**Phytochemical analysis of bioactive compounds of the Leaf Extracts of Ficus exasperata Vahl. (Moraceae)**

**ABSTRACT**

**Introduction:** Medicinal plants continue to be an interesting source of natural products for treating various health conditions. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. *Ficus exasperata Vahl*. (Moraceae) is a perennial herb with a long history of traditional medicinal use in the tropical and subtropical regions of the world.

**Aim**: The present study was designed to screen the major groups of phytochemicals found in the leaf parts of the plant.

**Methods:** The aqueous, hydroethanolic, infusion and decoction extracts of the leaves of Ficus exasperata were used for qualitative screening and quantitative analysis using specific standard methods. The identified classes of metabolites in the different extracts were then quantified using spectrophotometric methods.

**Results:** Phytochemical analysis of Ficus exasperata extracts revealed the presence of tannins, alkaloids, flavonoids, phenols, steroids, phlobatannins, saponins, and coumarins. The quantitative analysis of these extracts showed that the infusion extract of Ficus exasperata leaves had the highest content of polyphenols, flavonoids, and tannins, followed by the aqueous and decoction extracts, which exhibited similar concentrations. Ethanolic extracts had the lowest concentrations. Meanwhile, the total alkaloid content was highest in the ethanolic extract, followed by the aqueous and decoction extracts, with the infusion extract containing the least.

**Conclusion:** The quantitative phytochemical analysis of Ficus exasperata extracts revealed that the infusion and aqueous maceration extracts of leaves contained the highest levels of most of the metabolites. This study also demonstrates that the extracts of Ficus exasperata are rich in secondary metabolites, which may explain the plant's traditional use in treating various ailments. The presence of bioactive compounds such as polyphenols, flavonoids, tannins, saponins, and alkaloids underscores the potential therapeutic value of this plant in addressing inflammatory and other health conditions. The high concentrations of these bioactive compounds further support its pharmacological importance and justify its continued use in traditional medicine.

**Keywords:** *Ficus exasperata,* Plant extracts, Photochemical analysis, bioactive metabolites.

1. **INTRODUCTION**

“Natural products, such as plant extracts, in the form of pure compounds or standardized extracts, offer unlimited opportunities for the discovery of new drugs due to the availability of chemical diversity” (Badock et al., 2024). “Medicinal plants continue to be an interesting source of natural products for treating various health conditions. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines” [1]. “It is estimated that more than 150,000 plant species have been studied, many of which contain valuable therapeutic agents, and the applications of novel compounds from plants for pharmaceutical purposes have been gradually increasing in recent years” [2].

In modern medicine, plants continue to play an important role in healthcare even though we have the resources to improve drug synthesis in the laboratory. Due to their long history of usage in folk medicines and as preventative measures, medicinal plants, particularly in underdeveloped nations, have gained a lot of attention [3].

“Plants have played an important role in human health care since ancient times. In an adaptation against attacking pathogens and environmental stress, plants produce several substances that exert biological activities. These small organic molecules come from secondary metabolism and have several biological activities. Among the diverse functions, anti-inflammatory actions are highlighted” [4].

*Ficus exasperata*, also known as the "sandpaper tree" or "rough-leaved fig," is a species of fig tree native to tropical Africa. It is characterized by its rough, sandpapery leaves, which have a distinctive texture and are oval-shaped, growing up to 15 cm long. The bark of the tree is smooth and gray, with horizontal ridges. The fruits are small, greenish-yellow figs, measuring 1-2 cm in diameter [5]. “The leaves of *F. exasperata* have been employed in folkloric medicine for the treatment of various diseases such as ophthalmic and oral infections, venereal diseases, parasitic infection (cutaneous, subcutaneous), leprosy, and malaria” (Awala et al., 2017).

