Evaluation of gastroprotective and phytochemical properties of *Terminalia superba* engl. & diels (combretaceae) stem bark methanol extract

Michael O. Chukwuma*[1], Uchenna E. Odoh[1], Christopher O. Ezugwu[1], Theodora C. Mba[2], Austin Okorie[3]

[1]Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka
[2]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka
[3]Department of Pharmacognosy Enugu State University

Submitted: 7th Aug., 2022; Accepted: 22nd Oct.,2022; Published online: 31st Dec.,2022
DOI: https://doi.org/10.54117/jcbr.v2i6.3
**Corresponding author: Michael Onyegbulam Chukwuma; chukwuma.michael@unn.edu.ng*

Abstract
Peptic ulcer is a disease that is affecting a large part of the population world-wide irrespective of the race and tribe. Advances made in the search for an ideal or perfect antiulcer drug has been eroded by cost, side effects and treatment failure. Search for cheap, safe, and effective drugs continues. It is the aim of the research to identify the secondary metabolites present in *Terminalia superba* Engl. & Diels (Combretaceae) stem bark methanol extract and investigate its gastroprotective properties. Methanol extract of *T. superba* was prepared by cold maceration extraction method using methanol. The extract was concentrated and subjected to acute toxicity test using Lorke's method, and phytochemical investigation using standard methods. Gastro-protective activity was investigated by three models: aspirin-induced gastric ulcer, hypothermic restraint stress-induced ulcers and ethanol-induced gastric ulcer. The extract was safe in mice at doses below 2154 mg per Kg body weight and the extract contained the major secondary metabolites-alkaloids, flavonoids tannins, saponins, terpenoids, steroids. Pre-treatment with the extract reduced gastric ulceration in rats. There was a 77.3% reduction in the aspirin model, 75.2 % in the ethanol model and an 84 % in the Hypothermic restraint stress-induced ulcers model. There was a statistically significant difference at $p = .05$ between the treated groups and the un-treated group The methanol extract of *Terminalia superba* bark is rich in the major secondary metabolites and has gastro-protective a property.
Keywords: Terminalia, Phytochemistry, Gastric ulcer, Ulcer induction, Ulcer treatment

Introduction

The use of plants for medicinal uses by man is as old as man's stay on earth. The use of natural products in treatment of human diseases is generally characterized by very low / few, and their cost is highly attractive. Their efficiency is not in doubt, what may be lacking is information on these herbs. Peptic ulcer is a disease that is affecting a large part of the population world-wide irrespective of race and tribe. Despite substantial advances, this disease remains an important clinical problem (Maifertheine *et al*., 2009). Advances made in searching for an ideal or perfect anti-ulcer drug have been eroded by the high cost of the drugs and treatment failure. Search for cheap, safe, and effective drugs continue. The synonym is *Terminalia altissima* A. Chev (https://plants.jstor.org)

Botanical Description and Geographic distribution

Terminalia superba is a tree of about 30-50 m high. It is about 5 m in girth, cylindrical, long and straight with large, flat buttresses and 6 m above the soil surface. In Africa it is found in West and Central Africa, from Guinea Bissau east to DR Congo and south to Cabinda (Angola) (Burkill, 2009). In Nigeria it is Indigenous to Cross River State (Orewa *et al*., 2009).

Ethnomedicinal Uses

Terminalia superba is used in traditional medicine to treat bacterial, fungal and viral infections. The bark is used to treat intestinal worms and gastrointestinal disorders such as enteritis, abdominal pain, diarrhoea, fever, headache, conjunctivitis. In the Southwest of Côte d'Ivoire the bark of *T. superba*, called "tree of malaria". The bark is used as analgesics, for pulmonary troubles, antemetics, diarrhoea, dysentery, dropsy, swellings, oedema, and gout (Orewa *et al*., 2009).

Causes of Peptic ulcer

Peptic ulcers are caused by an imbalance between destructive and defensive factors in the stomach (Calam *et al*., 2001).There both endogenous destructive factors like HCl, pepsin, biliary reflux, lipid peroxidation, and ROS, and exogenous factors like ethanol, indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAID), stress, smoking, and infection by *Helicobacter pylori* bacteria (Alrashdi *et al*., 2012; Gasparetto *et al*., 2012). The defensive factors are mucus-bicarbonate barrier, mucin secretion, surface phospholipids, prostaglandins (PGs), nitric oxide (NO), mucosal blood flow, cell renewal, growth factors, and antioxidant enzymes (Alqasoumi, *et al*., 2009).

Classification of gastric ulcers

Gastric ulcers are classified on the basis of location and encoscopic findingsin to Type 1: Ulcer present at the body of stomach without involving duodenum, pylorus or prepyloric region and not as sociated with hyper-secretion of gastric acid. Type 2: Ulcer present at the body of stomach combined with duodenum and associated with gastric acid hyper-secretion Type 3: Ulcer close to pylorus and associated with gastric acid hyper-secretion (Johnson, 1957).

Treatment of Peptic Ulcers

The pharmacological basis for ulcer treatment is inhibition of proton pump activity and antagonization of histamine H2 receptors, elevation of GIT Ph, eradication of *H. pylori* infection, protection protection of gastrointestinal surface. Drugs for treatment of stomach ulcers are placed into the following groups; Proton pump inhibitors-Omeprazole, Rabeprazole, Esomeprazole, Lansoprazole; Hiatamin H2-receptor antagonists-Ranitidin, cimetidine, Famotidin, Nizatidine; Cytoprotective agents-Sucralfate, Bismuth chelate; Prostaglandin analogues-misoprosol; Antibiotics- clarithromycin, amoxicillin, metronidazole; Antacids-Aluminium hydroxide gel, magnesium trisilicate

The standard therapeutic regimen consist of a combination of three drugs, consisting of a proton pomp, clarithromycin, and metronidazole (Dan Greer, 2019).

