



Stem bark methanolic extract of *Bauhinia thonningii* (Schum) demonstrates gastroprotective properties against *in vivo* ethanol-induced injury

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ABSTRACT

Background: Stomach ulcer is one of the diseases that many patients suffer from all over the world, and it causes damage to the intestinal wall. Some research has shown that *Bauhinia thonningii* extract, which has been traditionally used to treat a variety of ailments, including stomach ulcers, has powerful antioxidant and mucus-increasing properties.

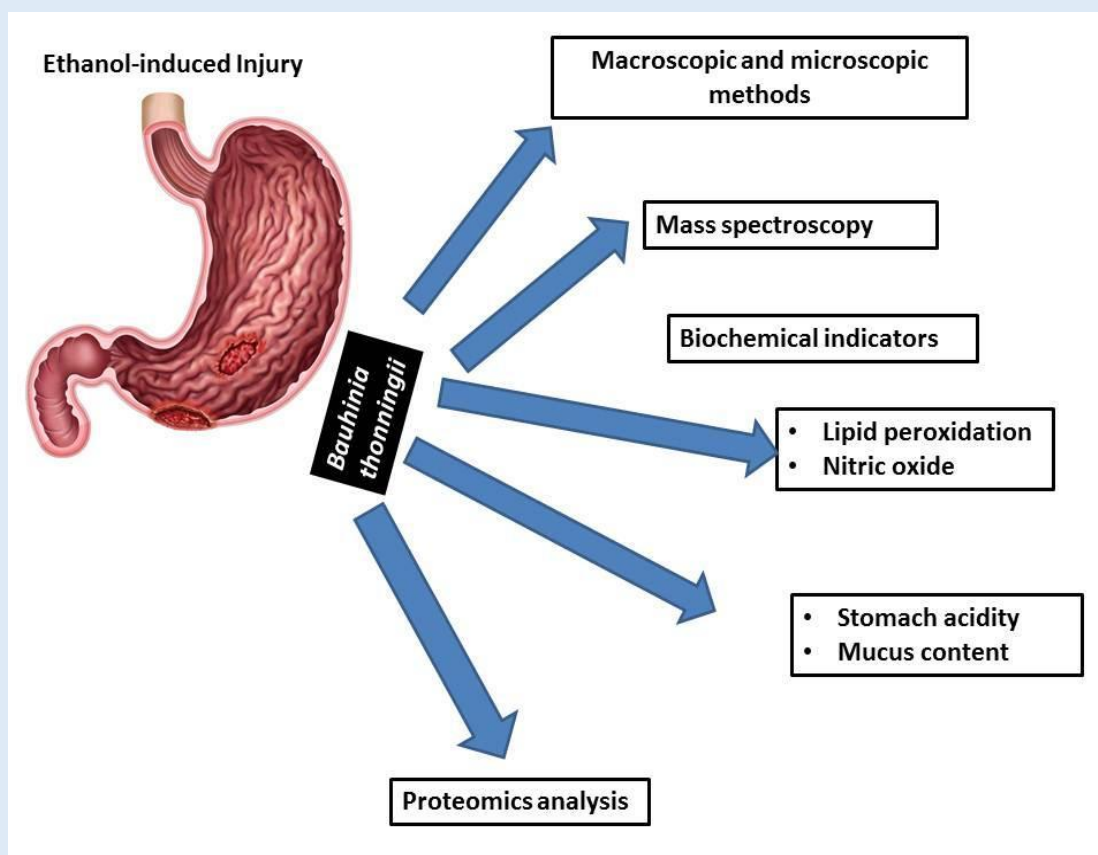
Objective: This research is aimed at studying the mechanism(s) through which methanolic (MBST) and chloroform (CBST) extracts of *B. thonningii* stem bark protects against ethanol-induced stomach damage in rats.

Material and Methods: MBST and CBST were evaluated *in vivo* for their cytoprotective qualities employing stomach acidity, mucus content, macroscopic and microscopic methods, lipid peroxidation, nitric oxide, mass spectroscopy, 2D gel electrophoresis, and biochemical indicators. These extracts were tested *in vitro* on lipopolysaccharide/interferon-stimulated rat cells. Additionally, *in-vivo*, and *in-vitro* toxicity investigations were conducted. FRAP, DPPH, and ORAC tests were used to determine the antioxidant activity of MBST and CBST. Chemical and spectroscopic approaches were used to analyze the phytochemistry of MBST and CBST.

Results: The gross and histological characteristics of *B. thonningii* proved its anti-ulcerogenic effects. MBST has gastroprotective effects by modulating lipid peroxidation and proteomics indicators [heat shock protein (Hsp60), disulfide isomerase (ERp60), protein disulfide-isomerase, creatine kinase B, cytosolic malate dehydrogenase, peroxiredoxin-6, and ATP synthase]. MBST and CBST have substantial impacts on nitric oxide both in-vitro and in-vivo. In comparison to CBST, methanolic extract (MBST) demonstrated greater gastroprotective properties, polyphenolic content, and antioxidant activity. The plant extracts did not show any toxicity in-vitro or in-vivo. MBST shows anti-ulcer action, which may be ascribed to its ability to reduce ethanol-triggered oxidative injury, to intervene in proteomic mechanisms, and to modulate nitric oxide.

Conclusion: The present study found that stem bark extracts of *Bauhinia thonningii* Schum have cytoprotective activities against in vivo ethanol-induced damage due to its powerful antioxidant and mucus-increasing capabilities. *Bauhinia thonningii* warrants more attention since it may provide an exciting novel medication for the treatment of acute erosive gastropathy.

