Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2025 June Vol 7 Issue 2: 168-179

Original Research

ANTIULCER ACTIVITY OF HYDRO-ETHANOL LEAF EXTRACT OF *FICUS AURICULATA* PRE-TREATMENT IN INDOMETHACIN-INDUCED GASTRIC ULCER IN WISTAR RATS

SWEM, T.F*., NYON, D.A. & AHUR, V.M.

Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University Makurdi, Benue State, Nigeria.

**Correspondence: swemfestus422@gmail.com* +2348165743539

ABSTRACT

This study was aimed at evaluating the anti-ulcer activity of hydro-ethanol leaf extract of *Ficus auriculata* in indomethacin-induced ulcer in Wistar rats. The extraction was done by cold maceration in 80% ethanol and phytochemical constituents were analyzed qualitatively. Twenty-five (25) adult Wistar rats were randomly divided into five groups of five rats each. The extract was tested orally at 250 and 500 mg/kg, while omeprazole was used as standard drug. The rats were humanely sacrificed and the ulcer lesions were observed with the aid of a magnifying glass while tissue antioxidant assay was carried out following standard methods. The extract contained alkaloids, saponins, tanins, phenols, phlobatannins, flavonoids and reducing sugars. Macroscopic observation of the stomach revealed obvious ulcer lesion in the negative group, which were greatly reduced in the extract pre-treated groups, evident by the significantly *($p \leq 0.05$)* low ulcer index of 2.2 ± 1.06 and 0.5 ± 0.00 in group 4 and 5 respectively when compared with the ulcer index of 8.2 ± 2.78 in group 2 (negative). The extract significantly increased total protein and all investigated antioxidant parameters, while malondialdehyde decreased significantly ($p \leq 0.05$), when compared with the negative and normal control group. Therefore, this study has provided evidence for the anti-ulcer activity of hydro-ethanol extract of *F. auriculata* leaf.

Keywords: Antioxidants, *Ficus auriculata*, Gastric ulcers, Indomethacin, Omeprazole

INTRODUCTION

A wide variety of plant species serve several purposes and of utmost importance are their therapeutic and or prophylactic uses (Abubakar and Haque, 2020) Medicinal plants provide a reliable source of both conventional and complementary medicine, and have been used extensively throughout history to treat and prevent the occurrence of various diseases and/or disorders both in humans and in domestic animals (Calzetta *et al.*, 2012). According to Lewis & Elvin-Lewis (2012), herbal medicines have been a significant part of human civilization throughout history. These medicinal plants have been traditionally and experimentally employed in the treatment of various ailments including gastrointestinal disorders such as ulcers which have been the principal cause of delays in weight gain, loss in productivity in livestock and consequently animal and human mortality (Calzetta *et al.*, 2012). Ulcers generally refer to sores that form in soft tissues (Catarina *et al*., 2020). Peptic Ulcer Disease (PUD) therefore refers to sores that form in the lining of the digestive tract (Catarina *et al*., 2020). Peptic ulcer disease (PUD) remains a significant health concern worldwide, affecting millions of people and posing substantial socioeconomic burdens. Depending on the site of ulcer, PUD can be gastric ulcer (stomach) or duodenal ulcer (duodenum) (Kaur *et al*., 2012). This condition is the most common gastrointestinal disease with a worldwide prevalence of about 40% in developed and 80% in developing countries (Adinortey *et al*., 2013). Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, being a major burden for healthcare organizations (Tanih *et al*., 2010). Factors

http://doi.org/10.54328/covm.josvas.2025.233

involved in the development of peptic ulcers are *Helicobacter pylori* infection, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), acid hypersecretory state (Zollinger-Ellison syndrome), and tumors as well as life-style factors like smoking and excessive use of alcohol (Malfertheiner *et al.*, 2009). Essential symptoms of peptic ulcer include episodes of distress, epigastric pain with burning sensation, pain during empty stomach or after food intake, provoked night time awakening, in addition to common symptoms like vomiting, loss of appetite, heart burn and intolerance to fatty diet (Ramakrishnan & Salinas, 2007). Despite advancements in pharmacological treatments, such as proton pump inhibitors and H2-receptor antagonists, the quest for effective and safer alternatives continues due to side effects and recurrence issues associated with these therapies (Kuna *et al*., 2019). Although medicinal plants are utilized often with some effectiveness, scientific research on many of them is inadequate.

Ficus is one such poorly studied genus of medicinal plants, with more than 800 species and 2000 variants (Mohammed *et al*., 2013; Yinxian *et al*., 2014; Satish *et al*., 2014). *Ficus auriculata,* also referred to as Elephant ear fig or Roxburgh fig (El-Fishawy *et al*., 2011), is a member of the *Moraceae* family. It naturally occurs in low-land tropical rainforests, typically found growing along streams or on rocks (Ahmed *et al*., 2011). It is renowned in traditional medicine for its diverse therapeutic properties. Squeezed leaves of the plant are applied to wounds, and they are also employed in treating diarrhoea and dysentery. People lop the leaves for use as fodder. The juice from the stem bark is effective for diarrhoea, cuts and wounds (Bhakta *et al*., 2011). The fruits are edible and can be used to make jams and curries. Roasted figs are consumed to alleviate diarrhoea and dysentery. The latex from the roots is utilized for mumps, cholera, diarrhoea, and vomiting. In Northern eastern India, particularly in Manipur, various tribes traditionally utilize the leaves of *Ficus auriculata* for managing diabetes, (Anitha *et al*., 2011; Rosalind *et al*., 2012). Ethnobotanical studies have highlighted its use in treating various ailments, including gastrointestinal disorders (Ahmed *et al*., 2011). Its raw extract has displayed antioxidant, antibacterial, antimicrobial and antihyperlipidemic properties (El-Fishawy *et al*., 2011; Thingbaijam *et al*., 2012). Previous phytochemical investigations of *Ficus auriculata* have revealed the presence of flavonoids, tannins, saponins, and other bioactive constituents, which are known for their antioxidant and anti-inflammatory properties (vu Nguyen *et al*., 2006). These attributes suggest that *Ficus auriculata* could potentially exhibit antiulcer activity.

Although the ulcer healing effect of *Ficus auriculata* has been reported in traditional medicine, comprehensive scientific evidence regarding its antiulcer activity especially in the study area is lacking, hence this study was undertaken to evaluate the anti-ulcer activity of hydro-ethanol leaf extracts of *Ficus auriculata* in Wistar rats.

METHODOLOGY

CHEMICALS AND REAGENTS

Distilled water, ethanol, omeprazole, indomethacin, normal saline, ketamine, potassium chloride, formalin, physiologic buffered saline, 1% hydrochloric acid, ammonium hydroxide, acetic anhydrid, dilute sulphiric acid, Wagner reagent, 2% sodium bicarbonate, 10% zinc sulphide, Dragendorffs reagent, Glacia acetic acid, 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH), Absolute Alcohol, Paraffin wax, Xylene.

ETHICAL CONSIDERATION

Ethical approval was gotten from the ethical Committee of the College of Veterinary Medicine, Joseph Sarwuan Tarka University Makurdi. Laboratory animals were handled in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and Organization for Economic Cooperation and Development (OECD) guidelines.

COLLECTION OF PLANT MATERIAL

The fresh leaves of *F. auriculata* were collected at 72 Army Barracks, North Bank Makurdi, Benue State and sent for authentication at the Department of Forest Production and Products, Joseph Sarwuan Tarka University Makurdi, Benue State. The plant sample was deposited at the department's herbarium for further reference (Voucher No: UAM/FH/0446).

PREPARATION AND EXTRACTION OF THE PLANT MATERIAL

The leaves of *F. auriculata* were washed under clean running water and air-dried under shade at room temperature. Thereafter, the dried leaves were pulverized to fine powder using mortar and pestle. Then, 300g of plant powder was weighed using an electronic weighing balance and soaked in 3000 ml of 80% ethanol for 48 hours at room temperature with occasional stirring. After 48 hours, the mixture was filtered with What-man filter paper No. 1. The filtrate was dried using a water bath at 40°C.