*"Ficus exasperata* is a medium-sized tree, growing up to 15 meters tall, with a spreading canopy. In African traditional medicine, different parts of this plant (fruit, leaf, sap, bark, and root) are considered medicinally important. In Africa, Yemen and India, various parts of the plant are used as analgesic, antiarthritic, diuretics, vermifuges, febrifuge, abortifacient, ecbolic, wound healing, animal fodder and also in general debility, malnutrition, parasitic infection (cutaneous, subcutaneous), leprosy, ophthalmic and oral infections, nasopharyngeal afflictions, arthritis, rheumatism, gout, edema, kidney disorders, diarrhea, dysentery, hemorrhoids and venereal diseases. The leaves of *F. exasperata* are much valued in the treatment of a variety of diseases/disorders. In French Guinea, a decoction of the leaves is used for stomach disorders. The leaves are used to treat hemostatic ophthalmia, coughs, hemorrhoids anxiety disorders, epilepsy, high blood pressure, rheumatism, arthritis, cancer, intestinal pains, colics, bleeding and wounds” [6].

Since *Ficus exasperata* has been widely used for several medicinal purposes, the goal of the current study was to identify and quantify the phytochemicals present in different leaf extracts of the plant. This study aims to provide a scientific basis for justifying its traditional therapeutic applications.

# MATERIAL AND METHODS

# Sample collection and authentication

Freshly collected leaves of Ficus exasperata were harvested from the locality of Obala in the Central Region of Cameroon, 45 km north of Yaoundé, the capital of Cameroon. The plant sample was identified by a botanist at the National Herbarium by comparing it with the voucher specimen: Botanical Collection No. 697, registered at the National Herbarium as No. 14506/SRF Cam. The leaves were shade-dried, and the dried leaves were pulverized into powder form using a clean mechanical grinder.

#  Preparation of Plants extracts

**Aqueous Maceration**

In this process, 100 g of the coarsely powdered crude plant material was placed in a stoppered container with 1000 mL of distilled water and allowed to stand at room temperature for 48 hours with frequent agitation until the soluble matter was dissolved. The mixture was then strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration using Whatman paper (No. 2). The supernatant was collected, and the filtrate was evaporated. The extract was then collected, and the percentage yield was calculated as follows.

$$Percentage Yield =\frac{Mass of extract obtained }{Mass of powder initially use} ×100$$

 **Hydroethanolic Maceration**

In this process, 100 g of the coarsely powdered crude plant material was placed in a stoppered container containing 500 mL of ethanol and 500 mL of water and allowed to stand at room temperature for 48 hours with frequent agitation until the soluble matter had dissolved. The mixture was then strained. The marc (the damp solid material) was pressed, and the combined liquid was clarified by filtration using Whatman paper (No. 2). The supernatant was collected, the filtrate was evaporated, and the extract was collected. The percentage yield was then calculated.

 **Infusion**

In this process, water was heated using a water bath. The warm water was then poured into a container containing 100 g of the plant material. The mixture was strained, the marc (the damp solid material) was pressed, and the liquid was filtered using Whatman paper (No. 2). The supernatant was collected. This filtrate was evaporated, and the extract was collected. The percentage yield was then calculated.

 **Decoction**

In this process, 100 g of the powdered plant material was dissolved in 1000 mL of water. The mixture was placed in a water bath to boil at 98°C for 30 minutes, then allowed to cool. The mixture was strained, pressed, and the combined liquid clarified by filtration using Whatman paper (No. 2), and the supernatant was collected. This filtrate was evaporated, and the extract was collected. The percentage yield was then calculated..

* 1. **Phytochemical Screening**

**Qualitative analysis**

The secondary metabolites that plants contain determine their biological activities. The expected biological activity can be ensured by phytochemical screening for the presence of specific metabolites. We screened for primary and secondary metabolites. The screening methods were carried out according to the general methods proposed by Trease and Evans.. [6].

 **Qualitative analysis of Primary Metabolites**

**Test for carbohydrates**

* **Fehling’s test**: About 1 mL of the filtrate was taken, to which 1 mL of Fehling’s reagent was added and boiled in a water bath. The appearance of a red precipitate indicates the presence of sugars
* **Molisch’s test**: To about 2 mL of the sample, 2 drops of an alcoholic solution of α-naphthol were added, and the mixture was shaken well. A few drops of concentrated H₂SO₄ were added along the sides of the test tube. A violet ring indicates the presence of sugars [7].

