PROPHYLACTIC AND CURATIVE EFFECTS OF DIOSPYROS MESPILIFORMIS (Jackalberry) LEAVES EXTRACT ON ETHANOL INDUCED GASTRIC ULCER IN RATS

LUKA, S., ADEFOLALU, F. S.*, JIGAM, A. A., & EGWIM, E. C.
Department of Biochemistry Federal University of Technology Minna, Nigeria
*E-mail: adeffun@gmail.com Phone No: +234-803-451-2717

Abstract

Gastric ulcer is known to be associated with alterations in some physiological parameters such as lipid peroxides, reactive oxygen species and gastric juice production causing great discomfort and harm to people worldwide. This study aimed at evaluating the prophylactic (protective) and curative antiulcer potential of 80 % methanol extract of Diospyros mespiliformis (D. mespiliformis) leaf on ethanol induced gastric ulcer in albino rats. Acute toxicity, ulcer index (UI), total acidity (TA), percentage protection from ulcer (PPU), percentage ulcer reversal (PUR) and hepatotoxicity were determined using standard methods. Ranitidine (100 mg/kg body weight) served as the standard antiulcer drug. No death was recorded for the acute toxicity test even at 5000 mg/kg bw of extract of D. mespiliformis leaves. There was a significant difference (p>0.05) in the ulcer index of the control (6.00±0.66) when compared with that of the animals treated for prophylactic with 200, 400 mg/kg bw of extract of D.mespiliformis leaf and ranitidine (0.83±0.06, 0.50±0.00 and 0.50±0.00 respectively). The PPU for 200, 400 mg/kg bw of plant extract were 86.16 and 91.66 % respectively while that of ranitidine was 55.53 %. The PUR for curative treatment for 200 and 400 mg/kg of the plant extract, and ranitidine were 80.66, 91.66 and 97.33 % respectively. The extract at 200 and 400 mg/kg bw decreased the TA Level both for prophylactic (56.54±5.35 and 53.45±3.45 mE/l) and curative (56.16±0.1 and 64.5±0.00 mE/l) in a dose dependent manner when compared both with the control (76.60±3.57mE/l) and ranitidine (41.45±4.67 and 21.34± 3.45 mE/L for prophylactic and curative respectively). Serum liver enzymes were not significantly different for the curative treatment and for ranitidine compared to the normal control. Diospyros mespiliformis leaf extract have both prophylactic and curative effects however, it exhibits better prophylactic antiulcer potential than the standard drug and may be toxic at higher concentration of prolonged use.

Keywords: *Diospyros mespiliformis,* Gastric ulcer, Prophylactic, curative and Ranitidine

Introduction

Ulcers are open sores of the skin or mucus membrane characterized by sloughing of inflamed dead tissue (Chan and Graham, 2004). Ulcers form lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. There are many known types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer (Meyer-Rosberg *et al.,* 2016), of these, peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum (Bhowmik *et al.*, 2010). The two most common types of peptic ulcer are called “gastric ulcer” and “duodenal ulcer.” The name refers to the site of ulceration. A person may have both gastric and duodenal ulcers at the same time. Gastric ulcers are located in the stomach, it is common in older age group and characterized by nausea, vomiting, weight loss and pain; Eating may increase pain rather than relieve pain. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in complete absence of acid (Vyawahare *et al.*, 2009).

Duodenal ulcers are found at the beginning of small intestine and are characterized by severe pain with burning sensation in upper abdomen that awakens patients from sleep. Generally, pain occurs when the stomach is empty and its relieved after eating. A duodenal ulcer is more common in younger individuals and predominantly affects males. In some cases, peptic ulcer

can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting blood (Bagalkotkar *et al*., 2011).

The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (Lau *et al*., 2011). Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors and the real causes have been found by research to include bacterial infection (*Helicobacter pylori*) or reaction to various medications, particularly NSAIDS (nonsteroidal anti-inflammatory drugs) (Kasper *et al*., 2005). Emotional stress, alcohol abuse, and smoking are also etiological factors associated with peptic ulcer (Malfertheiner *et al*., 2009).

