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Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2024**, 14(1), 42-49

Phytochemical analysis, evaluation of the antioxidant and antiplasmodial activities of the ethanolic extract of *Ficus elastica* Roxb. ex Hornem. (Moraceae) lianas

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Abstract: *Ficus elastica* Roxb. ex Hornem, also called the Rubber fig tree, is a plant of the Moraceae family used in traditional medicine to treat allergies and skin infections. Our study aims to perform the quantitative screening and the evaluation of the antioxidant and anti-plasmodial activities of the ethanolic extract of *Ficus elastica* lianas. The estimation of total polyphenols, flavonoids, tannins, alkaloids, and saponins has been evaluated by different methods described in the literature. The evaluation of the antioxidant activity was carried out using the DPPH scavenging method and that of the ferric-reducing antioxidant power (FRAP). The antiplasmodial activity was tested on the *Pf* 3D7 strain (sensitive to Chloroquine and artemisinin), and *Pf* Dd2 (chloroquine-resistant and sensitive to artemisinin), and the inhibitory concentration 50 (IC_{50}) was determined. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, polyphenols, and tannins with respective contents of 855.2 µg QiE/mg D.M., 179.99 µg Q.E./mg D.M., 68.24 µg SaE/mg D.M., 46.46 µg GAE/mg D.M. and 0.11 µg TAE/mg D.M. The extract of *Ficus elastica* lianas showed antioxidant activity with an IC_{50} = 25.30 µg/ml compared to that of gallic acid, which was 5.06 µg/ml. At 31,25 µg/ml, a 22,49 µg/g GAE concentration could reduce Fe^{3+} to Fe^{2+}. It also showed good antiplasmodial activity (IC_{50}: 11.98 ± 0.42 µg/ml) on strain Dd2 and moderate activity (IC_{50}: 23.17 ± 0.45 µg/ml) on strain 3D7. On the *Pf* Dd2 strain, the extract was more active than Chloroquine and artemisinin, while on the *Pf* 3D7 strain, the extract showed better activity than Chloroquine but less than artemisinin. These results demonstrate that the ethanolic extract of Ficus elastica lianas has antioxidant and antiplasmodial properties, probably due to its high alkaloids and flavonoids. It could be a natural alternative for discovering new antioxidant and antimalarial drugs.

Keywords: *Ficus elastica;* Lianas; phytochemicals; Antioxidant activity; Antiplasmodial activity.

1. Introduction

Oxidative stress corresponds to an imbalance between the production of activated oxygen species (AOS) and the body's antioxidant defenses in favor of the former. Our lifestyle (smoking, alcoholism, obesity, intense physical exercise), but also our bad eating habits, abnormally increase the production of AOS in our body. This exposes us to various pathologies such as cancers or cardiovascular diseases [1]. An antioxidant is a substance that prevents or slows down oxidation by neutralizing free radicals responsible for excess cell damage. The use of synthetic antioxidant molecules is currently questioned because of the potential toxicological risks. Now, new sources of natural antioxidants are being sought [2,3]. Indeed, natural compounds such as polyphenols are widely distributed in the plant kingdom and are of increasing importance thanks to their beneficial effects on health [4]. Their role as natural antioxidants is attracting more and more interest in preventing and treating cancer, inflammatory, and cardiovascular diseases [5]. They are also used as additives in the food, pharmaceutical, and cosmetic industries [2]. The increasing global burden of malaria incidence rate and death, as well as resistance development with all the available classes of antimalarial drugs, has become a matter of great concern. Malaria is a parasitic disease transmitted by

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DOI: http://dx.doi.org/10.13171/mjc02401291768mbosso_teinkela

Received November 28, 2023
Accepted January 19, 2024
Published January 29, 2024

the female *Anopheles* mosquito. In 2012, there were approximately 247 million cases and over 619,000 deaths of Malaria worldwide [6].

