hand for its low boiling temperature of around 65°C (a temperature that minimizes the risk of damage to secondary metabolites during evaporation on a rotary evaporator) and on the other hand for its capacity to dissolve a large proportion of polar and non-polar compounds [22].

Fractionation by liquid-liquid extraction

The yields resulting from this fractionation showed that the hexane fraction (HFFEl) had the highest yield with a value of 27.4%, followed by the aqueous fraction (AFFEl) with 23.5%, then the dichloromethane fraction (EDFEl) with 19.6%, then the ethyl acetate fraction (EAFEl) with 17.6% relatively to the total extract (ETFEl). These values would mean that *F. elastica* lianas contain more apolar than polar compounds. On the other hand, the work of Mbosso *et al.* [1] in 2016 on the wood of the aerial roots of *F. elastica* showed a higher yield of 77% obtained by methanol maceration, which could mean that the wood of the roots is rich in polar compounds.

Phytochemical tests

Phytochemical qualitative tests carried out on the total extract and the different fractions of *F. elastica* lianas revealed the presence of alkaloids in HFFEl, TEFEl, DFFEl, AFFEl, tannins in TEFEl, DFFEl, triterpenoids in TEFEl, saponins in TEFEl and not contain flavonoids and anthraquinones. The ethyl acetate fraction of *F. elastica* lianas was not screened due to the low yield obtained during fractionation. These results are consistent with those obtained by Phan *et al.* [23] in 2012 who showed the presence of the same classes of secondary metabolites in *F. elastica* leaves; Mbosso *et al.* [11] in 2012 who isolated compounds belonging to the same classes of secondary metabolites in addition to flavonoids from the bark of aerial roots, and Mbosso *et al.* [1] in

2016 reported that the wood of aerial roots contained the same classes of secondary metabolites.

Antimicrobial activity

According to Kuete [24] in 2023, for plant extracts, we can have outstanding activity when MIC ≤ 8 µg/mL; excellent activity when 8 < MIC ≤ 64 µg/mL; very good activity when 64 < MIC ≤ 128 µg/mL; good activity when 128 < MIC ≤ 256 µg/mL, average activity when 256 < MIC ≤ 512 µg/mL, weak activity when 512 < MIC ≤ 1024 µg/mL, and not active MIC > 1024 µg/mL. From this classification, total extract and different fractions are not active on different strains because the MICs values are > 1024 µg/mL. These results are close to those obtained by Mbosso *et al.* [11] in 2012 on the methanolic extract of the aerial roots of *F. elastic* on gram (+) bacteria (*Enterococcus faecalis, S. aureus, Staphylococcus saprophyticus, S. epidermidis*), gram (-) bacteria (*Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi*), a filamentous fungus (*Trichophyton rubrum*) and a yeast (*Candida albicans*) with MIC values between 0.02 and 6.25 mg/mL against Gram (+) bacteria and between 0.50 and 6.25 mg/mL against Gram (-) bacteria. This extract was even found to be more active than the reference drug (gentamicin) against *S. saprophyticus* with a MIC value of 0.02 mg/mL against 0.98 mg/mL of gentamicin. On the contrary, previous work by Mbosso *et al*. [1] in 2016 on *F. elastica* aerial root wood showed that the total extract and 4 isolated compounds (β-sitosterol glucoside, elasticamide, elastiquinone and ficusoside B exhibited significant antimicrobial activity with a MIC value of 0.04 mg/mL on *S. aureus* for the methanol extract, 0.08 mg/mL for β-sitosterol glucoside and elasticamide which showed moderate antimicrobial activity and 0.01 mg/mL for elastiquinone which was even found to be the most active compound. This could be justified by the presence of molecules with antagonistic effects in the extracts.

