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Evidence of circadian effects on phytochemical content and antioxidant potential of *Ficus bubu* extracts

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Abstract: *Ficus bubu* Warb. is a member of the Moraceae family, which includes species of the same genus with numerous therapeutic virtues. No other studies besides antimicrobial and antiproliferative activities have been conducted on *Ficus bubu*. Current research aims to observe the influence of circadian rhythms on the qualitative and quantitative phytochemical analysis and to evaluate the antioxidant activity of ethanolic extracts from leaves, stem bark, and roots, taken at different times of day (6 am, 12 pm, 6 pm), making 09 samples processed separately. Secondary metabolites were identified qualitatively using insoluble complex formation or color reactions, and quantitatively using a spectrophotometer at specific wavelengths. Antioxidant activity was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, iron reduction (FRAP), and phosphomolybdenum methods. The results show that leaves (10L1Fb) harvested in the morning show the most promising activity, followed by stem bark (10B3Fb) in the evening and roots (10R2Fb) at midday. These extracts demonstrate significant antioxidant potential, suggesting that *Ficus bubu* could be a promising natural source for developing new drugs, notably in treating rheumatoid arthritis, cancer, cardiovascular disease, malaria, etc.

Keywords: *Ficus bubu;* Leaves; Stem bark; Roots; Phytochemicals; Antioxidant activity.

1. Introduction

Medicinal plants are an essential source of natural bioactive compounds with numerous health-promising properties, including antioxidant properties [1,2]. However, the content of these phytochemicals can vary significantly depending on various environmental and physiological factors [3,4]. Among these factors, the circadian rhythm seems essential in regulating plants' secondary metabolites [5]. Using a multidisciplinary approach combining temporal, physiological and phytochemical analyses, qualitative and quantitative characterizations of circadian variations in the profile of secondary metabolites (polyphenols, flavonoids, carotenoids, etc.) in different plant organs; the evaluation of the impact of circadian fluctuations on bioactive compounds and antioxidant activities *in vitro* and finally, the identification of the underlying samples with the best antioxidant activities in the circadian regulation of secondary metabolism, would serve as common threads [5-7]. Oxidative stress is an imbalance between the production of oxidizing molecules, such as free radicals, and the body's ability to neutralize them through its antioxidant defense systems. This phenomenon can lead to significant cellular damage and plays a major role in the development of numerous pathologies, including cardiovascular and neurodegenerative diseases, cancer, and aging [8,9]. Throughout evolution, living organisms have developed complex antioxidant defense mechanisms, involving enzymes, vitamins, and other molecules capable of neutralizing reactive oxygen and nitrogen species. However, when the production of these oxidizing species exceeds the capacity of these detoxification systems, a state of oxidative stress sets in, with potentially deleterious consequences for health. When the body's system is compromised, external supplements can support and help neutralize oxidizing agents [10,11]. We then considered it essential to study *Ficus bubu* Warb., also called *Ficus kyimbilensis Mildbr. or Ficus pachypleura Warb.*, an endemic fig species of the Moraceae family, whose species of the same genus are reputed to have numerous therapeutic virtues [1-5]. The latex of various *Ficus* species is

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traditionally used for its antirheumatic properties [12,13]. However, no biological or phytochemical studies have been conducted on *Ficus bubu*, which is endowed with antimicrobial and antiproliferative activities. Its latex is traditionally used for treating hemorrhoids and asthma [14,15]. The present study, therefore, aims to investigate the circadian rhythm through several harvests made during the day of three organs of the chosen plant on the phytochemical content and antioxidant potential of *Ficus bubu* Warb.

2. Experimental

2.1 Materials and methods

2.1.1 Plant material

The plant material consists of leaves, stem bark, and roots of *Ficus bubu* Warb. harvested at 6 am, 12 pm, and 6 pm, making 09 samples for the study of the influence of circadian rhythm on secondary metabolites and antioxidant activity. These plant parts were collected in the vicinity of the Melen market in Yaoundé, in the Central region of Cameroon, then identified and confirmed at the Cameroon National Herbarium on January 10, 2024, in comparison with the material of R. Letouzey n° 12153 from Herbarium collection specimen N°29050/SRF/Cam.

2.1.2 Experimentation

2.1.2.1 Harvest and extraction

Leaves, stem bark, and roots were separated from the tree at the three defined times of the day, divided into 09 samples and weighed separately, then dried, weighed again, and processed into fine fibers by pulverization, using the methods described by Allal (2018) and Zaiter (2017) [6,7]. To prepare ethanol extracts of the harvested *Ficus bubu* Warb. (Moraceae) parts, we followed the method described here [8]. We used 1 kg of crushed material from each harvest, which we macerated in 5 L of 70% ethanol; then, we stirred the resulting mixture for 48 h at room temperature. The mixture was then filtered through 1 mm Wattman filter paper, and the filtrate was evaporated at reduced pressure using a rotary evaporator. All this was done separately for each extract, giving us extraction yields by formula:

$$\tau = \frac{m}{M} x\ 100$$

***τ*:** extraction rate**; M:** powder mass**; m:** extract mass

2.1.2.2 Qualitative phytochemical screening

Phytochemical screening is an identification method that uses qualitative techniques to detect specific chemical compounds. This can be achieved by forming insoluble complexes or colour reactions [9,10]. The various groups of compounds, such as alkaloids, flavonoids, sterols, polyterpenes, coumarins, saponins, phenols, tannins, and anthocyanins contained in stem bark extracts were highlighted for each harvest.

2.1.2.3 Quantitative phytochemical screening

The quantification of the various secondary metabolites (alkaloids, flavonoids, polyphenols, terpenoids, tannins, and saponins) was done by absorption spectrophotometry as described by the protocols below:

2.1.2.3.1 Alkaloids

Quantifying alkaloids in the plant extract was done using the method described in the literature, with a few modifications [10,11]. Briefly, 100 mg of the extract was dissolved in 10 mL of ethanol (80%). The whole was homogenized and centrifuged at 5000 rpm for 10 min. After centrifugation, 1 mL of the supernatant from each extract was removed and placed in a test tube, followed by the respective addition of 1 mL of acidified $FeCl_3$ solution (0.025 M) (0.5 M HCl) and 1 mL of 1,10-phenanthroline solution in ethanol (0.05 M). The mixture was incubated at 100°C in a water bath for 30 min.