The dried extract was weighed and transferred into vials and kept in a refrigerator and stored as hydro methanol leaf extract of *F auriculata* (HELEFA) until the actual study was carried out. Percentage yield of the plant was determined using the formula below:

$$\%\,Yield = \frac{Final\ weight\ of\ extract}{total\ weight\ of\ ground\ plant} \times 100$$

PHYTOCHEMICAL SCREENING

The hydro-ethanol leaf extract of *F. auriculata* was screened for the presence of active phytochemical constituents by using the methods by Trease & Evans (2002) as described by Salem *et al.,* (2016). The follwing phytochemicals were analyzed; alkaloids, saponins, flavonoids, terpenoids, phenols, steroids, glycosides, and tannins were performed.

EXPERIMENTAL ANIMALS

A total of thirty (30) healthy Wistar rats of either sex weighing 100 – 150 g, and aged 12 – 16 weeks were used for the study. The rats were housed in plastic cages with softwood shavings and chips as bedding at room temperature (25 ± 2°C) and were kept under a 12/12-hour light to dark cycle. They were allowed free access to water and standard commercial poultry feed (Chikun®) and were acclimatized to the working environment for two weeks before the actual experiment. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals and Organization for Economic Co-operation and Development (OECD) guidelines (NRC, 2011; OECD, 2022).

ACUTE TOXICITY TEST

An acute toxicity test was conducted according to OECD 425 guidelines for experimental animals. Five female, adult non-pregnant were used. All rats were fasted (food but not water) overnight before and 2 hours after the administration of the extract. A single female rat was first treated with a limit dose of 2000 mg/kg of the crude extract by oral gavage and the rat was closely observed every 30 minutes for 4 hours for any signs of toxicity and mortality within the first 24 hours. Following the outcome (i.e. no mortality), the remaining 4 female rats were then treated sequentially once with a similar dose of the extract and were observed for any signs of toxicity and mortality for 14 days (OECD, 2022).

INDOMETHACIN INDUCED GASTRIC ULCER EXPERIMENT

A total of 25 Wistar rats were used. All the rats were fasted for 24 hours, but allowed free access to water. They were randomly divided into five (5) groups, each containing five (5) rats and treated thus: Group one (Normal Control): received distilled water (5ml/kg, *p.os.*). Group two (negative group): received only indomethacin (30 mg/kg, *p.os* for two days). Group three (positive group): received Omeprazole (20 mg/kg, *p.o*s. for three days). Groups four (Test Group 1) and five (Test Group 2): were pre-treated with 250 mg/kg and 500 mg/kg*p.os* of the extract respectively for three days. On the third and fourth day, rats in each group were administered indomethacin (30 mg/kg, *p.os).* Four hours later, all the rats were sacrificed by an overdose of ketamine and the stomachs were removed and opened along the greater curvature. Ulcers formed in the glandular portion of the stomach were observed and scored under magnifying glass. The stomachs were then weighed, washed with ice cold saline and divided into two parts. One part was used to prepare a homogenate in Phosphate Buffered Saline (PBS) at the ratio 1g:4mls. An aliquot of the homogenate was used for the estimation of lipid peroxidation (LPO) and endogenous Antioxidants (Superoxide dismutase, GPx, Glutathione and catalase). The remaining part of the stomach was fixed in 10% buffered formalin and used for histopathology.

ULCER ASSESSMENT

The stomachs were harvested, opened along the greater curvature and the mucosa was exposed for macroscopic evaluation. The ulcerated area was assessed and the ulcer index (UI) was calculated for each treatment by the method of Kulkarni (2002).

MEAN SCORING

A score for the ulcer was made as follows:
0: Normal colouration, 0.5: Red colouration, 1: Spot ulcers, 1.5: Hemorrhagic streaks, 2: Ulcers > 3 mm but < 5 mm, 3: Perforation

$$\text{Ulcer index (UI)} = UN + US + UP\ x\ 10^{-1}$$

Where: UI = Ulcer Index, UN = Average of number of ulcers per animal, US = Average of severity score, UP = Percentage of animal with ulcer.

DETERMINATION OF % PROTECTION

Determination of percentage inhibition or protection against ulcer by the various agents used was calculated using the formula:

$$\%\ Protection = \frac{UC - UT}{UC} \times 100$$

Where
UC = Ulcer index of the negative control group
UT = Ulcer index of the test group.

HISTOLOGICAL EXAMINATION OF GASTRIC TISSUES

The section of the stomach tissues fixed in 10% formalin was prepared for histological examination according to the method described by Nowacek (2010) and Gill (2010).

STATISTICAL ANALYSIS

The collected data were analyzed using the software Statistical Package for Social Sciences (SPSS), version 20.

The results were expressed as mean ± standard error of means (SEM). The significant difference between groups was compared using one way ANOVA followed by post hoc Tukey's test. A *P* value of less than (<) 0.05 was considered statistically significant.

RESULTS

PERCENTAGE YIELD OF LEAF EXTRACT

The weight of the powdered plant material used was 300g, which yielded 37.09g of extract. Therefore, the percentage yield of the leaves of *F. auriculata* in 80% ethanol was calculated to be 12.36%.

QUALITATIVE PHYTOCHEMICAL SCREENING

Phytochemical analysis of the hydro-ethanol leaf extract of *F. auriculata* revealed the presence of various bio-active compounds as presented in the Table I.

TABLE I: RESULTS OF QUALITATIVE PHYTOCHEMICAL SCREENING OF HYDRO-ETHANOL LEAF EXTRACT OF *FICUS AURICULATA* (HELEFA)

S/No.	Bioactive compound	Reaction
1.	Alkaloids	++
2.	Saponins	++
3.	Tannins	+
4.	Phlobatannins	+
5.	Phenols	++
6.	Flavonoids	+
7.	Reducing sugars	++
8.	Volatile oil	-
9.	Steroid	-
10.	Trepenoids	-

Key: - = negative; + = positive; ++ = highly positive

INDOMETHACIN INDUCED ULCER MODEL

ULCER INDEX: Assessment of the indomethacin induced ulcers revealed varying degrees of ulceration in relation to the group and the treatment administered. Group 2 (negative control) had the highest ulcer index (8.2 ± 2.78) followed by extract treated group at 250 mg/kg b.w (2.2 ± 1.06) while treatment at 500 mg/ b.w gave the lowest ulcer index (0.5) as shown on Figure I. There was a significant ($p<0.05$) decrease in the ulcer index of the groups pre-treated with omeprazole and HELEFA at 250 mg/kg b.w and 500 mg/kg b.w when compared with the negative control group (Figure I).

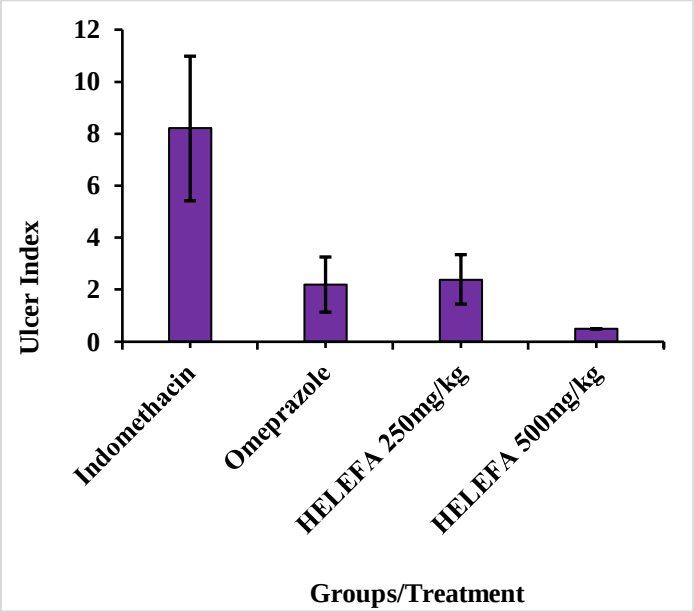


Figure I: Ulcer Index of Indomethacin-induced gastric ulcer in rats, following treatment with HELEFA
NB: Values are Mean ± Standard Error of Mean (SEM) at 95% confidence interval
HELEFA = Hydro-ethanolic extract of *Ficusauriculata*

PERCENTAGE ULCER INHIBITION

The Extract at 500 mg/kg offered the highest protection (93.90%) against ulcer followed by the standard drug, Omeprazole at 20 mg/kg (73.17%) while 250 mg/kg of the extract offered the least protection (70.73%) against ulcer (Figure II), which revealed significant ($p<0.05$) protection against ulcer when compared with the negative control group.

