Phytochemical characterization and systemic exposure, evaluation of the aqueous extracts of *Ficus thiningii* Blume (Moraceae) on albino Wistar rats.

ABSTRACT

Ficus thonningii (Blume) is considered a herbal plant with well documented biological activity in the management of several diseases in the tropics. However, there is a gap of information on its safety and proof of efficacy in evidence-based medicine. The objective of this study was to characterize the bioactive metabolites of the hydro-ethanolic extract of the stem bark of *Ficus. thonningii* and in vivo evaluation of the systemic exposure of the bioactive metabolite. Phytochemical screening was done using standard extraction techniques, and test according to methods adopted from Sofowora and collaborators. Quantitative analysis was done using spectrophotometer of plant extract with different reference standards.

Analysis of the animals' plasma following administration of the extract was used to investigate systemic exposure to confirmed the presence of absence of metabolites in systemic circulation.
This work shows that *F. thonningii* (Blume) stem bark hydro-ethanolic extract contains polyphenols, saponins, alkaloids, flavonoids, catechic tannins, gallic tannins, coumarins, quinones, phlobatannins. This study shows that the hydro-ethanolic extract of *F. thonningii* contains total phenolic content of 192,27 ± 3,40 mgEQ/MS g gallic acid and total flavonoid content of 103,59 ± 15,72 mgEQ/MS quercetin. This study shows that the secondary metabolites in the hydro-ethanolic extract of the stem bark of *F. thonningii* (Blume) were not detected in plasma and not bioavailable.

Keywords: *Ficus thonningii,* hydro-ethanolic extract, stem bark, Phytochemical screening, metabolites, systemic exposure.

INTRODUCTION.

Plants and plant extracts are an important source of food and medicine for humans. In fact, about 80 % of the world-wide population still relies on plants and plant extracts as a source of primary health care[1,2]. Nowadays, there is a resurging interest in exploiting herbal medicines for the treatment of diseases which may be due to the fact that they are less costly, less toxic and more readily available especially in developing countries as compared to orthodox pharmaceuticals. There is equally a growing belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective or

inadequate. Thus, medicinal plants are paving a new approach to improved medicine and are emerging as alternative therapeutic options [2,3]. Unfortunately, the lack of enough studies to provide information about their quality, efficacy and safety remains a major concern to the public.

Among the plants used in ethnomedicine is the *Ficus thonningii* (Blume) also known as the strangler or common wild fig. It is extensively used for the treatment of a number of diseases such as diarrhea, stomach pain, peptic ulcer, urinary tract infections, diabetes mellitus, gonorrhea, respiratory infections, and mental illnesses. It is an evergreen tree (about 6-21m tall) with a dense rounded to spreading crown [3] mainly distributed in the upland forests of tropical and sub-tropical Africa [4]. This plant contains various bioactive compounds which include alkaloids, terpenoids, flavonoids, tannins and active proteins, all of which contribute to its curative properties. *In vitro* and *in vivo* pharmacological studies revealed that *F. thonningii* has antimicrobial, antidiarrheal, anthelmintic, antioxidant, anti-inflammatory and analgesic properties.

A number of studies [5,6] have recently shown that *Ficus thonningii* has promising biologic activities, for instance, in the management of peptic ulcers. This has motivated the thought that it has the potential to be developed into an improved traditional medicine. Unfortunately, so far, there is still little scientific data in the literature on the possible toxic effects of the plant [7]. This prompted researchers like Tembe et al (2018) [8] to conduct an acute toxicity study on the hydro-ethanolic stem bark extract of the plant in order to contribute information in regards to its toxicity profile and safety pharmacology. Therefore, the present study was to determine the qualitative and quantitative composition of the secondary metabolites of the hydro-ethanolic extract of the stem bark of *F. thonningii* for a four-year preserved plant. and to evaluate the systemic exposure as a subsequent study to determine the stability of the phytochemicals, and systemic exposure.

METHODOLOGY

Study site and study design.

This was a preclinical experimental in vitro and *in vivo* study, conducted in *Wistar* albino rats from November 2021 to May 2022. The study was carried out at the laboratory of preclinical animal and pharmaco-toxicology research, of the Faculty of Medicine and Biomedical Sciences at the university of Yaoundé 1, Cameroon. Ethical approval was requested from the institutional review board of the Faculty of Medicine and Biomedical Sciences. An approval to work in the

animal house of the faculty was given by the Head of laboratory. The OECD Guidelines 420, 425, on the use of animals in preclinical studies was applied.

Collection and identification of the plant

The powdered stem bark of *F. thonningii* which was previously obtained from the work conducted by Pougoue (2017) [8] was used in the present study. Fresh stem barks were collected from the plant growing at Bafoussam on the 03 of January 2017. The barks were then identified taxonomically and authenticated at the National Herbarium of Cameroon by comparison with a sample having the number 44042/HNC by Tadjouteu F.

Rationale for the selection of the plant material

F. thonningii (Blume) is a medicinal plant which has been used for over the centuries by the Western region of Cameroon for the treatment of several ailments. *F. thonningii* remedies are commonly prepared as decoctions, macerations and infusions and the extracts are taken orally. This subacute toxicity study is a follow up of the study previously conducted by Tembe et al. (2018) [9] on the acute toxicity of *F. thonningii* (Blume). Therefore, the hydro-ethanolic stem bark extract of *F. thonningii* was equally used in the present study. The same doses of the stem bark extract of the plant used by Pougoue (2017) [8] were applied in this study.