**Test for proteins**

**Biuret test**: To 2 mL of filtrate, 1 drop of 2% copper sulfate solution was added, followed by the addition of 1 mL of 95% ethanol. This was followed by the addition of an excess amount of KOH. The appearance of a pink color indicates the presence of protein

**Millon’s test**: To 2 mL of filtrate was added a few drops of *Millon’s* reagent. The appearance of a white precipitate indicates the presence of proteins[8]

**Test for lipids**

To 1 mL of the extract, NaOH was added, and the mixture was then heated in a boiling water bath for 5 minutes. Ethanol was subsequently added to the mixture. The appearance of foam indicates the presence of lipids [9].

**Vitamin C**

To 1 mL of the plant extract, dinitrophenyl hydrazine dissolved in concentrated H2SO4 was added. The presence of yellow precipitates indicates the presence of vitamin C. [10].

**Carotenoids**

To two test tubes containing 2 mL of the plant extract and 2 mL of water each, 2 mL of HCl and 2 mL of H2SO4 were added. The appearance of a blue-green color in both test tubes confirms the presence of carotenoids. [11].

 **QUALITATIVE ANALYSIS OF SECONDARY METABOLITES**

**1-Test for alkaloids**

Extracts were dissolved in dilute hydrochloric acid with the use of a hot water bath and filtered. The following tests were carried out on the filtrate;

**Mayer’s test**: To 1 mL of the extract, 2 mL of Mayer’s reagent (Potassium Mercuric Iodide) was added, a dull white precipitate indicates the presence of alkaloids.

**Wagner’s test**: To 1 mL of the extract, 2 mL of Wagner’s reagent (Iodine/Potassium Iodide) was added. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

**Hager’s test:** 1 mL of filtrate was treated with Hager’s reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow precipitate [12].

**2-Test for phenolic compounds**

**Lead acetate test**: To the test solution, a few drops of 10% lead acetate solution are added. Formation of a white precipitate indicates the presence of phenolic compounds.

**Ferric chloride test:** To the test solution, a few drops of ferric chloride solution are added. A dark green or bluish-black colour indicates the presence of phenolic compounds [13].

1. **Test for flavonoids**

**Sulfuric acid test:** 2mL of aqueous extract was placed in a test tube.To this was added a few drops of concentrated sulphuric acid; being careful not toagitate. The presence of an orange color indicates the presence of flavonoids**.**

**Sodium hydroxide test:** A small quantity of dried extract was dissolved in water and filtered; to 2 mL of this 1 mL of 10 % aqueous Sodium Hydroxide was added to produce a yellow coloration. A change in color from yellow to colourless upon the addition of dilute hydrochloric acid is an indication of the presence of flavonoids [13].

 **4. Test for tannins**

**Reaction with ammoniacal Copper II Sulphate Solution**: 1 mL of 1% CuSO4 solution was added to 1 mL of the extract. To this mixture, two drops of ammonia were added. The formation of a black, blue, or green precipitate indicates the presence of tannins.

**Characterization of catechic tannins:** To 5 mL of the extract, 1 mL of concentrated HCl was added. The mixture was heated in a water bath for 15 minutes and then filtered. The formation of a red precipitate indicates the presence of catechic tannins.

**Gallic tannins**: To the filtrate, pulverized sodium acetate was added, followed by 1 mL of FeCl3. The presence of gallic tannins, not precipitated by the STIANYS test, is indicated by the appearance of a dark blue coloration [12].

1. **Test for Steroids**

1 mL of extract was mixed with 2 mL of Acetic Anhydride and 2 mL of $H\_{2}SO\_{4}$in a test tube. The presence of a violet to blue to green colour indicates the presence of steroids [14]

1. **Test for terpenoids**

1 mL of the extract was mixed with 2 mL of chloroform to which was carefully added 2 mL of Sulphuric Acid. The formation of a reddish-brown layer in the interface between the two liquids indicates the presence of terpenoids [15].

**7. Test for Resins**

1 mL of the extract was placed in a test tube. To this was added a few drops of acetic anhydride and 1mL of Sulphuric acid. The apparition of a yellow coloration indicates the presence of resins [15].

**8.Test for Betacyanin’s**

To 1 mL of extract in a test tube, 1 mL of 2N Sodium Hydroxide. The mixture was heated in a hot water bath for 5 minutes. The appearance of a yellow colour indicates the presence of betacyanin’s.

**9.Test for Phlobotanins**

To 1 mL of the extract in a test tube, a few drops of HCl were added, and the mixture was heated in a hot water bath. The presence of a red precipitate indicates the presence of phlobatannins [10].