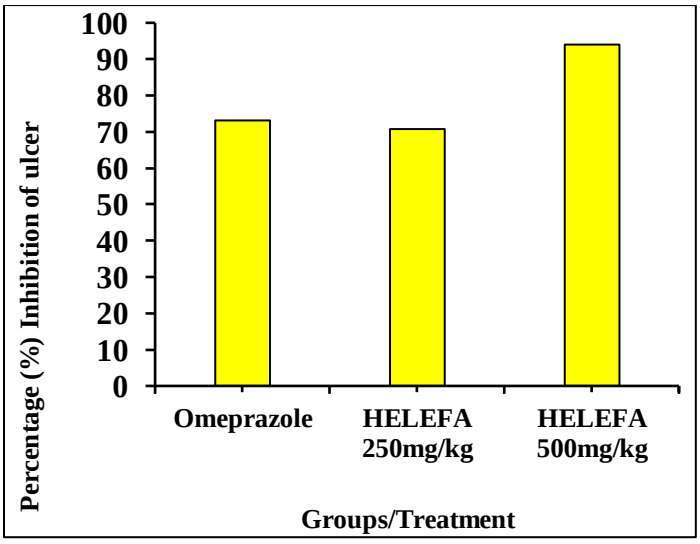


Figure II: Percentage inhibition of Indomethacin-induced gastric ulcer in rats, following treatment with HELEFA.
NB: Values are percentages (%), HELEFA = Hydro-ethanolic extract of *Ficusauriculata*

GROSS ULCER ASSESSMENT

Plates I – V shows the gross appearance of the stomach. Plate I showed the normal appearance of the gastric mucosa lining with its characteristic folds. Plates II-V showed varying degrees of gastric ulcer lesions. negative group (Plate II) had more severe gastric ulcer lesions evident by the wide area of smooth and thin mucosa surface and reddish appearance. In the omeprazole group (Plate III) reduction in the smooth mucosa area was observed with less reddening. Plates IV and V which represent the test group pre-treated with extract (250 and 500 mg/kg b.w), showed further reduction in the ulcer lesion, with little or no reddish appearance.

PLATE A-E: GROSS LESION OF INDOMETHACIN-INDUCED GASTRIC ULCER IN RATS, PRE-TREATED WITH HELEFA.