Preparation of the hydro-ethanolic plant extract

Preparation of medicinal plants for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality and quantity of bioactive constituents before proceeding with the intended biological testing. According to the work done by Pougoue et al. (2017) [9], the hydro-ethanolic maceration of the stem bark of the plant was most active. Hence, a hydro-ethanolic extract was also be used during this study.

Maceration was the extraction procedure in which coarsely powdered plant material, either leaves or stem bark or root bark, was placed inside a container; the menstruum was poured on top until it completely covers the drug material. The container was then closed and kept for at least two days. The content was stirred or agitated from time to time to ensure complete extraction. At the end of extraction, the micelle was separated from marc by filtration and from the menstruum by evaporation in an oven [5]. The extract was then weighed in order to determine the percentage yield obtained from the initial powder quantity and then stored in an air-tight container for subsequent experimental tests.

Yield determination

200 g of the powder was weighed and mixed with several fractions of a 50:50 hydro-ethanolic solution in order to obtain a final solution of 2000 mL in a flat-bottomed flask. This mixture was agitated several times within 48 h of maceration, after which the mixture was filtered using Whatman paper number 3. The macerate was then dried in an oven at 50°C for two days. The dried extract obtained was weighed in order to determine the percentage yield from the initial powder used. The yield (%) was obtained using the following formula [10]

$$Percentage\ yield = \frac{mass\ of\ the\ extract\ obtained}{mass\ of\ the\ initial\ powder} \times 100$$

PHYTOCHEMICAL SCREENING

The protocol of Sofowara (1993) [10] and Savithrama et al. (2011) [11] were applied for the screening of the various bioactive constituents. The filtrates of the hydro-ethanolic stem bark extract of *Ficus thonningii* were used for further phytochemical analysis which includes test for carbohydrates, proteins, glycosides, tannins, alkaloids, flavonoids, terpenoids, saponins, resins, quinones, cardiac glycosides, coumarins, steroids, phenols, anthraquinones and phlobatannins.

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 red precipitate indicates the presence of sugars.

Molisch's test: 2 drops of alcoholic solution of α-naphthol were added to about 2 mL of the sample, and to the mixture after being shaken well. Few drops of conc. H_2SO_4 was added along the sides of the test tube. A violet ring indicates the presence of sugars [10].

Test for proteins

Biuret test: To 2 mL of filtrate was added 1 drop of 2% copper sulphate solution; and 1 mL of 95% ethanol was added. This was followed by excess addition of KOH. The appearance of pink color indicates the presence of protein [18].

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.

Test for lipids

To 1 mL of the extract was added NaOH and the mixture was then be heated in a boiling water bath for 5 minutes. Ethanol was be added to the mixture. The appearance of foam indicates the presence of lipids [12].

Miscellaneous compounds

Test of resins

1 mL of extract was taken and to this few mL of acetic anhydride was added to 1 mL of conc. H_2SO_4. The appearance of orange to yellow color indicates the presence of resins.

Test for Gums and mucilage

To 1 mL of extract, distilled water, 2 mL of absolute ethanol was added accompanied by constant stirring white or cloudy precipitate indicates the presence of gums or mucilage.

Vitamin C

To 1 mL of the plant extract was added dinitrophenyl hydrazine dissolved in concentrated H_2SO_4. The presence of yellow precipitates indicates the presence of vitamin C.

QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

Test for anthraquinones

To 5 mL of extract, few mL of conc. H_2SO_4 was 1 mL of diluted ammonia added. The appearance of rose pink confirms the presence of anthraquinones[22].

Test for quinones

To 1 mL of extract, alcoholic KOH was added. The presence of red to blue color indicates the presence of quinones.

Test for alkaloids

1. Mayer's test: to a few mL of filtrate, 2 drops Mayer's reagent was added. A creamy or white precipitate shows a positive result for alkaloids.

2. Wagner's test: (iodine – potassium iodine reagent): To about an mL of extract few drops of Wagner's reagent was added. Reddish – brown precipitate indicates presence of alkaloids.

3. Hager's test: To a few mL of the filtrate was added 2 mL of Hager's reagent. A creamy white/ yellow precipitate shows a positive result for alkaloids.

4. Tannic acid test: To the acidified extract, 10% tannic acid solution was added. A buff color precipitate indicates the presence of alkaloids

Test for glycosides

2 mL of extract is mixed with a few drops of glacial acetic acid containing traces of ferric chloride and 0.5 mL of conc. H_2SO_4 was added. The production of blue color is positive for glycosides.

Test for cardiac glycosides (Keller-Killani test)

5mL of solvent extract was mixed with 2mL of glacial acetic acid and a drop of ferric chloride solution followed by the addition of 1mL of conc. H_2SO_4. A brown ring in the interface indicates the presence of deoxy sugars of cardenolides. A violet ring may appear beneath the brown ring while acetic acid layer a green ring may also form just gradually towards the layer.