Models for investigating anti-ulcer activity include the following:

Water-immersion stress or cold water-restraint, or cold-restraint stress (Levine, 1971), NSAID gastric ulcer induction (Bhargava, *et al*., 1973), ethanol-induced gastric ulcers (Oates and Hakkinen, 1988), acetic acid-induced gastric ulcers (Okabe and Amagase, 2005), histamine-induced gastric ulcers (Parsons, 1985), reserpine-induced gastric ulcers (Kagoshima and Uguro, 1982), pylorus-ligation (Shay, 1945), diethyldithiocarbamate-induced

ulcers (Oka *et al*., 1990), methylene blue-induced ulcers (Shah *et al*., 2006), ischemic perfusion model (Wada *et al*., 1996), cysteamin-induced stomach ulcers (Szabo, 1990), indomethacin-histamin-induced ulcers (Takeuchi *et al*., 1986), ferrous iron-ascorbic acid model (Nito, *et al*., 1995), [23], *Helicobacter pylori* and acetic acid-*H. pylori* induced ulcers (Megraud and Lehours, 2007).

Some *Terminalia* species with antiulcer activities

Terminalis species that have been investigated for anti-ulcer and gastroprotective activities include *Terminalia coriaceae* (Roxb) Wight & Arn (Khan, *et al*., 2017), *Terminalia Terminalia cheluba* Retz (Sharma, *et al*., 2011), (Roxb), *Terminali a arjuna* (Roxb) Wight & Arn (Devi *et al*., 2008), *Terminalia belerica* (Gaertn) Roxb (Jawanjahl, *et al*., 2012), *Terminalia fagifolia* Mart (Nunes, *et al*., 2014), and *Terminalia avicennioides* Guill & Perr. (Suleiman, *et al*., 2007).

Phytochemicals

Phytochemical simply means plant chemicals. They are components of plants that are chemical in nature produced by plant. Plant secondary metabolites are grouped into the following chemical groups: alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, glycosides, and phenolics. They may also be grouped according to their pharmacological activites. Their production involves building of complex compounds (molecules) from simple elements through biosynthetic pathways. The pathways involved are shikimic acid pathway, the mevalonate pathway, and the acetate pathway.

The shikimate pathway provides the aromatic amino acids L-phenylalanine, L-tyrosine and L-tryptophan. From L-phenylalanine *p*-coumarin-CoA is produced. This is the is precursor for a wide range of natural products such as tannins, flavonoids, and isoflavonoids, anthocyanidins, coumarins stibens etc (Vogt, 2010). The mevalonic acid pathway involves the condensation of isopentyl pyrophosphate (IPP) with its isomer dimethylallyl pyrophosphate (DMAPP) to yield geranyl pyrophosphate (Evans, 2009). This is the beginning of isopenoid biosynthesis. Addition of a molecule of IPP to geranyl pyrophosphate produces farnesyl. From geranyl and farnesyl, monoterpenes, aqualene, triterpenes, steroids tetrterpboids are produced (Evans, 2009). Flavonoids and tannins (proanthocyanidins) because of the presence of an aromatic nucleus and one or more hydroxyl groups, of many hydroxyl groups in their structures are generally referred to as poly-phenolics. Most phenolics exist as glycosides, hence, are water soluble. The polyphenolic nature of poly-phenolics gives them the antioxidant capability, scavenging free radicals and chelating metal ions (Derick, 2009).The antioxidant activity makes them beneficial to humans both as neutraceuticals and as therapeutic agents.

Alkaloids

Alkaloids are complex organic nitrogenous compounds of plant or animal origin, with significant pharmacological activities (El-Olemlyl, *et al*., 1994). If they are derived from an amino acid and the nitrogen atom is not part of a ring it is called a proto-alkaloid, if it is part of a ring it is called a true alkaloid. If it does not have an amino acid precursor it is called a pseudo-alkaloid (Derick, 2009). The nitrogen atom carries a lone pair of electrons and this imparts basicity to the molecule. In other words, alkaloids are basic in nature.

Glycosides

Glycosides are compounds which on chemical or enzymatic hydrolysis yield a sugar and non-sugar component. The non-sugar component if called the aglycone or genin while the sugar component is referred to as the glycine. Depending on the atom connecting the two component of the glycoside are described as O-glycosides, N-glycosides, or S-glycosieds if the connecting atom is oxygen, nitrogen, or sulphur, respectively.

Saponins

Saponins are high molecular weight compounds characterized by their frothing in aqueous solution. Saponins are glycosides which, and exhibit soap-like surfactant properties, even at low concentrations. They originate from the mevalonic acid biosynthetic pathway. They are divided into two groups, steroidal and the pentacyclic triterpenoid groups, based on the nature of the aglycone. A common feature is the presence of a C3 glycosidal linkage (Evans, 2002).

The value of plants as food and as agents for disease treatment and management is derived from their chemical composition. It is not enough to know what a plant can be used for, knowledge of what it contains is also a necessity. Phytochemical evaluation provides information on the chemicals present and the concentrations. Side effects and adverse reactions (major and minor) are major concerns in the use of orthodox medicines that may affect patient compliance and result to treatment failure. Diarrhoea and headache are the most common, and occasionally mental confusion and rashes have been reported. Cimetidine, due to its antiandrogenic effects, has been associated with gynaecomastia and impotence when used in high doses. Misoprostol can cause miscarriages when administered to pregnant women and should be avoided by women of childbearing age (Dan Greer, 2019). These side effects make the search for a safer anti-ulcer drug necessary.

It is the aim of the research to identify the secondary metabolites present in this plant (*Terminalia superba*) and investigate its gastroprotective properties.

Materials And Methods

Collection and Identification

The stem bark of *Terminalia superba* was collected in Nsukka, Enugu State, Eastern Nigeria during the month of July, 2019. The plant material was identified by Mr. A.O. Ozioko, a taxonomist at International Centre for Ethnomedicine and Drug Development (InterCEDD A specimen has been deposited with the herbarium of the Department of Pharmacognosy and Environmental Medicine of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka with voucher number PCG/UNN/0084. The names and family of the plant were cross-checked and confirmed through search on Medicinal Plant Names Services, https://mpns.science.kew.org/ and "The Plant List", http://www.theplantlist.org/.