Currently treatment of ulcers in developing countries has been largely by the suppression of pain, with little or no successful strategy aimed at a cure. Herbal medicine is fast emerging as an alternative to available synthetic drugs for the treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness from ethno medical practices. Some tropical herbs have been scientifically reported to possess potent antiulcer activity (Kumar, 2011; Singh *et al*., 2008).

Diospyros mespiliformis also known in Nigeria as Kanya (Hausa) and in Igidudu. (Yoruba) is commonly called Jackal-berry or African ebony (Mann, 2003). The plant is reputed for its medicinal values, and is used in ethno medical practice for treating various ailments that include sleeping sickness, malaria, headache, cough, leprosy, helminth infection toothache (Adzu *et al*., 2015; (Bagalkotkar *et al*., 2011 and Belemtougri *et al*., 2006). Its seeds are also known to have nutraceutical value in managing high cholesterol, reducing risk of type-2 diabetes and for weight control (Chivandi and Erlwanger, 2001). It is also used in Nigeria for the treatment of diabetes, high blood pressure, coronary heart diseases, and ulcer (Adefolalu *et al*., 2017). Useful biologically active compounds including naphthoquinone epoxide, α-amyrin, β-sitosterol, betulin and betulinic acid amongst others had been isolated from the plant (Mohammed *et al*., 2009).

This research work was aimed at determining the anti-ulcer effect of the methanol extract of *Diospyros mespiliformis* leaves on ethanol induced gastric ulcer in rats.

Materials and Methods

Collection of *D. mespiliformis* Leaves

Fresh leaves of *Diospyros mespiliformis* was obtained in October, 2017 from Agwara Local Government Area, of Niger State Nigeria. Taxonomic authentications of the plants were carried out by a Botanist at the Department of Biological science, Federal University of Technology, Minna, Niger State.

Chemicals

The solvent methanol, ranitidine (standard drug) and other chemicals used were obtained commercially and were of analytical grade.

Preparation of crude extract

The fresh leaves of *Diospyros mespiliformis* collected were destalked, washed with clean-water, dried at room temperature (27^{o}C) and grounded using a grinder mill. The powdered leaves (300g) was extracted with 80% methanol (600 ml) using the continuous cold extraction method for 72 hours (Kalra *et al*., 2011). The extract was filtered, concentrated using a rotary

evaporator. The resulting extract was allowed to dry in a water bath at 45 ^{o}C) and stored in refrigerator prior to use.

Experimental Animals

Apparently healthy albino rats weighting between 150-180 g were purchased from Biochemistry Department, Federal University of Technology, Minna, Niger State. Nigeria. The rats were kept in clean plastic cages and maintained under standard laboratory conditions in the animal house. They were allowed unrestricted access to poultry feed (grower mash) and water *ad-libitum*. The animals were allowed to acclimatize for two weeks. The study was carried out according to the Guide for the Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA.

Acute toxicity Study

The acute toxicity study was conducted in accordance with Lorke's method (Lorke's, 1983). The acute toxicity study was conducted to observe the range of toxicity so the proper dose level could be established. The study was conducted in two phases. In the first phase nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were orally given 10, 100 and 1000 mg/kg body weight of the extract respectively, in addition a fourth group served as control (and rat in the group were not given the extract. In the second phase the animals received 1600, 2900 and 5000 mg/kg body weight dose of the extract to group 1, 2, and 3 respectively.