Test for polyphenols

1. To the extract was added few drops of 5% ferric chloride. The formation of dark green color indicates presence of polyphenols.

2. To 1 mL of extract, few drops of 5% solution of lead acetate was added. The appearance of yellow precipitate indicates the positive results for polyphenols [13]

Test for tannins

To 5mL of extract, few drops of neutral 5% ferric chloride solution was added. The production of dark green color indicates the presence of tannins.

Differentiation of Catechic and Gallic tannins

It is obtained by STIASNY reaction, which is carried out in the following manner. To 30mL of the extract, we add 15 mL of STIASNY reagent (10 mL of 40 % formalin and 5 mL of concentrated HCl) and heated for 15 minutes in a water bath at 90 °C. Catechic tannins: The obtaining of a precipitate shows their presence;

Gallic Tannins: After filtration, we saturate the filtrate with powdered sodium acetate, Then 1 mL of a solution of 1 % ferric perchloride ($FeCl_3$). The presence of gallic tannins not precipitated by the STIASNY reagent is indicated by the development of a shade dark blue [19].

Test for flavonoids

In a test tube, put 2 mL of the 1 % extract. Add 1 mL of sodium hydroxide (NaOH) of 2N. The

formation of a yellow-orange coloration indicates the presence of flavonoids. The addition of a few drops of sulfuric acid and the change of coloring confirm the presence of flavonoids.

Test forPhlobatannins

Aqueous extract was boiled with diluted HCl leading to the deposition of reddish precipitate indicates the presence of phlobatannins.

Identification test for beta cyanide

2 mL of the extract was introduced in a test tube and 2 mL of 2N NaOH was added and heated the tube in a boiling water bath for 5 minutes. The appearance of a yellow coloration indicates the presence of betacyanin.

Test for saponins

0.5 mg of extract was vigorously shaken with few mL of distilled water. The formation of frothing is positive for saponins

Test for steroids

2 mL of extract with 2 mL of chloroform and 2mL of concentrated H_2SO4 was added, the appearance of red color and yellowish green fluorescence indicates the presence of steroids.

Test for anthocyanins

2 mL of aqueous extract was taken to which was added 2N HCl and it was followed by the addition of ammonia, the conversion of pink-red turns blue-violet indicates the presence of anthocyanins.

Test for coumarins

To 2 mL of the extract, 3 mL of 10% aqueous solution of NaOH was added. The production of yellow color indicates the presence of coumarins.

Test for oxalates

In a test tube, put 2 mL of the extract. A few drops of ethanoic acid were added. Obtaining a greenish-black color indicates the presence of oxalates.

Quantitative analysis of the metabolites of the plant

Depending on the qualitative results, the quantitative assay is carried out for carbohydrates, proteins, polyphenols and flavonoids [17].

Determination of total soluble carbohydrates

Principle

Picric acid is reduced into picramic acid by glucose. The intensity of the orange color of picramic acid will be proportional to the concentration of glucose. The orange color of picramic acid produced, whose maximum absorption is around 570 nm, is proportional to the quantity of phenolic compounds present in the plant extract [17].

Procedure

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 following calibration curve was used during the reading of the optical density of the extract and blank concentration at 570 nm.

Determination of total protein content

Principle

In an alkaline medium, copper ions are reduced and bind to proteins. In an acid medium, Folin-Ciocaltau reagent is reduced by the copper-protein complex. The stable blue complex formed is detected at an absorbance of 760 nm [18].

Procedure

BSA is used as 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 $1/10^{th}$) were 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 quantity of protein content After that the absorbance was measured at 660 nm and the amount of protein is expressed as µg/mL of BSA.

Evaluation of total phenolic contents

Principle

To determine the total phenolic content, we used the method of Folin-Ciocalteu [19] . Folin-Ciocalteu reagent is a yellow-colored acid consisting of a mixture of two acids: phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It is reduced during the oxidation of polyphenols to form a stable blue complex of tungsten and molybdenum oxides. The color produced, whose maximum absorption is around 760 nm, is proportional to the quantity of phenolic compounds present in the plant extract.

Procedure

1000 µL of the Folin-Ciocalteu reagent (diluted to $1/10^{th}$) were added to 1000 µL of the extract at 1000 µg/mL and the whole was incubated for two minutes at room temperature. Then, 1000 µL of sodium bicarbonate at 75 g/L added as 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 (mg EQ/g Ps), was determined by the calibration curve varying from 0 to 1000 mg/mL, made with different concentrations of gallic acid.

Evaluation of total flavonoid contents

Principle

The quantification of flavonoids was carried out using aluminum trichloride and sodium hydroxide. The aluminum trichloride forms a yellow complex with flavonoids and the soda forms a pink colored complex which absorbs in the visible electromagnetic spectrum at 510 nm [20].

Procedure

In each tube, we introduced 1000 µL of the extract at 1000 µg/mL, followed by the addition of 150 µL of sodium nitrite ($NaNO_2$) at 5%. After 5 minutes, 150 µL of aluminum trichloride ($AlCl_3$) 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 content. 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

studied was expressed in milligram equivalent of quercetin per gram of dry plant matter (mg EQ/g).