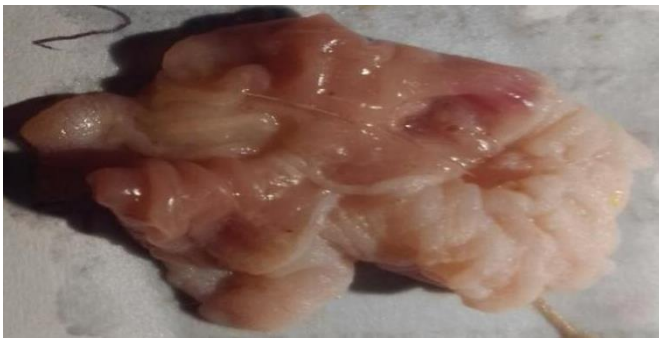


Plate A: Normal control

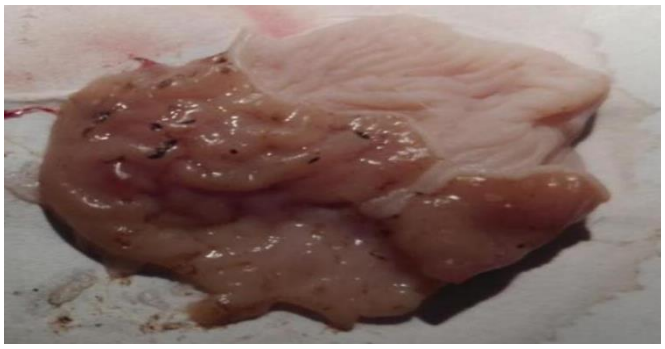


Plate B: Indomethacin 30 mg/kg

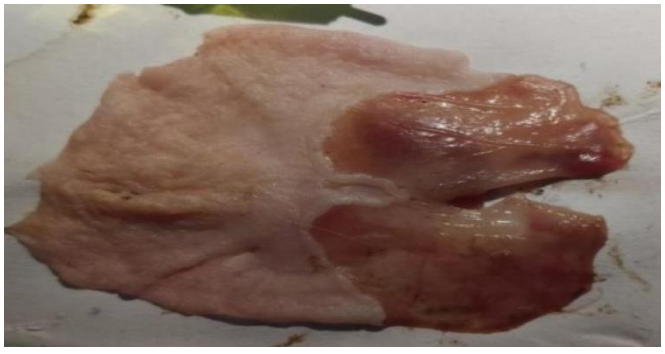


Plate C: Omeprazole 20 mg/kg

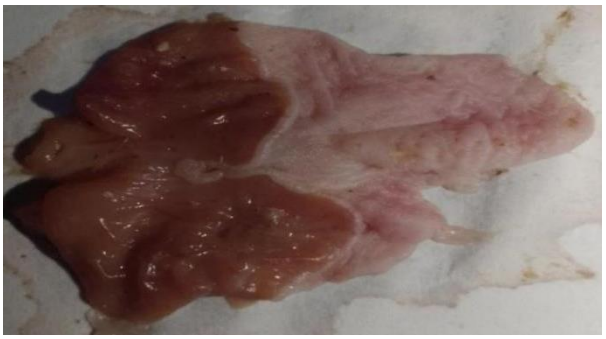


Plate D: HELEFA 250 mg/kg

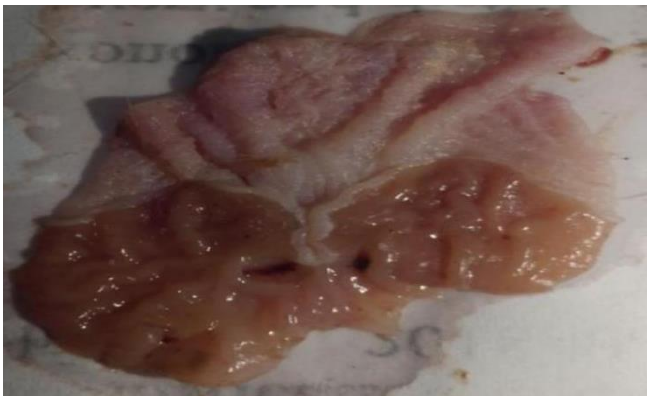
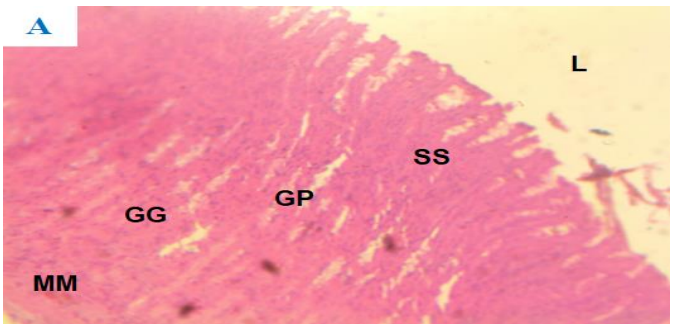
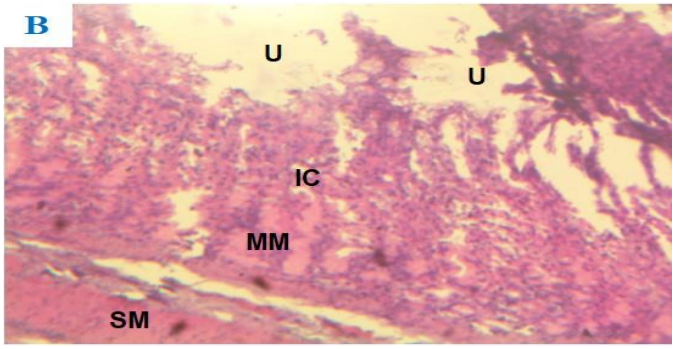
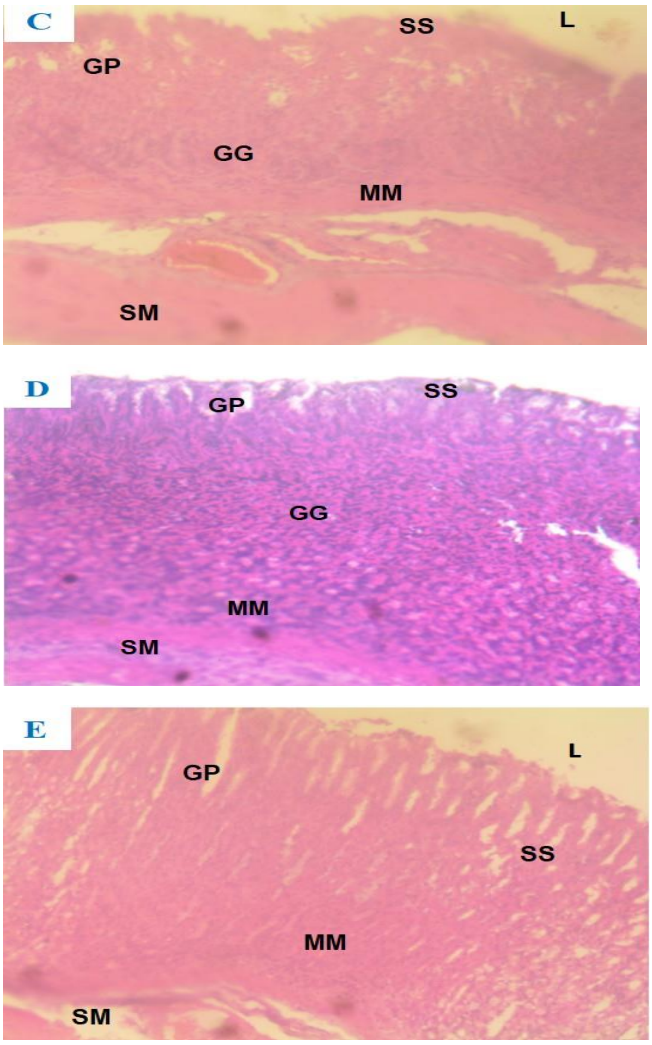


Plate E: HELEFA 500 mg/kg

SLIDES A-E: PHOTOMICROGRAPHS OF THE TRANSVERSE SECTION OF ULCERATED STOMACH OF RATS PRE-TREATED WITH HELEFA IN INDOMETHACIN-INDUCED GASTRIC ULCERS.







Key: A - Normal control, B - Indomethacin 30 mg/kg, C - Omeprazole 20 mg/kg, D - HELEFA 250 mg/kg, E- HELEFA 500 mg/kg. L = lumen; SS = Secretory sheath; GG = Gastric glands; MM = Muscularis mucosae; SM = Submucosa; GP = Gastric pit; IC = Inflammatory cells; U = Ulcers

ANTIOXIDANT ASSAY

MALONDIADEHYDE (MDA)

Result of the tissue concentration of malondialdehyde (MDA) is presented in Figure III. The negative group had the highest concentration of MDA, which was significantly (P>0.05) different when compared with the normal control. The standard group had a significantly (*P<0.05*) low concentration of MDA when compared with the normal control, the negative group as well the group pretreated with HELEFA at 250 mg/kg. The extract pre-treated group (250 mg/kg bw) showed significant (*P*>0.05) higher concentration when compared with the normal control, but significantly (*P*>0.05) low when compared with the negative groups. Pre-treatment with HELEFA at 500 mg/kg b.w, lowered the concentration of MDA significantly (*P<0.05*) when compared with the normal control, negative, standard groups as well as the group pre-treated with HELEFA at 250 mg/kg bw.

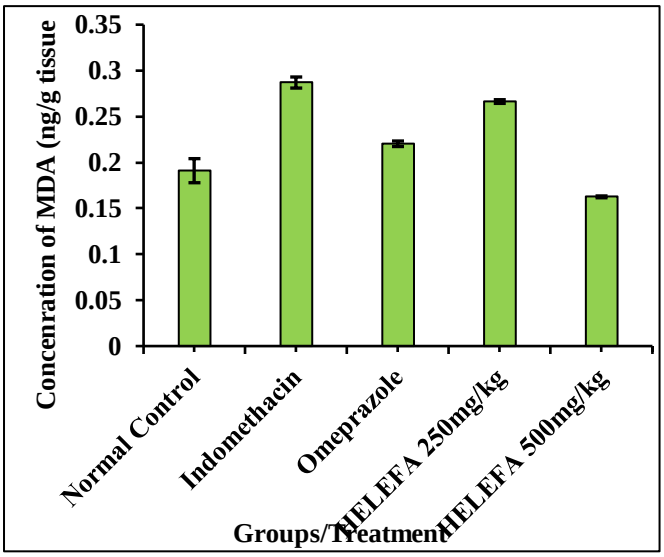


Figure III: Bar chart showing the effect of treatment with HELEFA on the concentration of MDA of indomethacin-induced gastric ulcer in rats.

NB: Values are Mean ± Standard Error of Mean (SEM) at 95% confidence interval.

CATALASE

Figure IV shows the result of tissue activity of catalase (CAT). The extract pre-treated group (-250 mg/kg b.w) showed significant ($p<0.05$) increase in tissue activity of catalase, when compared with the normal, negative and positive control groups, while a significant ($p<0.05$) decrease was observed in the group treated with 500 mg/kg of the extract, when compared with the normal, negative and positive control groups.

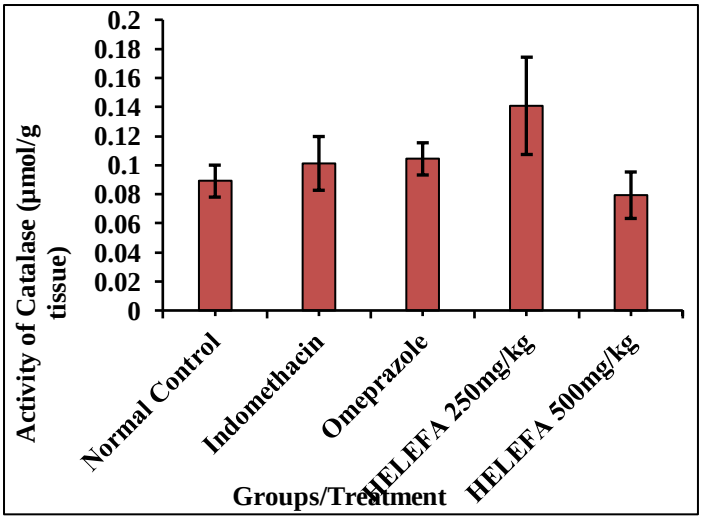


Figure IV: Bar chart showing the effect of treatment with HELEFA on activity of catalase of indomethacin-induced gastric ulcer in rats.