Children under five years of age account for 67% of malaria-related deaths worldwide, and more than 93% occur in sub-Saharan Africa [7], including 12.8% recorded in Cameroon for 2017 [8]. Geographical and economic inaccessibility to modern health care, as well as the inadequacy of the health care resources, are factors that result in more than 80% of the population in Africa relying on traditional medicine for the treatment of malaria [9]. Thus, *Ficus elastica* Roxb. ex Hornem (Moraceae) was chosen for our work. The selection of this plant was motivated, on the one hand, by its traditional use in the treatment of skin diseases such as allergies and microbial infections and as a diuretic, on the other hand, by the fact that a review of the literature did not reveal any study concerning the evaluation of the antioxidant activity of the ethanolic extract of *Ficus elastica* lianas to the best of our knowledge. However, the anti-proliferative, antimicrobial, and antimalarial activity of the total methanolic extract of *F. elastica* lianas and its fractions were done on *Plasmodium falciparum* 3D7 (sensitive to Chloroquine and artemisinin) [10]. The antimicrobial and cytotoxic activity of the leaves [11], the antimicrobial and anti-proliferative activities of the bark of aerial roots [12], and the anti-proliferative activity of the wood from aerial roots [13] were also studied. This paper, therefore, aims to carry out the quantitative screening, the evaluation of the antioxidant activity (DPPH and FRAP) and the antiplasmodial activity on the *Plasmodium falciparum* 3D7 strain (sensitive to Chloroquine and artemisinin) and *Plasmodium falciparum* Dd2 (chloroquine-resistant and sensitive to artemisinin) of the ethanolic extract of *Ficus elastica* lianas.

2. Experimental

2.1. Materials and methods

2.1.1. Plant material

The plant material consisting of *Ficus elastica* lianas was harvested in Bonaberi in the Douala 4th district, Littoral region of Cameroon, with the help of Botanist Dr TANKEU Sévérin. The plant was identified at the National Herbarium of Cameroon by comparison with specimen number 65646 HNC. The preparation of the ethanolic extract of the plant was performed according to the method described by Adesokan *et al.* [14]. The harvested lianas were washed several times with distilled water to eliminate any impurities. The plant material was then dried at room temperature in a dry place out of direct sunlight. After that, it was grounded and yielded a mass of 2912.8 g of powder. The obtained powder was mixed with 15 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 on 3 mm Wattman filter paper. Finally, the filtrate was evaporated at 60°C using a rotary evaporator. The crude extract obtained was weighed (44.4 g) and utilized for the various tests.

2.1.2. Experimentation

2.1.2.1. Quantitative phytochemical screening

The bioactive compounds in our sample were determined through estimates of the contents of total polyphenols, flavonoids, tannins, alkaloids, and saponins as described by the above protocols.

2.1.2.1.1. Total polyphenols

The total polyphenol content of the sample of interest was determined by Singleton and Rossi's (1965) method using the reagent Folin–Ciocalteu [15]. An aliquot of 0.1 ml extract (4 mg/ml) was added to 0.75 ml of Folin-Ciocalteu reagent (diluted 10 times). The entire mixture was incubated at room temperature (25 ± 1°C). After 5 min, 0.75 ml of sodium carbonate solution (Na_2CO_3, 6%) was added. The mixture was homogenized and incubated at room temperature (in the dark) for 90 min. The absorbance was read at 725 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu-Japan) against a reactive white. A gallic acid standard (0-1000 µg/ml) was used. The total polyphenol content of the extract was calculated using a calibration curve (r^2 = 0.97) and expressed as micrograms of gallic acid equivalent per gram of dry matter (µg GaE/mg D.M.).

