ejpmr, 2019,6(2), 121-129

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT PROPERTIES OF SECONDARY METABOLITES IN AQUEOUS EXTRACTS OF *FICUS THONNINGII BLUME TESTED ON WISTAR RATS*

Estella Tembe Fokunang[1], Jessica Ketchemen Pougoue[1], Borgia Njunkio[1], Joseph Ngoupayo[2], Palmer Masumbe Netongo[3], Patrice Mimche[4], Donatien Gatsing[5] and Charles Ntungwen Fokunang[1]*

[1]Department of Pharmaco-toxicology and Pharmacokinetics, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.
[2]Department of Pharmacognosy and therapeutic Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.
[3]Department of Biochemistry, Faculty of Science, University of Yaoundé 1, Cameroon.
[4]Department of Pathology, School of Medicine, University of Utah.

***Corresponding Author: Charles Ntungwen Fokunang**
Department of Pharmaco-toxicology and Pharmacokinetics, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.

Article Received on 03/12/2018 **Article Revised on 24/12/2018** **Article Accepted on 15/01/2019**

ABSTRACT

Introduction: Traditional medicine methods have had a very long history to treat every sort of health disorder. The World Health Organization estimates that around 80 % of the world population in developing countries relies on traditional plant medicines for primary health care needs, of which a major proportion corresponds to plant extracts or their active principles. Plants and herbs have been used since ancient times to treat different gastrointestinal illnesses, including peptic ulcers. **Objective**: To identify the major classes of secondary metabolites and evaluate the antioxidant property of the aqueous extracts of *Ficus thonningii.* **Methods**: The phytochemical screening of the extracts was done using the Odebiyi and Sofowora method. Various biochemical parameters such as the Malondialdehyde (MDA), Catalase, Glutathione, Superoxide dismutase (SOD), Xanthine Oxidase (XO), were quantified using standard regulatory techniques. **Results:** The phytochemical screening of the extract of the *F. thonningii* stem bark showed the presence of the sapononins, quinones, coumarins, catechic tannins, phlobotanins, anthocyanins, flavonoids and betacyanes. The extract resulted in a non-significant increase of SOD, Catalase and glutathione in the preventive and curative activity. There was a dose-dependent significant decrease in MDA $P<0.05$. The MDA content was higher in the group receiving the extract 500 mg/Kg, omeprazole and healthy animals, but lower in the negative control group. **Conclusion:**The phytochemical screening of the extract of the bark showed the presence of the saponins, quinones, coumarins, catechic tannins, phlobotanins, anthocyanin, polyphenols, flavonoïds and betacyanes. The extract resulted in a non-significant increase of SOD, Catalase and glutathione with a dose dependent decrease in MDA. The administration of this extract with 2000 mg/Kg dose was well tolerated with no lethality observed.

KEYWORDS: *Ficus thonningii* stem bark hydro-ethanolic extract, phytochemical screening, anti-oxidant, antacid, herbal medicine.

INTRODUCTION

For most of humankind's history, traditional methods of healing were used to treat every sort of health disorder. The World Health Organization estimates that around 80 % of the world population in developing countries relies on traditional plant medicines for primary healthcare needs, of which a major proportion corresponds to plant extracts or their active principles.[1,2] Plants and herbs have been used since ancient times to treat different gastrointestinal illnesses, including peptic ulcers. In China, Traditional Chinese Medicine is practiced in hospitals in addition to western medicine. In Germany, all medical physicians are also trained in the use of herbs.[3] Considering the several side effects of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. Recently, many efforts have been made in order to identify new anti-ulcer drugs from natural resources. Anti-ulcer drugs such as carbenoxolone from *Glycyrrhiza glabra*, solon from sophoradin and gefarnate from cabbage are some of such drugs.[3,5] Liquorice from the root and rhizome of different varieties of *Glycyrrhiza glabra* has been extensively used in medicine for its anti-ulcer activity. The principal constituent of liquorice, is a triterpenoid saponin. It is the substance responsible for its gastro

protective action against ulcers and has been extensively used in medicine. Zinc-carnosine, another natural supplement consisting of zinc and L-carnosine, strengthens the stomach's mucosal defences and harnesses the stomach's natural ability to fight disease, battle infection, and heal itself.[6,7] Its component L-carnosine, a dipeptide made up of L-histidine and β-alanine, demonstrates antioxidant properties that also add to its protective and healing effects. The medicinal properties of folk plants are mainly attributed to the presence of flavonoids, and other organic compounds such as coumarins, phenolic acids, tannins, antioxidants and inorganic micronutrients such as Cu, Mn and Zn.[3, 8] These secondary plant metabolites have been shown to scavenge free radicals and are viewed as promising therapeutic options. Therefore, by scavenging free radicals, antioxidants from plant metabolites might be useful in protecting the gastric mucosa from oxidative damage or in accelerating healing of gastric ulcers. The potential role and basic mechanisms of plant-originated gastro protective substances applied intragastrically are known to account for mucosal protection against various irritants and ulcerogens.[9] These materials might possess anti-inflammatory action by suppressing the neutrophil/cytokine cascade in gastrointestinal tract, promoting tissue repair through expression of various growth factors, exhibiting antioxidant activity, scavenging reactive oxygen species (ROS), showing anti-nucleolytic, anti-necrotic and anti-carcinogenic activities.[10-13]