**10.Test for Anthocyanins**

1 mL of the extract was placed in a test tube. To this, 1 mL of concentrated H2SO4 was added, followed by 1 mL of ammonia. In the presence of anthocyanins, the color intensifies in an acidic medium and then turns purplish-blue in a basic medium, indicating the presence of anthocyanins

**11.Test for Saponins**

**Foam tes**t: Crude extract was mixed with 5 mL of distilled water in a test tube. The presence of a stable foam after 15 minutes was considered an indication of the presence of saponins [16]

**12.Test for Coumarins**

In a test tube containing 1 mL of the plant extract and 1 mL of distilled water, a few drops of 10% FeCl3 were added. The appearance of a green or blue coloration, which turns yellow upon the addition of nitric acid (HNO3), indicates the presence of coumarins [16].

**13.Test for Quinones**

To 1 mL of extract was added 1 mL of conc. H2SO4. The presence of a red coloration indicates the presence of quinones. [17]

**14.Test for Cardiac Glycosides**

0.5 mL of the extract was mixed with 2 mL of glacial acetic acid and a few drops of 5% ferric chloride solution (FeCl3), followed by the addition of 1 mL of concentrated sulfuric acid. The formation of a greenish or brown ring at the interface indicates the presence of cardiac glycosides [17].

**15.Test for Oxalates**

To 1 mL of the extract, a few drops of glacial acetic acid were added. The formation of a blue-black coloration indicates the presence of oxalates. [18].

**16.Test for Mucilage**

To 1 mL of the extract, 5 mL of absolute ethanol was added. The formation of a fluffy precipitate upon agitation indicates the presence of mucilage [18]

**3. QUANTITATIVE ANALYSIS**

####  Quantitative estimation of carbohydrates by the picric acid method

Picric acid is reduced to picramic acid by glucose. The intensity of the orange color of picramic acid is proportional to the concentration of glucose. The orange color of picramic acid, which has a maximum absorption around 570 nm, is proportional to the number of phenolic compounds present in the plant extract. The reaction is as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Glucose**  | **+ Picric acid** |  | **Oxydation byproducts**  | **+ Picramic acid** |
|  | **Yellow color** |  |  | **Orange or brown color**  |

In a test tube, 1000 µL of 13% picric acid and 1000 µL of 4% sodium hydroxide were added to 1000 µL of the extract at a concentration of 1000 µg/mL. The tube was placed in a boiling water bath for 10 minutes. The optical density at 570 nm was measured, and the calibration curve was used to determine the concentration of the extract and the blank. The amount of carbohydrate content was expressed as equivalent glucose per mg of dry extract of the plant (EG/g) [7].

### Quantitative estimation of proteins of plant extract by Lowry's method

Lowry's method is another colorimetric protein assay, complementary to the Biuret method. In this method, the protein first reacts with an alkaline cupric reagent (Gornall's reagent from the Biuret method), followed by the addition of a second reagent, phosphotungstomolybdic acid (Folin-Ciocalteu's reagent). This reagent allows the reduction of aromatic amino acids (tyrosine and tryptophan), leading to the formation of a dark blue-colored complex, whose absorbance is measured between 650 and 750 nm. BSA was used as a standard reagent for preparing the standard curve, against which the unknown concentration of proteins was estimated. In a test tube, 1000 µL of alkaline reagent was added to 1000 µL of the extract at 1000 µg/mL and incubated for 10 minutes, followed by the addition of 500 µL of the Folin-Ciocalteu reagent (diluted to 1/10th). This was followed immediately by incubation at room temperature for 20 minutes in darkness to allow for the development of the blue color. The absorbance was read at 760 nm on a spectrophotometer. The amount of protein was expressed as µg/mL of BSA (EBSA/g) [22].

**Quantitative estimation of total phenolic content**

To determine the total phenolic content, we used the Folin-Ciocalteu method. The Folin-Ciocalteu reagent is a yellow-colored acid consisting of a mixture of two acids: phosphotungstic acid (H₃PW₁₂O₄₀) and phosphomolybdic acid (H₃PMo₁₂O₄₀). It is reduced during the oxidation of polyphenols to form a stable blue complex of tungsten and molybdenum oxides. The color produced, with a maximum absorption of around 760 nm, is proportional to the quantity of phenolic compounds present in the plant extract.