2.1.2.1.2. Total flavonoids

The estimation of the flavonoid content in the extract was performed using the method of Aiyegoro and Okoh (2010) [16]. An aliquot of 0.5 ml extract (4 mg/ml) was added to 1.5 ml methanol solution. Then, 0.1 ml of aluminum chloride solution ($AlCl_3$, 10%), 0.1 ml of potassium acetate (CH_3COOK, 1M), and 2.8 ml of distilled water were added. The whole mixture was well homogenized and incubated for 30 min at room temperature (25 ± 1°C), and the absorbance was read at 415 nm (UVmini-1240, UV-Vis Spectrophotometer, ShimadzuJapan) against the reactive white. Quercetin at different concentrations (0-1000 µg/ml) was standard. The flavonoid content was calculated using a standard curve (r^2 = 0.99) and expressed in micrograms of quercetin equivalent per gram of dry matter (µg Q.E./mg D.M.).

2.1.2.1.3. Tannins

Tannins were quantified in the extract using Sun et al. (1998) [17] method. A volume of 0.3 ml of chloridric acid (HCl, 1N) is added to 0.6 ml of vanillin dissolved in ethanol (4% v/v) and 0.1 ml of extract (4 mg/ml). The solution is homogenized and incubated at room temperature (25 ± 1°C) for 15 min. The absorbance is read at the wavelength of 500 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu Japan) against white. Tannic acid is used as a standard at different concentrations (0-1000 µg/ml) to establish the calibration range.

The results are expressed in micrograms of gallic acid equivalent per gram of dry matter (µg TAE/mg D.M.).

2.1.2.1.4. Alkaloids

Determining alkaloids in the extract was done using the method described by Diouf *et al.* (2014) [18] with some modifications. 100 mg of extract powder is extracted in 10 ml of 80% ethanol, then filtered and centrifuged at 5000 rpm (Rotofix 32 A, HettichZentrifugen, Germany) for 10 min. In the supernatant obtained, 1 ml was taken from which is added 1 ml of $FeCl_3$ (0.025 M) + HCl (0.5 M) and 1 ml of 1.10 phenanthroline (0.05 M) in ethanol. The mixture was incubated in a water bath for 30 minutes at 70 ± 2°C. The absorbance of the red coloration of the formed complex was read at the wavelength of 510 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu-Japan) against white. Quinine has been used as a standard at different concentrations (0-1000 µg/ml) to establish the calibration range. Results are expressed in micrograms of Quinine equivalent per gram of dry matter (µg QiE/mg D.M.).

2.1.2.1.5. Total saponin content

The determination of saponins in plant extract is carried out using the method described by Hiai *et al.* (1976) [19]. 200 µl of extract were introduced into a test tube; 200 µl of vanillin was prepared by dissolving vanillin in ethanol (80%), and then 2000 µl of a sulfuric acid (72%) solution were added. The mixture was homogenized and placed in a water bath at 60°C for 10 minutes. The absorbance of this prepared solution was read after incubation at a wavelength of 535 nm relative to the blank. To establish the calibration range, saponins were used as standards at different concentrations (0-1 mg/ml). The results are expressed in milligrams of saponin equivalent per gram of dry matter of the sample (mg SaE/mg D.M.). A total of three repetitions are performed for each.