Preparation of plant material

Fresh barks were collected, foreign materials were removed and the barks were dried at room temperature for seven days and powdered mechanically.

Extraction: A portion of the powdered material (1100 g) was extracted by cold maceration for 3 days in large amber bottles with intermittent shaking. It was filtered after 24 hours and a fresh solvent was added and the procedure repeated for another 24 hours.

The filtrates were pooled and concentrated using a rotary evaporator. The extract was then stored in air-tight container and kept in fridge at 4°C till needed.

Equipments

Shimadzu ATX224 Analytical Balance *(*Shimadzu, Kyoto 604-8511, Japan) , EIE – 213EP Soxhlet extractor (EIE Instruments, Ahmedabad - 380006, Gujarat, India, RE-5003 50L Rotary Evaporator (Henan Lanphan Industry Co., Ltd Zhengzhou, Henan, China), centrifuge (Eppendorf Centrifuge 5425 Eppendorf AG, Hamburg, Germany), Stuart Reciprocating Shaker Model SSL2 by Stuart (Cole-Parmer Staffordshire, UK). spectrophotometer, Model UV 7 UV/Visible Spectrophotometer by Mettler-Toledo Inc., Manual single channel micropipette (Pipet-Lite XL Model, Mettler-Toledo Inc., Columbus, USA), Homogenizer (Frain Industries, Inc, IL, USA).

Reagents

Dragendorff's reagent, picric acid, Fehling's solutions A and B, rutin,, Methanol, conc. Sulphuric acid concentrated ammonia (Merck KGa A, Darmstadt, Germany), gallic acid, potassium ferricyanide, NaOH, acetic anhydride, (Reagents, Charlotte, NC 28214,USA), ferric chloride (Xilong Scientific Co., Ltd, China) Na_2CO_3, Zouping Zhijin New Material Technology Co., Ltd Shandong, China), Cholesterol, Fissions Chemicals (United Kingdom), atropine and vanillin (Sigma Chemical, USA), diosgenin, Xiangyang Wellbeing Pharmchem Co., Ltd, China), linalool (Basf Se, Belgium)

Quantitative and qualitative phytochemical analysis

The quantitative and qualitative phytochemical analysis of the plant material was performed according to the methods of Evans (2009) and Harborne (1998), with slight modifications. Garlic acid was used to calculate the total phenolic content of the extracts, rutin was used for flavonoids, atropine was used for alkaloids, KCN was used for cyanogenic compounds, cholesterol standard reagent for steroids, Tannic acid was used to quantify tannins, Linalool as standard reagent for terpenoids, standard saponnin was used as a standard for saponins.

Acute toxicity Test

Acute toxicity was evaluated by the method of Lorke (1983. Albino mice of both sexes, weighing between 97 – 136 g and albino mice weighing between 18.5 – 24.5 g purchased from University of Nigeria Nsukka, animal house, were used in this study. The animals were housed in cross ventilated room in cages at 22 ± 2.5°C) with 12 h dark/12 h light cycles and were feed with standard growers mash feeds. Animals were acclimatized for one week and fasted overnight, with free access to water, prior to experiments. The treated animals were observed for lethality or signs of acute intoxication for 24 hours (Lorke1983). The LD_{50} calculated as the geometric mean of the highest non-lethal dose (1600mg) and the lowest lethal dose (2900mg).

Evaluation of Antiulcer Activity

Animals

Ninty rats (*Rattus rattus*) rats of either sex weighing between 145g and 156 (non-pregnant females) were bought from the Department of Pharmacology and Toxicology of the University of Nigeria, Nsukka. They were housed in steel cages at room temperature 30 $\pm$ 2ºC under twelve hours light and dark cycle and were well fed with standard feed and water given freely for 10 days. Each treatment was performed by gavage administration.

Preparation of plant extract

The crude extract of *T. superba* stem bark, the extract was used to make 100 mg/ml stock solution. The solution was administered in volumes equivalent to 100 mg/kg, 200mg/kg, and 400 mg/kg to the

different groups of animals. This dose was based on the LD_{50} value obtained from the acute toxicity test. Ranitidine 50 mg/ml was used as standard.

Aspirin- induced gastric ulcer

Thirty were well fed and water given freely for three days, then fasted for 24 hours.
The method adopted was that used by Goel *et al*. (1985) slightly modified. The rats were randomly divided into five groups of with six animals in each. It was ensured that the difference in group means was not significant. Group Group 1 received extract 100 mg / Kg body weight, Group 2 received extract 200 mg / Kg body weight, Group 3 received extract 400 mg / Kg, Group 4 received Ranitidine received 50 mg / Kg body weight, Group 5 (Un-treated) received 5 ml of water. Both the test substance and the standard (ranitidine) were administered orally 30 minutes prior to administration of 200mg aspirin. Aspirin was suspended in 1% carboxy methyl cellulose in water. Four hours later the rats are sacrificed by using anaesthetic ether and their stomachs dissected and examined for the determination of gastric lesions.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with normal saline to remove gastric contents and blood clots and examined by a 100x magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted and scored.

Scoring of ulcer was made as follows: Normal stomach (0), Pinhole (1.0), Spot ulcer (1.5), Haemorrhagic streak (2.0), Small erosion (2.5), Large erosions (3.0), Perforation (3.5)
Mean ulcer score for each animal was used to express ulcer index. The Protection Index (PI) percentage of ulcer protection was determined as follows:

$$\text{Protection Index (PI)} = \frac{Uc - Ut}{Uc} \times 100$$

u_t = ulcer index of treated group. U_c = ulcer index of control group.

Hypothermic restraint stress-induced ulcers model

The procedure adopted was that of (Gurbuz and Yesilada, 2007), slightly modified. The modification was in the dose of extract used, the composition of the solvent administered to the un-treated group.