KEYWORDS: *Bauhinia thonningii* Schum; polar and non-polar extraction; peptic ulcer; nitric oxide; proteomics



INTRODUCTION

Peptic ulcer distresses a considerable proportion of the global statistics of human diseases. Ulcers can be a serious ailment because it causes damage to the gastrointestinal tract (GIT) wall. Pharmacotherapy of gastric ulcer is widespread and acid-suppressing agents (ranitidine and cimetidine) are the most used antiulcer drugs. Various unwanted side effects were observed for antacids, anticholinergics, Hydrogen-receptor antagonists, and liquorice derivatives. Treatment with these pharmacological agents is sometimes ineffective and does not eliminate the principal causative agents producing GIT ulcers. Experimental single dose studies of anti-acid drugs have demonstrated changed disposition of many drugs when used with antacids. The development of selective antiulcer should provide antisecretory agents with minimal side effects [1,2].

Interdisciplinary efforts are required to investigate the perception and use of pharmaceuticals in developing countries. Emphasis has long been on medicinal plants. *Bauhinia thonningii* Schum. (Cesalpiniaceae) is traditionally well-known as *Tambarib* in central Sudan [3-5]. The use of *B. thonningii* as a treatment for stomach ulcers, fever, dysentery, blennorrhagia, malaria, leprosy, sore throat, hemoglobinuria, and painful teeth is common [3]. Despite these extensive ethno-pharmacological usages, there have been minimal biological and phytochemical investigations. In this case the plant's antiviral, antifungal, and wound-healing abilities have been evaluated. Studies on *B. thonningii*'s phytochemistry found that the stem bark of the plant contains alkaloids, flavonoids, anthocyanins, betacyanins, anthraquinones, saponins, and steroids [6-8]. Therefore, the purpose of the present study was to

investigate the gastroprotective mechanism(s) of *B. thonningii* extracts in relation to ethanol-induced gastric ulcer using Male Sprague Dawley rats. Comparative research was also undertaken on the biological and antioxidant properties of the chloroform (CBST) and methanolic (MBST) extracts of this plant.

MATERIALS AND METHODS

Method of collection and extraction of phytochemicals:

B. thonningii (Cesalpiniaceae) was obtained in May 2015 at Ennohoud, Sudan's Wadelmak Botanical Garden. The bark was sloughed off by one of the researchers so that the stem tissue does not peel off from the tree. A Senior Botanist at Sudan's National Research Centre's Herbarium recognized the voucher specimen (BT-E-2015-654). *B. thonningii* stem bark was washed, shade-dried for one week, and ground into powder. The powdered material was extracted progressively with chloroform and methanol for three days in each solution. Chloroform and methanol were extracted using a rotary evaporator, and the yielded extracts were stored at 4°C until required. The yields of methanolic (MBST) and chloroform (CBST) extracts were 26.4 % and 5%, respectively.

Induction of ulcer: Male Sprague Dawley rats (160-200 g) were fasted for 24 hours, followed by two hours of dehydration. Individual rats were kept in cages and were divided into seven groups of six rats each. Plant extracts were supplied at dosages of 250 and 500 mg/kg for methanolic and chloroform extracts respectively (Groups III and IV) (Group V and VI, respectively). Group VII was the control group used as the benchmark. After this pretreatment, rats in Groups I-VI were gavaged with 5 ml/kg of 95% ethanol one hour later following Mahmood

et al. [9]. The study was approved by the Ethical Committee of the University of Malaya, Kuala Lumpur, Malaysia.

Determination of Acidity and Mucus weights: The opening was made in the greater curved portion of each stomach. A digital pH meter was used in conjunction with a 0.1N solution of sodium hydroxide in order to conduct a titration that measured the quantity of hydrogen ions in the stomach content. The amount of acid was measured in milliequivalents per liter [10]. The mucosa lining of the gastrointestinal tract of each rat was carefully scraped with a glass slide, and the mucus that was produced was measured using an accurate digital scale (U S Solid, USA) [10].

Macroscopic assessment of ulcer: Using a dissecting microscope, the length (mm) and breadth (mm) of the stomach ulcer region (10x10 mm²) were measured (1.8x). Each ulcer lesion's area was determined by counting 2mm by 2mm squares throughout its length and breadth. The ulcer area (UA) was estimated by summing the areas of all lesions for each stomach. The inhibition percentage (I) was calculated using the method below [12], with some adjustments

The inhibition percentages (I%) = $\frac{[(UA_{\text{control}} - UA_{\text{treated}}) \div UA_{\text{control}}] \times 100\%}{}$

Lipid peroxidation assay: Malondialdehyde (MDA), a biomarker of lipid peroxidation, was evaluated using a thiobarbituric acid reactive substances test (TBARS) [11]. In brief, 10% (w/v) of stomach homogenate was centrifuged at 4°C for 10 minutes in 0.1 mol/L PBS. Two milliliters of supernatant were cooked in a water bath at 95°C for 30 minutes with a solution containing 0.66% 2-thiobarbituric acid and 20% trichloroacetic acid. After

centrifuging the mixture, the supernatants were analyzed spectrophotometrically at 532 nm for MDA concentrations in nmol/mg.

Griess assay: Griess reagent was used to assess total nitrate/nitrite levels in the stomach mucosa. In a 50mM potassium phosphate buffer, stomach homogenates were centrifuged at 4000rpm for 30 minutes at 4°C (pH 7.8). About 50 microliters of Griess reagent (0.1% N/(1 - naphthyl) ethylenediamide dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) was added to 50L supernatant and agitated for 10 minutes. Standard curves utilized sodium nitrite. Calculations utilized micromoles of nitrate/nitrite per gram of protein.