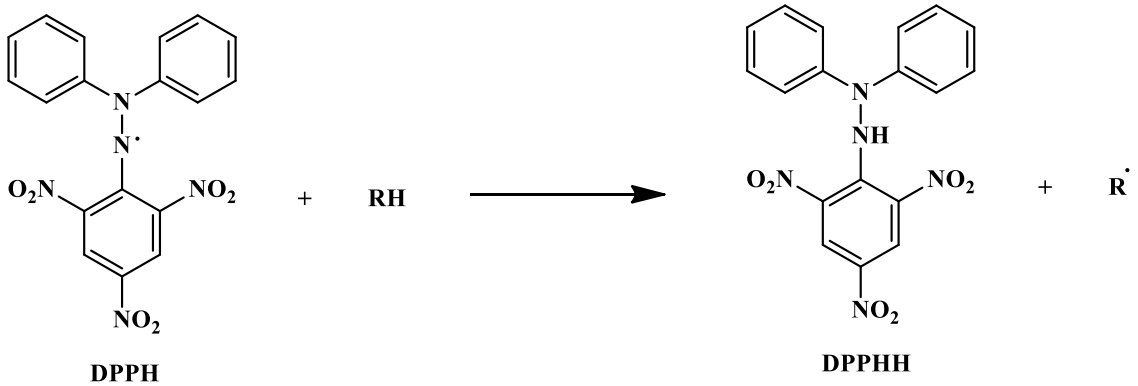
2.1.2.2. Antioxidant activity

2.1.2.2.1. DPPH (2,2-diphenyl-1-pycrylhydrazyl) activity

Based on the ability of an antioxidant to reduce the violet-colored DPPH· radical by transfer of hydrogen to a yellow compound (DPPH-H). The test was performed as proposed by Makris *et al.* with minor modifications [20]. DPPH free radical scavenging test. In each spectrophotometric cuvette, we mixed the extract (2500 µg/ml) prepared at different concentrations (500, 250, 125, 62.5, 31.5 µg/ml) with the DPPH reagent (0.02 mg/ml) in the ratio 1:5 (extract 20 µl; DPPH 80 µl) and curved at 27°C in the dark for 30 min. The positive control was gallic acid (1 mg/ml), which was also prepared at different concentrations (200, 100, 50, 25, 12.5, 6.25 µg/ml) under the same conditions (gallic acid 20 µl DPPH 80 µl). Radical scavenging activity was determined by measuring U.V. absorbance at 517 nm against white (DPPH° + ethanol). Each experiment was performed in triplicate, and the anti-radical activity was expressed as a percentage of inhibition according to the formula below:

$$RSA = (A_0 - A_s / A_0)\ 100$$

RSA: Radical Scavenging Activity; A_0: control absorbance; A_S: absorbance of the extract at 517 nm.



DPPH **DPPHH**

2.1.2.2.2. FRAP test (Ferric Reducing Antioxidant Power)

This method measures the ability of antioxidants to reduce ferric iron to ferrous form at low pH. The reducing power of the extracts was determined by the method described by Yefrida *et al.* [21]. 25 µl of each test sample were prepared at different concentrations (3.125, 6.25, 12.5, 25, and 50 µg/ml) in the test plates, and 25 µl of iron (III) chloride (1.2 mg /ml) were added to the samples. Gallic acid served as a positive control. The plates were incubated at room temperature for 15 minutes. After incubation, 50 µl of 1,10-phenanthroline (0.02%) was added, then the absorbance of the mixture was determined at 510 nm using a spectrophotometer. The control consisted of iron (III) chloride, distilled water, and 1,10-phenanthroline. Each experiment was performed in triplicate. The sample concentration required to scavenge 50% of Fe^{3+} (50% inhibition concentration, IC50) was determined from dose-response curves obtained from different concentrations of the samples.

2.1.2.3. Antiplasmodial activity

Plasmodium falciparum 3D7 (chloroquine-sensitive/artemisinin-sensitive) and *Plasmodium falciparum* Dd2 (chloroquine-resistant/artemisinin-sensitive) strains were obtained from the Biodefense and Emerging Infections (BEI) Research Resources (Manassas, VA). They maintained 5% CO_2 at 37°C using a modified Trager and Jensen method [22]. 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 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) before the drug activity assays. EtOH extract of *F. elastica* lianas dissolved in dimethylsulfoxide (DMSO) at 0.2% was diluted in RPMI 1640 and mixed with the parasite cultures (1.5% hematocrit and 1% parasitemia) 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 *F. elastica* lianas 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 a 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 [23]. The experiments were performed in triplicate, and each one was repeated at least once.

2.1.2.4. Statistical Analysis

The concentrations at which 50% inhibition of growth (IC_{50} values) was obtained 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. Data were expressed as a mean ± Standard error on Mean (SEM).