Thirty *Rattus rattus* rats of either sex were randomly divided into five groups of six rats in group. The rats were well fed and water given freely for three days, then fasted for 24 hours. Group 1 received extract 100 mg/Kg body weight, Group 2 received extract 200 mg/Kg body weight, Group 3 received extract 400 mg/Kg, Group 4 received Ranitidine 50 mg/Kg body weight, Group 5 (un-treated (received 5 ml of water).

Immediately after the last extract administration, animals were placed into restraint cages to avoid any movement. Cold-restraint-tress ulcer was induced by strapping the rats on a wooden plank and keeping them for 4 hours at 7°C. At the end of the time the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with normal saline to remove gastric contents and blood clots and examined by a 100x magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted and scored as follows normal stomach (0), Pinhole (1.0), Spot ulcer (1.5), Haemorrhagic streak (2.0), Small erosion(2.5), Large erosions (3.0), Perforation (3.5). Mean ulcer score for each animal was used to express ulcer index. The

percentage of ulcer protection was determined as follows:

$$\text{Protection Index (PI)} = \frac{Uc - Ut}{Uc} \times 100$$

u_t = ulcer index of treated group. U_c = ulcer index of control group.

Ethanol-induced ulcer model.

The rats were well fed and water given freely for three days, then fasted for 24 hours.
The method adopted was that used by Hollander *et al*. (1985), slightly modified.
Thirty *Ratus ratus* rats were randomly divided into five groups of six rats in group. Group 1 received extract 100mg/Kg body weight, Group 2 received extract 200mg/Kg body weight, Group 3 received extract 400mg/Kg, Group 4 received Ranitidine 50mg/Kg body weight, Group 5 Un-treated (received 5ml of water).

The animals were sacrificed one hour after alcohol administration by using anaesthetic ether and their stomachs dissected and examined for the determination of gastric lesions and scoring.

Macroscopic evaluation of stomach
The stomachs were opened along the greater curvature, rinsed with normal saline to remove gastric contents and blood clots and examined by a 10x magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted and scored and scored as follows: Normal stomach (0), Pinhole (1.0), Spot ulcer (1.5), Haemorrhagic streak (2.0), Small erosion (2.5), Large erosions (3.0), Perforation (3.5)

Mean ulcer score for each animal was used to express ulcer index. The percentage of ulcer protection was determined as follows:

$$\text{Protection Index (PI)} = \frac{Uc - Ut}{Uc} \times 100$$

u_t = ulcer index of treated group. U_c = ulcer index of control group.

Statistical analysis
The experimental data was analysed with SPSS 23 package by means of analysis of variance with one way ANOVA, followed by Turkey contrast analysis. Results were expressed as Mean ± SEM and $P \leq 0.05$ was considered significant. Missing data was handled by "Mean/Mode Substitution" approach, (Replace missing value with sample mean).

Results

Yield of extraction
The weight of the extract was 145g. This is equivalent to 13.8% of the weight of material extracted.

Acute toxicity test

This result showed that *Terminalia superba* stem bark methanol extract is non-toxic at doses below 2154mg / Kg body weight.

Macroscopy
The bark has a rough outer surface and a striated inner surface (Fig. 1a and 1b).





Fig 1a: Internal surface

Fig 1b: Outside surface

Qualitative Phytochemical Evaluation

The extract contained the major secondary metabolites-alkaloids, flavonoids tannins, saponins, terpenoids, steroids (Table 1).

Table 1: Qualitative Phytochemical Evaluation

Constituent	Results
Alkaloids	+
Glycosides	+
Tannins	+
Saponins	+
Flavonoids	+
Carbohydrates	+
Steroids	+
Terpenoids	+
Reducing sugars	+
Anthraquinone glycosides	+

(+) Indicates Presence; (-) Indicates Absence.

Quantitative phytochemical analysis

The extract had alkaloids 1046.23±16.56 mg atropine equivalent /g of extract, flavonoids 1191.74±29.25 mg rutin equivalent /g of extract, phenolics 1388.19±83.66 mg gallic acid equivalent /g of extract, saponins 885 mg diosgenin equivalent /g of extract, terpenoids 133.64 mg linalool equivalent /g of extract, steroids 257.55±13.98 mg cholesterol equivalent /g of extract, 0.323.68±25.37 mg tannic acid equivalent /g of extract.

Table 2: Results of the quantitative phytochemical analysis of methanol extract of *Terminalia superba* stem bark

Parameter	Conc. mg/ 100g
Alkaloids	100.05±16.56
Saponins	88.5.29±13.55
Tannins	32.37±25.37
HCN	Nil
Flavonoids	119.17±29.25
Phenolics	138.82±83.66
Terpenoids	13.37±19.14
Steroids	25.76±13.98

HCN = cyanogenic compounds

Evaluation of Gastroprotective Activity

Pre-treatment with the methanol extract of *Terminalia superba* stem bark reduced gastric ulcer in the three models. There was a statistically significant difference (at p = .05) between the treated groups and the un-treated group (Tables 4, 6, 8). The mean

unalcer score was significantly lower in groups pre-treated with the extract at than in the un-treated group,. in the three models used for three evaluation (Tables 3, 5, 7). There was a 77.3% reduction in the aspirin model, 75.2 % in the ethanol model and an 84 % in the Hypothermic restraint stress-induced ulcers model group (Table 3). In each model ranitidine demonstrated it role as standard drug with consistent higher activity (83%, 87.5,%, 84.06 %, in the aspirin, ethanol,and stress models respectively).