Proteomics analysis: This examination was done to analyze the mechanism(s) of MBST on ethanol-induced gastric ulceration. Two-dimensional gel electrophoresis (2-D) was done as previously described [12], with the exception of the utilization of precasted immobilized dry strips (GE-Healthcare Biosciences, Sweden). Using the Multiphor Flatbed electrophoresis equipment (G GE-Healthcare Biosciences, Sweden), proteins isolated from gastric homogenized tissues (30 g) were exposed to isoelectric solution. The other dimension was electrophoresis of the samples in the strips using an 8 - 18% gradient polyacrylamide gel using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS). Silver staining or western blotting was used on the gels. All of the samples were tested twice. The silver staining of the 2-DE gels was done in accordance to Heukeshoven and Dernick's procedure [13]. A modified silver staining methodology based on the previously published method was used for mass spectrometry [14]. Protein bands were manually removed from silver-marked gels and maintained hydrated in clean 1.5ml tubes containing distilled water prior to gel incorporation. After that, the gel plugs were de-stained with 15 mM K₃[Fe(CN)₆] in 50

mM ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) until they were clear. Utilizing 10 mM (DTT) in 100 mM (NH_4HCO_3) and 55 mM (iodoacetamide) in 100 mM NH_4HCO_3 , they were further reduced and alkylated. The gel plugs were thoroughly washed with 50% ACN in 100 mM NH_4HCO_3 and 100% ACN before being dehydrated by vacuum centrifugation. The dried plugs were then incubated at 37°C for an overnight period in 25 l of a solution containing 6 ng/l trypsin and 50 mM NH_4HCO_3 . After it had been extracted with 50% and 100% ACN, the peptides were then dried in a vacuum centrifuge for mass-spectrometry analysis.

MALDI TOF/TOF Mass spectrometry: After being reconstituted with 0.1% formic acid and desalted using ZipTip reversed-phase media containing C18, the peptides that had been dried out were used (Millipore, Massachusetts, USA). On the Opti-ToF 384 well insert (Applied Biosystems/MDS Sciex, Canada), 0.7 L of the sample peptide and cyano-4-hydroxycinnamic acid (10 mg/ml) combination was promptly detected. The samples were analyzed using an Applied Biosystems/MDS Sciex 4800 Plus MALDI TOF/TOF analyzer. The mass standard kit (Applied-Biosystems/MDS Sciex, Canada) was used as a calibrator for the MS and MS/MS mass spectra scales throughout the analysis.

Protein identification: To find protein spots against a database, MASCOT (Matrix Science Ltd, London, UK; release version 2.1) was employed. The database search criteria were as follows: Trypsin was used; variable modifications like carbamidomethylation of cysteine and oxidation of methionine were allowed; up to one missed cleavage was allowed; the mass tolerances for the MS precursor ion and MS/MS fragment ion were set to 50 ppm and 0.1 Da, respectively; and the search only included monoisotopic masses. In Hercules, California, the Bio-Rad Laboratories Imaging Densitometer GS690

was used to scan 2-DE gels that had been dyed silver. The Image Master Platinum 7.0 application was used to calculate the volume of protein spots (GE Healthcare Biosciences, Uppsala, Sweden). A protein's spot volume is expressed as a percentage of the combined spot volumes of all detected proteins under the phrase "volume contribution by percentage". The Student T-test was used to assess the significance of differences between animal groups. A p value of less than 0.01 was considered significant ($P < 0.01$). Proteins from treated and controlled stomach tissues were analyzed using MS, and significant alterations were found.

Cell-based nitric oxide assay: In DMEM with 10% FBS, L-glutamine (2 mM), sodium pyruvate (1 mM), 4.5 g/L glucose, streptomycin (50 g/ml), and penicillin (50 U/ml) as well as, the murine monocytic macrophages cell line (RAW 264.7) was cultured. Confluent cells were centrifuged at 120 x g for 10 min at 4 °C and the cell concentration was adjusted to 2×10^6 cells/ml. Cell viability was always greater than 90%, as demonstrated by trypan blue exclusion. A 96-well plate containing 50 L of cell suspension (4×10^5 cells/well) was cultured at 37°C with 5% CO_2 to promote cell adhesion. The cells were subsequently stimulated with 5 g/ml LPS and 100 L/well with 100 U/ml IFN- in the presence or absence of plant extract. The plant sample was introduced into the culture medium using DMSO at a final concentration of 0.1% for 17–20 h at 37°C followed by 5% CO_2 . Griess nitrite analysis was performed on the supernatant [7].

Nitrite determination: Nitric oxide (NO) generation in culture media was tested to see if *B. thoningii* had any inhibitory effects using the Griess reaction. Color development was assessed at 550 nm after adding an equivalent volume of Griess reagent to the culture supernatant (SpectraMax Plus, Molecular Devices Inc., Sunnyvale, CA, USA). The concentration of nitrite in the

culture supernatant was determined using a standard curve (0-100 M) of sodium nitrite freshly produced in deionized water. The percentage of NO inhibition was calculated using the nitrate concentration of the IFN/LPS-induced group as a reference.

$$\text{NO inhibitory (\%)} = \frac{[\text{NO}_2^-]_{\text{control}} - [\text{NO}_2^-]_{\text{sample}}}{[\text{NO}_2^-]_{\text{control}}} \times 100$$

Murine macrophage *in-vitro* toxicity and *in-vivo* toxicity:

The cytotoxicity of *B. thoningii* on cultured cells was evaluated by converting MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagents to formazan salts [8]. After the supernatant was eliminated, the MTT reagents (0.05 mg/ml diluted in sterile PBS, pH7.0) were added to each well. After 4 hours of growth at 37 °C with the remaining cells, the formazan salts were dissolved in each well by adding 100 µL of 100% dimethyl sulfoxide (DMSO). The absorbance was calculated using a SpectraMax Plus microplate reader at 570 nm (Molecular Devices, USA). The percentage of cell viability was calculated using the IFN-gamma/LPS-induced group's cell viability as a control.

$$\text{Cell Viability (\%)} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100 \%$$

Male and female Sprague Dawley rats (6-8 weeks old; 150–180 g) were used for acute toxicity. Animals were observed for 14 days after receiving dosages of 1 and 2 g/kg b.w. of plant extract. The animals were starved the night before dosing. Following treatment, meals were avoided for a further three to four hours. After that, the animals were observed for 0.5, 2, 4, 8, 24, and 48 hours to see whether any clinical or toxicological symptoms appeared. Any mortality, if any, was tracked during a two-week period. The animals were offered up on day fifteen. The biochemical parameters were determined using conventional methods.