3. Results and Discussion

3.1. Quantitative phytochemical screening

The yield of the ethanolic extract of *Ficus elastica* lianas at the different stages was 65.30% after grinding and 1.52% after maceration. The other contents are shown in Figure 1. The results revealed that the extract has a higher content of alkaloids (855.20 µg QiE/mg D.M.), followed by flavonoids (180.00 µg Q.E./mg D.M.), then saponins (68.24 µg SaE /mg D.M.), then polyphenols (46.46 µg GAE/mg D.M.), and finally tannins (0.11 µg TAE/mg D.M.).

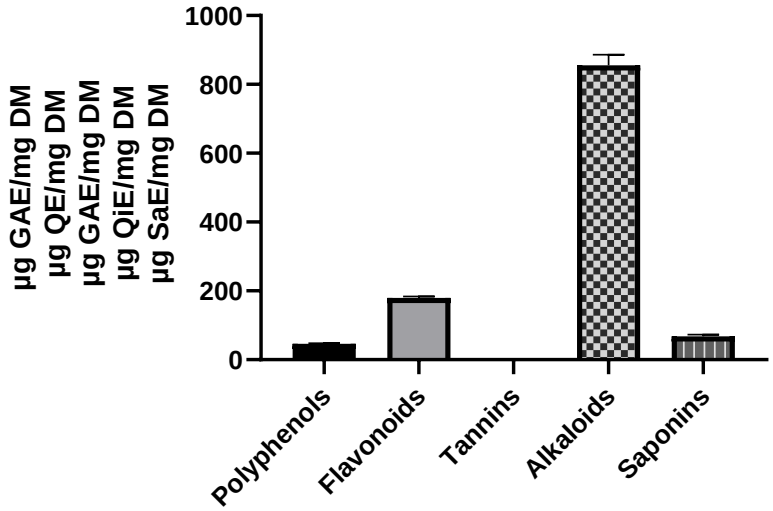


Figure 1. Contents of bioactive compounds in the ethanolic extract of *F. elastica* lianas. Values expressed as mean ± standard deviation; *F. elastica*: *Ficus elastic*a; GAE: Gallic acid equivalent; mg: milligram; D.M.: dry matter; Q.E.: Quercetin Equivalent; QiE: Quinine Equivalent; SaE: Saponins equivalent.

The different groups of secondary metabolites present in the ethanolic extract of *F. elastica* lianas were alkaloids (855,2 ± 31,03µg QiE/mg D.M.), flavonoids (179,99 ± 3,84µg Q.E./mg D.M.), saponins (68,24 ± 5,02µg SaE/mg D.M.), polyphenols (46,46 ± 1,83µg GAE/mg D.M.), and tannins (0,11 ± 0,009µg GAE/mg D.M.). Our results are close to those of Mbosso et al. [10], who, according to the qualitative phytochemical screening of the methanolic extract of *F. elastica* lianas, noted the presence of alkaloids, saponins, tannins, triterpenes, and the absence of flavonoids and anthraquinones. This difference may be related to the place of harvest (Yaoundé in their study and Bonabéri in ours) and to the difference in the extraction solvent (Methanol in their research and ethanol in ours).

3.2. Antioxidant Activity

3.2.1. DPPH activity

Figure 2 shows the evolution of the inhibition percentage as a function of the concentration of the extract and the Gallic Acid. We see that the percentage of inhibition increases with concentration. Nevertheless, because the inhibition percentage of Galic acid is higher than that of the extract, he presents better inhibition than the ethanolic extract of *F. elastica* lianas.