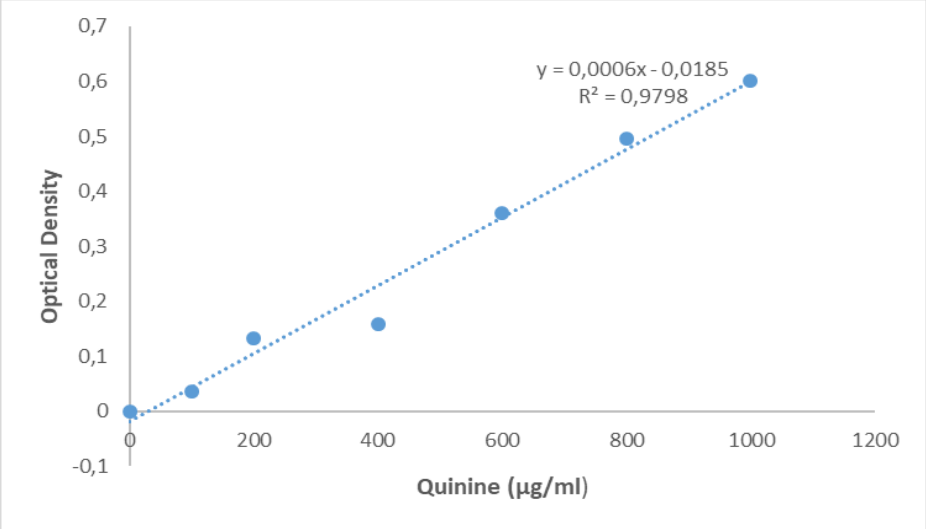

Figure 1. Alkaloids calibration curve.

The absorbance of the reddish complex formed was read at 510 nm against the blank. Quinine at 25 µg/mL concentration was used as the primary standard, and alkaloid content was expressed as micrograms quinine equivalent per milligram dry matter (µg EQi/mg DM). Alkaloid contents of the various extracts were determined from the Quinine calibration line (Y = 0.0006 X- 0.0185; R^2 = 0.9798). The values of each extract were obtained and appended by the calibration curves of the assay of each bioactive compound, as shown in Figure 1.

2.1.2.3.2 Total flavonoids

The colorimetric method used aluminum chloride to assess total flavonoids [12,13]. Briefly, 0.5 mL of the extract (2 mg/mL) was added to 1.5 mL of methanol; subsequently, 0.1 mL of aluminum chloride ($AlCl_3$, 10%), 0.1 mL of potassium acetate (CH_3COOK, 1 M), and 2.8 mL of distilled water were added. The mixture was thoroughly homogenized and incubated for 30 min at room temperature, and the absorbance was read at 415 nm against the reagent blank. Quercetin (0-1000 µg/mL) was used as a reference and total flavonoid content was expressed as microgram quercetin equivalent per milligram dry matter (µg QE/mg DM). The flavonoid content of the various extracts was determined from the calibration line for quercetin (Y = 0.0017 X - 0.0341; R^2 = 0.9956). The values of each extract were obtained and appended by the calibration curves of the assay of each bioactive compound, as shown in Figure 2.

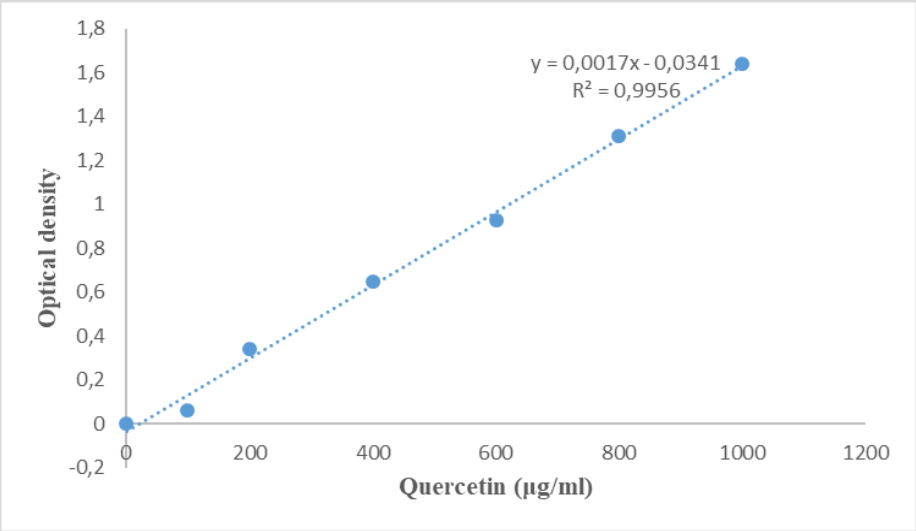

Figure 2. Flavonoids calibration curve.

2.1.2.3.3 Total polyphenols

Total polyphenols were assessed using the Folin-Ciocalteu reagent according to procedure [14,15]. 0.1 mL of extract (2 mg/mL) was mixed with 0.75 mL of Folin-Ciocalteu reagent (diluted 10-fold). The whole mixture was incubated at room temperature. Five minutes later, 0.75 mL sodium carbonate solution (Na_2CO_3, 6%) was added to the mixture. The mixture was homogenized and incubated for 90 min at room temperature (in the dark), followed by an absorbance reading at 725 nm against a reagent blank. Gallic acid (0-1000 µg/mL) was used as a reference.

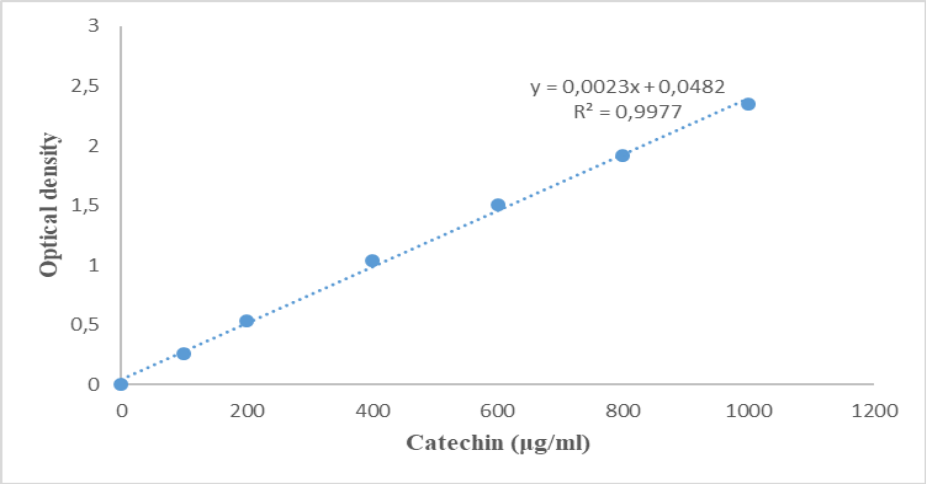

Figure 3. Polyphenol's calibration curve.

Total polyphenol content was expressed in micrograms of Gallic acid equivalent per milligram of dry matter (µg EAG/mg DM). The total phenol content of extracts was determined from the calibration line for Gallic acid (Y = 0.0023 X + 0.0482; R^2 = 0.9977). The values of each extract were obtained and appended by the calibration curves of the assay of each bioactive compound, as shown in Figure 3.

2.1.2.3.4 Total terpenoids

The total terpenoid content (TTC) of extracts was determined by the following method [16,17]. To 1 mL of extract, we added 2 mL of chloroform. The sample mixture was then carefully vortexed before being left for 3 minutes. Subsequently, 200 µl of concentrated sulfuric acid (H_2SO_4) was poured into the mixture, followed by incubation at room temperature for 1.5 to 2 hours in the dark. A reddish-brown precipitate formed in the mix during incubation. After this, the supernatant was carefully decanted without disturbing the precipitate, and 3 mL of absolute methanol was added and vortexed well until complete dissolution of the precipitate in methanol. Absorbance was read at 538 nm using a visible spectrometer. The TTC of each extract was calculated using mg linalool per gram of extract. The equation of the standard curve was Y = 0.0036X - 0.001; R^2 = 0.9927.