Treatment of this disease requires in most cases a combination of several molecules with specific mechanisms of action. This treatment has 4 goals: relieve pain, accelerate healing, prevent complications and reduce the frequency of relapses. But while effective, treatment using conventional medicines is not usually well attended by patients.[9,14] The reasons included their high cost and low availability to a large majority of the population especially those living in rural areas. In many developing countries, the health infrastructure is poor and a large majority of the population, mainly rural, has no access to primary health care and modern medicines. These patients use the resources of traditional herbal medicine as an alternative treatment.[11,15]

However, traditional herbal medicine is facing a number problems for its vulgarisation including lack of sufficient studies on therapeutic properties as well toxicity tests to provide sufficient guarantees for their rational use.

The common wild plant, *F. thonningii*, is extensively used in African ethno medicine for treating a number of disease conditions which include diarrhoea, urinary tract infections, diabetes mellitus, gonorrhoea, respiratory infections, and mental illnesses. The leaf of *F. thonningii* 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* possesses antimicrobial, antidiarrheal, antihelmintic, antioxidant, anti-inflammatory and analgesic properties. Scientific research has validated the ethno medicinal claims that *F. thonningii* is useful in disease management. However, there is need to continue identifying, isolating and quantifying the active principles and possibly determine the mechanisms underlying the curative properties of its bark.[10,16]

It is in this context that the current study was conducted to investigate phytochemically screen, evaluate the anti-oxidant activity the stem bark extract.

MATERIALS AND METHODS

This was an experimental *in vitro* and *in vivo* preclinical study on wistar rats conducted from the 11 November 2016 to the 25th May 2017. The study was done in the Preclinical Animal toxicology and Pharmacology Laboratory of the Faculty of Medicine and Biological Sciences, of the University of Yaoundé 1, Cameroon, while the quantification of biochemical parameters was done in the biochemistry laboratory of the same university.

Ethical consideration: Ethical approval was given by the institutional review board (IRB) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 and administrative authorization was obtained to conduct study in the animal house of this faculty.

Collection, identification preparation of plant material: Fresh stem barks were harvested after identification by a botanist from the plant growing at Bafoussam on the 03rd of January 2017. The identified plant was authenticated at the National Herbarium of Cameroon by comparison with a sample having the voucher reference number 444042/HNC. The barks were dried under shade at room temperature for a period of three weeks in order to avoid solar radiations from altering the API. These barks were spread on plastic bags while avoiding their stacking. Every day we turned these barks upside down so as to favour a homogenous drying process. The dried barks were ground in a clean electric grinding machine in such a way to obtain a fined powder which was stored in an airtight container.

Plant extract preparation: Three types of extraction procedures were used in order to evaluate the *in vivo* activity and selected the extract with the best activity since there were no studies with respect the evaluation of the antiulcer activity of the bark of *Ficus thonningii* Blume. These methods of extraction were:

Extraction by Maceration, Infusion and decoction: In this process, the coarsely powdered crude plant was placed in a stoppered container with the solvent (distilled water, ethanol and hydro-ethanolic solution 50:50) and allowed to stand at room temperature for a period of 48 hours with frequent agitation until the soluble matter has

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.[7]

By infusion, fresh infusion was prepared by mixing the crude plant or part of it for a short period of time specifically 10 to 15 minutes with initially boiling water[8] and by decoction, the crude plant was boiled in a specified volume of water for a defined time generally 10 to 15 minutes; it was then cooled and filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. The starting ratio of crude plant to water was fixed, 1:4 or 1:16; the volume was then brought down to one-fourth its original volume by boiling during the extraction procedure and the concentrated extract was filtered.[8]

Yield determination of the extract: The best activity was shown with the hydro-ethanolic maceration hence after 48 hours the macerate was filtered with Whatman No. 3 filtered paper and the collected filtrate was evaporated in an oven at 50 °C. This extract was weighed in order to determine the yield obtained from the initial powder quantity and then stored in an air-tight container for subsequent experimental tests.

Phytochemical screening: The protocol of Odebiyi and Sofowora[7], Soforowa,[9] was used to carry out the different chemical tests. This screening process did not only allow us to test and evaluate the various solutions prepared but also to have an idea of the secondary metabolites present in these solutions.

Preparation of the hydro-ethanolic plant extract
The powder obtained after the grinding period were weighed and then 10g of the powder were mixed with several fractions of a 50:50 hydro-ethanolic solution in order to obtain a final solution of 1000ml in a flat bottomed flask. This mixture was mixed several times within 48 h of maceration after which the mixture was filtered using Whatman paper number 3. The macerate was dried in an oven at 50 ^{o}C for three days. The dried extract obtained was then weighed in order to determine the yield from the initial powder used. The yield (%) was obtained from the formula below

$$\text{percentage yield} = \frac{\textit{mass of the extract obtained}}{\textit{mass of the initial plant powder}} * 100$$

Metabolite identification test
Alkaloid Identification was done using the Mayer Waltz test[11], Hager Test and the Wagner[12] test respectively.