According to Marmonier *et al.* [25] in 1990 when the ratio CMM/CMI < 4, the extract is considered microbicidal and if CMM/CMI ≥ 4, the extract is considered microstatic. From this classification, it appears that the dichloromethane fraction is microbicidal on *S. aureus* and *S. epidermidis* as it presented a ratio of 2 on both strains. The hexane fraction is bactericidal on *S. aureus* with a ratio of 2 and bacteriostatic on *S. epidermidis* with a ratio of 4. The total extract would be bacteriostatic on both strains with a ratio of 4 on *S. aureus* and 16 on *S. epidermidis.* These results are contrary to those of Mbosso *et al.* [1] in 2016 which had shown that the ratio CMM/CMI of the methanolic extract of *F. elastica* root wood had a bactericidal action on susceptible strains. The hexane fraction with lowest MIC value, and bactericidal effect on *S. aureus* would be considered the most active fraction with respect to antimicrobial activity. Since the hexane fraction is more active than the total extract, it could be assumed that there are antagonistic compounds within the phytocomplex.

Antiplasmodial activity

Total extract and fractions were tested for antiplasmodial activity against chloroquine-sensitive *Plasmodium falciparum* strains (3D7) using Chloroquine as drug reference. An extract or fraction is considered to be highly active when IC_{50} ≤ 5 µg/mL [26]. Only hexane fraction was able to decrease the viability of *Pf*3D7 under 50% (38.46%) (Figure 2). The hexane fraction showed moderate activity with an IC_{50} value of 26.41 µg/mL (Figure 3). These results suggest that the hexane fraction contains compounds responsible for this activity, the presence of alkaloids. In addition, the difference between the activity of the total extract the dichloromethane fraction, and the hexane fraction could be due to the presence of the inhibitory effect. Moreover, the work carried out by Mbosso *et al.* [1] in 2016 on the wood of the aerial roots of *F. elastica* had shown that the methanolic extract, at the same concentration, had very significantly reduced the viability of the 3D7 cells of *P. falciparum* by 100 % with an inhibitory concentration 50 (IC_{50}) of 9.49 µg/mL, a value close to that of chloroquine (IC_{50} = 10 µM) which had been used as the reference drug.

Antiproliferative activity

According to the NCI protocol for crude extract and fractions, is considered to have significant antiproliferative activity if the IC_{50} value is less than 30 µg/mL (IC_{50} < 30 µg/mL) in the preliminary assay [27]. The dichloromethane fraction tested by the MTT technique showed significant antiproliferative activity with IC_{50} values of 13 µg/mL against mouse melanoma (B16F10); 13.5 µg/mL against breast cancer (MCF7) and 16.16 µg/mL against human oligodendroglioma (Hs683). This is consistent with the work of Mbosso *et al.* [1] in 2016 who had shown that wood from the aerial roots of *F. elastica* exhibited significant antiproliferative activity against some cancer cell lines with IC_{50} values ranging from 3 to 8 µg/mL. Similarly, El-hawary *et al.* [28] in 2012 had also shown that the methanolic extract of *F. elastica* leaves possessed significant antiproliferative activity with a Lethal Concentration (LC_{50}) of 149.7 µg/mL against breast cancer. Surprisingly, these results are opposed to those of Mbosso *et al.* [11] in 2012 which showed that the bark of the aerial roots of *F. elastica* has no antiproliferative activity. Given that the total extract and the other fractions could not be tested due to solubility, we will not be able to compare the activity of the total extract with that of the fractions. According to the phytochemical screening, the activity of the dichloromethane fraction could be linked to the presence of alkaloids, knowing that an alkaloid (bisindole) is used in anti-tumor chemotherapy on rat hepatoma in cell culture [29].

Toxicity assessment

The study of the acute toxicity of the flowers of *F. elastica* lianas has shown that the extract administered by the oral route does not cause any mortality at dose limits of 2000 mg/kg and 5000 mg/kg of body weight. According to Clarke's [20] work, a LD_{50} greater than 5000 mg/kg of body weight is considered non-toxic for animal testing. Therefore, these results corroborate with those conducted by Shripad *et al.* [30] in 2014 who showed that the methanolic extract of the leaves of *Ficus microcarpa* showed no signs of acute or even oral toxicity. Similarly, Vinad *et al.* [31] in 2011 also showed that the ethanolic extract of the leaves of *Ficus benjamina* at doses of 800 to 2000 mg/kg bw did not cause any sign of acute toxicity by the oral route in rats and over a period of 72 hours.