Evaluation of the systemic exposure of the bioactive components of the plant

The systemic exposure provides information concerning the absorption of the active components of the plant. In this study, the systemic exposure of the bioactive compounds in the hydro-ethanolic extract of the stem bark of *F. thonningii* (Blume) was evaluated. The blood samples were taken from animals 30 mins, 45 mins and one hour following the administration of a dose of 500 mg/kg. Also, blood samples were taken from the test group which had received a daily dose of 500 mg/kg for a period of 28 days. The positive control used was the plant extract while the negative control was the blood sample obtained from the animal which received only distilled water. The plasma was prepared by centrifugation of the blood samples. The same qualitative and quantitative phytochemical analysis previously described in 3.6.2. was applied for the assessment of the bioactive components of the plant present in the systemic circulation of the animals [8].

Statistical analysis

The results are expressed in terms of mean ± standard deviation. The comparison between the groups were analyzed using one-way analysis of variance, the ANOVA test followed by *Dunnett's* post-hoc multiple comparison test using the GraphPad Instat version 5.0 software. A p-value of less than 0.05 was considered statistically significant [16].

RESULT

EXTRACTION

The extraction yield of the extract was 15 %. Table 1 shows the extraction yield of the hydro-ethanolic extract of *F. thonningii*.

Table 1: Extraction yield of the hydro-ethanolic extract of *F. thonningii*

Solvent	Hydro-ethanol (50:50)
Mass of powdered plant (g)	200
Mass of the extract (g)	30.32
Extraction yield (%)	15.16

Phytochemical analysis

A comparison between the phytochemical screening conducted by Pougoue (2017) [9] and this study (2021) revealed that only two phytochemicals changed after four years of conservation of the powdered stem bark of *F. thonningii* as shown in table 2. In this study (2021), anthocyanes were absent and gallic tannins were present whilst these phytochemicals were respectively found present and absent according to the work conducted by Pougoue (2017).

Table 2: Comparison between the phytochemical screening conducted by Pougoue (2017) and from this study (2021)

Test	Specific test	Hydroethanolic maceration from Pougoue (2017)	Hydroethanolic maceration from this study (2021)
Polyphenols	$FeCL_3$	++	++
	Lead acetate	++	++
Saponins	Distilled water	++	++
Mucilage	Absolute ethanol	-	-
	Wagner	++	++
Alkaloids	Hager	++	++
	Valse-Mayer	++	++
Flavonoids	$AlCL_3$	++	++
Tannins **Catechic tannins**	Stiasny	+	+
Gallic tannins	STIASNY	+	-
Steroids	Acetic anhydride	-	-
Coumarines	HNO_3	+	+
Oxalate	Ethanoic acid	-	-
Quinones	Sulfuric acid	+	+
Betacyane	NaOH	-	-
Phlobotannins	HCl	+	+
Anthocyane	NH_4OH	+	-
Cardiac glycosides	Glacial acetic	-	-
Resins	Anhydrous acetic	-	-
	Molisch test	*	+
Carbohydrates	Fehling's test	*	+
	Biuret test	*	+

Proteins	Million's test	*	+
Lipids	NaOH/Ethanol	*	-

– represents the absence of metabolites, + represents the presence of metabolites, ++ abundant

*Represents not conducted by researcher

Spectrophotometric procedures were used to quantify the carbohydrates, proteins, polyphenols and flavonoids content which have been previously detected by the qualitative analysis of the hydro-ethanolic extract of the stem bark of *F. thonningii* (Blume). Total carbohydrates content was confirmed to be 10,24 ± 0,53 mgEQ/g PS using the calibration curve in figure 1.

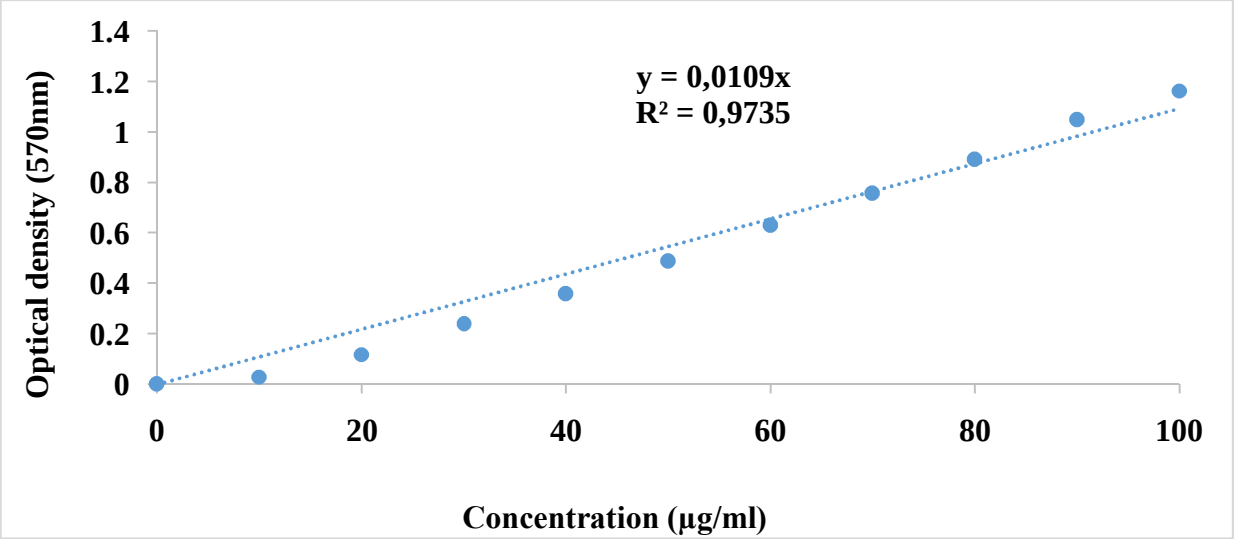

Figure 1: Standard line for total carbohydrate content analysis.