Polyphenol Identification; Tests was done using the Iron per chloride test[15], lead acetate test: In a test tube was added 2 ml of the 1% extract followed by a few drops of plead acetate to 10%. The formation of a white precipitate indicated the presence of polyphenols.

Flavonoid Identification Tests; In a test tube, 2 ml of the 1% extract was poured and a few drops of Sulfuric acid added by allowing them to flow over the tube wall. The formation of an orange coloration Orange indicated the presence of flavonoids.[17]

Identification test of anthocyanins: To 5 ml of 5% extract was added 5 ml of 10% H_2SO_4 and then 5 ml of ammonium hydroxide (NH_4OH) was diluted to half. In the presence of anthocyanin, the colouring was accentuated by acidification then turn to blue-purplish in basic medium.[21]

Test for identification of tannin ($FeCl_3$ Test)
In a test tube was introduced 5 ml of the 5% infused in which was added 1 ml of dilute aqueous solution of 1% ferric perchloride ($FeCl_3$). The presence of Tannins was indicated by blackish-blue or greenish coloration.

Differentiation of catechic and gallic tannins
It was obtained by the reaction of STIASNY, which was carried out in the following manner. To 30 ml of infused, was added 15ml of STIASNY reagent (10ml of 40% formalin more 5 ml of concentrated HCl) and heated for 15 minutes in a water bath at 90 ° C. Catechic tannins was obtained by the presence of a precipitate. The obtaining of precipitate showed their presence; Gallic Tannins: After filtration, the filtrate sodium acetate powder was saturated, Then 1 ml of a solution of 1% ferric perchloride ($FeCl_3$). The presence of gallic tannins was not precipitated by the STIASNY reagent was indicated by the development of a shade dark blue.

Mucilage Identification Test
To 1 ml of decoction extract at 10%, 5 ml of absolute ethanol was added to obtain a precipitate that was fluffy to indicate the presence of mucilages.

Test for the identification of saponins
Steroid Identification Test: In 1 ml of extract was added 2 ml of acetic anhydride then 2 ml of sulfuric acid to obtain a violet colour turning blue or green indicated the presence of steroids.[21]

Test for identification of resins: In a test tube, was add 2 ml of the 1% extract and a few drops of solution of anhydrous acetic acid and 1 ml of sulfuric acid (H_2SO_4) The appearance of a yellow colour indicated the presence of resins.

Test for identification of cardiac glycosides
In 0.5 ml of the extract were added 2 ml of glacial acetic acid and a few drops of 5% Ferric Chloride ($FeCl_3$) solution, then 1 ml of concentrated sulfuric acid. The Formation of a greenish or brown ring, at the interface indicated the presence of glycosides heart.[21]

Test for identification of quinones: In a test tube, 2 ml of 1% extract was added, 2 ml of concentrated $H_2 SO_4$ to obtaining a red colour indicated the presence of the quinones.

Identification test for betacans: In a test tube, put 2 ml of the 1% extract. Add 2 ml of 2N NaOH and Heat the tube in a boiling water bath for 5 minutes. The appearance of coloration A yellow color indicated the presence of beta-cyane.

Identification test for coumarins: In a test tube containing 1 ml of the plant extract was added 1 ml of distilled water and a few drops of 10% $FeCl_3$. Obtaining a green or blue coloration that turned yellow by addition of nitric acid (HNO_3) indicated the presence of coumarins.[14]

Oxalate Identification Test: In a test tube was added 2 ml of the 1% extract, a few drops of ethanoic acid to obtain a greenish-black color indicating the presence of oxalates.

Catalase: The catalase assay was performed according to the method described by Victor.[11]

0.9 ml of phosphate buffer (0.01M, pH 7) and 0.4 ml of H 2 O 2 were introduced into each tube to initiate the reaction. The reaction was interrupted after 30 seconds by the introduction of 2 ml of dichromic acetic acid. The whole was heated at 100 ° C. for 10 minutes. After cooling, the optical density was read at 570 nm. The amount of hydrogen peroxide remaining in the solution after addition of the perchloric acid was evaluated using the calibration curve. The specific activity of catalase was expressed in µM H2O2 / min / mg protein.

Glutathione: Reduced glutathione assay was performed according to the method described by Lipnick et al., 1995.[12] The 2,2-dithio-5,5'-dibenzoic acid (DTNB) reacted with the SH groups of the glutathione to form a yellow coloured complex which absorbed at 412 nm.

Malondialdehyde (MDA): The method used for the MDA assay was that of Ahur et al., 2010.[27] Carbonyl compounds such as malondialdehyde from the decomposition of fatty acid hydroperoxides react with thiobarbituric acid (TBA) to give pink chromophores whose concentration was determined by reading the absorbance at 532 nm.

superoxide dismutase (SOD): Principle according to Pathon et al., 2008.[14] Adrenaline (epinephrine), in the presence of the superoxide anion O 2, was oxidized spontaneously to adrenochrome; A coloured compound which absorbed at 490 nm. SODs, whose role was to reduce the O 2 anion, inhibited this reaction

Expression of results

$$\text{inhibition } \% = \frac{100 - \Delta A\ sample}{\Delta A\ White}\ x\ 100$$

The specific activity of SOD was evaluated in units of SOD/mg of protein. A unit of SOD was defined as the amount of SOD required to cause an inhibition of 50%Of the oxidation of adrenaline to adrenochrome for one minute.