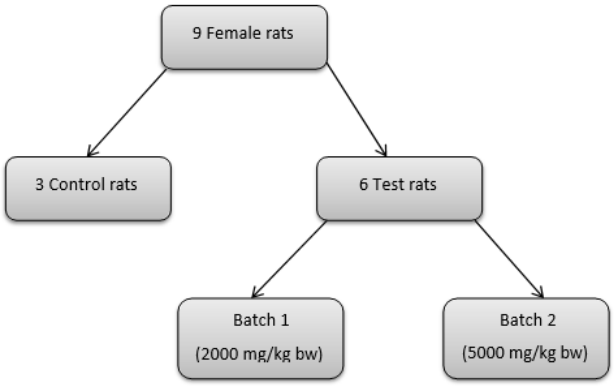

Figure 1. Rat allocation protocol for acute toxicity assessment

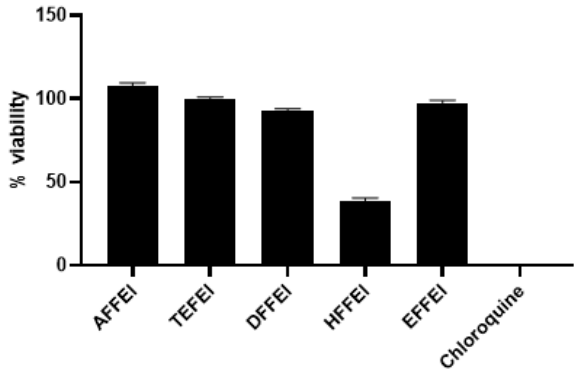

Figure 2. Viability percentage of *F. elastica* lianas total extract and fractions on *Plasmodium falciparum* 3D7 cells. The values are the means of 3 independent experiments with standard error bars indicated.

TEFEI = total extract of *F. elastica* lianas; HFFEI = hexane fraction of *F. elastica* lianas; DFFEI = dichloromethane fraction of *F. elastica* lianas; EFFEI = Ethyl acetate fraction of *F. elastica* lianas; AFFEI = aqueous fraction of *F. elastica* lianas; Chloroquine: reference drug.

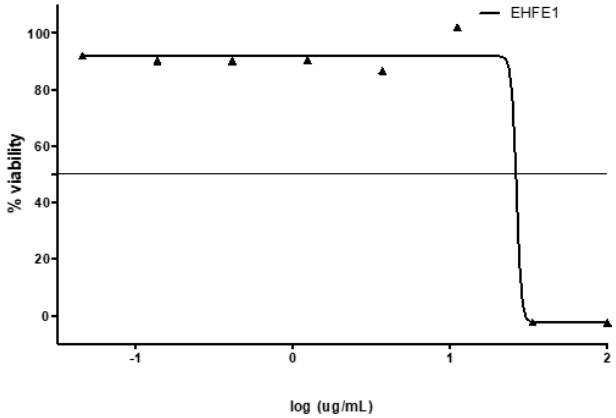

Figure 3. Dose-response curves of the anti-malarial assay of hexane fraction of *Ficus elastica* lianas. GraphPad Prism program version 5.02 was used for data analysis.

EHFEI = hexane fraction of *F. elastica* lianas

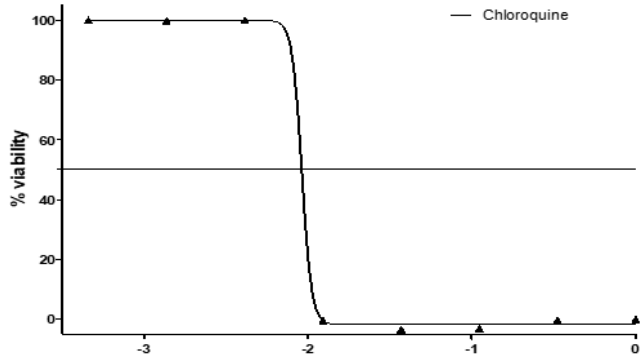

Figure 4. Dose-response curves of chloroquine compound. GraphPad Prism program version 5.02 was used for data analysis.