2.1.2.3.5 Tannins

The following method of was used to determine the total tannin content of the extract [18]. Briefly, 1 mL of extract (2 mg/mL) was mixed with 5 mL of working solution (50 g vanillin + 4 mL HCl in 100 mL distilled water), then incubated at 30°C for 20 min. Absorbance was read at a wavelength of 500 nm against the blank (without extract). Gallic acid (0-1000 µg/mL) was used to establish the calibration range, and tannin content's results were expressed in microgram Gallic acid equivalent per milligram dry matter (µg GAE/mg DM). The tannin content of the various extracts was determined from the calibration line for Gallic acid (Y = 2*10-5X - 0.0009; R^2 = 0.9454). The values of each extract were obtained and appended by the calibration curves of the assay of each bioactive compound, as shown in Figure 4.

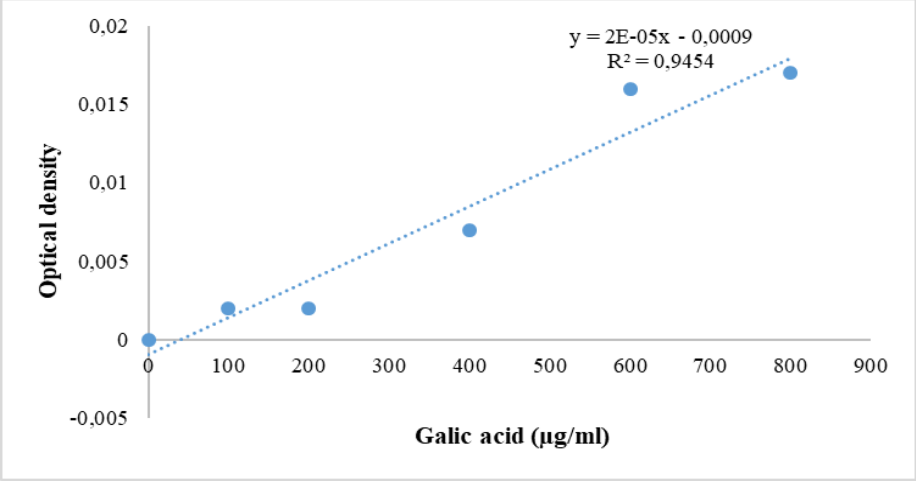

Figure 4. Tannin's calibration curve.

2.1.2.3.6 Total saponin content

The determination of saponins in the extract was carried out using the method described by Hiai *et al.* (1976) [19]. 200 µl of the extract was introduced into a test tube, then 200 µl of alcoholic vanillin solution

(prepared in 80% ethanol) and 2000 µL of a sulfuric acid solution (72%) 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 against the blank. Saponin was used at different concentrations (0-1 mg/mL) to establish the calibration range. Results were expressed as micrograms of saponin equivalents per milligram of sample dry matter (µg Esa/mg DM). A total of three replicates were performed for each extract. Total saponin levels in plant extracts were determined from the calibration line for saponin standard (Y = 0.002 X + 0.09; R^2 = 0.9517). The values of each extract were obtained and appended by the calibration curves of the assay of each bioactive compound, as shown in Figure 5.

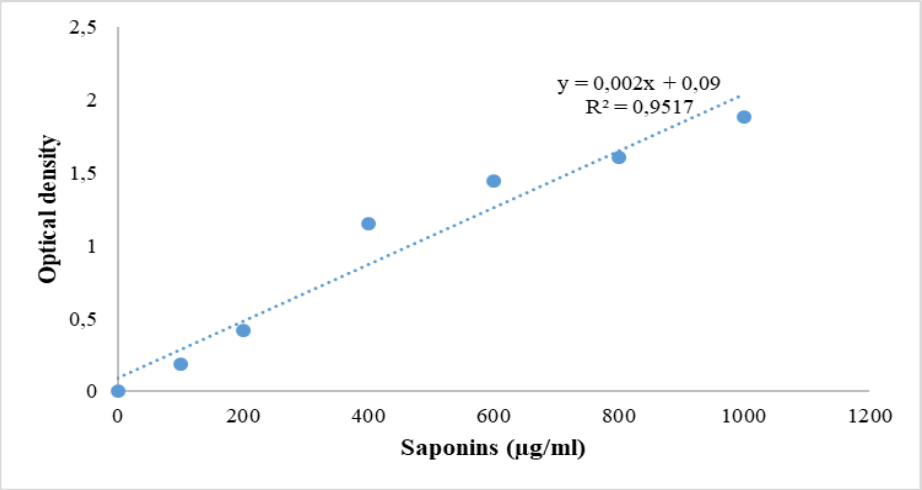

Figure 5. Saponins calibration curve.

2.1.2.4 Antioxidant activity

2.1.2.4.1 DPPH (2,2-diphenyl-1-pycrylhydrazyl) activity

DPPH radical scavenging capacity was assessed for each extract according to the method described as follows [20]. Briefly, 50 µl of methanolic solution of each extract at different concentrations (0.5-4 mg/mL) was added to 1.95 mL of DPPH methanolic solution (25 µg/L). A negative control was prepared in parallel, containing 50 µL of methanol and 1.95 mL DPPH methanolic solution. After 30 minutes of incubation at room temperature in the dark, absorbance was measured at 515 nm, using a reagent blank. Ascorbic acid was used as a reference compound and treated in the same way as the samples. All assays were performed in triplicate. Results were expressed as percentage inhibition (I%) of the DPPH radical, calculated according to the formula of Yen and Duh (1994) [21]:

(I%) = [(Abs control - Abs test)/ Abs control] x 100

The values for trapping capacity 50 (CP_{50} or CI_{50}) were determined graphically by linear regression (logarithmic curve).

2.1.2.4.2 FRAP test (Ferric Reducing Antioxidant Power)

The iron (Fe^{3+}) reducing power of each extract was determined according to the method described by Oyaizu (1986) [22,23]. 1 mL of each extract at different concentrations (0.5-4 mg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $K_3Fe(CN)_6$ solution. The mixture was incubated in a water bath at 50°C for 20 minutes. Next, 2.5 mL of 10% trichloroacetic acid (TCA) solution was added to stop the reaction, and the tubes were centrifuged at 3000 rpm for 10 minutes. An aliquot (2.5 mL) of the supernatant was combined with 2.5 mL distilled water and 0.5 mL 0.1% aqueous iron chloride ($FeCl_3$). The absorbance of the reaction medium was measured at 700 nm with a UV-VIS spectrophotometer, using a blank prepared in the same way but replacing the extract with distilled water.

Ascorbic acid at 0.25 to 10 µg/mL concentrations was used as a reference and treated under the same conditions as the samples. An increase in absorbance indicates an increase in the reducing power of the extract tested. Iron (Fe^{3+}) reducing capacity was expressed in micrograms of ascorbic acid equivalent per gram of dry matter (µg EAA/mg DM).