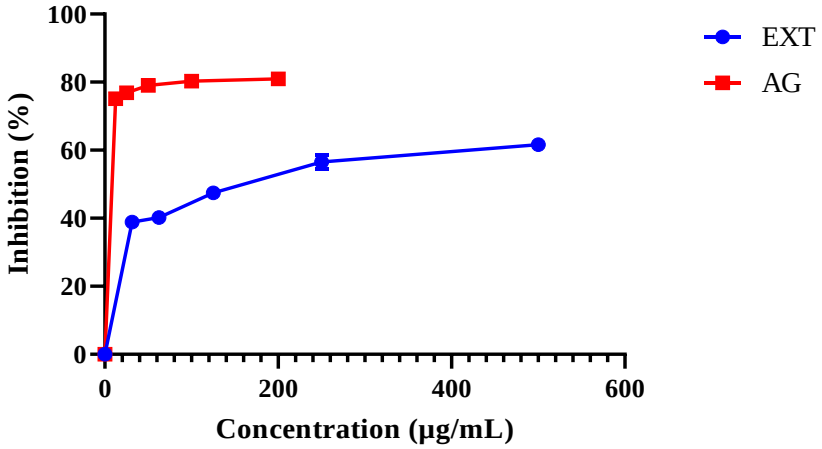


Figure 2. Percentages of inhibitions as a function of the concentration of the ethanolic extract of *F. elastica* lianas and gallic acid. Ext: ethanolic extract of *F. elastica* lianas; GA: gallic acid

Table 1 presents the effects of the ethanolic extract of *F. elastica* lianas on the DPPH radical. The results show inhibition of the DPPH radical by Gallic Acid and extract-dependent concentration, with 5.06 ± 0.36 and 25.30 ± 0.11 µg/ml inhibitory concentrations, respectively.

Table 1. Antioxidant activity of *F. elastica* lianas extract on the DPPH radical.

Samples	IC_{50} (µg/ml)
Gallic acid	5.06 ± 0.36
Ethanolic extract of *F. elastica* lianas	25.30 ± 0.11

3.2.2. FRAP (Ferric Reducing Antioxidant Power)

The reducing power of iron by the ethanolic extract of *F. elastica* lianas is summarized in Figure 3. The results show a reducing power of Fe^{3+} to Fe^{2+}, increasing depending on the extract concentration. At a concentration of 31.50, 62.50, 125, 250, and 500 µg/ml, we obtained the reducing power of 22.49 ± 2.42, 43.71 ± 0.48, 107.93 ± 2.21, 133.10 ± 1.92 and 142.86 ± 1.79 respectively.

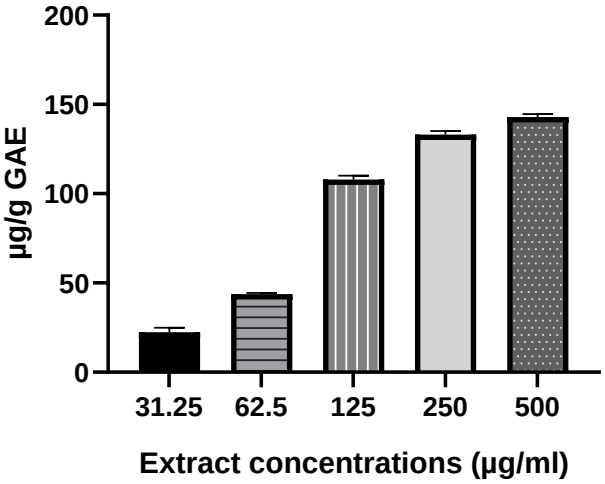


Figure 3. Ferric reducing power

The *in vitro* evaluation of the antioxidant activity of the ethanolic extract of *F. elastica* lianas showed that the extract possesses this activity and can reduce the DPPH with an IC_{50} = 25.30 µg/ml. This value is