Induced Ulcer and Treatment

Twenty four apparently healthy Wister albino rats were divided into eight groups with each group comprising of three (3) animals. All animals were deprived of food (but not water) for 24 hours prior to inducing ulcer with ethanol. Gastric ulcer was induced by the oral administration of 70 % ethanol at a dose of 0.5 mL/100 g body weight on 24 hour empty stomach. Group 1 was not induced and not treated while group 2 was induced with ulcer but not treated (positive control for the curative). Groups 3 after inducing ulcer received 10 mg of ranitidine (standard) while groups 3 and 4 received 200 and 400 mg/kg body weight of methanol extract of *Diospyros mespiliformis* leaf extract (curative groups) for seven days respectively. Groups 6 to 8 (prophylactic groups) received 200, and 400 mg/kg body weight of extract of *D. mespiliformis* leaf. Rantidine (100 mg/kg body weight) served as reference anti-ulcer drug (Suleyman *et al*., 2004; Vasudeva *et al*., 2012; Mard *et al*., 2008).

Measurement of Ulcer Index (UI)

The animals were placed under anesthesia (diethylether) one after the other prior to being dissected. The stomachs were cut opened along the greater curvature, rinsed with saline to remove gastric contents, blood clots and examined with a microscope (10X magnifier lens) to assess the formation of ulcers. The numbers of ulcers were counted as described by Vasudeva *et al*. (2012)

Table 1: Ulcer Scoring Index

Degree of ulcer	Scores index
Normal stomach colour	0
Red stomach coloration	0.5
Spot ulcer	1.0
Hemorrhagic streak	1.5
Ulcer > 3 mm < 5mm	2
Ulcers > 5mm	3

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined using the formula of Samaresh *et al.,* (2013) as given below.

$$\text{Protective Ratio (PR)} = 100 - \frac{\text{Ulcer index of treated animal}}{\text{Ulcer index of control animal}} \times 100$$

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was recorded. The total acidity is expressed as mEq/L by the following formula:

$$\text{Total Acidity} = \frac{\text{Volume of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}$$

Where N= Normality of NaOH

$$\text{Prophylactic/Curative Effect Calculation} = 100 - \frac{\text{Ulcer index of treated}}{\text{Ulcer index of control}} \times 100$$

Hepatotoxicity of methanol extract of *Diospyros mespiliformis* leaf in ethanol induced ulcer in rats

The methods of Reitman and Frankel 1957 and Wright *et al*. 1972) were used. Blood was collected by cardiac puncture into EDTA bottles and centrifuged at 3,000 rpm for 10 minutes and serum was store below 4 °C for assay. Enzyme assay kits (Randox Laboratories, Co-Antrim, UK) were used to assay for Alanine aminotransferase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP) activities.

Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities

The sample (50 µl) and 500 µl of the ALT and AST reagents were each seperately mixed in a test tube, and the initial absorbance at 340 nm was read after 1 minute and further readings of the absorbance were taken after 1, 2, and 3 minutes and calculations made from as shown below.

$$\text{ALT/ AST activity (nm/min)} = 1\,746 \times \Delta A\ 340\ \text{nm/min}$$

Where:

ΔA 340 nm/min =change in absorbance per minute for the homogenate sample,

1746 = Extinction coefficient of Absorbance.

Alkaline phosphatase (ALP) Activity

In a cuvette, 10 µl of sample was mixed with 500 µl of Randox reagent (a solution containing p-NPP (Para- Nitrogen pyrophosphate). The initial absorbance was read at 405 nm, and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation as below:

$$\text{ALP activity (IU/l)} = 2742 \times \Delta A\ 405\ \text{nm/min};$$

Where: 2742 = Extinction coefficient of Absorbance;

ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

Data Analysis

Values were analyzed using statistical package for social sciences (SPSS) version 16 and presented as means ± SE of the mean. Comparisons between the different groups were

carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $p < 0.05$ (Adamu and Johnson, 1997).

Results

Acute Oral Toxicity Study

Table 2 shows the acute toxicity studies result. No death was recorded in the animals receiving 80 % methanol extract of *Diospyros mespiliformis* leaves up to a dose of 5000 mg/kg body weight. Furthermore, the animals did not show any changes in general behavior and other physiological activities such as giddiness, sniffing, aggressiveness, tachypnoea, or convulsion. Lethal dose (LD_{50}) of the plant extract could not be calculated because no death was recorded even at 5000 mg /kg body weight of plant extract in rats.