1000 µL of the Folin-Ciocalteu reagent (diluted to 1/10th) was added to 1000 µL of the extract at 1000 µg/mL, and the mixture was incubated for two minutes at room temperature. Then, 1000 µL of sodium bicarbonate (75 g/L) was added, followed immediately by incubation at room temperature for 90 minutes. The absorbance was read at 760 nm on a spectrophotometer. The quantity of phenolic compounds, expressed in milligrams of gallic acid equivalent per gram of dry weight of the plant (GAE/g), was determined by the calibration curve, which varied from 0 to 1000 mg/mL, made with different concentrations of gallic acid [23].

#### Quantitative estimation of total flavonoid content

The quantification of flavonoids was carried out using aluminum trichloride and sodium hydroxide. Aluminium trichloride forms a yellow complex with flavonoids, while sodium hydroxide forms a pink-colored complex that absorbs in the visible electromagnetic spectrum at 510 nm [23].

In each tube, 1000 µL of the extract at 1000 µg/mL was introduced, followed by the addition of 150 µL of sodium nitrite (NaNO₂) at 5%. After 5 minutes, 150 µL of aluminium trichloride (AlCl₃) at 10% (m/v) was added to the mixture. After 6 minutes of incubation at room temperature, 500 µL of 4% sodium hydroxide (NaOH) was added. The mixture was stirred immediately to homogenize its contents. The absorbance of the pinkish-colored solution was read at 510 nm against a blank. A calibration curve was produced in parallel under the same operating conditions using quercetin as a positive control. The total flavonoid content of the plant extracts was expressed in milligrams equivalent of quercetin per gram of dry plant matter (mg EQ/g) [24].

Quantitative estimation of flavanol content

The sample containing flavonols results in the formation of green color when reacted with aluminium chloride and sodium acetate, and the samples are read at 440 nm in a UV-Vis spectrophotometer [25].

In a 10 mL test tube, 1000 µL of extracts at a concentration of 1000 µg/mL, 1000 µL of AlCl₃·6H₂O (2%), and 600 µL of sodium acetate (50 g/L) were added and the volume was completed with distilled water to 3000 µL. The tubes were incubated at room temperature for 2.5 hours. The solution was mixed well, and the absorbance was measured against a blank that did not contain the extract at 440 nm. A standard curve for flavonols was constructed using a standard solution of quercetin (0 to 200 μg/L), following the same procedure as previously described. The total flavonols were expressed in milligrams of quercetin equivalents per gram of dried fraction (mg EQ/g) [24].

#### Quantitative estimation of total tannin content

The technique for assaying condensed tannins by the Folin-Ciocalteu method is based on the reduction of phosphomolybdic and tungstic acid in an alkaline medium, in the presence of tannins, to give a blue color whose intensity is measured between 640 and 760 nm.

In a 10 mL test tube, 1000 µL of extracts at a concentration of 1000 µg/mL, 200 µL of Folin's reagent diluted to one-tenth, and 1000 µL of 35% Na₂CO₃ were added. The solution was mixed well, and the absorbance was measured against a blank containing no extract at 700 nm. A standard curve for total tannins was produced using a standard solution of tannic acid (0 to 500 µg/mL) following the same procedure as described previously. Total tannins were expressed in milligrams of tannic acid equivalents per gram of dried fraction (TAE/g) [27].

**Quantitative estimation of the total content of alkaloids**

The alkaloid, in contact with concentrated sulfuric acid and potassium dichromate, develops a violet line which then turns blue and finally green. The maximum absorption, proportional to the intensity of the color developed, is 650 nm.

In a glass test tube, 1000 µL of the sample at 1000 µg/mL was added to 1000 µL of 5% potassium dichromate, and incubation was allowed for 5 minutes at 30°C. Then, 800 µL of concentrated sulfuric acid was added to the tube's contents and mixed. The tubes were left at room temperature for 20 minutes to observe any color change, after which the absorbance was measured at 650 nm against the blank or the sample. The alkaloid concentration was obtained from the calibration curve and expressed in milligrams of quinine hydrochloride equivalents per gram of dried fraction (QHE/g) [27].