Table 3: Antiulcer Activity of Methanol Extract of *Terminalia superba* (% Protective Index)

Treatment	**Dose**	**Total ulcer score**	**Mean score (UI)**	**% Protective index**
Aspirin				
	100mg	27	4.5 ± 1.4 [a]	55
	200mg	20	3.3 ± 0.7 [a]	67
	400mg	14	2.3 ± 0.3 [a]	77
	Ranitidine	4	0.67 ± 0.33 [a]	93
	Water	60	10.1 ± 2.5	

Data in the same column with the same superscript are not significantly different

Table 4: Ulcer score (Aspirin Model)

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	302.667	4	75.667	7.276	.000
Within Groups	260.000	25	10.400		
Total	562.667	29			

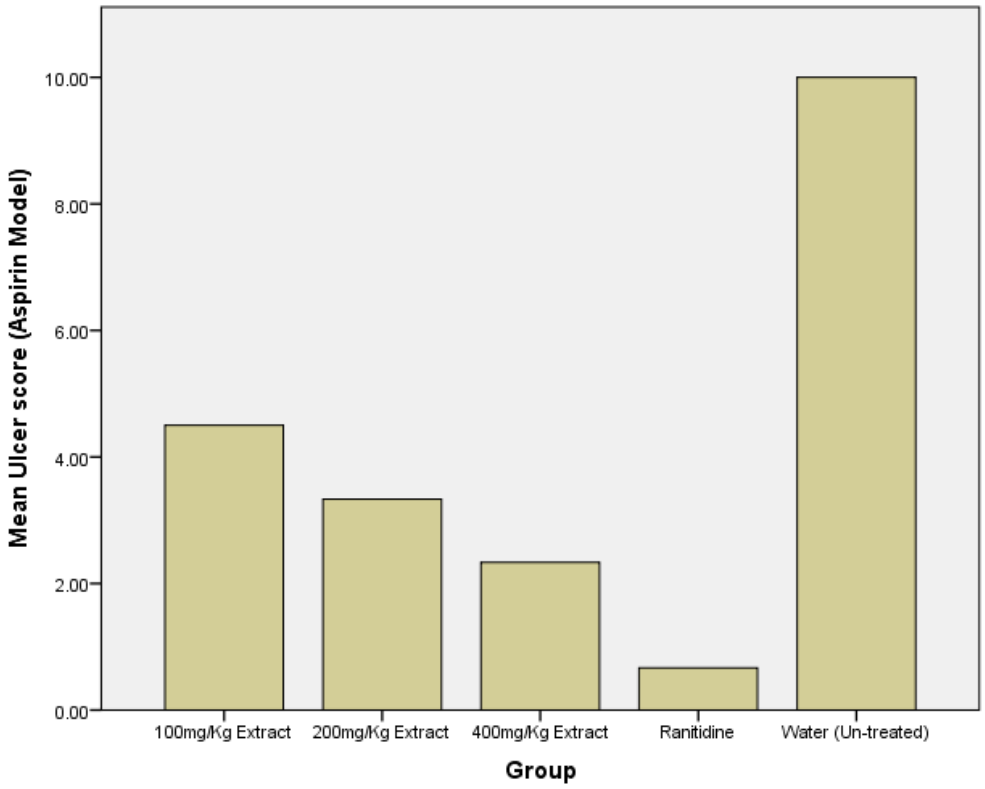

Fig 2: Histogram showing mean ulcer score in the aspirin model

Table 5: Antiulcer Activity of Methanol Extract of *Terminalia superba* (% Protective Index)

Treatment Ethanol	Dose	Total ulcer score	Mean score (UI)	% Protective index
	100mg of extract	100	20 ± 1.9	47.8
	200mg of extract	63.5	13.2 ± 3.0	66.8
	400mg of extract	47.5	9.5 ± 1.6 [a]	75.2
	Ranitidine (50mg/Kg b.wt.)	24	4.8 ± 0.96 [a]	87.5
	Distilled water (5ml)	191.5	38.3 ± 1.81	

Data in the same column with the same superscript are not significantly different

Table 6 : Ulcer score (Ethanol Model)

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3410.460	4	852.615	44.419	.000
Within Groups	383.900	20	19.195		
Total	3794.360	24			

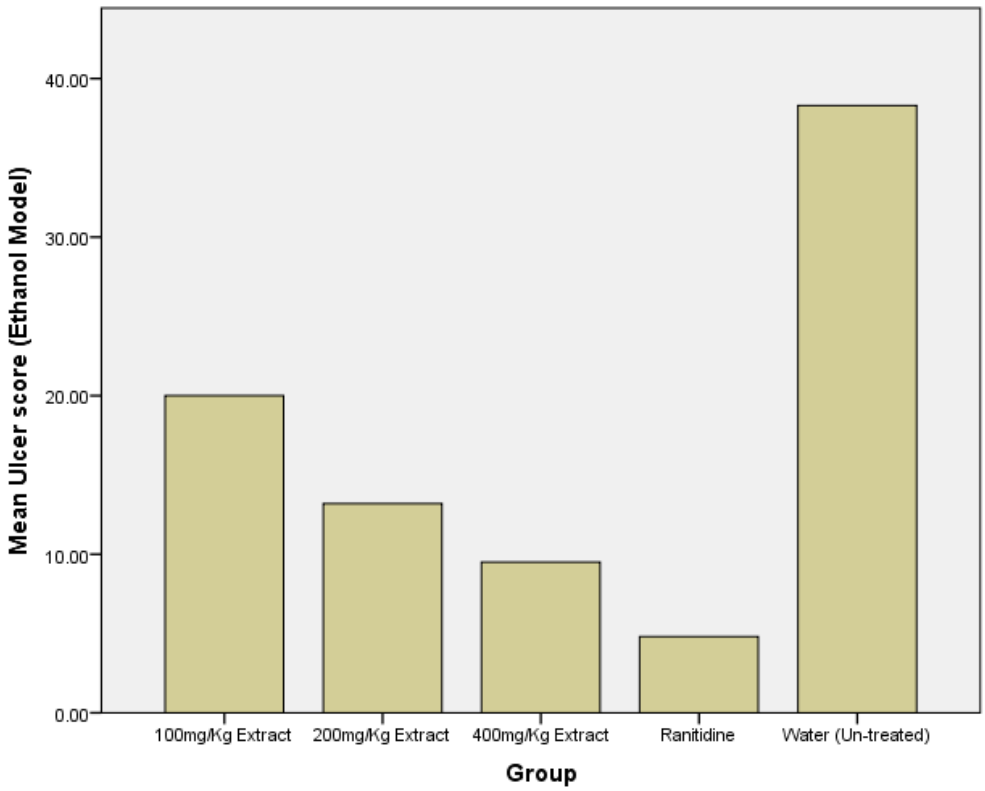

Fig 3: Histogram showing mean ulcer score in the ethanol model

Table7: Antiulcer Activity of Methanol Extract of *Terminalia superba* (% Protective Index)