Prophylactic effect of methanol extract of *D. mespiliformis* leaves in ethanol ulcer induced rats

Table 3 presents the prophylactic effect of extract *of Diospyros mespiliformis* leaf on ethanol ulcer induced animals. Ulcer protection for the animals treated with 200 and 400 mg/kg body weight of extract were 86.16 % and 91.66 % respectively while 55.53 % was ranitidine treated animals. The total acidity of the gastric secretions was found to be 76.60±3.57 mEq/l for control. Pretreatment with 200 and 400 mg/kg body weight of extract of *D. mespiliformis* leaf and ranitidine significantly (*p<0.05*) reduced the total acidity compared to the control. Treatment with extract of *D. mespiliformis* leaf however resulted in higher protection from ulcer and also reduced the total acidity more than ranitidine.

Curative effect of methanol extract of *D. mespiliformis* leaf in ulcer induced rats

Table 4 presents the result for curative antiulcer effect of extract of *Diospyros mespiliformis* leaf. Curative effect was 80.66 % and 91.66 % for the 200 and 400 mg/kg body weight of leaf extract respectively, whereas ranitidine showed 97.33 % ulcer curative effect. The total acidity of the gastric secretions were found to be 76.60±3.57 mEq/l. Prophylactic treatment with the extract significantly ($p<0.05$) reduced the total acidity for the 200 and 400 mg/kg doses respectively. While the antiulcer effect of ranitidine was lower than that of the plant extracts for curative assay.

Table 5 presents the hepatoxicity effect of methanol extract of *D. mespiliformis* leaf in ethanol induced ulcer in rats for prophylaxis and curative. There was a significant ($p<0.05$) increase in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the ulcer induced untreated animals (negative control). The prophylactic, curative extract and ranitidine treatments caused a significant decrease in serum enzyme activities when compared with the negative control.

Table 2: Acute Oral Toxicity effect of methanol extract of *Diospyros mespiliformis* leaf *in* wister albino rats

Group	Dosage (mg/kg)	No of animals	Mortality
Group 1	10	3	0/3
Group 2	100	3	0/3
Group 3	1000	3	0/3
Group 1	1600	3	0/3
Group 2	2900	3	0/3
Group 3	5000	3	0/3

Table: 3: Prophylactic Effect of Methanol Extract of *D. mespiliformis* Leaf in Ethanol Induced Ulcer in Rats

Treatments.	Ulcer Index	Total Acidity (mEq/l)	% Ulcer Protection (PPU)
Control	6.00±0.66^{c}	76.60±3.57^{c}	-
Ranitidine	2.67±0.21^{b}	41.45±4.67^{a}	55.53
DME 200 mg/kg	0.83±0.06^{ab}	56.54±5.35^{b}	86.16
DME 400 mg/kg	0.50±0.00^{a}	53.45±3.45^{b}	91.66

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).
DME = Methanol extract of *D. mespiliformis* leaf

Table 4: Curative Effect of Methanol Eextract of *D. mespiliformis* Leaf in Ethanol Induced Ulcer in Rats

Treatments	Ulcer index (UI)	Total Acidity (mEq/l)	% Ulcer Reversal (PUR)
Control	6.00±0.66^{c}	76.60±3.57^{c}	-
Ranitidine (100mg/kgbw)	0.16±0.09^{a}	21.34±3.45^{a}	97.33
DME 200 mg/kgbw	1.16±0.10^{b}	56.16±0.10^{b}	80.66
DME 400 mg/kgbw	0.50±0.00^{a}	64.50±0.00^{b}	91.66

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).
DME = Methanol extract of *D. mespiliformis* leaf

Table 5: Hepatotoxicity Effect of Methanol Extract of *D. mespiliformis* Leaf for Prophylaxis and Curative in Ethanol Induced Ulcer in Rats