2.1.2.4.3 Total antioxidant capacity

The total antioxidant capacity of each extract was assessed using the phosphomolybdenum method described by Prieto ***et al.*** (1999) [24]. A 0.2 mL volume of each extract at different concentrations (0.5-4 mg/mL) was mixed with 2 mL of the working reagent solution, consisting of 0.6 M H_2SO_4, 28 MM NaH_2PO_4, and 4 MM ammonium molybdate. Tubes were capped and incubated at 95°C for 90 minutes. After cooling, the absorbance of the solutions was measured at 765 nm, using the reagent blank (2 mL reagent solution + 0.2 mL distilled water) as a reference, treated under the same conditions as the samples. Ascorbic acid at concentrations ranging from 0.25 to 10 µg/mL was used as a standard. Total antioxidant capacity was expressed in micrograms of ascorbic acid equivalent per milligram of dry matter (µg EAA/mg DM).

2.1.2.5. Statistical Analysis

Data analysis was carried out using Excel 2013, GraphPad Prism 8.0.1, and statistical analysis of the following applications: One-way ANOVA and two-way ANOVA. All this enabled us to collect and process data through tables, diagrams, and curves.

3. Results and Discussion

3.1 Plant material and circadian rhythm

Several harvests were carried out to study the biological behavior of the plant. Weighing was carried out at each stage of the extraction process, from the harvest of fresh parts, dried in the shade, to

the crushed parts and extracts obtained. Table 1 shows the extraction yield.

The extraction yield gave us the following values (%): 7.41; 11.56; 8.29; 8.8; 5.65; 7; 7.65; 10.67 and 8.77 respectively for extracts: 10L1Fb; 10L2Fb; 10L3Fb; 10B1Fb; 10B2Fb; 10B3Fb; 10R1Fb; 10R2Fb and 10R3Fb (Table 1). Compared with work carried out on methanol extracts of *Ficus bubu* fruits (5.3%), leaves (4.2%), and stem bark (3.7%) [25,26], the yields obtained from all the current extracts are higher.

Table 1. Extract yield.

	Leaves			**Stem bark**			**Roots**		
	6 h	12 h	18 h	6 h	12 h	18 h	6 h	12 h	18 h
	10L1Fb	10L2Fb	10L3Fb	10B1Fb	10B2Fb	10B3Fb	10R1Fb	10R2Fb	10R3Fb
Fresh parts (g)	2011	863	976	2627	4311	2981	2197	1210	2420
Dried parts (g)	1300	400	400	1000	2100	1400	900	600	1000
Grindings (g)	1200	320	700	750	2000	1300	810	450	650
Extracts (g)	89	37	58	66	113	91	62	48	57
Yield (%)	7.41	11.56	8.29	8.8	5.65	7	7.65	10.67	8.77

3.2 Qualitative phytochemical screening

Table 2 shows the classes of secondary metabolites contained in each extract obtained and coded after phytochemical screening. These compounds were detected in each extract based on precipitation, turbidity, and colour change tests.

Table 2. Results of qualitative phytochemical screening of *Ficus bubu* Warb extracts.

	Leaves			**Stem bark**			**Roots**		
Harvest time	6 h	12 h	18 h	6 h	12 h	18 h	6 h	12 h	18 h
Code	10L1Fb	10L2Fb	10L3Fb	10B1Fb	10B2Fb	10B3Fb	10R1Fb	10R2Fb	10R3Fb
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	-	-	-
Steroids	+	+	+	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	+	+	+	+	+	+
Reducing sugars	-	-	-	-	-	-	+	+	+

+ (presence of metabolites); - (absence of metabolites)

Regardless of the time of harvest in the day, qualitative phytochemical screening of extracts revealed the presence of alkaloids, flavonoids, coumarins, saponins, polyphenols, steroids, anthraquinone, and triterpenes in the three parts of the plant. Tannins would be absent in the roots; glycosides would be lacking in the leaves, while reducing sugars would be present only in the roots. Some metabolites may also be absent due to their low concentration in the extracts. Our results are close to those found in the literature, which, based on qualitative phytochemical screening of the methanolic extract of *Ficus elastica* lianas noted the presence of alkaloids, saponins, tannins, triterpenes, and flavonoids. This similarity may be linked to the fact that they belong to the same genus [25,26].

3.3 Quantitative phytochemical screening

The bioactive compounds in the extracts were determined by estimating the content of alkaloids, flavonoids, polyphenols, terpenoids, tannins, and saponins, as shown in Table 3.

Table 3. Bioactive compound content of extracts.

Extracts	Alkaloids (µg EQi/g DM)	Flavonoids (µg EQ/g DM)	Polyphenols (µg ECa/g DM)	Terpenoids (µg Li/g DM)	Tannins (µg EAT/g DM)	Saponins (µg ES/g DM)
10B1 Fb	53.71 ± 6.77	71.12 ± 1.76	105.63 ± 4.77	68.18 ± 10.67	0.232 ± 0.005	55.56 ± 100
10B2 Fb	195.75 ± 2.59	84.98 ± 2.11	114.10 ± 9.35	124.20 ± 8.47	0.099 ± 0.005	51.11 ± 1.54
10B3 Fb	233.75 ± 2.61	94.67 ± 7.07	156.49 ± 4.22	112.39 ± 2.84	0.243 ± 0.005	51.23 ± 0.85
10L1 Fb	275.97 ± 7.38	124,46 ± 7,67	156,9 ± 10,11	128,83 ± 5,30	0,068 ± 0,003	44,62 ± 0,45
10L2 Fb	217.80 ± 3.61	112.58 ± 1.10	97.44 ± 4.05	124.66 ± 2.41	0.042 ± 0.000	41.44 ± 1.56
10L3 Fb	199.66 ± 2.97	85.94 ± 1.75	85.16 ± 5.66	69.80 ± 7.93	0.034 ± 0.003	17.27 ± 0.96
10R1 Fb	191.62 ± 4.08	43.46 ± 1.84	58.76 ± 5.13	67.25 ± 2.94	0.045 ± 0.000	24.21 ± 0.63
10R2 Fb	199.31 ± 1.54	70.37 ± 3.52	58.76 ± 12.68	82.30 ± 3.06	0.146 ± 0.006	41.35 ± 5.64
10R3 Fb	198.33 ± 1.87	76.65 ± 2.31	65.281 ± 0.34	68.87 ± 3.86	0.095 ± 0.005	35.39 ± 1.37

EQi: Quinine Equivalent; EQ: Quercetin Equivalent; ECa: Catechin Equivalent; Li: Linalol; EAT: Tannic Acid Equivalent; ES: Saponin Equivalent; g: Gram; DM: Dry Matter; 10L1Fb: Leaves harvested at 6 am; 10L2Fb: leaves harvested at 12 pm; 10L3Fb: Leaf harvested at 6 pm; 10B1Fb: Stem bark harvested at 6 am; 10B2Fb: Stem bark harvested at 12 pm; 10B3Fb: Stem bark harvested at 6 pm; 10R1Fb: Roots harvested at 6 am; 10R2Fb: Roots harvested at 12 pm; 10R3Fb: Roots harvested at 6 pm.