GLUTATHIONE PEROXIDASE (GPX)

Figure V shows the result of tissue activity of glutathione peroxidase (GPx). The standard drug, Omeprazole (group 3) revealed a significant ($p<0.05$) high activity of GPx when compared with the normal control and negative, while there appeared to be no significant difference between normal control and negative group. Pre-treatment with 250 mg/kg b.w and 500 mg/kg b.w of extract increased GPx activity significantly ($p<0.05$), when compared with normal control and negative groups, but significantly ($p<0.05$) decreased, compared with the group pre-treated with standard drugs.

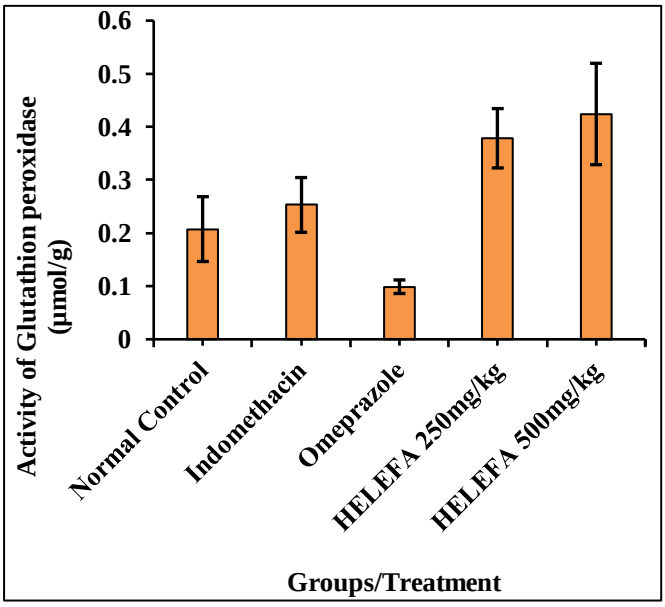


Figure V: Bar chart showing the effect of treatment with HELEFA on activity of glutathione peroxidase of indomethacin-induced gastric ulcer in rats

GLUTATHIONE REDUCTASE (GSH)

Figure VI shows the result of tissue activity of glutathione reductase (GSH). Indomethacin (negative group) showed no significant difference ($p<0.05$) in the activity of GSH when compared with the normal control. positive control group showed significant ($p<0.05$) increase in the activity of GSH when compared with normal control and negative groups. Extract pre-treated groups (250 mg/kg b.w and 500 mg/kg b.w) had significantly ($p<0.05$) higher activity compared to normal control, negative and positive control groups.

SUPEROXIDE DISMUTASE (SOD)

Figure VII shows the result of tissue activity of superoxide dismutase (SOD). Significant ($p<0.05$) increases in the activity of SOD was seen in the negative, positive control, and extract treated groups (250 mg/kg), when compared with the normal control group. The activity of SOD was significantly ($p<0.05$) low in the groups pre-treated with omeprazole 20 mg/kg b.w and extract at 250 mg/kg, when compared with the negative control group. The activity of SOD in the group pre-treated with the extract at 500 mg/kg b.w was significantly ($p<0.05$) reduced SOD, when compared with negative, positive control groups and group pre-treated with extract at 250 mg/kg b.w, but statistically similar to that of normal control group.

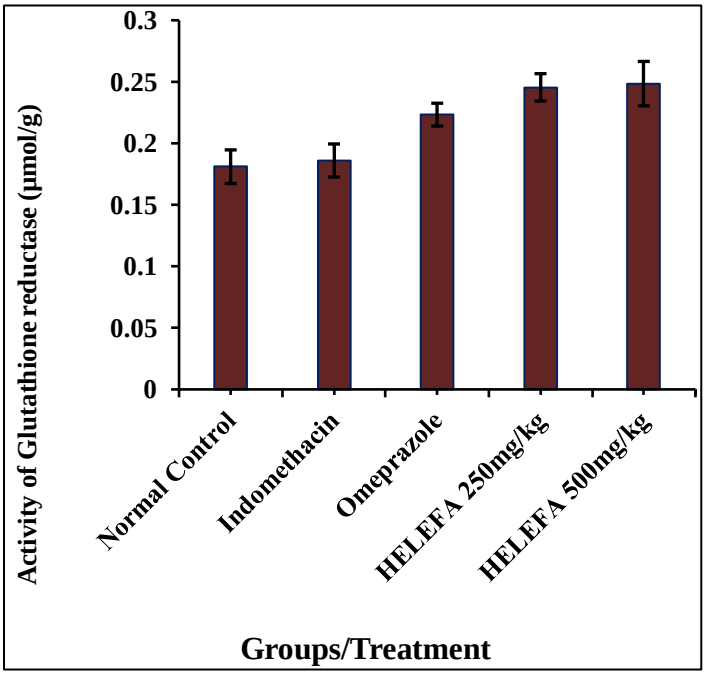


Figure VI: Bar chart showing the effect of treatment with HELEFA on activity of glutathione reductase of indomethacin-induced gastric ulcer in rats

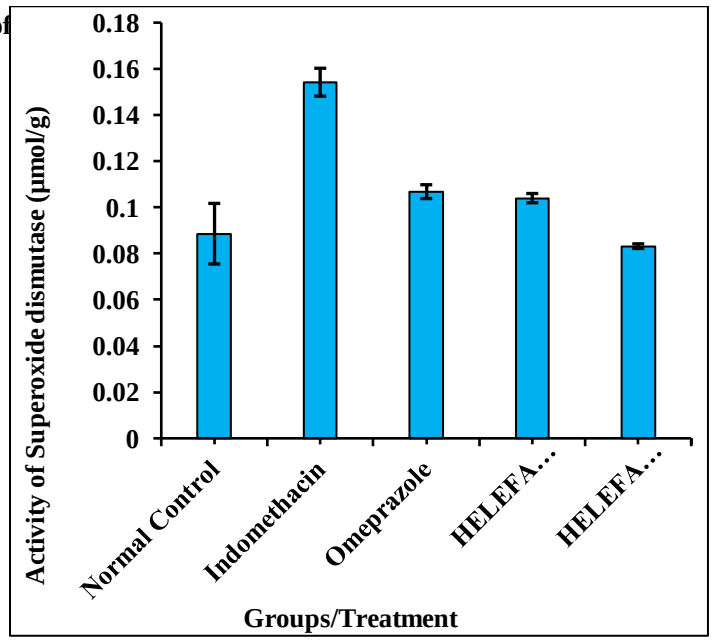


Figure VII: Bar chart showing the activity effect of treatment with HELEFA on activity of SOD of indomethacin-induced gastric ulcer in rats

TOTAL PROTEIN

Result of the tissue total protein concentration is shown on Figure VIII. There was a significant (P<0.05) decrease in the concentrations (g/L) of total protein in the negative, standard, extract pre-treated groups when compared with the normal control. A significant increase was observed in the omeprazole pre-treated group (positive control), when compared with the extract pre-treated (250 mg/kg bw and 500 mg/kg bw) groups.

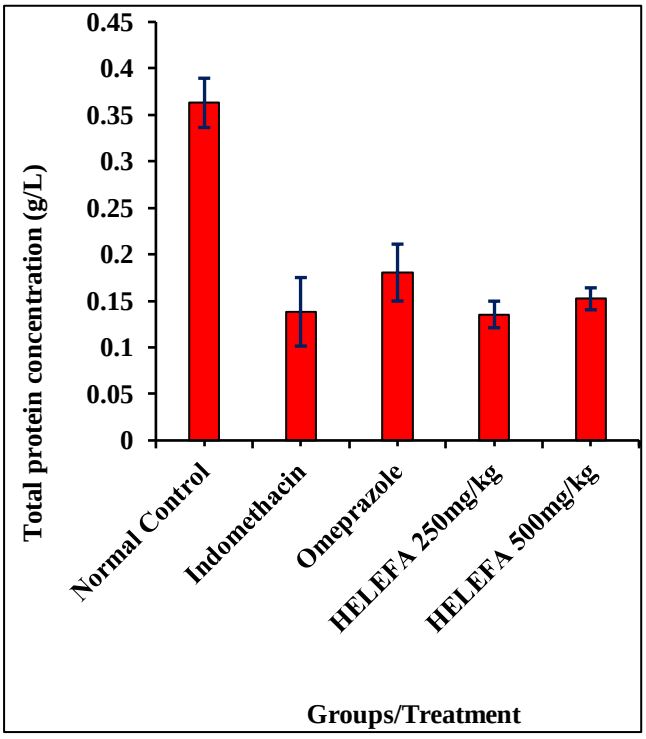


Figure VIII: Bar chart showing effect of treatment with HELEFA on total protein concentration of indomethacin-induced gastric ulcer in rats

DISCUSSION

The model for the present study was selected based on the actions of prostaglandins on the disease (Melese *et al*., 2011). Inhibition of prostaglandin synthesis by blocking the enzyme Cyclooxygenase-1 (COX-1) by indomethacin, a non staeroidal anti-inflammatory drug (NSAID) is the second most common etiologic agent of PUD after *H. pylori* infection (Melese *et al*., 2011).