Xanthine Oxidase: The quantification of xanthine oxidase was done following the procedure described by Arneson et al., 2002.[15]

STATISTICAL ANALYSIS

The results were expressed in terms of mean ± standard deviation. The comparisons between the groups were analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test using the GraphPad Instat version 5.0 software. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Extraction Yield

The extraction yield of the hydro-ethanolic extract (50:50) of the bark of *F. thonningii* Blume was 17%.

Phytochemical Screening: The phytochemical screening of the extract of the *F. thonningii* stem bark showed the presence of the sapononins, quinones, coumarins, catechic tannins, phlobotanins, anthocyanins, flavonoids and betacyanes (Table 1).

Table. 1: Presentation of the secondary metabolites in the aqueous extract of the bark of *F. thonningii* Blume.

Test	Specific test	Decoction	Infusion	Ethanolic maceration	Hydroethanolic maceration	Aqueous maceration
Polyphenols	$FeCl_3$	++++	++++	+	++	+
	Lead acetate	+++	++	++	++	-
Saponin distilled water		+	++	+++	++	-
Mucilage absolute EtOH		-	-	-	-	-
Alkaloids	Wagner	-	-	+	+	-
	Mayer	-	-	+	+	-
	Hager	-	-	+	+	-
Flavonoids	NaOH	+	+	+	+	-
	H_2SO_4	+	+	+	++	+
Tannins	Cu_2SO_4/NH_3	+	+	+	++	-
	Catechic	+	+	+	+	-
	Gallic	-	-	-	-	-
Steroids	Acetic anhydride	-	-	-	-	-
Coumarines	$FeCl_3$	+	+	-	-	-
	HNO_3	+	+	+	+	-
Oxalate Ethanoic acid		-	-	-	-	-
Quinones H_2SO_4		+	+	-	+	+
Betacyane NaOH		-	-	-	-	-
Phlobotannins HCl		+	+	+	+	-
Anthocyane H_2SO_4, NH_4OH		+	+	+	+	-
C Glycosides glacial acetic acid		-	-	-	-	-
Resins anhydrous acetic acid		+	+	-	-	-

– represents the absence of metabolites, + represents the presence of metabolites, ++ abundant and +++ very abundant, ++++ extremely abundant.

Quantification of the biochemical parameters to evaluate the oxidative stress: There was no significant variation in the glutathione level, but amongst the test groups, the 500mg/Kg group has the highest level as compared to the negative control group. There was a gradual increase of the catalase concentration as we move from the negative control group to the 500mg/Kg group (Fig 1). A non-significant dose-dependent decrease in MDA was observed in the groups receiving the extract as compared to the NC, though it was high in the 500mg/Kg group. The MDA content was lowest in healthy groups. We observed a non-significant dose-dependent decrease in MDA in the groups receiving the extract as compared to the NC (Fig 1).

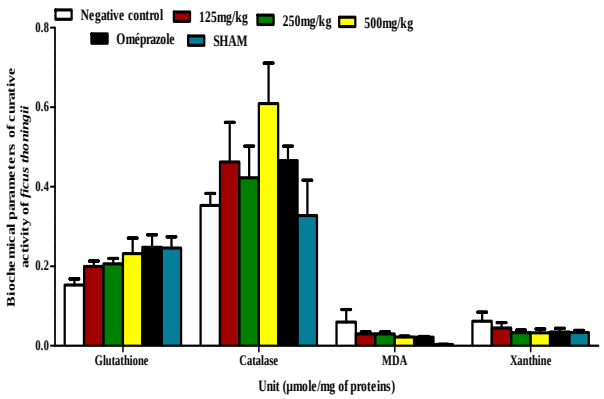

Figure. 1: Effect of the hydro-ethanolic extract on the concentration of glutathione, catalase, MDA, and Xanthine Oxidase (P<0.05).

Quantification of biochemical parameters to evaluate the oxidative stress: There was a gradual increase of the catalase concentration as we move from the negative control group to the healthy animal group. A significant dose-dependent decrease of MDA was determined in the groups receiving the extract as compared to the NC, though it was highest in the 500 mg/Kg group (Fig 2). The MDA content was lowest in healthy groups than omeprazole. There was no significant variation in the glutathione level, but amongst the test groups, the 500 mg/Kg group has the highest level as compared to the negative control group. There was no significant variation in the glutathione level, but the HA group has the highest level as compared to the negative control group (Fig 2).

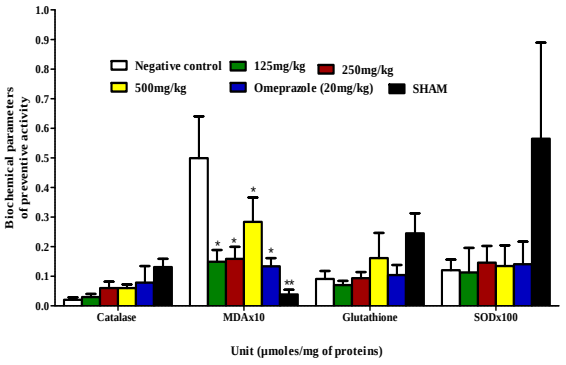

Figure. 2: Effect of the hydro-ethanolic extract on the concentration of catalase, MDA, glutathione and SOD (* for P<0.05 and ** for P<0.01).