The quantitative phytochemical analysis followed the positive qualitative results of the bioactive compounds, revealing the concentrations of the metabolites present: Alkaloids, Flavonoids, Polyphenols, Terpenoids, Tannins, and Saponins. These classes of secondary metabolites were chosen for their high antioxidant, antihelminthic, and antiplasmodial activities. The values revealed alkaloids, flavonoids, polyphenols, and terpenoids were highly concentrated; saponins were moderately concentrated, and tannins were lowly concentrated in all extracts. The extracts selected from each plant part as being the most characterized were leaves harvested at 6 am (10L1Fb), stem bark harvested at 6 pm (10B3Fb), and roots harvested at noon (10R2Fb). In the context of the biological clock [27], these results reveal the best times to harvest these different plant parts, and the Figures below illustrate explicitly the influence of the circadian rhythm on bioactive compounds:

Alkaloids

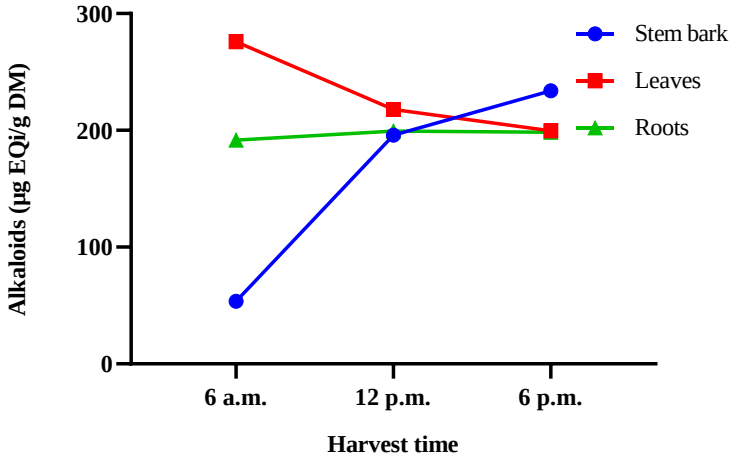

Figure 6. Alkaloid content of *F. bubu* extracts at different harvest times.

The alkaloid concentrations of ethanolic extracts decreased in the leaves, increased in the stem bark, and remained almost constant in the roots. The concentration in the leaves is highest in the morning, and in the stem barks in the evening. Concentrations in the roots are almost constant and relatively average throughout the day. The content of alkaloids and other bioactive compounds in plants varies according to several factors, including the plant's stage of development, the part of the plant, the time of day, and environmental conditions. For alkaloid optimization, the best times are early mornings, when humidity levels are generally lower, and optimal concentrations are reached before the sun's heat rises. Also in the late afternoon, temperatures drop, which can help preserve the compounds. Plants may have accumulated more nutrients during days [25,29]. This corroborates the results obtained.

Flavonoids

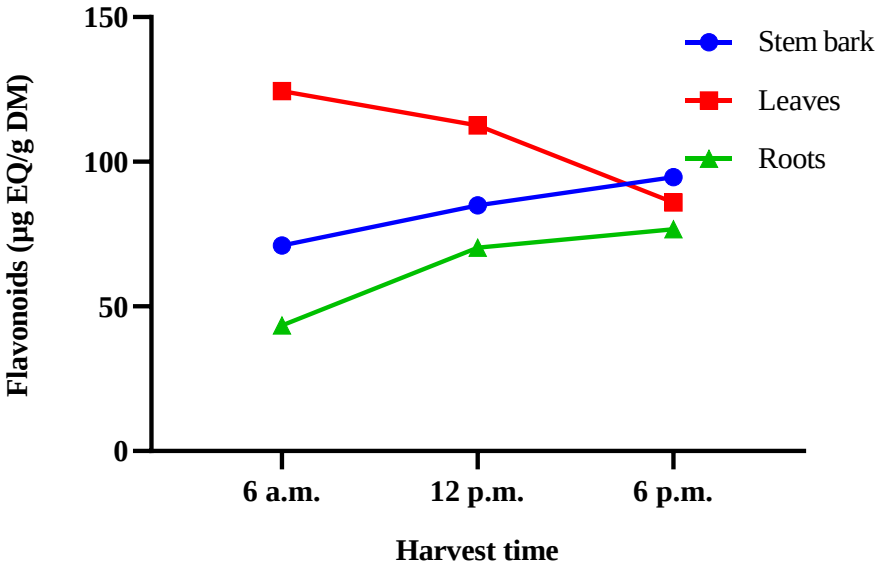

Figure 7. Flavonoid content of *F. bubu* extracts according to harvest time.

Flavonoid concentrations in ethanolic extracts decreased in the leaves and increased in the stem bark and roots. Leaf levels are high in the morning and average in the evening. Levels in the stem bark increase moderately and are slightly higher than those in the roots, which increase similarly. Like alkaloids, the best harvest times are early mornings and/or late afternoons [13,25].

Polyphenols

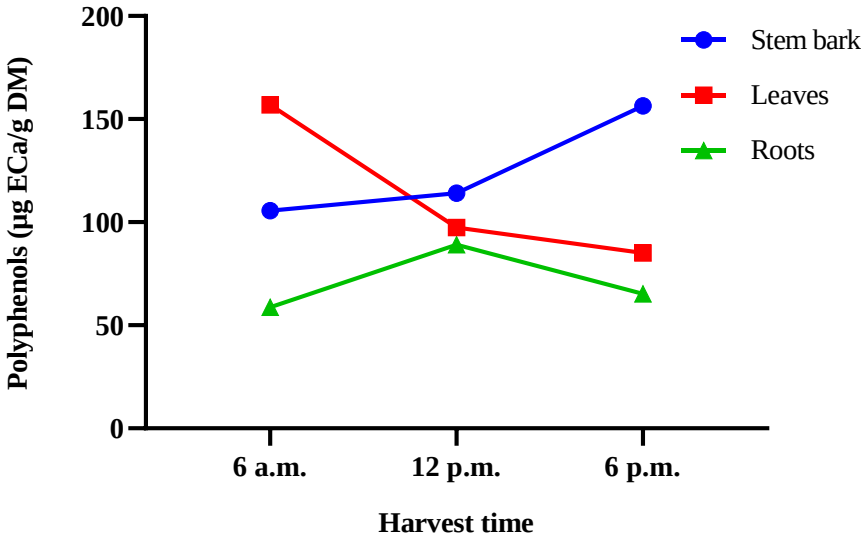

Figure 8. Polyphenol content of *F. bubu* extracts according to harvest time.