higher than that of gallic acid, which is 5.06 µg/ml, and therefore more active than the extract. At 31,25 µg/ml, a 22,49 µg/g GAE concentration can reduce ferric iron to ferrous iron. These results are consistent with those of Chougale *et al.* [24], which showed that *Ficus elastica* leaves ethanolic extract has lowest activity on DPPH scavenging activity (IC_{50} =13.82 µg/mL), moderate activity with IC50 value 23.29 µg/mL in ABTS scavenging activity, moderate activity with value 241.58 µM Fe(II)/µg in FRAP activity and moderate activity with IC_{50} = 83.97 µg/mL in H_2O_2 scavenging activity and El-Hawary et al. [25], which showed that the methanolic extracts of both leaves and branches of *Ficus elastica* showed DPPH• Scavenging activity with ED_{50} = 15.4 and 50 26.9 respectively in comparison with vitamin C using different concentration. This antioxidant activity could be linked to polyphenols, tannins, alkaloids, and flavonoids in the ethanolic extract of *F. elastica* lianas. These classes of secondary metabolites are recognized as potential antioxidant substances that can scavenge radicals and reactive oxygen species [26,27].

3.3. Antiplasmodial Activity

Ethanolic extract of *Ficus elastica* lianas was subjected to an antiplasmodial test on multi-drug-resistant *Plasmodium falciparum* Dd2 and chloroquine-sensitive/artemisinin-sensitive) 3D7 strains. The IC_{50s} for the crude extract tested against the chloroquine-sensitive/artemisinin-sensitive *Pf* 3D7 and the chloroquine-resistant/artemisinin-sensitive *Pf* Dd2 strains were 23.17 + 0.45 and 11.98 + 0.42 µg/ml respectively (Table 3). This value is close to that obtained for Chloroquine to which the strain is resistant (23.42 + 0.44 µg/ml with *Pf* 3D7) and lower than the IC_{50} obtained for Chloroquine to which the strain is not resistant (130.29 + 13.98 µg/ml with *Pf* Dd2) (Table 3).

Table 2. Sensitivity of 3D7 *P. falciparum* and multi-drug-resistant Dd2 *P. falciparum* strains to crude extract of *Ficus elastica* lianas and known antimalarial drugs.

Samples	*Pf* 3D7	*Pf* Dd2
	IC_{50} (µg/ml)	
Chloroquine	23.42 + 0.44	130.29 + 13.98
Artemisinine	16.23 + 10.01	15.75 + 0.46
***Ficus elastica* lianas**	23.17 + 0.45	11.98 + 0.42

Values are from one representative experiment; *Pf* 3D7: *Plasmodium falciparum* 3D7; *Pf* Dd2: *Plasmodium falciparum* Dd2

Chemosensitivity tests carried out on strains 3D7 and Dd2 only led to the suggestion that ethanolic extract of *F. elastica* lianas is not highly active based on reference IC_{50} values according to the scale of Singh *et al.*, en 2015 [28] which classified the plants extract for their antiplasmodial potential as (a) highly active (IC_{50} ≤ 5 mg/ml), (b) promisingly active (IC_{50} 5.1–10 µg/ml), (c) good activity (IC_{50} 10.1 – 20 µg/ml), (d) moderate activity (IC_{50} 20.1 – 40 µg/ml), (e) Marginal potency (IC_{50} 40.1 – 70 µg/ml), (f) poor or inactive (IC_{50} > 70.1 µg/ml). On the other hand, this extract presented good activity according to the same scale on the chloroquine-resistant/artemisinin-sensitive *Pf* Dd2 (11.98 + 0.42 µg/ml) and moderate activity on the chloroquine-sensitive/artemisinin-sensitive *Pf* 3D7 strain (23.17 + 0.45 µg/ml). The tests with the two molecules (Artemisinin and Chloroquine) taken as a reference molecule confirmed their effectiveness or ineffectiveness on the strain 3D7 (sensitive to both molecules) and Dd2 (resistant to Chloroquine), which validates our chemosensitivity tests (Table 2). In our preview study, only the hexane fraction significantly reduced the viability of *Plasmodium falciparum* 3D7 by 38.46% at a concentration of 25 µg/mL (IC_{50} 26.41 µg/ml). The total methanolic extract of *F. elastica* lianas and other fractions did not reduce viability. It showed percentages of viable cells of 107.27% for aqueous fraction, 97.18% for ethyl acetate fraction, 100% for total extract, and 92.81% for dichloromethane fraction [10]. These results show that the ethanolic extract of *F. elastica* lianas is more active than the methanolic extract, possibly linked to the absence of flavonoids in the methanolic extract. We obtained an IC_{50} of 11.98 + 0.42 µg/ml for the ethanolic extract of *F. elastica* lianas, a value well below for certain plant extracts, including leaf ethanol extract of *Alstonia scholaris* (L.) R. Br, *Albizia procera* (Roxb.) Benth, *Saraca indica* L., *Azadirachta indica* A.Juss, *Pongamia pinnata* (L.) Pierre, bark ethanol extract of *Alstonia scholaris* (L.) R. Br., *Ziziphus jujube* Mill., *Madhuca longifolia* var. *latifolia* (Roxb.) A. Chev., seed ethanol extract of *Carum carvi* L., whole plant ethanol extract of *Eclipta prostrate* (L.) L., used in Cameroon against malaria and/or associated symptoms, presented antiplasmodial activities with IC_{50} values greater than 20.1 µg/ml [28]. These results suggest ethanolic extract of *F. elastica* lianas could be used as an antiplasmodial drug on people with chloroquine-sensitive and chloroquine-resistant strains in combination with another molecule exhibiting a