DISCUSSION

Belonging to the family of Moraceae, the genus *Ficus* is among the largest genera of angiosperms, from about 60 are present in Cameroun.[13,17] In our search of bioactive compounds from Cameroonian medicinal plants of the *Ficus*, we examined the hydro-ethanolic extract of the bark of *F. thonningii* Blume on peptic ulcers induced by absolute ethanol.

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 the prepared extract solution included; polyphenols, saponins, alkaloids, flavonoids, catechic tannins, coumarins, quinones, phlobotanins, anthocyanins which corroborates with the work done by Dangarembizi et *al.* in 2013 on the leaves of *F. thonningii*[10] and Usman et *al.* in 2010.[15] These metabolites are similar to those found in *F. sycomorus*[18-21]; Phytochemicals such as alkaloids have anti-depressive and anti-inflammatory effects in which some have preventive and curative anti-ulcer activities[22], flavonoids favours blood circulation, are antioxidants as well increase the production of prostaglandins in the gastric mucosa.[25-27]

For most of humankind's history, traditional methods of healing were used to treat every sort of health disorder. The World Health Organization estimates that around 80 % of the world population in developing countries relies on traditional plant medicines for primary healthcare needs, of which a major proportion corresponds to plant extracts or their active principles. Plants and herbs have been used since ancient times to treat different gastrointestinal illnesses, including peptic ulcers.[28-30] In China, Traditional Chinese Medicine is practiced in hospitals in addition to western medicine. In Germany, all medical physicians are also trained in the use of herbs.[26] Considering the several side effects of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. Recently, many efforts have been made in order to identify new anti-ulcer drugs from natural resources. Anti-ulcer drugs such as carbenoxolone from *Glycyrrhiza glabra*, solon from sophoradin and gefarnate from cabbage are some of such drugs.[34,35] Liquorice from the root and rhizome of different varieties of *Glycyrrhiza glabra* has been extensively used in medicine for its anti-ulcer activity. The principal constituent of liquorice, is a triterpenoid saponin. It is the substance responsible for its gastro protective action against ulcers and has been extensively used in medicine.[37] Zinc-carnosine, another natural supplement consisting of zinc and L-carnosine, strengthens the stomach's mucosal defences and harnesses the stomach's natural ability to fight disease, battle infection, and heal itself. Its component L-carnosine, a dipeptide made up of L-histidine and β-alanine, demonstrates antioxidant properties that also add to its protective and healing effects.[21,38] The medicinal properties of folk plants are mainly attributed to the presence of flavonoids, and other organic compounds such as coumarins, phenolic acids, tannins, antioxidants and inorganic micronutrients such as Cu, Mn and Zn. These secondary plant metabolites have been shown to scavenge free radicals and are viewed as promising therapeutic options.[4,38] Therefore, by scavenging free radicals, antioxidants from plant metabolites might be useful in protecting the gastric mucosa from oxidative damage or in accelerating healing of gastric ulcers. The potential role and basic mechanisms of plant-originated gastro protective substances applied intragastrically are known to account for mucosal protection against various irritants and ulcerogens.[40] These materials might possess anti-inflammatory action by suppressing the neutrophil/cytokine cascade in gastrointestinal tract, promoting tissue repair through expression of various growth factors, exhibiting antioxidant activity, scavenging reactive oxygen species (ROS), showing anti-nucleolytic, anti-necrotic and anti-carcinogenic activities.[26]

Treatment of this disease requires in most cases a combination of several molecules with specific mechanisms of action. This treatment has 4 goals: relieve pain, accelerate healing, prevent complications and reduce the frequency of relapses. But while effective, treatment using conventional medicines is not usually well attended by patients.[9,41] The reasons included their high cost and low availability to a large majority of the population especially those living in rural areas. In many developing countries, the health infrastructure is poor and a large majority of the population, mainly rural, has no access to primary health care and modern medicines. These patients use the resources of traditional herbal medicine as an alternative treatment.[3,42]

However, traditional herbal medicine is facing a number problems for its vulgarisation including lack of sufficient studies on therapeutic properties as well toxicity tests to provide sufficient guarantees for their rational use. The common wild plant, *F. thonningii*, is extensively used in African ethno medicine for treating a number of disease conditions which include diarrhoea, urinary tract infections, diabetes mellitus, gonorrhoea, respiratory infections, and mental illnesses. The leaf of *F. thonningii* contains various bioactive compounds which include alkaloids, terpenoids, flavonoids, tannins and active proteins, all of which contribute to its curative properties.[2,43] *In vitro* and *in vivo* pharmacological studies revealed that *F. thonningii* possesses antimicrobial, antidiarrheal, antihelmintic, antioxidant, anti-inflammatory and analgesic properties. Scientific research has validated the ethno medicinal claims that *F. thonningii* is useful in disease management. However, there is need to continue identifying, isolating and quantifying the active principles and possibly determine

the mechanisms underlying the curative properties of its bark.[10,44]