Treatment Hypothermic restraint stress-induced ulcers model	**Dose**	**Total ulcer score**	**Mean score (UI)**	**% Protective index**
	100mg of extract	142	28.4 ± 5.13	12.88
	200mg of extract	76.5	15.3 ±6.56	53.07
	400mg of extract	25	5.0 ± 1.61[a]	84.66
	Ranitidine (50mg/Kg b.w.)	30.5	6.1 ± 1.72[a]	81.29
	Water (5ml)	163		

Data in the same column with the same superscript are not significantly different

Table 8: Ulcer score (Stress Model)

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3189.340	4	797.335	8.069	.000
Within Groups	1976.400	20	98.820		
Total	5165.740	24			

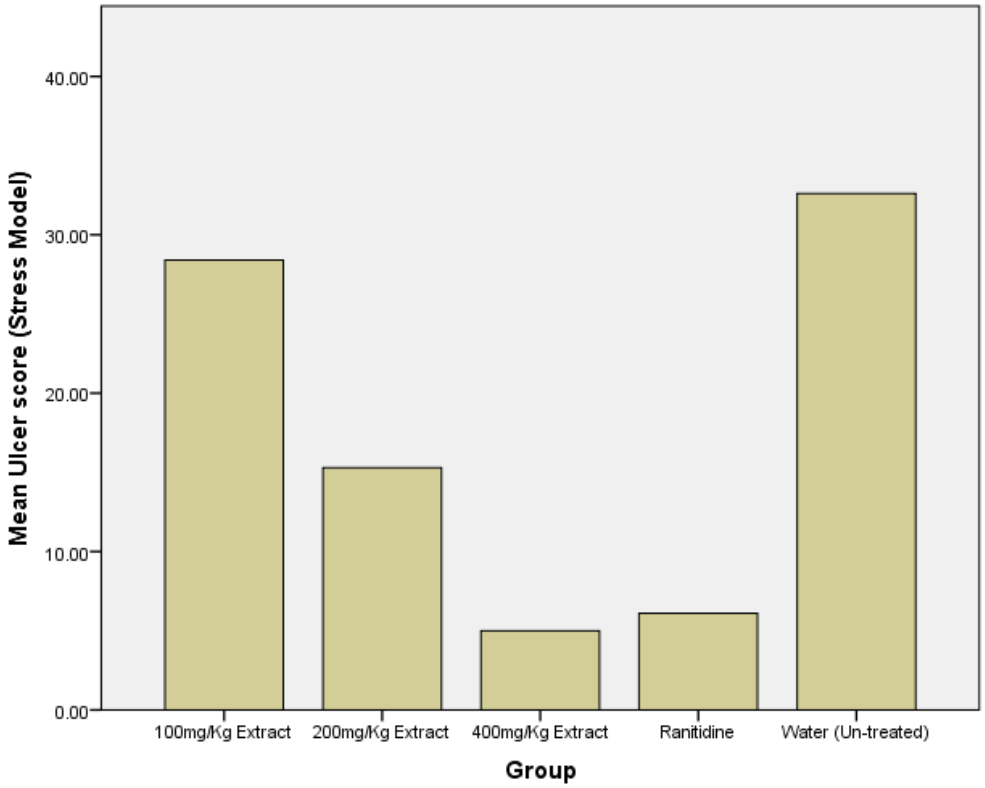

Fig 4: Histogram showing mean ulcer score in the stress model

DISCUSSION

The results of this study demonstrated that the methanol extract of *Terminalia superba* stem bark possess gastro-protective against aspirin-induced ulcer in mice. The groups pre-treated with the various doses of the bark extract showed a progressive decline in ulcer indices. The preventive index (percentage protection) conferred by the increased in a dose-dependent manner. Our results are in line with that of works by other researchers. The aqueous extract has been shown to have antiulcer effect (Kouakou, *et al* (2013). Kougnimon *et al.* (2017) obtained an LD_{50} figure of 2000 mg/kg body weight which is comparable to our result of 2150 mg/Kg.

The ranitidine group had the lowest ulcer score (4.8) and high percentage protective index (87.5), in other words ranitidine was very effective in protecting against aspirin-induced gastric ulcer. In the three models the extract demonstrated gastro-protective activity that is comparable to that of ranitidine. In the extract had ethanol 85. 94%, the activity of ranitidine, in the aspirin model it had 82.80% the activity of ranitidine, while in the stress model it had better activity than ranitidine, 104.45%. This indicates that the extract may possess antioxidant activity since oxidative stress has been implicated in a number of diseases including peptic ulcer. Also the high comparison to ranitidine in the aspirin model points to a possible antihistamine activity and implies that ulcer due to aspirin may involve elevation of histamine level in the stomach and the extract's activity involves acting as a histamine–receptor antagonist.

Looking at the quantitative phytochemical analysis it can be said that the gastroprotective property of *T. superba* may be due to its high phenolic and/or alkaloid content. Polyphenols like quercetin, kaempferol, and other flavonoids possess many pharmacological activities including anti-ulcer activities, and have demonstrated high level of protection against gastric ulceration. A suggested mechanism for this activity is stimulation of PGE2 formation (Hamedi *et al*., 2015).
A possible mechanism of action of the extract is by increasing mucus secretion in the stomach. The stomach of members of the group that received 400mg per kg body weight, of the extract, in the aspirin model was swollen and contained yellowish mucous substance. Mucus is the first line of defence of the mucous membrane, being secreted by epithelial cells and consisting of 95% water and 5% mucin, a polymerized glycoprotein that forms a gel (Laine *et al*., 2008). This group also experienced the highest protection against aspirin induced ulceration, they had the highest protective index.