Antioxidant activities of *B. thoningii* on, ORAC antioxidant activity, and FRAP assays: The scavenging activity of *B. thoningii* on DPPH (diphenyl picrylhydrazine) was determined using the method described by Choi [15]. The conversion of purple DPPH to yellow diphenyl picrylhydrazine is the basis for this approach. ORAC antioxidant activity assay was conducted based on the procedure described previously [16]. The total antioxidant activity (FRAP assay) of *B. thoningii* is determined using a modified Benzie and Strain [6] technique.

PHYTOCHEMICAL STUDY

Total phenolic content (TPC): The Folin-Ciocalteu method [17] is used to determine the TPC of the extracts. The TPC was calculated as gallic acid equivalent (GAE) in mg/g extract using the gallic acid standard curve.

Total flavonoid content: The AlCl₃ method was used to quantify total flavonoid content (TFC), with quercetin as a standard [18]. The quercetin equivalent (QE) of the total flavonoid content was calculated.

Qualitative phytochemical screening: Phytochemical tests were carried out on plant extracts using the protocols that were considered to be standard [19]. Investigations with nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy were carried out to verify the presence of functional chemical groups in the extracts.

Statistical analysis: The provided values were all mean±S.E.M. One-way ANOVA was used to evaluate the statistical significance of differences between the various groups. P less than 0.05 was regarded as a significant value.

RESULTS

***In-vitro* and *in-vivo* toxicity studies:** During the experiment, no clinical or behavioral symptoms were

noticed. The animals' body weights were not considerably dissimilar in any of the treated groups. After 14 days of testing, plant extracts demonstrated no death in the doses examined. There were no substantial changes between the groups when it came to histomorphological evaluation of the kidney and liver, hematology, and biochemical parameters (Data not shown and available upon request). The mitochondrial reduction of MTT was used to determine the viability of murine macrophages 17–20 hours after treatment with plant extracts at a concentration of 50 mg/mL. The results showed that macrophage viability was always more than 88%.

GASTROPROTECTIVE EXPERIMENT

Gastric mucus and pH of the gastric juice: The delivery of ethanol resulted in a decrease in mucus formation, as demonstrated in Table 1. Mucus content has augmented because of the pre-administration of MBST and CBST. In comparison to the ulcer control group, plant extracts considerably ($P<0.05$) and dose-dependently increase stomach mucus content. The pH of the gastrointestinal juice was considerably elevated ($P<0.05$) in animals pre-treated with MBST, CSBT, and omeprazole with the values of, 6.82 ± 0.3 , 5.2 ± 0.5 , and 5.60 ± 0.5 , respectively. When compared to CBST, MBST exhibited considerable gastroprotective effect ($P<0.05$) in both parameters as shown in Table 1.

Table 1: Gastroprotective activities of MBST and CBST ethanol-induced gastric injury

Animal groups **	Pretreatment (5ml/kg)*	pH of Gastric tissue	Mucus weight (g)	Ulcer Area (mm)(mean \pm S.E.M)	% Inhibition	Malondialdehyde (μ mol/g Tissue)	Nitric oxide (μ mol)	ALT(IU/L)	AST (IU/L)
I	Tween20 (ulcer control)	2.95 ± 0.4^a	0.112 ± 0.01^a	850 ± 14.43^a	-	30 ± 3.10^a	5.1 ± 0.60^a	232 ± 7.55^a	251 ± 2.7^a
II	omeprazole(20mg/kg)	5.60 ± 0.5^b	0.46 ± 0.02^b	178 ± 9.6^b	79	15.5 ± 1.2^b	9.2 ± 0.30^b	43.2 ± 0.9^b	63.1 ± 1.6^b
III	MSBT (250mg/kg)	6.51 ± 0.4^b	0.2 ± 0.026	68.2 ± 9.27^c	92	$11\pm 0.1.4^c$	10.5 ± 0.8^b	28 ± 5.2^c	20 ± 1.8^c
IV	MSBT (500mg/kg)	6.82 ± 0.3^b	0.39 ± 0.03^b	0.6 ± 2.92^d	100	9.9 ± 0.5^d	10.2 ± 1.2^b	19.5 ± 2.7^d	25 ± 1.5^d
V	CSBT (250mg/kg)	4.5 ± 0.2^b	0.28 ± 0.03^c	212.52 ± 20^f	24.9	18.2 ± 2.5^b	6.2 ± 0.94^b	57.5 ± 8.3^c	20.5 ± 2.1^c
VI	CSBT (500mg/kg)	5.2 ± 0.5^b	0.15 ± 0.02^b	521.1 ± 81.2^e	49.24	12 ± 0.14^c	8.2 ± 0.18^b	46.4 ± 6.1^c	39 ± 2.9^d
VII	Normal	7.05 ± 0.6^c	0.58 ± 0.03^b	-	-	9 ± 0.98^d	10.34 ± 0.12^b	19 ± 3.37^d	15 ± 4.02^c

*MEBT: methanolic extract of *B. thonningii*; CEBT: chloroform extract of *B. thonningii*; ALT: aspartate aminotransferase; AST: alanine aminotransferase. **Groups with different alphabets are statistically significant.