Polyphenol concentrations in the ethanolic extracts decreased in the leaves, increased in the stem bark, and remained almost constant in the roots, with a slight peak at midday. The concentration in the leaves is highest in the morning, and in the stem bark in the evening. Concentrations in the roots were almost constant and low throughout the day, on dry, sunny days, early mornings, when temperatures are cooler and humidity levels are lower. Also in the mornings, just before peak heat, plants may have accumulated nutrients and beneficial compounds. Also, in the late afternoon, as with alkaloids, temperatures begin to drop, which can also preserve polyphenol quality. Hence, the peaks observed as a favorable harvesting time [18,29].

Terpenoids

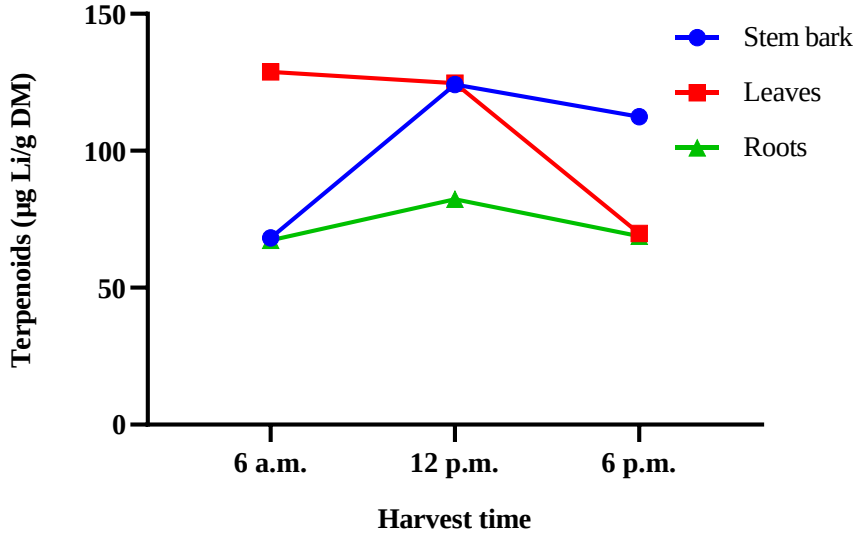

Figure 9. Terpenoid content of *F. bubu* extracts according to harvest time.

Terpenoid concentrations in the ethanolic extracts decreased in the leaves, increased in the stem bark, and remained almost constant in the roots, with a slight peak at midday. The concentration in the leaves is highest in the morning, and in the stem bark in the evening. Concentrations in the roots were almost constant and low throughout the day. Terpenes, aromatic compounds found in many plants, including *Ficus bubu*, have optimal harvesting times depending on various factors, including temperature, humidity, and light cycle. Favorable harvest times are early morning and late afternoon. As the temperature rises, some terpenes can evaporate in the middle of the day. However, some growers prefer to harvest at this time to capture specific terpene profiles [16, 29]. Hence, the peaks of the stem bark occur at midday.

Tannins

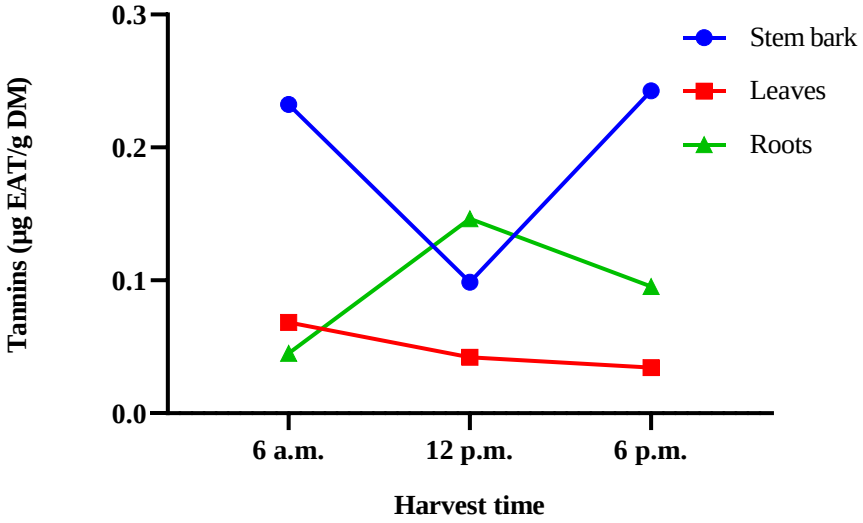

Figure 10. Tannin content of *F. bubu* extracts according to harvest time.

Tannin concentrations in the ethanolic extracts were highest in the morning and evening, with a considerable decrease at midday. Leaves decrease with low concentrations, and roots are moderately concentrated in the stem bark at midday and remain almost constant in the roots with a slight peak at midday. Like terpenes, the best times to harvest tannins are early morning, noon, and late afternoon [29].

Saponins

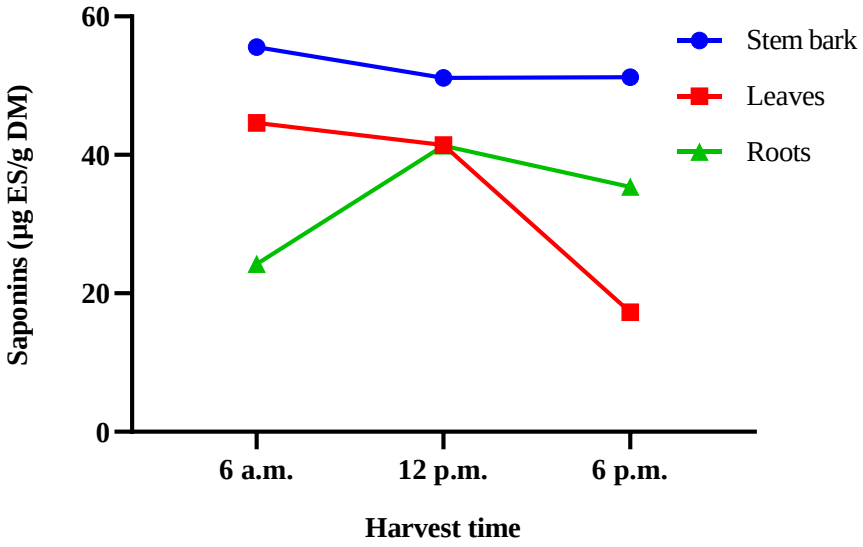

Figure 11. Saponin content of *F. bubu* extracts according to harvest time.

Saponin concentrations in the ethanolic extracts of the stem bark are higher and decrease slightly over the day. Average concentrations in leaves also decrease over the day, and those in roots, which are lower, increase to a certain average. Harvesting saponins, which are found in various plants, requires careful attention to environmental conditions. The best times to harvest saponins during the day are early morning, midday, and late afternoon, depending on the plant parts [19, 29].

The curves illustrated in the previous six Figures clearly show the influence of circadian rhythms on the bioactive compounds quantified during phytochemical screening. These results corroborate those obtained on *Ficus elastica* lianas, which revealed in the ethanolic extract the presence of alkaloids (855.2 ± 31, 03 µg QiE/mg DM), flavonoids (179.99 ± 3.84 µg QE/mg DM), saponins (68.24 ± 5.02 µg SaE/mg DM), polyphenols (46.46 ± 1.83 µg GAE/mg DM), and tannins (0.11 ± 0.009 µg GAE/mg DM) [25].