Some of them have been scientifically investigated and proven to posse the claimed activities. Some investigations have even gone as far as identification an isolation of the compounds responsible for the activity. Examples include zingeron fron ginger (Karampour et al., 2019), rutaecarpine, and alkaloid fron Evodia rutaecarpa Benth (Wang et al., 2005), rohitukine from Armoora rohikut (Roxb.) Wight & Arn and *Dysoxylum binectariferum* (Roxb.) Hook (Harmon *et al*., 1979). Further phytochemical evaluation and pharmacological screening will revealing interesting and active compounds.

Conclusion: *Trminalia superba* Engl. & Diels (Combretaceae) stem bark methanol extract is rich in the major secondary metabolite classes-phenolics, flavonoids and alkaloids-, and has gastro-protective activity as it showed low ulcer index in the treated groups in comparison to the un-treated group.

Acknowledgments
We thank the laboratory staff of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Nigeria

Conflict of interest
None to declare

References

Alqasoumi S, Al-Sohaibani M, Al-Howiriny T, Al-Yahya M, Rafatullah S. (2009). Rocket "*Eruca sativa*": a salad herb with potential gastric anti-ulcer activity. *World journal of gastroenterology*, *15*(16), 1958–1965. https://doi.org/10.3748/wjg.15.1958

Alrashdi AS, Salama SM, Alkiyumi SS, Abdulla MA, Hadi AH, Abdelwahab SI, *et*

al (2012). Mechanisms of Gastroprotective Effects of Ethanolic Leaf Extract of *Jasminum sambac* against HCl/Ethanol-Induced Gastric Mucosal Injury in Rats. *Evidence-based complementary and alternative medicine : eCAM*, 786426 https://doi.org/10.1155/2012/786426

Bhargava KP, Gupta MB, Tangri KK. (1973). Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. *European journal of pharmacology*, 22(2): 191–195. https://doi.org/10.1016/0014-2999(73)90012-5

Burkill HM. (1985). *The useful plants of West Tropical Africa*. 2nd Edition. Volume 1, Families A–D. Richmond, United Kingdom: Royal Botanic Gardens, Kew; pp960

Calam J, Baron JH. (2001). ABC of the upper gastrointestinal tract: Pathophysiology of duodenal and gastric ulcer and gastric cancer. *BMJ (Clinical research ed.)*, 323(7319): 980–982. https://doi.org/10.1136/bmj.323.7319.980

Dan Greer (1957). Dyspepsia, Peptic Ulcer Disease and Gastro-Oesophageal Reflux Disease. In: *Clinical Pharmacy and Therapeutics*. Whittlesea C, and Hodson K (Eds.), 6th edition. London, UK: Elsevier, 2019:174-180

Devi RS, Kist M, Vani G, Devi CS. (2008) Effect of methanolic extract of *Terminalia arjuna* against Helicobacter pylori 26695 lipopolysaccharide-induced gastric ulcer in rats. *The Journal of pharmacy and pharmacology*; 60(4):505–514. https://doi.org/10.1211/jpp.60.4.0014

Dewick PM. (2009). *Medicinal Natural Products: A Biosynthetic Approach*, 3rd Edition. John Wiley & Sons, Ltd, UK.

El-Olemyl MM, Al-Muhtadi FJ Afifi AA. (1994) *Experiomental Phytochemistry. A laboratory Manual* College of Pharmacy King Saudi University Press. Pp. 1 – 134

Evans WC. *Trease and Evans Pharmacognosy*. 16th Edition, Saunders Elsivier, London. (2009): 171-427

Evans WC. *Trease and Evans Pharmacognosy*, 15TH Edition, Elsevier India, (2002), 516 - 536.

Gasparetto M, Pescarin M, Guariso, G. (2012). Helicobacter pylori Eradication Therapy: Current Availabilities. *ISRN gastroenterology*. https://doi.org/10.5402/2012/186734

Goel RK, Das DG, Sanyal AK. (1985). Effect of vegetable banana powder on changes induced by ulcerogenic agents in dissolved mucosubstances of gastric juice. *Indian journal of gastroenterology*. ; 4(4):249–251 PMID: 3850848

Gurbuz I, Yesilada E. (2007). Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from *Centaurea solstitialis* L. ssp. solstitialis by using various *in vivo* and biochemical techniques. *J Ethnopharmacol*. 112:284–91. https://doi.10.1016/j.jep.2007.03.009.

Hamedi S, Arian AA, Farzaei MH. (2005). Gastroprotective effect of aqueous stem bark extract of ziziphus jujuba l. Against hcl/ethanol-induced gastric mucosal injury in rats. *J. Tradit. Chin. Med.*; 35:666–670.https://doi.org/10.1016/S0254-6272(15)30157-6

Harborne JB. (1998). *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis*. 3rd Ed. London, UK. Springer.

Harmon AD, Weiss U, Silverton JV (1979). The structure of rohitukine, the main alkaloid
of *Amoora rohituka* (Syn. *Aphanamixis po*

lystachya) (meliaceae). Tetrahedron Letters; 20(8):721-724. https://doi.org/10.1016/S00404039(01)93556-7

Hollander D, Tarnawski A, Krause WJ, Gergely H. (1985). Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. Macroscopic, histologic, ultrastructural, and functional time sequence analysis. *Gastroenterology*, 88: 366–374. https://doi.org/10.1016/s00165085(85)80191-8

https://plants.jstor.org/stable/10.5555/al.ap.upwta.1_813 Accessed on 24/02/2021

Jawanjal H, Rajput MS, Agrawal P, Dange V. (2012). Pharmacological evaluation of fruits of *Terminalia belerica* Roxb. for antiulcer activity. *Journal of complementary & integrative medicine* ;9, https://doi.org/10.1515/1553-3840.1556.