Gross and microscopic lesions: The ulcer index and microscopic inspection were used to assess morphologically the gastroprotective properties of MBST and CBST. In comparison to rats pre-treated with only vehicle (Figures 1F and 1G), MBST (Figures 1D & 1E), and omeprazole (Figures 1B), macroscopic evaluation of stomachs obtained from rats pre-administered with CBST (Figures 1F and 1G), MBST (Figures 1D & 1E), and

omeprazole (Figures 1B) revealed clearly decreased areas of gastric ulceration (ulcer control group, Figure 1C). The stomach mucosa of rats pre-treated with CBST and MBST flattened as well. When compared to CBST, MBST provided better protection. The ulceration indices decreased considerably ($P<0.05$) as seen in Table 1. MBST at doses of 500 and 250 mg/kg b.w. has been shown to lower the incidence of ulcer by 100% and 96%,

respectively. Oedema and leucocyte infiltration of the sub-mucosal region of the stomach wall were histological abnormalities generated by ethanol, as illustrated in Figure 2. (Figure 2C). Pre-administration of CBST (Figures 1F and 1G), MBST (Figures 1D & 1E), or omeprazole

(Figures 2B) avoided the formation of these lesions, as evidenced by decreased or non-appearance of sub-mucosal oedema and leucocyte infiltration. Both MBST and CBST were found to have a dose-dependent impact.

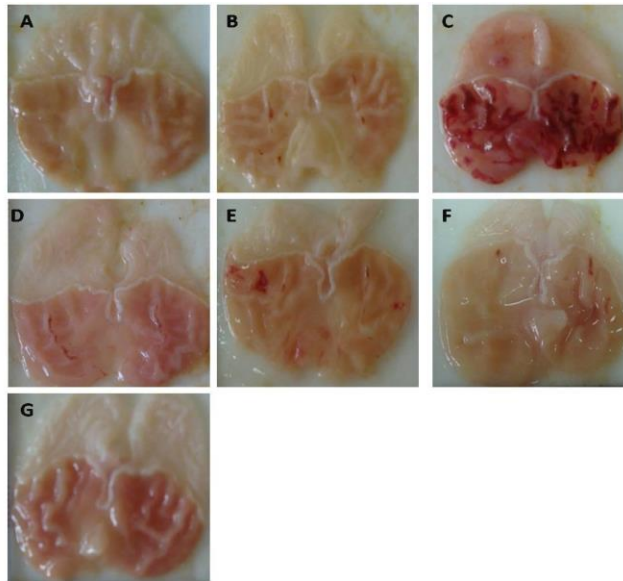


Figure 1. Examination of the gastric wall at the macroscopic level. According to the findings, rats that were pre-treated with methanolic extract of *B. thonningii* (D & E; 250 & 500 mg/kg respectively), chloroform extract of *B. thonningii* (F & G; 250 & 500 mg/kg respectively), and omeprazole (B; 20 mg/kg) of *B. thonningii* had significantly fewer areas of ulcer in comparison to rats that were pre-administered with only (ulcer group, Figure A). Figure 1B exhibits tissue from normal animals.

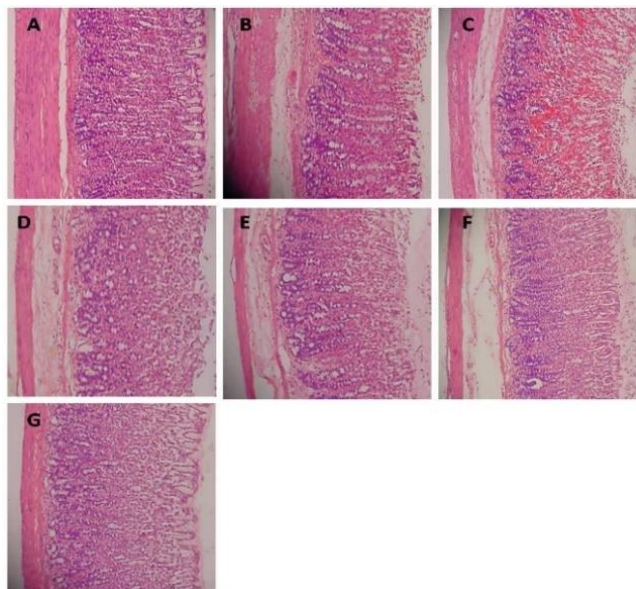


Figure 2. Stomach stained with hematoxylin and eosin. The findings showed that rats pre-treated with *B. thonningii* methanolic extract (D & E; 250 & 500 mg/kg), chloroform extract (F & G; 250 & 500 mg/kg), and omeprazole (1B; 20 mg/kg) had better stomach histology compared to rats pre-treated with just vehicle (ulcer control group, Figure A). Oral MBST reduced inflammation and neutrophil infiltration. 2B shows typical animal tissue. 10x H&E stain.

Role of MBST and CBST on malondialdehyde, nitric oxide and biochemical parameters: The treatment of rats with MBST and CBST against ethanol-induced stomach damage was evaluated using a lipid peroxidation test. The ethanol-induced ulcer group had greater ($P < 0.05$) levels of MDA ($30 \pm 3.10 \mu\text{mol/g Tissue}$) than the other groups as shown in Table 1. Using Griess reagent, the effect of the extracts on NO levels in the stomach was measured and represented as total nitrate/nitrite. Rats in Group I had the lowest levels of NO in the fundus region of their stomach. When the animals in Group II were given omeprazole and the animals in Groups III, IV, V, and VI were given extracts, there was a significant difference ($P < 0.05$) when compared to the rats in Group I. ALT and AST levels were increased in

ethanol-induced rats' serum (Table 1). The pre-treatment of extracts to the animals in Groups III, IV, V, and VI stabilized these liver damage indicators.