Chloroquine = reference drug used

Table 1. Results of the screening carried out on *Ficus elastica* lianas.

Chemical compound family	TEFEI	HFFEI	DFFEI	AFFEI
Alkaloids	+	+	+	-
Gallic Tannins	+	-	+	-
Flavonoids	-	-	-	-
Triterpenoids	+	-	-	-
Anthraquinones	-	-	-	-
Saponins	+	-	-	-

+ = presence; - = absence; TEFEI = total extract of *F. elastica* lianas; HFFEI = hexane fraction of *F. elastica* lianas; DFFEI = dichloromethane fraction of *F. elastica* lianas; AFFEI = aqueous fraction of *F. elastica* lianas

Table 2. Inhibition parameters (MIC, MBC and MBC/MIC) of total extract of *Ficus elastica* lianas and its fractions (mg/mL)

Tested extract/fraction	Parameters	Bacteria	
		S. aureus	***S. epidermidis***
TEFEl	MIC	12.5	3.13
	MBC	50	50
	MBC/MIC	4	16
HFFEl	MIC	3.13	6.25
	MBC	6.25	25
	MBC/MIC	2	4
DFFEl	MIC	6.25	6.25
	MBC	12.5	12.5
	MBC/MIC	2	2

TEFEl = total extract of *F. elastica* lianas; HFFEl = hexane fraction of *F. elastica* lianas; DFFEl = dichloromethane fraction of *F. elastica* lianas.

Table 3. *In vitro* growth inhibitory activity of dichloromethane fractions on a panel of three cancer cell lines

Cancer cell lines	IC_{50} (µg/mL)
	DFSCf[b]
B16F10	13 ± 3
MCF7	13.5 ± 1.8
Hs683	16.16 ± 5.5

Hs683 = human oligodendroglioma, primary brain cancer; MCF7 = human breast carcinoma; B16F10 = mouse melanoma; DFFEl = dichloromethane extract of *F. elastica* lianas.

Table 4. General observations in acute toxicity

Parameters	Witness batch (distilled water 10 mL/kg)	Batch 1 (extract at 2000 mg/kg)	Batch 2 (extract at 5000 mg/kg)
Number of rats	3	3	3
Coat modification	A	N	N
Eyes modification	A	A	A
Regurgitation	A	A	A
Trembling	A	A	A
Convulsions	A	A	A
Impaired gait	A	A	A
Nervousness	N	N	N
Mating attitude	A	N	N
Salivation	A	N	N
Lethargy	A	N	N
Slumber	N	N	N
Itching	N	N	N
Agitation	N	N	N
Vomiting	A	A	A
Intense thirst	N	N	N
Nutrition	N	N	N
Bizarre behaviours	A	A	A

A= absence; N= normal.

Conclusion

The results of the present studies provide clear evidence that the methanolic crude extract of *F. elastica* lianas and its hexane as well as dichloromethane fractions possess low antibacterial activity. The dichloromethane fraction possesses antiproliferative activity while the hexane fraction possesses antiplasmodial activity and shows no signs of acute toxicity by the oral route. These results support the use of *F. elastica* in the treatment of various diseases such as skin infections and could also be suggested after other studies in the treatment of malaria and cancer.

Abbreviations

BW: body weight
DMSO: dimethyl sulfoxide
HNC: Herbier National du Cameroun
MMC: Minimal Microbicidal Concentration
MHB: Mueller Hinton Broth
MHB: Mueller-Hinton Agar
MIC: Minimal Inhibitory Concentration
NCI: National Cancer Institute

Authors' Contribution

JETM designed the study and supervised the work while GWT conducted the experiments; CNN wrote the first draft of the manuscript; YF analyzed the results and plotted the curves; EMM carried out the bibliographic research; JCAN corrected the first draft of the manuscript; all authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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