The total amount of protein was found to be 316,41 ± 7,75 µg/mL of BSA using the calibration curve in figure 2

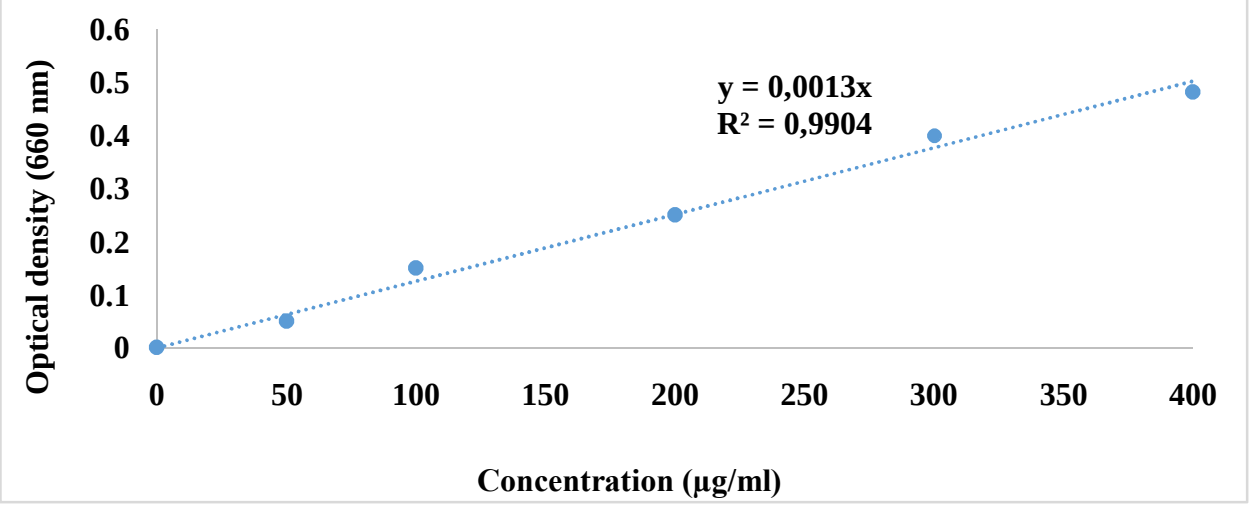

Figure 2: Calibration curve for protein content.

Total phenolic content was found to be 192,27 ± 3,40 Gallic acid (mgEQ/MS g) using the calibration curve in figure 3.

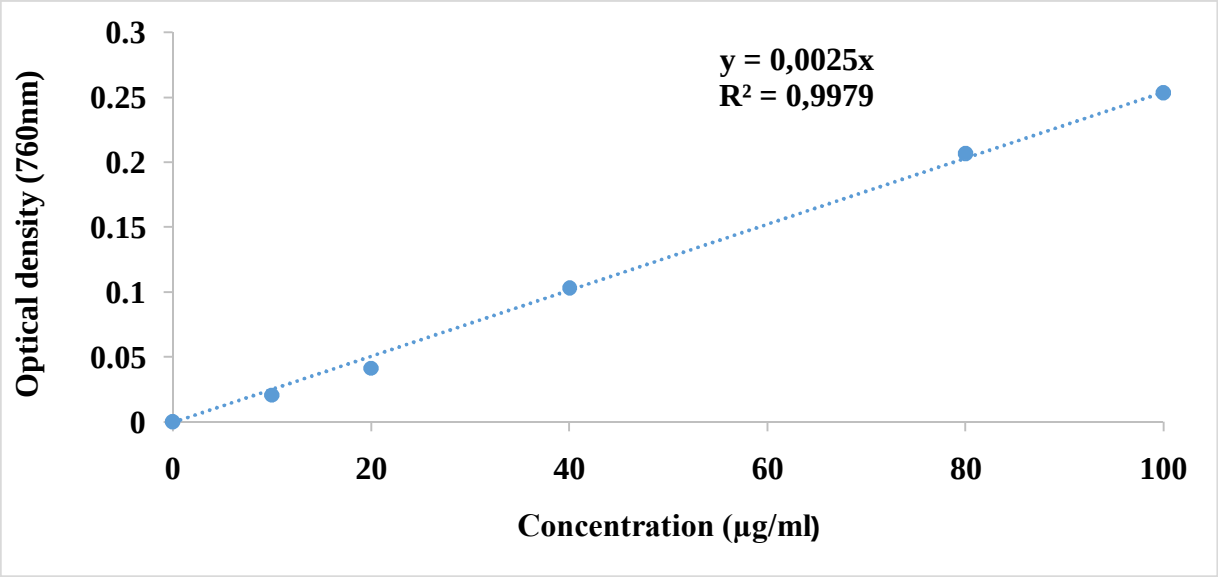

Figure 3: Standard line of gallic acid concentration for total polyphenol content analysis.