Proteomic analysis: Protein spots no. 13 (Hsp60), 14 (ERp60), 15 (Prolyl 4-hydroxylase subunit beta), 23 (B-CK), 46 (Cytosolic malate dehydrogenase), 47 (1-Cys peroxiredoxin), and 49 (ATP synthase D chain, mitochondrial) were found to be considerably up-regulated in the test group compared to the controls. 2D-gel and mass spectroscopy were used to successfully analyze protein expression. Densitometry study revealed substantial variations in protein bands volume utilizing Photo analyzers and IBM SPSS software (Figure 3 and Table 2).

Table 2. Identification of proteins by mass spectrometry. Proteins as seen on Figure 4 were identified by tandem mass spectrometry.

Well No	Protein No	Protein ID	Mass	Peptide score	Matched peptide	PI	Seq coverage	Accession No	Fold differences (M4/Control)
L9	13	60 kDa heat shock protein, mitochondrial precursor (Hsp60)	61016	274	4	5.7	10%	P10809	2.806709443
K10	14	Protein disulfide-isomerase A3 precursor (EC 5.3.4.1) (Disulfide isomerase ER-60) (ERp60)	56747	144	3	5.98	6%	P30101	2.728400547
K11	15	Protein disulfide-isomerase precursor (EC 5.3.4.1) (PDI) (Prolyl 4-hydroxylase subunit beta)	57081	287	4	4.76	11%	P07237	2.201575314
K13	23	Creatine kinase B-type (EC 2.7.3.2) (Creatine kinase B chain) (B-CK)	42617	671	11	5.34	35%	P12277	6.502403648
K16	46	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37) (Cytosolic malate dehydrogenase)	36403	258	4	6.91	14%	P40925	3.142794234
K17	47	Peroxiredoxin-6 (EC 1.11.1.15) (Antioxidant protein 2) (1-Cys peroxiredoxin)	25019	172	3	6	13%	P30041	1.778346779
K18	49	ATP synthase D chain, mitochondrial (EC 3.6.3.14)	18479	153	2	5.21	8%	O75947	1.97625616

* Fold change is significant at $P < 0.05$.

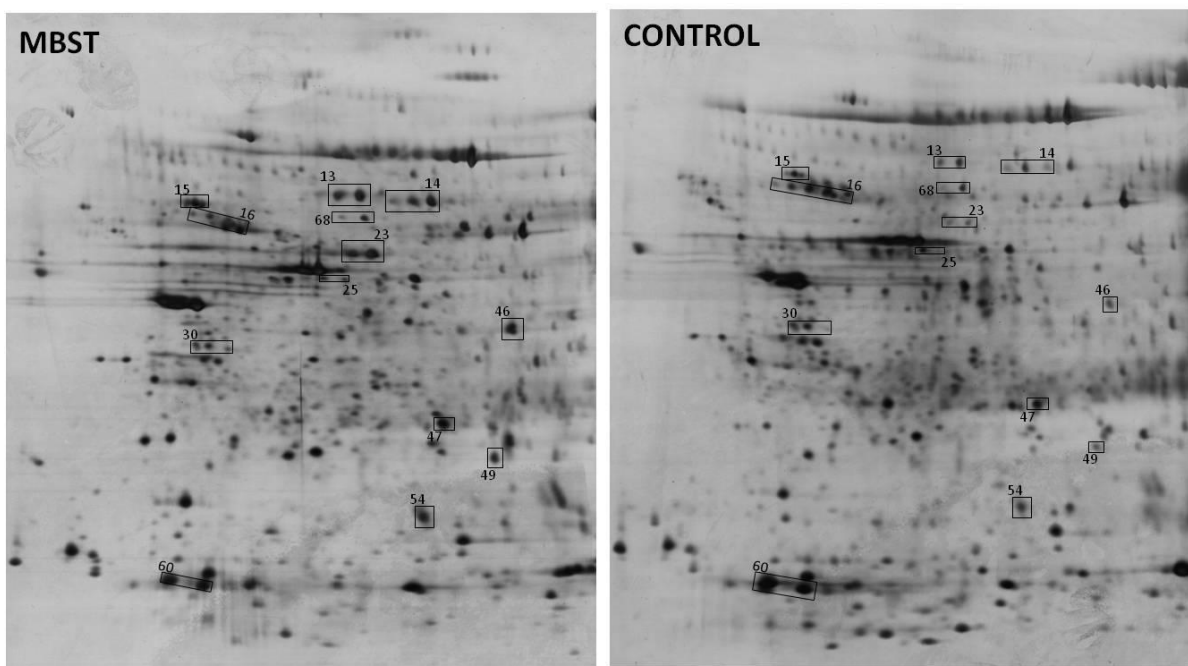


Figure 3. Representative photos of the rat’s gastric homogenates’ 2D gels. The photos of 2D real gels illustrate the resolved separation modalities of protein bands. The picture processing using Image-Master and following statistical analyses revealed that the intensities of the locations indicated by the rectangles varied between the two gel groups.