Ethanol also reduces the activity of antioxidant enzymes such as SOD, catalase and glutathione. It significantly increases the amount of MDA than that the healthy control. The role of these enzymes in the defense of the organism against oxidative stress is well known. SOD, for example, provides the first line of defense by converting superoxide free radicals to hydrogen peroxide. The latter is then degraded by catalase in water.[13,45] As for glutathione, it interacts directly with active oxygen species and ensures the elimination of peroxidized lipids such as MDA.[34] Thus, the increase in the level of these enzymes (glutathione, SOD. catalase) in animals that received *F. thonningii* hydro-ethanolic stem bark extract and omeprazole show that the inhibition of gastric ulcers in these gastric ulcers is also due to an antioxidant activity. Jing-Yang Wong et *al*. in 2013 did similar studies in which the aqueous extract of *H. Erinaceus* significantly decreased lipid peroxidation and resulted in increased SOD and catalase activity, indicating its efficacy in the Prevention of gastric ulcers induced by ethanol in rats.[13]

In the present alcohol-induced gastric ulcer model, the levels of glutathione, catalase and MDA increased in the 500mg/Kg group as compared to the negative control. These compounds are important for maintaining the integrity of the gastric mucosa and mediating the protective effects of prostaglandins against gastric mucosal injury.[6,31]

CONCLUSION

We can conclude from this work that the *F. thonningii* Blume stem bark hydro-ethanolic extract- Contains flavonoids, saponins, quinones, alkaloids, coumarins, catechic tannins, , polyphenols, flavonoids, phlebotanins and anthocyanides. The phytochemical screening of the extract of the bark showed the presence of the saponins, quinones, coumarins, catechic tannins, phlobotanins, anthocyanin, polyphenols, flavonoïds and betacyanes. The extract resulted in a non-significant increase of SOD, Catalase and glutathione with a dose dependent decrease in MDA. The administration of this extract with 2000 mg/Kg dose was well tolerated with no lethality observed.

ACKNOWLEDGEMENTS

This work was partly funded by the Centre for Biopolymer and Bio-molecular Research, Athlone Institute of Technology, County Westmeath, Republic of Ireland, and the research mobilization grant from the Ministry of Higher Education, Cameroon. Thanks to the Pougoue family for the financial support and the Preclinical Animal Toxicology laboratory for the technical support.

REFERENCES

1. Togola A., Karabinta K., Dénou A., Haidara M., Sanogo R., Diallo D. Effet protecteur des feuilles d'Opilia celtidifolia contre l'ulcere induit par l'ethanol chez le rat. Int. J. Biol. Chem. Sci., 2014; 8(6): 2416-2423.
2. Fokunang CN, Ndikum V, Tabi OY, Jiofack RB, Ngameni B, Guedje NM, Tembe-Fokunang EA, et al. Traditional medicine: past, present and future research and development prospects and integration in the national health systemof cameroon. Afr J Tradit Complement Altern Med., 2011; 8(3): 284-295 284.
3. Rachael D., Kennedy H. Erlwanger D. M., Eliton C. Phytochemistry, pharmacology and ethnomedicinal uses of Ficus thonningii (blume moraceae): A review. Afr J Tradit Complement Altern Med., 2013; 10(2): 203-212.
4. 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. Tradi., Comple. Alt. Med., 2009; 6(3): 289 – 295.
5. Lichtenberger LM. The hydrophobic barrier properties of gastrointestinal mucus. Annu Rev Physiol, 1995; 57: 565-583.
6. Kato ST Kawase J, Alderman N, Inatomi CS, Lieber B. Role of xanthine oxidase in ethanol- induced lipid peroxidation in rats," Gastroenterology, 1990; 98: 203-210,
7. Odebiyi O. and Sofowora E. phytochemical screening. Nigeria medical plants, 1978; 41-234.
8. Sofowora A. Plantes médicinales et médecine traditionnelle dAfrique. Paris: KARTHALA,1996; 378p.
9. Brzozowski I, Konturek P.C, Brzozowski T, Konturek S.J, Kwiecien S, Pajdot R, Drozoowicz D, Pawlik N, Ptak A et Hahn E.G. Role of prostaglandin's, nitric oxide sensory nerves and gastrinin acceleration of ulcer healing by melantonin and its precursor, L-trytophan: J Pineal. Res., 2002; 32: 149-162.
10. Alcaraz MJ, HoultJR. Actions of flavonoids and the novel anti-inflammatory flavones hypolaetin-8-glucoside, on prostaglandine biosynthesis and inactivation. Biochem pharmacol, 1985; 34: 2477-2482.
11. Victor OO. Phytochemical Screening and Anti-diarrhoeal Activity of the Leaves of the Plant Ficus sycomorus Family: Moraceae. Unpub. Undergrad. Proj. Fac. of Pharm. Sciences, A.B.U., Zaria, Nigeria, 2006; 27.
12. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP et al. Comparison of the up-and-down, conventional LD_{50} and fixed dose acute toxicity procedures. Food Chem. Toxicol. 1995; 33(3): 223-231.
13. Ahur VM, Madubunyi I, Adenkola AY, Udem SC. The effect of acetyl acetate extract of Ficus thonningii (Blume) leaves on erythrocyte osmotic