Total flavonoids content was confirmed to be 103,59 ± 15,72 Quercetin (mgEQ/MS g) using the calibration curve in figure 4.

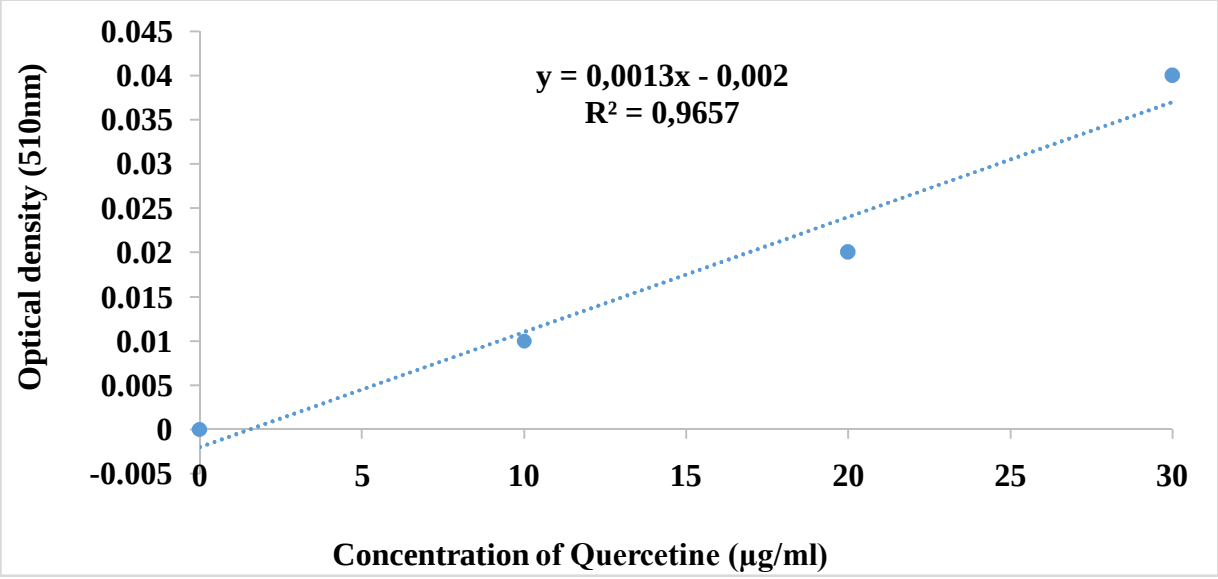

Figure 4: Standard line of quercetin concentration for total flavonoid content analysis.

Systemic exposure

The phytochemical analysis of the blood obtained from animals which were administered *F. thonningii* hydro-ethanolic stem bark extract showed that no metabolite was present in their blood (see table 3).

Table 3: Phytochemical analysis of metabolites in blood.

Time lapse after extract administration	Phytochemicals in plasma	
	Polyphenols	alkaloids
30 mins	-	-
45 mins	-	-
1 hour	-	-
28 days daily	-	-

DISCUSSION

Despite the popularity of medicinal plants, few scientific studies have been undertaken to determine the safety of traditional medicinal plants. It is argued, that plants are not only effective but also very safe as compared to conventional drugs for similar indications. The claim that natural plant product is safe should be accepted only after the plant product has passed through toxicity testing using modern scientific methods. The screening of metabolites and testing the bioactivity is important for the categorization of improved traditional medicine, as stipulated by WHO [13-16]

Ficus genus belongs to Moraceae family and includes extremely different plants, from trees exceeding 30 m high in their countries of origin, to dwarf, crawling species. *F. thonningii* (Blume) has been used in several countries as a medicinal plant in the treatment of several ailments such as urinary tract infections, diabetes mellitus, gonorrhea, respiratory infections, mental illnesses and peptic ulcers [12-15]. This study has been done to confirm bioactive metabolites in the plant after preservation for four years and to evaluate thesystemic exposure of *F. thonningii* stem bark considering the fact that several researchers [16-22] have shown that it has promising biological activity in the management of peptic ulcers, a globally prevalent disease, with little attention accorded to the study of its toxicity.

In this study, the results of the phytochemical screening showed that the hydro-ethanolic stem bark of *F. thonningii* (Blume) contained various biologically active compounds called phytochemicals, which are naturally produced by the plant as protection against biotic and abiotic

stresses. The main groups of phytochemicals isolated from this extract included; polyphenols, saponins, alkaloids, flavonoids, catechic tannins, gallic tannins, coumarins, quinones, phlobatannins which corroborates with the work done by Pougoue (2017) [8, 9] and is also coherent with report of other researchers such as Dangarembizi et al. (2013) [4] on the leaves of *F. thonningii* and Usman *et al.* (2010) [1]. The plant material used in this study was prepared five years ago by Pougoue (2017) [5], it is evident that there has been little change in the constituent phytochemicals indicating good stability. The minor differences in the results obtained from this study and that which was reported by Pougoue (2017) may be due to the susceptibility of some phytochemicals' stability and degradation to some factors such as light, temperature, processing, storage, oxygen etc. as explained by Enaru et al. (2021) [22].