These aggressive factors can increase the susceptibility of gastrointestinal mucosa by luminal irritants, alter the microcirculation that is critical to the pathogenesis of ulceration, and damage the mucus and bicarbonate secretion (Shawon & Gautam, 2012). Indomethacin has also been shown by previous studies to initiate lipid peroxidation by functioning as an oxidant thus, producing reactive oxygen species such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), nitric oxide ($NO_2^{\cdot}$) which cause mucosa damage (Yoshikawa *et al*., 1993; Melese *et al*., 2011).

The 80% ethanol solvent was used to dissolve both polar and nonpolar phytochemicals since many organic substances are not extracted from aqueous solvents only (Galanakis *et al*., 2013). Shahinuzzaman *et al.* (2020) explained that aqueous ethanol has low toxicity and better extraction ability; accordingly, studies by Wang *et al.* (2013) and Shahinuzzaman *et al.* (2020), found that the combination of water with pure solvent is more effective in extraction of the active compounds from plants than the pure solvent alone, hence, the use of aqueous ethanol in this current study. The preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins, phenols, phlobatannins, flavonoids and reducing sugars. Similar findings were reported by Adane *et al.* (2021) and Tamta *et al.* (2021). Rekka *et al.* (2021) also reported a similar finding in their study on evaluation of phytochemical screening and antibacterial activity of *F. auriculata* in India. They also reported the presence of terpenoids which was absent along with volatile oil and steroids in this current study. Kavitha *et al.* (2017) on the contrary, reported the absence of flavonoids, alkaloids and tannins but the presence of phenols. This disparity could be due to the difference in the type of solvent used, the soil and climatic condition of the region as well as the aim of the research. The method of drying of plant material could also have contributed to the disparity seen in these results.

The phytochemicals in *F. auriculata*, especially phenols and flavonoids, exhibit optimal structural chemistry for antioxidant effects, aiding in free radical scavenging (Dave, 2017). The antioxidant activity, observed in the study, maybe due to these secondary metabolites working with gastric antioxidants to decrease free radical accumulation and prevent mucosal damage caused by the peroxidative effect of indomethacin (Adane *et al.*, 2021). Their effectiveness is attributed to their high reactivity as electron donors. Saponins contribute to antiulcer activity by facilitating mucous membrane formation and inhibiting acid secretion (Shankar, 2017; Awaad *et al*., 2023). Alkaloids show gastroprotective effects possibly involving sulfhydryl compounds, nitric oxide (NO), prostaglandin (PG), and reducing interleukin 1 beta (IL-1β) and tissue necrosis factor (TNF-α), while increasing glutathione reductase (GSH). The study found that the extract significantly increased GSH activity compared to omeprazole, aligning with the findings of Hazarika *et al*. (2015). Alkaloids prevent ulcers by stimulating mucus production and acting as antioxidants (Awaad *et al*., 2023). Tannins, with their astringent properties, protect the gastric mucosa by precipitating proteins, suppressing gastric secretion, and promoting the mucus layer (Benchikh, 2008). Phenolic compounds are known for their anti-ulcerogenic activities, enhancing prostaglandin synthesis and having anti-secretory and

cytoprotective effects (Awaad *et al.*, 2023). Quercetin, a well-studied flavonoid, shows gastroprotective effects by protecting the gastrointestinal mucosa from acute injuries and facilitating the healing of gastric ulcers through various mechanisms such as influencing platelet activation factor, enhancing mucus production, exhibiting antihistaminic properties, inhibiting *H. pylori* growth, and impacting gastric acid secretion (Alarcon De La Lastra *et al*., 1994; Izzo *et al*., 1994; Kahraman *et al*., 2003).

The gastroprotective effects of flavonoid is also attributed to its antioxidant properties, such as free radical scavenging, chelation of transition metal ions, inhibition of oxidizing enzymes, enhanced levels of protein and non-protein antioxidants, and reduced lipid peroxidation (Kahraman *et al*., 2003). The current study supports these findings, showing that the extract decreased Malondialdehyde (MDA) concentration, a marker of oxidative cellular damage. Flavonoids also enhance blood flow in the stomach, promote the synthesis of muco-substances in the gastric mucosa, and amplify the effects of prostaglandins on gastric tissues (Saziki *et al*., 1984). They also exhibit a direct bactericidal effect on *H. pylori* (Saziki *et al*., 1984). Additionally, flavonoids display anti-urease activity and reduce the adhesion of *H. pylori* to gastric epithelial cells (Sunairi *et al*., 1984). Other flavonoid compounds, such as rutin, monomeric leucocyanidin, and garcinol, contribute to gastro and cytoprotective activities by inhibiting mucosal platelet-activating factor content, reducing lipoperoxide levels, and boosting glutathione peroxidase (GPx) activity (Saziki *et al*., 1984). The current study supports this, showing increased activity in extract-treated groups. Flavonoids also enhance mucosal prostaglandin secretion, decrease histamine secretion by inhibiting histidine decarboxylase, improve mucus secretion, and inhibit *H. pylori* growth (Eriyamremu & Iorliam, 2014), as demonstrated in the gross and histologic sections of the study (plates A-E and slides A-E).

Antioxidants play a vital role in neutralizing harmful free radicals and protecting cells (Atoui *et al*., 2005). Organisms use both enzymatic and non-enzymatic defense mechanisms to combat the toxicity of reactive oxygen species (ROS) (Zaghlool *et al.*, 2015). Preventive antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione reductase (GSH), help mitigate gastric mucosal damage caused by NSAIDs (Kim *et al*., 2011). Lipid peroxidation, resulting from ROS interactions with cell membranes, produces Malondialdehyde (MDA), which damages the gastric mucosa (Zheng *et al*., 2014). Indomethacin and similar agents induce lipid peroxidation by generating ROS, leading to gastric mucosa damage (Polat *et al*., 2011).

In this study, indomethacin significantly increased lipid peroxidation (MDA), which is consistent with the findings of Zaghlool *et al.* (2019). It decreased non-enzymatic (GSH) levels ($P > 0.05$) while increasing enzymatic (SOD) antioxidant activity in the gastric mucosa of indomethacin-induced ulcer rats, which contrasts with Allam *et al.* (2017) and Suleyman *et al.* (2009), who reported a decrease. The decreased GSH concentration in the negative group was associated with higher lipid peroxidation, as indicated by elevated MDA levels, in agreement with Zaghlool *et al.* (2019) and Hazarika *et al.* (2015). However, the study observed a decrease in MDA levels in extract pre-treated groups. The increase in SOD activity caused by indomethacin administration which is contrary to the report of Hazarika *et al.* (2015), suggests that more superoxide radicals are converted to hydrogen peroxide by SOD, which catalase then breaks down into water and oxygen, explaining the increased catalase activity observed and is similar to the findings of Zaghlool *et al*. (2019).