**Data Analysis**

Raw data were collected and entered in Microsoft Excel 365. The GraphPad Instat version 5.1 software was used for comparison between the groups which were analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test. The results were expressed in terms of mean ± standard deviation. P-values ≤0.05 were considered as statistically significant

**4.RESULTS**

**Yield of extraction**

The Percentage yield of the different extracts were obtained as illustrated in table 1. The highest yield was obtained from decoction of 17.64

Table I: Percentage yield of the different extracts*.*

|  |  |
| --- | --- |
| **Extracts** | **Percentage yield (in %)** |
| Aqueous | 15.42 |
| Hydroethanolic | 10.85 |
| Decoction | 17.64 |
| Infusion  | 13.63 |

Decoction had the greatest percentage yield followed by aqueous, then infusion. Hydroethanolic extract had the lowest percentage yield.

PHYTOCHEMICAL ANALYSIS

### Quantitative Phytochemical analysis

Phytochemical screening of the different extracts of *Ficus exasperata* indicated the presence of the following primary and secondary metabolites: Polyphenols, flavonoids, tannins, mucilage, phlobotanins, quinones, coumarins , alkaloids, carbohydrates, protein and Vitamin C (Figure 2). Other important secondary metabolites such as oxalates, resins, Anthocyanin and Cardiac glucosides, Betacyanin, Chalcone, Anthocyanin, anthraquinone were absent.

Table 2: Phytochemical screening of the different extracts of *Ficus exasperata.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test** | **Reagents** | **Aqueous extract** | **Hydro ethanol**  | **Decoction** | **Infusion** |
| **Polyphenol** | $$FeCl\_{3}$$ | + | + | + | + |
| Lead acetate | + | + | + | + |
| **Mucilage**  | Ethanol | + | + | + | + |
| **Saponins**  | Foam test | + | - | - | - |
| **Oxalates**  | Ethanoic acid | - | - | - | - |
| **Resins** | Acetic acid/$NH\_{3}$ | - | - | - | - |
| **Coumarins**  | $FeCl\_{3}$ /$HNO\_{3}$ | + | + | + | + |
| **Cardiac glycosides**  | Acetic acid | - | - | - | - |
| **Flavonoids**  | $$AlCl\_{3}$$ | + | + | + | + |
| NaOH/H2SO4 | + | + | + | + |
|  **Total Tannins**  | $$CUSO\_{4}/NH\_{3}$$ | + | + | + | + |
| **Gallic Tannins** | STIASNY | + | + | + | + |
| **Alkaloids**  | Wagner | + | + | + | + |
| Hager | + | + | + | + |
| Valse mayer | + | + | + | + |
| Talic-acid | + | + | + | + |
| **Anthocyanins**  | $H\_{2}SO\_{\begin{array}{c}4\\\end{array}}$/$NH\_{3}$ | - | - | - | - |
| **Phlobotannin**  | HCl | + | + | + | + |
| **Betacyanine** | NaoH | - | - | - | - |
| **Chalcone**  | NH3 | - | - | - | - |
| **Quinones**  | H2SO4 | + | + | + | + |
| **Anthraquinone** |  | - | - | - | - |
| **Carbohydrate** | Molish test | + | + | + | + |
| Fehling test | + | + | + | + |
| Picric test | + | + | + | + |
| **Protein**  | Biuret test | + | + | + | + |
| Xanthoprotein |  |  |  |  |
| **Vitamin C** | $H\_{2}SO\_{\begin{array}{c}4\\\end{array}}$/DNPH | + | + | + | + |
| **Carotenoids** | HCL/$H\_{2}SO\_{\begin{array}{c}4\\\end{array}}$ | - | -- | - | - |
| **Lipid** | NaoH /Ethanol | - | - | - | - |
| Ethanol/NaOH | - | - | - | - |
| **Steroids**  | H2SO4 | - | - | - | - |

**KEY: +:** Positive ; -: Negative

**Total carbohydrates contents**

The amount of carbohydrates is expressed as mg of glucose E/ g of dry weight.

*Figure 1: Standard Curve for glucose*

The distribution of carbohydrates in the different extracts shows that the aqueous extract had the highest concentration of carbohydrates, followed by the infusion, with the decoction and hydroethanolic extracts having similar concentrations (Fig 2).