3.4 Antioxidant Activity

Determination of the antioxidant potential of the extracts followed a circadian rhythm logic consisting of progressively studying the behaviour of several plant parts harvested at different times of the day and selecting those which showed the most promising phytochemical and antioxidant profiles i.e. Stem bark harvested at 6 pm (10E3Fb); Leaves harvested at 6 am (10F1Fb) and Roots harvested at 12 pm (10R2Fb).

.

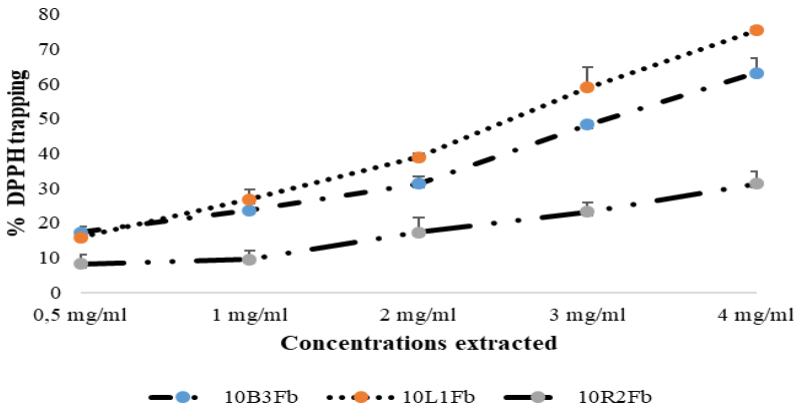

Figure 12. DPPH radical scavenging by extracts. 10L1Fb: Leaves harvested at 6 a.m.; 10R2Fb: Roots harvested at 12 p.m.; 10B3Fb: Stem bark harvested at 6 p.m.

Logarithmic equations

$Y_{(10B3Fb)} = 26.879 \ln(x) + 10.909$; $R^2 = 0.8298$ $IC_{50} = 4.28$ mg/mL

$Y_{(10L1Fb)} = 35.673 \ln(x) + 8.988$; $R^2 = 0.886$ $IC_{50} = 3.16$ mg/mL

$Y_{(10R3Fb)} = 13.836 \ln(x) + 4.6071$; $R^2 = 0.8351$ $IC_{50} = 26.60$ mg/mL

3.4.1 DPPH activity

Figure 12 shows the radical scavenging activity of the various extracts' DPPH (2,2-diphenyl-1-picrylhydrazyl). After observation, we noted that the different extracts trapped this synthetic radical in a concentration-dependent manner, and activity increases in parallel with increasing extract concentrations. Extract 10L1Fb with an IC_{50} of 3.16 mg/mL exhibited the best activity. Figure 12 shows the evolution of DPPH inhibition percentages as a function of extract concentrations. We can see that the inhibition percentages increase with concentration.

3.4.2. Iron reduction (FRAP test)

The data recorded in Figure 13 are those for the capacity of the various extracts at different concentrations to reduce ferric iron using the FRAP method. After observation, we noted that the iron (Fe^{3+}) reducing capacity was expressed in µg ascorbic acid equivalent per gram of dry matter (µg EAA/mg MS) and increased in parallel with increasing extract concentrations. Extract 10L1Fb reduced ferric iron (Fe^{3+}) with the best activity, followed by extract 10E3Fb, compared with extract 10R2Fb, which was the least characterized at all concentrations.

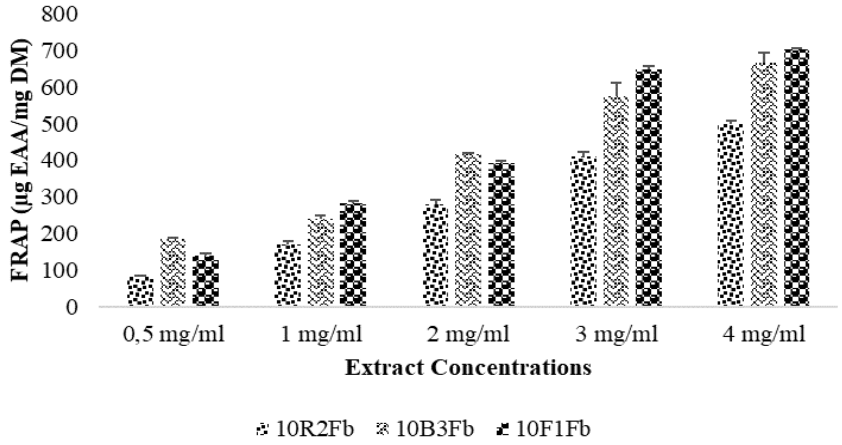

Figure 13. Ferric iron reducing capacity (FRAP) of different extracts. 10L1Fb: Leaves harvested at 6 a.m.; 10R2Fb: Roots harvested at 12 p.m.; 10B3Fb: Stem bark harvested at 6 p.m.

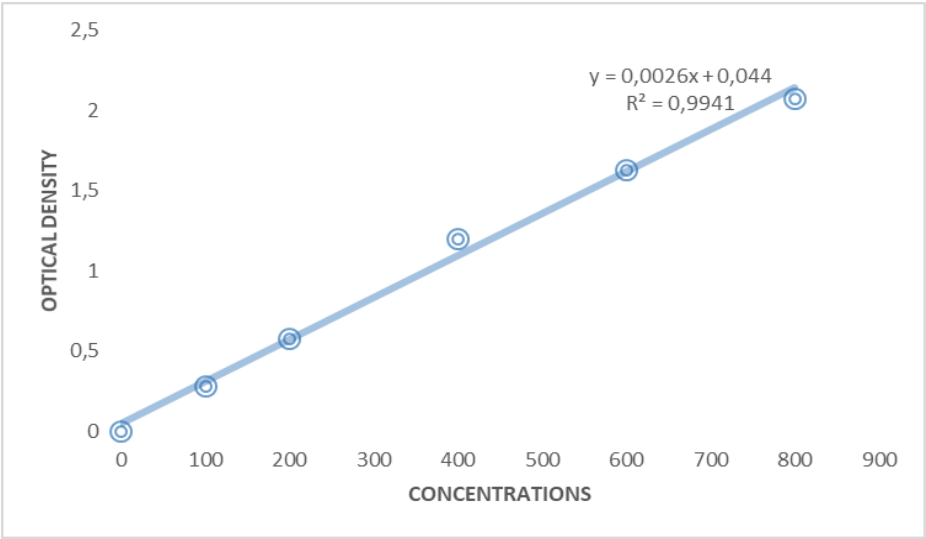

Figure 14. FRAP calibration graph.

3.4.3 Total antioxidant capacity of different extracts

The results shown in Figure 15 are those of the total antioxidant capacity (TAC) of the various extracts at different concentrations. Ascorbic acid at a concentration of (0.25-10) µg/mL was used as the standard. Total antioxidant capacity was expressed as micrograms of ascorbic acid equivalent per milligram dry matter (µg EAA/ mg DM). Extract 10B3Fb reduced molybdate with the best activity at concentrations of 2, 3, and 4 mg/mL; followed by extract 10L1Fb, which was higher at 0.5 and 1 mg/mL, compared with extract 10R2Fb, which was the least characterized at all concentrations.