In-vitro effect on NO: The inflammatory condition of RAW264.7 cells was validated by elevated NO levels, which indicated sensitivity to LPS/IFN- therapy (Figure 4). NO₂⁻, a by-product of NO, was colorimetrically quantified in DMEM medium at an average level of 38.2 ± 1.94 M. (Figure 4). NO was released in trace levels by cells that

were not stimulated. The generation of NO from LPS/IFN-triggered RAW 264.7 cells was decreased dose-dependently (P<0.05) by MBST. L-NAME, a typical NOS inhibiting agent, was utilized as a positive agent and decreased NO significantly (751.82%) at 250

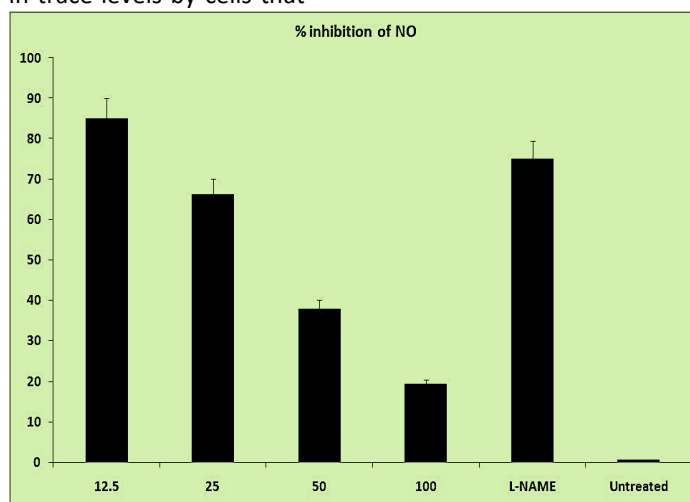


Figure 4: The effect of *B. thoningii* chloroform and methanolic extracts on NO levels. Macrophages from mice were either left un-treated or pre-treated with the appropriate MBST concentrations. Following that, the RAW264.7 cells were either left in the media or pre-treated with LPS/IFN. The data represents the mean of three separate trials.

Antioxidant activities of *B. thonningii*: ORAC, DPPH scavenging, and FRAP tests were used to determine the antioxidant properties of *B. thonningii*. The methanolic extract of *B. thonningii* inhibited DPPH activity in a time and concentration-dependent mode ($P < 0.05$), with a 50% inhibition (IC_{50}) at a concentration of 5.231.1 g/mL (Table 3) after 120 minutes. As demonstrated in Table 3, this extract has a substantial concentration-dependent FRAP value ($P < 0.05$). The ORAC test was performed to assess the antioxidant potential of *B. thonningii*, and the potency of the extracts was compared to that of a

positive control, quercetin. For the plant extracts, trolox, and quercetin, the area under the curve (AUC) was computed. Table 3 shows the ORAC findings. The extract, at a concentration of 20g/ml, was comparable to a trolox levels of 864.3415.2 M. At 5g/ml, quercetin is comparable to a Trolox concentration. (Table 2). The total phenolic content (g GAE/mg extract) of the extract, on the other hand, was determined to be 452.15 GAE/mg, respectively (Table 3). Methanolic extract (4.120.4 g QE/mg extract) was found to have the highest total flavonoid $AlCl_3$ content.

Table 3. Antioxidant activities, extractable yield and total phenolic and flavonoid contents of methanolic extract of *B. thonningii* stem park.

Sample		Yield% (W/W)	TPC μ g GAE/1mg Extract	TFC (μ g QE 1mg extract)	FRAP value \pm SD	DPPH radical activity (IC_{50} μ g/mL)	ORAC [Equivalent concentration to Trolox at 100 μ g/ml (μ M)]
CBST		0.51 ^a	4.1 ^a	58.3 \pm 2.5 ^a	1246.0 \pm 14	1.1 \pm 0.21	58.2 \pm 2.15 ^a
MBST		26.44 ^b	45 \pm 2.15 ^b	4.12 \pm 0.40 ^b	4684.44 \pm 142.19 ^b	5.23 \pm 1.1 ^a	864.34 \pm 15.2 ^b
Positive controls	Galic acid	-	-	-	2885.56 \pm 121.50 ^b	2.9 \pm 0.62 ^b	-
	Ascorbic acid	-	-	-	461.11 \pm 11.25 ^a	7.2 \pm 0.33 ^c	-
	Retin	-	-	-	825.00 \pm 68.95 ^c	12 \pm 1.4 ^d	-
	Querciten	-	-	-	2561.11 \pm 90.26 ^d	3.12 \pm 0.50 ^b	219.49 \pm 0.48 ^c
	Trolox	-	-	-	922.78 \pm 81.575 ^c	-	-

TPC: total phenolic content; TFC: total flavonoid content; GAE: gallic acid equivalent; QE: quercetin equivalent. **Groups with different alphabets are statistically significant.

Phytochemical investigations: Standard techniques were used to test MBST for alkaloid, terpenoid, and saponin content. The presence of saponin and terpenoid was detected by MBST. The existence of distinct functional groups was discovered by the IR spectra and 1H -NMR. The sample's infrared spectrum revealed the occurrence of hydroxyl groups and aromatic hydrocarbons (Data not shown). The 1H -NMR analysis of the material revealed the existence of methylene, methyl, and aromatic CH, with multiple signal peaks ranging from 3.23 to 4.18 ppm in the strong magnetic field. These peaks might suggest the existence of

glycosides units in the extract, which correspond to the existence of saponins.

DISCUSSION

Inherited knowledge is the starting point for some functional food sciences. It represents the stock of knowledge of the communities that still practice folk medicine and consume local food. This study represents a positive exploitation and starts from inherited knowledge to root it into a functional food that can benefit humanity. The aim of this research was to identify the mechanism(s) by which stem bark chloroform (CBST)

and methanolic (MBST) extracts of *B. thoningii* protect rats from ethanol-induced ulcers. In addition, a comparison of the biological, phytochemical, and antioxidant qualities of CSBT and MSBT was conducted. Animal models were frequently used to study experimental ulcerogenesis when ethanol is present. Alcohol works by penetrating the gastrointestinal mucosa, increasing mucosal permeability, and releasing vasoactive substances. Vascular damage, cell death, and oxygen free radicals are all included in the pathogenesis of ethanol-triggered ulcers [20]. In this setting, the application of medicinal plants to the treatment of human illnesses, which is the focus of this investigation, is making rapid progress [21]. Previous phytochemical tests conducted on the stem bark of *B. thoningii* revealed the presence of anthraquinone, alkaloid, flavonoid, saponin, anthocyanin, betacyanin and steroid [7,8,22,23].