Johnson HD. (1957). The Classification and Principles of Treatment of Gastric Ulcers. *The Lancet* ; 273(6994) :518-520. https://doi.org/10.1016/S0140-6736(57)90825-5

Kagoshima M, Uguro NS. (1982). Gastric movements and reserpine- induced ulcersin rats. *Nippon Yakurigaku Zasshi*, 80: 231–238

Karampour NS, Arzi A, Rezaie A, Pashmforoosh M, Kordi, F. (20119). Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. *Medicina (Kaunas, Lithuania)*; 55(3): 64. https://doi.org/10.3390/medicina55030064

Khan MS, Nazan S, Matjais AM. (2017). Flavonoids and anti-oxidant activity mediated gastro-protective action of leathery murdah, *Terminalia coriacea* (Roxb.) Wight & Arn. Leaf methanolic extract in rats. *Arq. Gastroenterol*. 54(3); 183-191 https://doi.org/10.1590/s00042803.2017000000-21

Kouakou KL, Goze NB, Bleyere NM, Andre KB, Augustin K, Kouakou A, *et al* (2013). Acute toxicity and anti-ulcerogenic activity of an aqueous extract from the stem bark of *Terminalia superba* Engl. and Diels *(Combretaceae). World Journal of Pharmaceutical Sciences*; 1(4), 117-129.

Kougnimon E, Ahouangninou C, Akpovi CD. (2107). Evaluation of the toxicological effect of stem bark extracts of *Terminalia superba* Engler & Diels (Combretaceae). *International Journal of Biosciences*;11(1):182-189. https://dx.doi.org/10.12692/ijb/11.1.182-189

Laine L, Takeuchi K, Tarnawski A. (2008). Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*, 135:41–60, https://doi.org/10.1053/j.gastro.2008.05.030

Laine L, Takeuchi K, Tarnawski A. (2008). Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*; 135(1):41–60. https://doi.org/10.1053/j.gastro.2008.05.00

Levine RJ. (1971). A method for rapid production of stress ulcers in rats. In: Pfeiffer CJ (Ed.), Peptic Ulcer. Munksgaard, Copenhagen,:92–97.

Lorke D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology* 54(4): 275–287. https://doi.org/10.1007/BF01234480.

Maifertheine P, Chan FKL, Kenneth EL, McCOLL KEL. *The lancet*. 2009;374(9699):1449-1461. https://doi.org/10.1016/S01406736(09)60938-7.

Megraud F, Lehours P. (2007). *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clinical Microbiology Reviews*; 20(2):280–322. https://doi.org/10.1128/CMR.00033-06.

Naito Y, Yoshikawa T, Yoneta T, Yagi N, Matsuyama K, Arai M, Tanigawa T, Kondo M. (1995). A new gastric ulcer model in rats produced by ferrous iron and ascorbic acid injection. *Digestion* 56(6); 472–478. https://doi.org/10.1159/000201278

Nunes PH, Martins MC, Oliveira RC, Chaves MH, Sousa EA, Leite JR, et al (2014). Gastric Antiulcerogenic and Hypokinetic Activities of Terminalia fagifolia Mart. & Zucc. (Combretaceae). BioMed Research International, vol. 2014, Article ID 261745. https://doi.org/10.1155/2014/261745

Oates PJ, Hakkinen JP. (1988). Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology*; 94(1):10–21.https://doi.org/10.1016/0016-5085(88)90604-X.

Oka S, Ogino K, Hobara T, Yoshimura S, Yanai H, Okazaki Y, *et al* (1990). Role of active oxygen species in diethyldithiocarbamate-induced gastric ulcer in the rat. *Experientia*, 46(3) :281–283. https://doi.org/10.1007/BF01951766

Okabe S, Amagase K. (2005). An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. *Biological & pharmaceutical bulletin* 28(8):1321–1341. https://doi.org/10.1248/bpb.28.1321

Orewa C, Mutua A, Kindt R , Jamnadass R, Simons A. 2009. *Agroforestree Database: A tree reference and selection guide*

Parsons ME. (1985). Histamine and the pathogenesis of duodenal ulcer disease. *Gut*;26(11):1159–1164. https://doi.org/10.1136/gut.26.11.1159

Shah DI, Santani DD, Goswami SS. (2006). A novel use of methylene blue as a pharmacological tool. *Journal of Pharmacological and Toxicological Methods* 54(3):273–277. https://doi.10.1016/j.vascn.2005.12.003

Sharma P, Prakash T, Kotresha D, Ansari MA, Sahrm UR, Kumar B, *et al* (2011). Antiulcerogenic activity of Terminalia chebula fruit in experimentally induced ulcer in rats. *Pharmaceutical biology*, 49(3) : 262–268. https://doi.org/10.3109/13880209.2010.503709

Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. (1945). A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology*; *5*:43–61

Suleiman MM, Isife OR, Sadiq Y. (2007). Gastroprotective effect of the crude methanol extract of *Terminalia avicennioides* in rats. *Veterinarski Arhiv*; 77(4); 45–354. https://hrcak.srce.hr/24777.

Szabo S. (1978). Animal model of human disease. Duodenal ulcer disease: cysteamine-induced acute and chronic duodenal ulcer in the rat. *American Journal of Pathology*; 93(1): 273–276. PMC2018340.

Takeuchi T, Furukawa O, Tanaka H, Okabe S. (1986). A new model of duodenal ulcers induced in rats by indomethacin plus histamine. *Gastroenterology*. *90*(3), 636–645.

Vogt T (2010). Phenylpropanoid Biosynthesis. *Molecular Plant*, 310: 2-20. https://doi.org/10.1093/mp/ssp106.

Wada K, Kamisaki Y, Kitano M, Kishimoto Y, Nakamoto N, Itoh TB. (1996). A new gastric ulcer model induced by ischemia reperfusion in the rat: role of leukocytes on ulceration in rat stomach. *Life Sciences* 59(19):295–301. https://doi.org/10.1016/00243205(96)00500-0

Wang L, Hu CP, Deng PY, Shen SS, Zhu HQ, Ding JS, *et al* (2005). The protective effects of rutaecarpine on gastric mucosa injury in rats. *Planta medica*, ;71(5):416–419.https://doi.org/10.1055/s-2005-864135