fragility and hematological parameters in acetaminophen-treated rats. Com. Clin. Pathol, 2010; 10: 1107-1111.
14. Pathom J, Krisana P, Yanee P, Chadarat D, Prasit T. Acute and repeated dose 28-day oral toxicity study of Garcinia mangostana Linn. Rind extract, 2008: 202.
15. Arneson W, Brickell J. Assessment of the renal function. In: Clinical chemistry: A laboratory perspective. Philadelphia, USA, 201-232.
16. Hegazi AG, Abd El Hady FK. Egyptian Propolis: Antioxidant, Antimicrobial Activities and Chemical Composition of Propolis from reclaimed lands.Z. Naturforsch, 2002; 57: 395-402.
17. Bronner C and Landry Y. Kinetics of the inhibitory effect of flavonoids on histamine secretion from mast cells. Agents Actions, 1985; 16: 147-151.
18. Morikawa T, Li N, Nagatomo A, Matsuba H, Li X, and YoshikawaM. Triterpene saponins with gastroprotective effects from tea seed the seeds of Camellia sinensis. J Nat Prod., 2006; 69(2): 185-190.
19. Sun H, Fang W WS, Wang WZ and Hu C. Structure activity relationships of oleanane and ursane-type triterpenoids. Botanical Studies, 2006; 47: 339-368.
20. Harju E and Sajanti J. The protective effects of nutrients against stress induce gastric ulcers in the rat. In vivo, 1991; 5: 397-400.
21. Tan PV and Nyasse B. Anti-ulcer compound from Voacanga africana with possible histamine H 2 receptor blocking activity. Phytomedicine, 2000; 7(6): 509-515.
22. OECD: OECD Guidelines for the testing of chemicals acute oral toxicity – method by acute toxic class. OCDE 420 adopted 17 December 2001. 312pp.
23. Mahmood AA, Mariod AA, Al-Bayaty F, Abdel-Wahab SI. Antiulcerogenic activity of Gynura procumbens leaf extract against experimentally induced gastric lesions in r ats. J. Med. Plants Res., 2010; 4(8): 685-691.
24. Tan P.V, Lyonga E. L, Nditafon G.N, Njimi CK et Bopelet M: Gastric cytoprotective anti-ulcerogenic actions of the aqueous bark extract of leaf extract of Eremomastax spesiosa in rats. J. Biol.Biochem.Sci., 1997; 7(1): 69-77.
25. Mishra, H.P. and Fridovich, I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of Biological Chemistry, 1972; 247: 3170-3175.
26. Ibrahim G, Abdulmumin S, Musa, KY, and Yaro AH. Anticonvulsant activities of Crude Flavonoid Fraction of the Stem bark of Ficus sycomorus (Moraceae). J. Pharmacol. Toxicol, 2000; 3(5): 351-356.
27. Ahur, V.M., Madubunyi, I., Adenkola, A.Y. and Udem, S.C. The effect of acetyl acetate extract of Ficus thonningii (Blume) leaves on erythrocyte osmotic fragility and haematological parameters in acetaminophen-treated rats. Com. Clin. Pathol, 2010; 10: 1107-1111.
28. Ukwe C. V, Ubaka C. M, Adibe M. O. Okonkwo C. J and Akah P. A. antiulcer activity of roots of Zapoteca portoricensis (Fabiaceae): J. Basic.Clin. Pharm, 2010.
29. Benjamin KN, Joseph FM, Mesfin BD, Joseph A. Antiulcerative properties and acute toxicity profile of some African medicinal plant extracts: Journal of Ethnopharmacology, 1994; 42: 13-18.
30. Miller, T.A., Henagan, J.M. Indomethacin decreases resistanceof gastric barrier to disruption by alcohol. Dig. Dis. Sci., 1984; 29: 141–149.
31. Tembe-Fokunang EA, Fokunang CN, Ngameni B, Barkwan S, Hoare G, Gatsing D, Ngadjui BT, Tomkins P. Pre-clinical in vitro investigation of the cytotoxic effect of Ficus species on hepatoma G2 cells using two standard toxicity assays. Int. J. Biol. Chem. Sci. 2018; 12(1): 11-23, February 2018 ISSN 1997-342X (Online), ISSN 1991-8631 (Print). http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int.
32. Nguele RL, Fokunang CN, Etoundi C, Chakokan RM, Ngondi JL, Tembe EA, Kechia FA, Ngameni B, Gatsing D, Oben EJ. Utilisation des especes du genre Aframomum (Aframomum aulacocorpus,. A citratum, A. daniellii) pour le contrôle du poids, le profil lipidique et le statut antioxydant chez les rats Wistar nouris avec une diete atherogene. Int. J. Biol.Chem. Sci., 2016; 10(6): 2575-2586. DOI : http://dx.doi.org/10.4314/ijbcs.v19i6.14.
33. Eteme FL, Fokunang CN, Tchuenguem F, Nolna D, Boula A, Ndze VN, Kemadjou G, Tembe-Fokunang EA, Gatsing D. Epidémiologie moléculaire du Rotavirus du groupe A associé aux gastroentérites chez les enfants de moins de 5 ans dans la ville de Yaoundé (Cameroun) Int. J. Biol. Chem. Sci. 2015; 9(5): 2561-2573, October 2015. ISSN 1997-342X (Online), ISSN 1991-8631 DOI : http://dx.doi.org/10.4314/ijbcs.v9i5.25. http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int.
34. Jiofack, T Ayissi I, Fokunang C, Guedje N and Kemeuze V. Ethnobotany and phytomedicine of the Upper Nyong valley forest in Cameroon. Afric. J. Pharm. Pharmacol, 2009; (4): 144-150.
35. Focho DA, Anjah MG, Nwana FA, Ambo FB. Ethnobotanical survey of trees in Fundong, Northwest Region, Cameroon. J Ethnobiol Ethnomed, 2009; 25; 5: 17.
36. Dongmo MSN, Fokunang CN, Fekam FB, Asonganyi T. Anticonvulsant activity of extracts from six Cameroonian plants traditionally used to treat epilepsy Int. J. Biol. Chem. Sci., 2014; 8(6): 2407-2415, ISSN 1997-342X (Online), ISSN 1991-8631, International Formulae Group. DOI: http://dx.doi.org/10.4314/ijbcs.v8i6.4.
37. Emmanuel E Haule, Mainen J Moshi, Ramadhani SO Nondo, Dennis T Mwangomo, Rogasian LA Mahunnah. A. A study of antimicrobial activity,