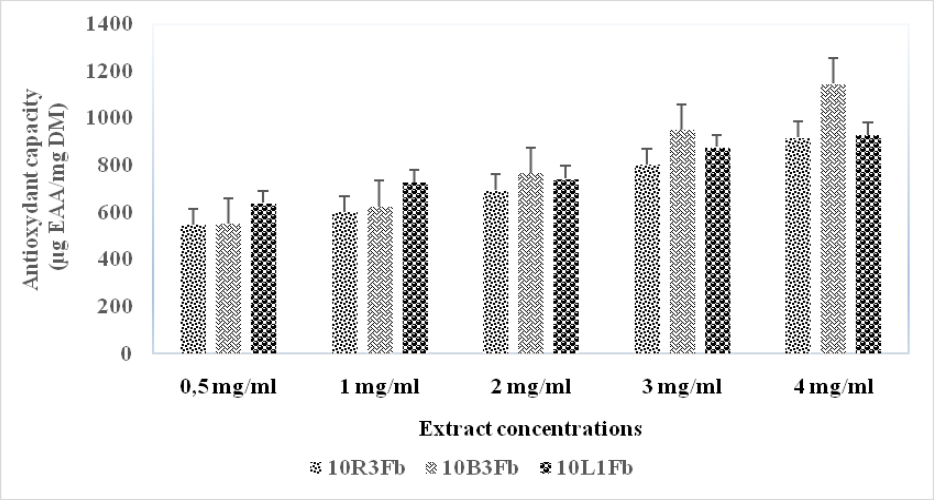

Figure 15. Total antioxidant capacity (TAC, molybdate reduction) of different extracts. 10L1Fb: Leaves harvested at 6 a.m.; 10R2Fb: Roots harvested at 12 p.m.; 10B3Fb: Stem bark harvested at 6 p.m.

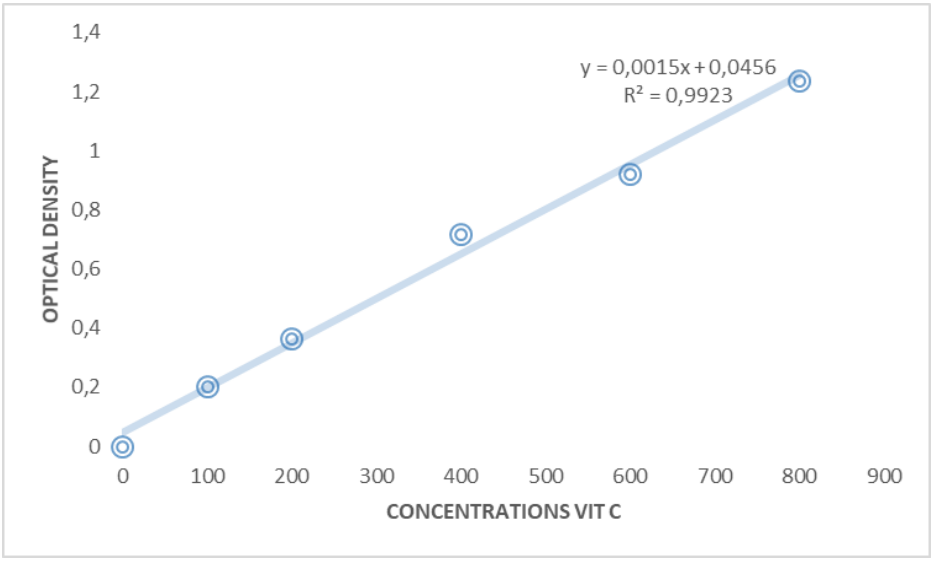

Figure 16. Total antioxidant capacity calibration curve.

Extracts with the highest bioactive potentials (10L1Fb, 10R2Fb, 10B3Fb) enabled us to monitor biological activities and obtain interesting antioxidant powers. DPPH radical scavenging tests determined antioxidant activity. Extract 10L1Fb, with an IC_{50} of 3.16 mg/mL, showed the best activity. The ferric iron reduction capacity (FRAP) test with extract 10L1Fb reducing ferric iron (Fe^{3+}) with the best activity, followed by extract 10B3Fb, compared with extract 10R2Fb, which is the least characterized at all concentrations. Finally, the total antioxidant capacity test with extract 10B3Fb reducing molybdate with the best activity at concentrations 2, 3, and 4 mg/mL, followed by extract 10L1Fb, higher at 0.5 and 1 mg/mL in comparison with extract 10R2Fb, which is the least characterized at all concentrations. These results show that *Ficus bubu* leaves have a high antioxidant capacity, followed by bark and roots. These results are consistent with those of Mbosso *et al.* (2024) [25] and Ginting *et al.* (2020) [28], who showed that ethanol extracts of lianas (IC_{50} = 25.30 µg/mL) [25] and leaves (IC_{50} =13.82 µg/mL) [25] of *Ficus elastica* have low DPPH scavenging activity and moderate activity with a value of 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. There is also consistency with El-Hawary *et al.* [29], who showed that methanolic extracts of *Ficus elastica* leaves and branches exhibited DPPH scavenging activity with ED_{50} = 15.4 and 26.9 [29]. These antioxidant activities of leaves harvested at 6 am. (10L1Fb), stems bark at 6 pm (10B3Fb) and roots at 12 am. (10R2Fb) could be linked to the presence of bioactive compounds revealed during quantitative phytochemical screening with the contents of Alkaloids = 275.97 ± 7.38 µg EQi/g DM (10L1 Fb); Flavonoids = 124.46 ± 7.67 µg EQ/g DM (10L1Fb); Polyphenols = 156.9 ± 10.11 µg ECa/g DM (10L1 Fb). These classes of secondary metabolites are recognized as potential

antioxidant substances that can scavenge free radicals and reactive oxygen species [30,31]. This corroborates the results, which reveal that the levels of polyphenols and flavonoids are responsible for antioxidant activities independently of the methods used [34].

4. Conclusion

Plants are living beings in their own right. Although static, they function and behave differently from day to day and season to season. Studying the influence of *Ficus bubu's* circadian rhythm on secondary metabolites and antioxidant activity enabled us to understand the impact of the biological clock on the various parts of the plant, and then to know that the best times to harvest the leaves (10L1Fb) would be at 6 am, the stem bark (10B3Fb) at 6 pm and the roots (10R2Fb) at 12 pm. Their decreasing contents of bioactive compounds, respectively, justified this. From the nine extracts analyzed, leaves (10L1Fb), stem bark (10B3Fb) and roots (10R2Fb) extracts of *Ficus bubu* respectively showed the most promising antioxidant powers, probably due to their high contents of alkaloids, flavonoids and polyphenols, and could constitute a natural alternative for the discovery of new antioxidant drugs essential in the treatment of rheumatoid arthritis, cancer, cardiovascular diseases, malaria.

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