Although, phytochemicals such as flavonoids, tannins and coumarins are known for their numerous pharmacologic effects, they have also been found to possess toxic effects. A few studies have shown that they cause liver and kidney injury as well as dose-dependent carcinogenic activity especially flavonoids and coumarins through mechanisms which are yet to be explained scientifically [18, 19].

Quantitative analysis of the metabolites found in the hydro-ethanolic extract of *F. thonningii* revealed a total proteins content of 316,41 ± 7,75 µg/mL of BSA, total carbohydrate content of 10,24 ± 0,53 mgEQ/g PS, total phenolic content of 192,27 ± 3,40 gallic acid mgEQ/MS g, total flavonoid content of 103,59 ± 15,72 mgEQ/MS quercetin. The preliminary study of the systemic exposure of the blood samples of animals following the administration of *F. thonningii* stem bark extract shows that no secondary metabolites were present in blood. This may be due to the non-specificity of the method for plasma samples.

CONCLUSION

This work shows that *F. thonningii* (Blume) stem bark hydro-ethanolic extract contains polyphenols, saponins, alkaloids, flavonoids, catechic tannins, gallic tannins, coumarins, quinones, phlobatannins. This study also showed that the hydro-ethanolic extract of *F. thonningii* contained total phenolic content of 192,27 ± 3,40 mgEQ/MS g gallic acid and total flavonoid content of 103,59 ± 15,72 mgEQ/MS quercetin. This study also indicated that systemic exposure of the secondary metabolites of the hydro-ethanolic extract of the stem bark of *F. thonningii* (Blume) were not bioavailable on analysis of rat plasma samples.

REFERENCES

1. Usman H, Abdulrahman FI, Usman A. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of Ficus thonningii (Moraceae). Afr J Tradit Complement Altern Med., 2009 May 7;6(3):289-95. doi: 10.4314/ajtcam. v6i3.57178.
2. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae). J Ethnopharmacol. 2009 Jul 30;124(3):556-61. doi: 10.1016/j.jep.2009.05.003. Epub 2009 May 18. PMID: 1945067.
3. Tekwu EM, Pieme AC, Beng VP. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. J Ethnopharmacol. 2012 Jun 26;142(1):265-73. doi: 10.1016/j.jep.2012.05.005. Epub 2012 May 11. PMID: 22583961.
4. Dangarembizi R, Erlwanger KH, Moyo D, Chivandi E. Phytochemistry, pharmacology and ethnomedicinal uses of Ficus thonningii (Blume Moraceae) a review. .Afr J Tradit Complement Altern Med. 2012 Dec 31;10(2):203-12. doi: 10.4314/ajtcam. v10i2.4. eCollection 2013.PMID: 24146443
5. Adane H, Atnafie SA, Kifle ZD, Ambikar D. Evaluation of *In Vivo* Antiulcer Activity of Hydro-Methanol Extract and Solvent Fractions of the Stem Bark of *Ficus thonningii* (Moraceae) on Rodent Models. .Biomed Res Int. 2021 Apr 9;2021:6685395. doi: 10.1155/2021/6685395. eCollection 2021.PMID:
6. Dangarembizi R, Erlwanger KH, Chivandi E Effects of Ficus thonningii extracts on the gastrointestinal tract and clinical biochemistry of suckling rats. Afr J Tradit Complement Altern Med. 2014 Jan 28;11(2):285-91. doi: 10.4314/ajtcam. v11i2.10. eCollection 2014.PMID: 25435610.
7. Koukouikila-Koussounda F, Abena AA, Nzoungani A, Mombouli JV, Ouamba JM, Kun J, Ntoumi F In vitro evaluation of antiplasmodial activity of extracts of Acanthospermum hispidum DC (Asteraceae) and Ficus thonningii Blume (Moraceae), two plants used in traditional medicine in the Republic of Congo. .Afr J Tradit Complement Altern Med. 2012 Dec 31;10(2):270-6. eCollection 2013.PMID: 24146450
8. Tembe E.F., Pougoue K.J., Borgia N., Ngoupayo J., Gatsing D., Tomkins P., et al. Phytochemical screening and in vivo evaluation of antiulcer properties of secondary metabolites in aqueous extracts of *Ficus thonningii* Blume tested on Wistar rats. Int J Biol Chem Sci. 13(1). 2019;475–92.
9. Pougoue K.J. Investigation of the antiulcer, and toxicity of the stem bark extract of *Ficus thonningii* Blume Linn. (Moraceae) on Wistar rats. university of Yaounde 1; 2017.
10. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd; 1993. 191–289 p.
11. Savithrama N., Rao M.L., Suhrulatha D. Screening of Medicinal Plants for Secondary Metabolites. Middle East J Sci. Res. 8th ed. 2011;579–84.
12. Cox-Georgian D., Ramadoss N., Dona C., Basu C. Therapeutic and Medicinal Uses of Terpenes. Med Plants. 2019;333–59.