Glutathione reductase (GSH) and GSH-associated enzymes, particularly glutathione peroxidase, are proposed as chemo-preventive agents due to their antioxidant and detoxification properties (Zaghlool *et al*., 2019). They likely protect against indomethacin-induced damage. Studies have reported reduced levels of these antioxidants in gastric tissues treated with indomethacin (Zaghlool *et al*., 2019). This study's findings on GSH levels in both damaged and healthy tissues are consistent with earlier research. Antioxidants like GSH, melatonin, and vitamins help prevent tissue damage by regulating reactive oxygen species levels (Ajaikumar *et al*., 2005). Co-administration of omeprazole or hydro-ethanolic extract of *F. auriculata* with indomethacin significantly increased these antioxidants, with the crude extract showing potential in preventing gastric tissue damage through the activation of antioxidative defense mechanisms and enhanced antioxidant enzyme activity, rather than direct antioxidant action, aligning with Zaghlool *et al.* (2019).

The study found that *F. auriculata* extract significantly decreased the ulcer severity score and ulcer index in a dose-dependent manner, inhibiting the increase in the area of gastric mucosal lesions induced by indomethacin in rats, as evidenced by histological sections. The hydro-ethanolic extract at 250 mg/kg significantly ($P<0.05$) reduced the ulcer index from 8.2±2.78 to 2.4±0.9, and to 0.5±0.00 at 500 mg/kg, similar to Adane *et al.* (2021) and Zaghlool *et al.* (2019). The extract also provided dose-dependent ulcer inhibition of 70.73% (250 mg/kg) and 93.9% (500 mg/kg). The antiulcer activity of the extract may be due to sustaining prostaglandin release through cyclooxygenase-1 (COX-1) activation, maintaining gastric mucosa integrity (Shawon & Gautam, 2012; Zaghlool *et al.,* 2019; Adane *et al.,* 2021). Additionally, the antioxidant activity of the extract likely counteracted the peroxidative effect of indomethacin, inhibiting its ulcer-causing capability (Zaghlool *et al.,* 2019; Adane *et al.,* 2021).

CONCLUSION

In conclusion, this study has provided evidence for the antiulcer activity of hydro-ethanolic extract of *F. auriculata* leaves. The extract showed a potential antiulcer activity that could be due to its effect on prostaglandin secretion via cyclooxygenase activation and its antioxidant stimulating effect. The extract can be further developed into a better cost-effective antiulcer drug due to its high percentage ulcer inhibition in the rat models.

ACKNOWLLEDGEMENTS

The technical support of Mr. Vincent Upev, Mr. Martins Ishu is strongly acknowledged and appreciated. We also acknowledge Prof. C.O. Ezihe, the Head of Department of Veterinary Physiology and Biochemistry, Joseph Sarwuan Tarka Univeristy Makurdi for making some equipment and reagents available for the work. This research was Self funded.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this work.

REFERENCES

Abubakar, A.R. & Haque, M. (2020). Preparation of medicinal plants: basic extraction andfractionation procedures for experimental purposes. *Journal of Pharmacy andBioallied Scienc*e, 12, 1-10.

Adane, H.,Seyfe, A.A.,Zemene, D.K. & Digambar, A. (2021). Evaluation of In Vivo Antiulcer Activity of Hydro-Methanol Extract and Solvent Fractions of the Stem Bark of *Ficus thonningii* (Moraceae) on Rodent Models. *Biomedical Research International*, 3, 23-50.

Adinortey, M.B., Ansah, C., Galyuon, I., Nyarko, A. (2013). In vivo models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, 2013, Article ID 796405.

Ahmed, F., Chavan, S., Satish, A. & Punith, K.R. (2011). Inhibitory activities of *Ficus benghalensis* bark against carbohydrate hydrolyzing enzymes - an in vitro study. *Pharmacognosis Journal*, 3(20), 33–37.

Ajaikumar, K.B., Asheef, M.B., Babu, H. & Padikkala, J. (2005) . The inhibition of gastric mucosal injury by P*unica granatum* l. (pomegranate) methanolic extract. *Journal of Ethnopharmacology*, 96, 171–176.

Alarcon De La Lastra, C., Martin, M.J. & Motilva, V. (1994). Antiulcer and gastroprotective effects of quercetin, a gross and histologic study. *Pharmacy*, 48, 56-62.

Allam, M.M. & El-Gohary, O.A. (2017). Gastroprotective effect of ghrelin against indomethacin-induced gastric injury in rats: Possible role of heme oxygenase-1 pathway. *General Physiology and Biophysics*, 36, 321–330.

Anitha, R.S. & Stuti, M. (2011). Ficus racemosa: Phytochemistry, Traditional Uses and Pharmacological Properties: A Review. *International Journal of Research in Ayurveda and Pharmacy*, 4, 6-15.

Atoui, A.K., Mansouri. A., Boskou, G. & Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry*, 89, 27–36.

Awaad, A.S., El-Meligy, R.M. & Soliman, G.A. (2023). Natural products in treatment ofulcerative colitis and peptic ulcer. *Journal of Saudi Chemical Society*, 17, 101–124.

Benchikh, F. (2008). *Pharmacological E_ects of Myrtus Communis L. on the Gastrointestinal Tract of Rats and Mice*. Ph.D. Thesis, Department of Biology and Animal Physiology, Faculté des Sciences de la Nature et de la Vie, Université Ferhat Abbas Sétif 1, Setif, Algeria.

Bhakta, P.G., Ramakanta, L., Chitra, B.S., Amrita, S., Sabita, N. & Sushil, P. (2011). Phytochemical Screening and Analysis of Antiacterial and Antioxidant activity of *Ficus auriculata* (Lour.) Stem bark. *Phamacology Journal*, 3(21), 49-55.

Calzetta, L., Pistocchini, E., Leo, A., Roncada, P., Ritondo, B.L. & Palma, E. (2012).Anthelminthic medicinal plants in veterinary ethnopharmacology: a network meta-analysis following the PRISMA-P and PROSPERO recommendations, *Heliyon*, 6, e03256.

Catarina, S., Maria, E.A., Edvaldo, A.J., Margareth, D., Clélia, H. & Leônia, B. (2020). A review of the role of flavonoids in peptic ulcer (2010–2020). *Molecules*, 25, 5431.

Dave, R. (2017). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. *African Journal of Microbiology Research*, 3(13), 981–996.

El-Fishawy, A., Zayed, R. & Afifi, S. (2011). Phytochemical and pharmacological studies of *Ficus auriculata* Lour. *Journal of Natural Product*, 4, 184-195.

Eriyamremu, G. & Iorliam, B.E. (2014). Biochemical evaluation of ulcer curative effect of ethanolic and aqueous extracts of Schwenkia americana Linn on experimental rats. *Journal of Pharmacy and Pharmacology*, 3 (4), 125–130.

Galanakis, C., Goulas, V., Tsakona, S., Manganaris, G.A. & Gekas, V. (2013). A knowledge baseforthe recovery of natural phenols with different solvents. *International Journal of Food Properties*, 16(2), 382–396.

Gill, G.W. (2010). Haematoxylin and Eosin staining. Education Guide: Special Stains and Haematoxylin

and Eosin (2nd ed.). Dako North America, pp. 119-128.

Hazarika, I., Hussain, M. & Das, A. (2015).Anti-Ulcer Activity of Ethanolic Extract of *Sesamum indicum* Seed on Indomethacin-Induced Ulcer Model and Its Antioxidant Property in Gastro-Protection. *Research & Reviews: Journal of Pharmacy*, 18-25

Izzo, A.A., Di Carlo, G., Mascolo, N. & Capasso, F. (1994). Antiulcer effect of flavonoids: role of endogenous PAF. *Phytotherapy Research*, 8, 179-181.

Kahraman, A., Erkasap, N., Koken, T., Serteser, M., Aktepe, F. & Erkasap, S. (2003). The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. *Toxicology*, 183, 133-142.

Kaur, A., Singh, R., Sharma, R. & Kumar, S. (2012). Peptic Ulcer: A review on ethiology and pathogenesis. *International Research Journal of Pharmaceutics*, 2230- 8407.