**Figure 2** : Result of quantitative estimation of Carbohydrates content.

**Total protein contents**

The amount of protein is expressed as mg BSAE/ g of dry weight

Figure 3: Standard Curve for BSA*.*

The distribution of proteins in the leaf extracts showed a high percentage of protein in the aqueous extract, followed by the hydroethanolic extract, and then the infusion and decoction, which had similar concentrations (Fig 4)



*Figure 4 : Result of Protein content in the different extracts*

**Total Phenolic content**

The amount of total phenolic is expressed as mg of gallic acid E/ g of dry weight

Figure 5 : Standard Curve for gallic acid.

The distribution of phenolic compounds in the different leaf extracts showed that the infusion extract had the highest concentration of phenolic compounds, followed by the aqueous extract and decoction, which had similar concentrations. The hydroethanolic extract had the lowest concentration (Fig 7).



**Figure 6** : Result of polyphenol content in the different extracts

**Total flavonoids content**

The amount of Total flavonoids content (mg quercetin/g of dry sample)

**Figure 7**: Total flavonoids content (mg quercetin/g of dry sample)

The distribution of flavonoids in the different leaf extracts showed that the infusion extract had the highest percentage of flavonoids, followed by the aqueous and hydroethanolic extracts, which had similar concentrations. The decoction had the least concentration (Fig 8).



Figure 8 : Flavonoids content in the different extract.

**Total flavanoid content**

The amount of Total flavonoids content (mg quercetin/g of dry sample)

Figure 9: Total flavanoid content (mg of quercetin/g of dry sample)

The distribution of flavanoid in the different extracts shows that the infusion extract had the highest concentration of flavanoid, followed by the aqueous extracts, with the hydroethanolic extracts and decoction showing similar concentrations (Figure 10).



Figure 10: Result of Flavonol contents in the different extracts.

**Total tannin content**

The amount of total Tannin content is expressed as (mg of tannic acid/g of dry sample).

Figure 11: Total Tannin content (mg of tannic acid/g of dry sample)

The distribution of tannins in the different extracts shows that the infusion extract had the highest concentration of tannins, followed by the decoction extract and aqueous extracts, with the lowest concentration in the hydroethanolic extracts (Fig 12).



Figure 12 : Result of Total Tannins contents in the different extracts

**Total alkaloids contents**

The amount of total alkaloids content is expressed as (mg of tannic acid/g of dry sample).

Figure 13 : Calibration curve of quinine hydrochloride

The distribution of alkaloids in the different extracts shows the highest concentration in the ethanolic extract, followed by the aqueous extract, then the decoction, with the lowest concentration in the infusion extract (fig 14)

**Figure14:** Total Alkaloids content (mg tannic acid /g of dry sample) among of extract leaves

Table 3: Result from quantitative Phytochemistry*.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Metabolites**  | **Aqueous** | **Hydro-ethanolic** | **Decoction**  | **Infusion**  |
| **Total proteins** | 304,36 ± 35,89 | 301,79 ± 7,27 | 269,74 ± 7,15 | 272,56 ± 7,39 |
| **Carbohydrates** | 8,93 ± 0,41 | 7,51 ± 0,26 | 7,52 ± 0,16 | 8,87 ± 0,37 |
| **Polyphenols** | 1,37 ± 0,01 | 1,20 ± 0,04 | 1,36 ± 0,01 | 1,66 ± 0,02 |
| **Flavonoids** | 0,26 ± 0,02 | 0,25 ± 0,02 | 0,24 ± 0,02 | 0,39 ± 0,03 |
| **Flavonols**  | 0,41 ± 0,05 | 0,35 ± 0,04 | 0,36 ± 0,03 | 0,58 ± 0,02 |
| **Total tannin**  | 251,74 ± 8,00 | 218,49 ± 3,05 | 271,52 ± 10,68 | 324,86 ± 2,86 |
| **Alkaloids** | 247,22 ± 61,67 | 278,33 ± 58,59 | 206,11 ± 136,72 | 180,56 ± 91,67 |

All values are expressed as mean ± standard error of the mean, n=3; Statistical analysis is done by one-way One-Way Analysis of Variance followed by *Dunnet’s “t” test*; \*p<0.05 (considered significant when compared to normal group).