Mucus production has historically been increased by antiulcer drugs as a coping mechanism [24]. Herbal remedies and medicinal herbs enhance the formation of mucus on the stomach wall. The pathophysiology of ethanol is associated with decreased epithelial mucus production. In these cases, pathologists used mucus production as a diagnostic tool. In this study, weight and mucus production were employed as predictors. This study's findings of increased mucus production demonstrate that MBST has cytoprotective properties, which may result from the development of a mucosal barrier against a variety of stomach-intravenously delivered necrotizing substances. As a result, it appears that *B. thoningii* therapy improves the mucosal barrier, the body's first line of defense against external chemicals that cause ulcers [25,26].

Inflammatory conditions that cause the production of specific proinflammatory markers are the root cause of gastric ulcers [27]. Alcohol administration caused neutrophil infiltration. Both inflammation and neutrophil infiltration were decreased in MBST after oral treatment.

On the other hand, neutrophils produce reactive oxygen species, or free radicals (ROS). These reactive oxygen species (ROSs) encourage the production of malondialdehyde and lipid peroxidation (MDA). MDA is a marker for reactive oxygen species-induced mucosal damage (ROS). This research showed that ethanol taken orally increased MDA levels. However, MBST pretreatment led to a decrease in the tissue level of MDA. Our findings show that MBST protects the stomach mucosa of rats by reducing inflammation and inhibiting lipid peroxidation. MBST reduced free radical activity in the DPPH, ORAC, and FRAP tests in our in vitro experiment in a concentration-dependent manner. Conversely, CBST had less success preventing the rise in lipid peroxidation.

Previously, it was revealed that nitric oxide has an intervention in the development of stomach ulcers triggered by alcohol. Without a doubt, L-inhibition NAME's of nitric oxide generation entirely eliminated the gastroprotective action of many prospective antiulcer drugs. Indeed, NO-stimulating agents protect against ethanol-induced gastric ulceration, whereas inhibiting NO production increases the gastric mucosa's vulnerability to ethanol damage [28]. Additionally, because NO is involved in the ulcer healing process, it will be interesting to investigate the possible action of *B. thoningii* in protecting the stomach mucosa against ethanol damage. As seen in Table 1, pretreatment with *B. thoningii* increases nitric oxide levels in animals given ethanol. Moreover, the NO findings were confirmed using an in vitro cellular test. Whereas, when murine macrophage cells were triggered with LPS/IFN- and pretreated with the extract, the NO level was not considerably different from that of LPS/IFN- stimulated cells. These data indicate that *B. thoningii* had no effect on the natural release of NO in vitro or in vivo.

When exposed to ethanol, distinct alterations in protein expression were found using quantitative proteomics on stomach homogenate. MBST therapy

caused the development of certain proteins in ulcerogenic rats, according to a proteomic investigation. The MBST group had significantly higher levels of Hsp60, ERp60, Prolyl 4-hydroxylase subunit beta, B-CK, cytosolic malate dehydrogenase, 1-Cys peroxiredoxin, and mitochondrial ATP synthase D chain. Polyphenol-rich phytochemicals may prevent mitochondrial ATP synthase from working [29]. HSP60 is an inflammatory and oxidative stress mediator in adipose tissue that is employed as an early biomarker of cellular damage [30].

Additionally, flattening of mucosal folds were found in the current research, suggesting that the cytoprotective effect of MBST may be attributed to decreased stomach motility. Our findings corroborate prior research indicating that pre-administration with herbal preparations decreases stomach motilities [1]. Thioredoxins are a family of tiny redox proteins found in all animals. They serve a critical function in humans and are becoming more associated with medicine due to their sensitivity to reactive oxygen species ROS. The triggering of a stomach damage in rats is systematically linked to ROS [31]. Thus, the present study demonstrated that antioxidant-rich MBST is capable of inducing thioredoxin expression in ethanol-induced ulcers. In a knowledge-based economy, ethnopharmacological heritage is viewed as a source of income and innovative medications. As a result of the current findings, the extract of *B. thonningii* may be a candidate for the development of a novel antiulcer medication. TFC, TPC, ¹H-NMR, phytochemical screening, and IR studies of the methanolic extract of *B. thonningii* revealed the presence of functional groups and a significant number of polar polyphenols. The scavenging of ROS and anti-lipoperoxidant activity of MBST are thought to be largely dependent on its polar polyphenol concentration. Additionally, plant extracts have been shown to protect against ethanol-induced stomach damage due to their antioxidant properties [26].

CONCLUSION

Functional Food Science uses inherited knowledge. It represents the community's knowledge of folk medicine and local food. This study uses inherited knowledge to create a functional food for humanity. Due to its potent antioxidant and mucus-increasing properties, stem bark extract of *Bauhinia thonningii* Schum was found to have cytoprotective actions against in- vivo ethanol-induced damage in the current investigation. More research should be done on *B. thonningii* since it has the potential to yield an innovative and interesting new drug for the treatment of acute erosive gastropathy. People who are at high risk of developing stomach ulcers can benefit from taking a dietary supplement that was produced from an extract of this plant.

Abbreviations: MBST: methanolic *Bauhinia* stem bark, CBST: chloroform *Bauhinia* stem bark, FRAP: Ferric Reducing Antioxidant Power, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ORAC: Oxygen Radical Absorbance Capacity, GIT: gastrointestinal tract, MDA: Malondialdehyde, TBARS: thiobarbituric acid reactive substances, DTT: dithiothreitol, CAN: acetonitrile, DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl sulfoxide, NO: Nitric oxide, MTT: (3-(4,5-Dimethylthiazol-2-yl), TPC: Total phenolic content, TFC: total flavonoid content, ANOVA: analysis of variance.

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