mechanism of action other than the extracts. Interestingly, the crude extract showed high flavonoid and alkaloid content. These two classes of secondary metabolites could be responsible for the antiplasmodial activity observed. This hypothesis is supported by the results of Bero Joanne *et al.* in 2011, who demonstrated promising antiplasmodial activity (IC_{50} < 2 µM) with alkaloids such as flinderole B isolated from *Flinderiaam boinensis* Poir (Rutaceae) and astiphyllanin B isolated from *Alstonia macrophylla* Zall (Apocynaceae) with the reference strains Dd2 and 3D7 [29]. In another study conducted by Cho *et al.* in 2018, acetylcaranine, an alkaloid isolated from *Atropa belladonna* (Amaryllidaceae), also showed antiplasmodial activity on the Dd2 strain with an IC_{50} of 1.1 µg/ml [30]. Marked antiplasmodial activities have also been reported for plant flavonoids. Common dietary flavonoids, including quercetin and kaempferol, have been documented to inhibit the *in vitro* viability of 3D7 *P. falciparum* [31]. Quercetin has shown a higher antiplasmodial activity than isoquercitrin, suggesting that glycosylation, which makes isoquercetin more polar, reduced the antiplasmodial activity. Also, four flavonoids, namely nymphaeol, solophenol, nymphaeol, and nymphaeol B, isolated from ethyl acetate fraction of *Macaranga tanarius* leaves showed antiplasmodial activity against *Plasmodium falciparum* strain 3D7 with an IC_{50} values of 0.30, 0.24, 0.31, 0.05, and 0.05 µg/mL, respectively [32].

4. Conclusion

The results of the present studies provide clear evidence that the crude ethanolic extract of *F. elastica* lianas possesses alkaloids, flavonoids, saponins, polyphenols, and tannins. The extract showed antioxidant activity against DPPH and could reduce ferric iron to ferrous iron. This extract showed good activity on the chloroquine-resistant/artemisinin-sensitive *Pf* Dd2 and moderate activity on the chloroquine-sensitive/artemisinin-sensitive *Pf* 3D7 strain. It would be more active than the methanolic extract, probably due to its high content of alkaloids and flavonoids. These results show that ethanolic extract of *F. elastica* lianas could be a natural alternative for synthesizing new antioxidant and antimalarial drugs. However, the next step is the *in vivo* study, followed by preparing standardized traditional medicine while keeping the work on active ingredient isolation.

Acknowledgments

The authors are grateful to Dr TANKEU Sévérin for the botanical identification, Dr Thierry Fokou for his support, and the Malaria Research unit of Centre Pasteur du Cameroun (CPC) for the antiplasmodial tests.

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