Kavitha, C.C.I. & Revikuma, K.G. (2017). *Ficus auriculata* (elephant ear fig): a phytochemical and pharmacological review. *World Journal of Pharmaceutical and Pharmaceutical Sciences*, 6 (5).

Kim, J.H., Kim, B.W., Kwon, H.J. & Nam, S.W. (2011). Curative effect of selenium against indomethacin-induced gastric ulcers in rats. *Journal of Microbiology & Biotechnology*, 21, 400–404.

Kulkarni, K. (2002). Hand Book of Experimental Pharmacology (3rded.). New Delhi, India: Vallabh Prakashan.

Lewis, W.H. & Elvin-Lewis, M.P. (2012). Medical Botany: Plants Affecting Human Health, (2nd ed.) New York, John Wiley and Sons, PP. 812.

Malfertheiner, P., Chan, F.K.L. & McColl, K.E.L. (2009). Peptic ulcer disease. *Lancet* 374, 1449–1461.

Melese, E., Asres, K., Asad, M. & Engidawork, E. (2011). Evaluation of the antipeptic ulcer activity of the leaf extract of Plantago lanceolata L. in rodents. *Phytotherapy Research*, 25 (8), 1174-1180.

Mohammed, R., Mohammed, U. & Patil, R.S. (2013). Medicinal Uses of *Ficus racemosa* Linn. *International Journal of Pharmceutical & Biologiy Archive,* 2(3), 33-42.

National Research Council. (2011). Guide for the Care and Use of Laboratory Animals, Washington, DC, USA. National Academies.

Nowacek, M.J. (2010). Fixation and tissue processing. Education Guide: Special Stains and Haematoxylin and Eosin (2nd ed). Dako North America, pp. 141 – 152.

OECD. (2022).Guidelines for the Testing of Chemicals. Vol. 4. pp. 1–27, OECD, Paris, France.

Polat, B., Albayrak, Y., Suleyman, B., Dursun, H., Odabasoglu, F., Yigiter, M. & Suleyman, H. (2011). Antiulcerative effect of dexmedetomidine on indomethacin-induced gastric ulcer in rats. *Pharmacological Reports*, 63, 518–526.

Ramakrishnan, K. & Salinas, R.C. (2007). Peptic ulcer disease. *Amarican Family Physician*, 76, 1005-112.

Rekka, R., Nirubama, K.& Moorthy, D. (2021). Evaluation of phytochemical screening and antibacterial activity of *ficus auriculata* lour. (moraceae) – an traditional medicinal plant. *Kongunadu Research Journal,* 8 (1), 22-29.

Rosalind, T., Biman, K.D. & Satya, B.P. (2012). Invitro antioxidant capacity, Estimation of Total Phenolic and Flavonoid content of *Ficus auriulata* Lour. *International Journal Pharmacy & Pharmceutical Science*, 4(4), 518-521.

Salem, M.E., Atega, A., Afifa, O. & Fouzy, A. (2016). Qualitative and quantitative analysis of phytochemicals of various extracts for Ephedra altissima from Libya. *Journal of Medicinal Plant Studies*, 4(3), 119 – 121.

Satish, A., Bhalerao, A. & Amit, S. (2014). Sharma. Ethenomedicinal, Phytochemical profile of Ficus religiosa Roxb. *International Journal of Current Microbial & Applied Science*, 3(11), 528-538.

Saziki, R., Arai, I., Isobe, Y., Hirose, H. & Aihara, H. (1984). Effects of sofalcone on necrotizing agents-induced gastric lesions and on endogenous prostaglandins in rat stomachs. *Journal of Pharmacobiodynamics*, 7, 791-797.

Shahinuzzaman, M., Akhtar, P., Amin, N., Ahmed, Y., Anuar, F.H., Misran, H. & Akhtaruzzaman, M. (2020). New insights of phenolic compounds from optimized fruit extract of *Ficus auriculata.Scientific Reports*,11, 12503.

Shankar, B.R. (2017). Anti-ulcer potential of saponin fraction of Trichopus zeylanicus on a various experimental animal models. *International Journal of Green Pharmacy*, (11), 1.

Shawon, L. & Gautam, P.(2012). An overview of the current methodologies used for evaluation of gastric and duodenal antiulcer agents. *Pharmacologia*, 3 (8), 249-257.

Suleyman, H., Cadirci, E., Albayrak, A., Polat, B., Halici, Z., Koc Ahmet, F. & Hacimuftuoglu, Y.B. (2009). Comparative study on the gastroprotective potential of some antidepressants in indomethacin-induced ulcer in rats. *Chemico-Biological Interactions*, 180, 318–324.

Sunairi, M., Watanabe, K., Suzuki, T., Tanaka, N., Kuwayama, H. & Nakajima, M. (1984). Effects of

anti-ulcer agents on antibiotic activity against *Helicobacter pylori*. *European Journal of Gastroenterology and Hepatology*, 6(Suppl 1), S121-S124.

Tamta, G., Mehra, N. & Tandon, S. (2021). Traditional Uses, Phytochemical and pharmacological properties of *Ficus auriculata*: A Review. *Journal of Drug Delivery and Therapeutics*, 11(3), 163-169.

Tanih, N.F., Okeleye, B.I., Naido, N., Clarke, A.M., Mkweshana, N., Green, E., Ndip, L.M. & Ndip, R.N. (2010). Marked susceptibility of South African *Helicobacter pylori* strains to ciprofloxacin and amoxicillin: *Clinical Implication*,100, 49–52.

Thingbaijam, R., Dutta, B.K. & Paul S.B. (2012). In vitro antioxidant capacity, estimation of total phenolic and flavonoid content of *Ficus auriculata lour*. *International Journnal of Pharmaceutical Science*, 4(4), 518–521.

Trease, G.E. & Evans, W.C. (2002). *Pharmacognosy.* (15th ed). London: Saunders Publishers; . pp. 42-393.

vu Nguyen, T., Le H.C. & Nguyen, G.K. (2006). Weintraub A. Etiology and epidemiology ofdiarrhoea in children in Hanoi, Vietnam. *International Journal of Infectoius Diseases*, 10(4), 298-308.

Wang, L., Wang, Z. & Li, X. (2013). Optimization of ultrasonic-assisted extraction of phenolic antioxidants from *Malus baccata* (Linn.) Borkh. using response surface methodology. *Journal of Seperation Science*, 36(9–10), 1652–1658.

Yinxian, S., Huabin, H., Youkai, X. & Aizhongli U. (2014). An ethnobotanical study of the less known wild edible figs (genus Ficus) native to Xishuangbanna, Southwest China, *Journal of Ethnobiology & Ethno-medicine*, 10(68), 1-10.

Yoshikawa, T., Naito, Y., Kishi, A., Tomii, T., Kaneko, T., Iinuma, S., Ichikawa, H., Yasuda, M., Takahashi, S. & Kondo, M. (1993). Role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut*, 34, 732–737.

Zaghlool, S.S., Abo-Seif, A.A., Rabeh, M.A., Abdelmohsen, U.R. & Messiha, B.A.S. (2019). Gastro-protective and anti-oxidant potential of Althaea o_cinalis and Solanum nigrum on Pyloric Ligation/Indomethacin-Induced Ulceration in Rats. *Antioxidants*, 8, 512.

Zaghlool, S.S., Shehata, B.A., Abo-Seif, A.A. & El-Latif, H.A.A. (2015). Comparison between the protective effects of famotidine, ginger and marshmallow on pyloric ligation-induced peptic ulcer in rats. *Journal of Biochemistry and Biophysics*, 7, 170–178.

Zheng, Y.F., Xie, J.H., Xu, Y.F., Liang, Y.Z., Mo, Z.Z., Jiang, W.W., Chen, X.Y., Liu, Y.H., Yu, X.D. & Huang, P. (2014). Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats. *Chemico-Biological Interactions*, 222, 27–36.