1. **DISCUSSION**

“Phytochemical screening of the different extracts of Ficus exasperata indicated the presence of the following primary and secondary metabolites: polyphenols, flavonoids, tannins, mucilage, phlorotannins, quinones, coumarins, alkaloids, carbohydrates, protein, and vitamin C. This is similar to the studies conducted by Adekeye et al. in Nigeria” [28]. “Only cardiac glycosides were present in their studies but absent in ours. The presence of saponins, flavonoids, and polyphenols in Ficus exasperata leaves, as confirmed by the phytochemical screening, indicates that this plant is of pharmacological importance. The significant amount of flavonoids is particularly noteworthy, as flavonoids act as powerful protective agents against inflammatory disorders. They reduce edema formation and inhibit the synthesis of prostaglandin E, prostaglandin F, and thromboxane B. It has been reported that the saponins and flavonoids present in the plant extracts have various uses, including antiulcerogenic, anti-inflammatory, fibrinolytic, antipyretic, analgesic, and anti-edematous effects” [29].

The total carbohydrate content ranged from 8.93 to 7.51. The highest carbohydrate content was detected in the aqueous maceration extract with a concentration of (8.93 ± 0.41), followed by the infusion extract with a concentration of (8.87 ± 0.37), then the decoction and ethanolic extracts, both with concentrations of (7.51 ± 0.16) and (7.51 ± 0.26), respectively. The carbohydrate content was lower compared to the studies conducted by Bello et al. in 2014 in Nigeria, where they reported (72.81 ± 2.56) [30]. “Carbohydrates are ubiquitous and perform a wide array of biological roles. Carbohydrate-based or carbohydrate-modified therapeutics are used extensively in cardiovascular and hematological treatments, ranging from inflammatory diseases and anti-thrombotic therapies to wound healing” [31].

The total protein content was detected, with the maximum concentration observed in the aqueous maceration (304.36 ± 35.89). Decoction had the lowest concentration (269 ± 7.15). The amount of phenolic compounds was expressed as mg of gallic acid per gram of extract (mg GAE/g). The highest polyphenol content was detected in the infusion extract (1.66 ± 0.02), followed by aqueous and decoction extracts, which had similar concentrations of (1.37 ± 0.01) and (1.36 ± 0.01), respectively. The ethanolic extract had the lowest concentration (1.20 ± 0.04).

The total content of flavonoids was measured using a colorimetric method and expressed as catechin equivalent (CAE) per gram of dry extract weight. In our study, the infusion extract revealed the highest flavonoid content (0.39 ± 0.03 g CAE/g of extract), while the lowest was observed in the decoction (0.24 ± 0.02 mg CAE/g of extract).

The total flavonol content ranged from 0.35 to 0.58. The highest flavonol content was detected in the infusion extract (0.58 ± 0.02), followed by the aqueous extract (0.41 ± 0.05), then decoction (0.36 ± 0.03). Ethanol had the lowest concentration (0.35 ± 0.04).

The total tannin level ranged from 218 to 324. The highest tannin content was detected in the infusion extract (324.86 ± 2.86), followed by the decoction extract (271.52 ± 10.68), then the aqueous extract (251.71 ± 0.03). The lowest tannin content was found in the ethanolic extract (218.49 ± 3.05). The significant amounts of polyphenols, flavonoids, and tannins detected in the extracts of *Ficus exasperata* justify its use in the treatment of inflammatory diseases and inflammation-related conditions.

**CONCLUSION**

In conclusion, this study demonstrates that the various leaf extracts of *Ficus exasperata*, including aqueous, hydroethanolic, infusion, and decoction, contain polyphenols, flavonoids, tannins, mucilage, quinones, coumarins, alkaloids, carbohydrates, protein, and vitamin C. The present work also shows that most primary and secondary metabolites are predominantly found in the aqueous and infusion extracts. This study could serve as a benchmark for further pharmacological studies on the plant that may be harnessed for drug development in the future.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

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**ETHICAL APPROVAL**

All experiments are in phase with the terms of the Institutional Ethical Review Board of Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. Ethical clearance approval reference No 277/UY1/FMBS/VDRC/ESD of 09 May 2023.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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