acute toxicity and cytoprotective effect of a polyherbal extract in a rat ethanol-HCl gastric ulcer model. BMC Res Notes, 2012; 5: 546.doi:10.1186/1756-0500-5-546.

38. Mainen J Moshi, Ramadhani SO Nondo, Emmanuel E Haule, Rogasian LA Mahunnah, Abdul W Kidukuli. Antimicrobial activity, acute toxicity and cytoprotective effect of Crassocephalum vitellinum (Benth) S. Moore extract in a rat ethanol-HCl gastric ulcer model. BMC Res Notes, 2014; (7): 91. Published online 2014 Feb 19.doi:10.1186/1756-0500-7-91.
39. Nahla Saeed AL-Wajeeh, Maryam Hajrezaie, Nawal Al-Henhena, Sareh Kamran, Elham Bagheri, Maryam Zahedifard, Kamelia Saremi, Suzita Mohd Noor, Hapipah Mohd Ali, Mahmood Ameen Abdulla. The antiulcer effect of Cibotium barometz leaves in rats with experimentally induced acute gastric ulcer. Drug Des Devel Ther. 2017; 11: 995–1009.Published online 2017 Mar 30.doi:10.2147/DDDT.S107018.
40. Margaret O, Sofidiya LA, Abidemi JA, Johnson AO, Oluwole BF. Effect of Flabellaria paniculata Cav. Extracts on gastric ulcer in rats. BMC Complement Altern Med. 2012;12: 168. Published online 2012 Oct 2.doi:10.1186/1472-6882-12-168.
41. Noraziah NS, Munir SS, Shahram GM, Maryam HA. Anti-ulcerogenic effect of methanolic extracts from Enicosanthellum pulchrum (King) Heusden Mehran., PLoS One.2014;9(11): e111925. Published online 2014 Nov 7.doi:10.1371/journal.pone.0111925.
42. Nguele LR, Fokunang CN, Etoundie C, Chakokan RM, Ngondi JL, Tembe EA, Kechia, FA, Ngameni B, Gatsing D, Oben JE.2016. Utilisation des espèces du genre Aframomum aulacocorpus, A. citratum, A. daniellii) pour le contrôle du poids, le profil lipidique et le statut antioxydant chez les rats Wistar nourris avec une diète athérogène. Int. J. Biol. Chem. Sci., 2016; 10(6): 2575-2586. DOI: http://dx.doi.org/10.4314/ijbcs.v10i6.14.
43. Vemo BN, Kenfack A, Ngoula F, Kodjio N, Nounamo GA, Megnimeza M, Teguia A. 2017. Effects of ethanol extract of Bersama engleriana leaves on oxidative stress and reproductive parameters in male Guinea pig (Cavia porcellus) exposed to cypermethrin. Int. J. Biol. Chem, Sci., 11(5): 2243-2253. DOI: http://dx.doi.org/10.4314/ijbcs.v11i5.23.
44. Yapi AB, Kassi DJ, N'Guessan BY,Zirihi GN. 2015. Etude ethnobotanique des Asteraceae medicinales vendues sur les marches du district autonome d'Abidjan (Cote d'Ivoire). Int J. Biol. Chem., Sci., 9(6): 2633-2647.
45. Zakari AH, Mahamadou CI, Hachimou ZTA. 2016. Efficacité de l'huile de neem (Azadirachta indica) et de Bacillus thuringiensis (Biobit 2X) sur la dynamique de la population de Bemisia tabaci (Gennadius 1889) et Helicoverpa armigera (Hubner, 1808) dans une plantation. Int. J. Biol. Chem. Sci., 10(2): 497-505. DOI: http://dx.doi.org/10.4314/ijbcs.v10i2.4.