13. Smeriglio A., Barreca A., Bellocco B., Trombetta D. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. Br J Pharmacol. 2017;174(11):1244–62.

14. Saeed A., Huma R., Muhammad A., Irshad A., Muhammad M.H., Zafar I., and Nisar-ur-R. Phytochemical composition and pharmacological prospectus of *Ficus bengalensis* Linn. (Moraceae)- A review. J Med Plants Res. 2011;5(28):6393–400.

15. Fongang Y.S.F., Bankeu J.J.K., Ali M.S., Awantu A.F, Zeeshan, Assob C.N., et al. Flavonoids and other bioactive constituents from *Ficus thonningii* Blume (Moraceae). PHYTOL [Internet]. 2015 Mar [cited 2022 Jun 15]; 11:139–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S187439001400233X.

16. Ahur V.M, Madubunyi I., Adenkola A.Y., Udem S.C. The effect of ethyl acetate extract of Ficus thonningii (Blume) leaves on erythrocyte osmotic fragility and haematological parameters in acetaminophen-treated rats. Comp Clin Pathol [Internet]. 2012 [cited 2021 Nov 23];21(4):409–13. Available from: http://link.springer.com/10.1007/s00580-010-1107-1.

17. Thomas w., Dutcher R.A. The colorimetric determination of carbohydrates in plants by the picric acid reduction method I. The estimation of reducing sugars and sucrose. J Am Chem Soc [Internet]. 1924 [cited 2022 Jun 15];46(7):1662–9. Available from: https://pubs.acs.org/doi/abs/10.1021/ja01672a015.

18. Lowry OH, Rosebrough NJ, Farr AL, Randall R J. Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1). 1951;265–75.

19. Galvão M.A.M., Arruda A.O., Bezerra I.C.F., Ferreira M.R.A., Soares L.A.L. Evaluation of the Folin-Ciocalteu Method and Quantification of Total Tannins in Stem Barks and Pods from Libidibia ferrea (Mart. ex Tul) L. P. Queiroz. Braz Arch Biol Technol [Internet]. 2018 [cited 2022 Apr 22];61(0). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-89132018000100431&lng=en&tlng=en.

20. Kosalec I. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharmaceutica. 54(1). 2004;65–72.

21. OECD Guidelines for the testing of chemicals. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure | OECD Guidelines for the Testing of Chemicals, Section 4 : Health Effects | OECD iLibrary [Internet]. [cited 2022 Mar 14]. Available from: https://www.oecd-ilibrary.org/environment/test-no-420-acute-oral-toxicity-fixed-dose-procedure_9789264070943-en.

22. Enaru B, Drețcanu G, Pop TD, Stănilă A, Diaconeasa Z. Anthocyanins: Factors Affecting Their Stability and Degradation. Antioxidants [Internet]. 2021 [cited 2022 Jun 16];10(12):1967. Available from: https://www.mdpi.com/2076-3921/10/12/1967.

23. OECD Guidelines for the testing of chemicals. Repeated dose 28-day oral toxicity study in rodents. In 2008. Balehegn M, Eik LO, Tesfay Y. Replacing commercial concentrate by Ficus thonningii improved productivity of goats in Ethiopia.Trop Anim Health Prod. 2014 Jun;46(5):889-94. doi: 10.1007/s11250-014-0582-9. Epub 2014 Apr 9. PMID:

24. Ango PY, Kapche DW, Fotso GW, Fozing CD, Yeboah EM, Mapitse R, Demirtas I, Ngadjui BT, Yeboah SO.Z Thonningiiflavanonol A and thonningiiflavanonol B, two novel flavonoids, and other constituents of Ficus thonningii Blume (Moraceae).Naturforsch C J Biosci. 2016 Mar;71(3-4):65-71. doi: 10.1515/znc-2015-0147.PMID: 26959540.

25. Ahur VM, Adenkola YA, Saganuwan SA, Ikye-Tor JT. Vet Res Forum. 2013 Fall;4(4):207-12. PMID: 25568673 Ameliorative properties of aqueous extract of Ficus thonningii on erythrocyte osmotic fragility induced by acetaminophen in Rattus norvegicus.

26. Falade MO, Akinboye DO, Gbotosho GO, Ajaiyeoba EO, Happi TC, Abiodun OO, Oduola AM.J In Vitro and In Vivo Antimalarial Activity of Ficus thonningii Blume (Moraceae) and Lophira alata Banks (Ochnaceae), Identified from the Ethnomedicine of the Nigerian Middle Belt.Parasitol Res. 2014; 2014:972853. doi: 10.1155/2014/972853. Epub 2014 May 14. PMID: 24955248

27. World Health Organization (WHO). (2008). Traditional medicine; Fact sheet number 134.

28. Egharevba O.H., Carew O., Kunle O.F. Phytochemical and Pharmacognostic Analysis of *Ficus thonningii* Blume Leaves for Monograph Development. Int. j. basic appl. 4(2). 2015;94–100.

29. Dangarembizi R., Kennedy H.E., Davison M., and Eliton C. Phytochemistry, pharmacology and ethnomedicinal uses of *Ficus thonningii* (Blume Moraceae): a review. Afr J Tradit Compl Altern Med. 2013;10(2):203–12.