Treatments	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control	59.33±3.45^{a}	55.67±3.46^{a}	45.83±3.45^{a}
Negative Control	98.00±3.25^{bc}	126.08±4.56^{d}	187.00±0.09^{d}
RSD (Prophylactic)	58.00±5.67^{a}	100.98±3.45^{c}	61.90±2.34^{b}
RSD (Curative)	53.50±4.56^{a}	63.08±3.45^{a}	90.75±1.24^{c}
DMELD (Prophylactic)	105.50±6.07^{c}	117.50±5.67^{cd}	38.50±3.55^{a}
DMELD(Curative)	57.50±3.45^{a}	51.90±4.23^{a}	50.50±3.45^{b}
DMEHD (Prophylactic)	54.06±4.56^{a}	102.33±3.45^{c}	87.00±3.09^{c}
DMEHD (Curative)	89.33±3.56^{b}	80.50±4.45^{b}	56.83±5.67^{b}

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).
RSD = Ranitidine Standard Drug (100 mg/kgbw)
DMELD = Methanol extract of *D. mespiliformis* lower Dose (200 mg/kgbw)
DME = Methanol extract of *D. mespiliformis* Higher Dose (400 mg/kgbw)

Discussion

Lethal dose (LD_{50}) of extract of *Diospyros mespiliformis* leaf could not be determined due to the absence of death among the experimental animals even at the administration of 5000 mg/kg body weight of extract of *Diospyros mespiliformis* leaf during and 72 hrs after the acute

oral toxicity test. This suggest that *Diospyros mespiliformis* leaf extract could be safe as established by Lorkes, (1983) that doses beyond 5000 mg/kg body weight of any substance orally administered is safe.

The higher value of the ulcer index (UI) for the control animal compared to the animals which received extract of *Diospyros mespiliformis* leaf (200 and 400 mg/kg body weight) and ranitidine (100 mg/ kg b.w) for seven days in both the curative and prophylactic treatment groups suggest the extract and ranitidine may have antiulcer impact on the stomach tissues resulting in the reduced production of free radicals after the extracts were administered. This finding is also in line with Tarek *et al.,* (2012) who recorded a corresponding high ulcer index in the control of an antiulcer study of the protective and therapeutic effect of *Argyreia speciosa* against ethanol induced gastric ulcer in rats. Furthermore, the significant ($p< 0.05$) antiulcer effect of the extract of *Diospyros mespiliformis* leaf may be attributed to the active phytochemicals. It is reported that plants with promising antioxidant properties are able to either suppress the production of free radicals and/or reduce the activity of reactive oxygen species which are responsible for ulceration (Nordmann, 2014). Many tropical herbs including *Diospyros mespiliformis* have been scientifically reported to possess potent antioxidant activity (Kumar, 2011; Singh *et al.*, 2008).

Phytochemicals in *Diospyros mespiliformis* are capable of gastroprotective activity by preventing damage to the stomach wall from ethanol toxicity or any other substance that can induce ulcer possibly through the stimulation of mucus membrane. The presence of flavonoid a phytochemical with antioxidant properties in *Diospyros mespiliformis* plant reported by Adefolalu, *et al.*, (2017), supports the plants use for the prevention of stress mediated cell injury including ulcer. The presence of flavonoid a phytochemical with antioxidant properties in *Diospyros mespiliformis* plant reported by Adefolalu, *et al.*, (2017), supports the plants use for the prevention of stress mediated cell injury including ulcer. The gastroprotective effect displayed by the extract and ranitidine agrees with the report of Tarek *et al.,* (2012) who reported a dose dependent decrease in ulcer lesion count for *Argyreia speciosa*.

The curative effect for ulcer (97 %) recorded for ranitidine which is significantly higher than that recorded for extract of *Diospyros mespiliformis* leaf (91.66 %) while the prophylactic antiulcer effect displayed by the extract was better than ranitidine suggest that ranitidine may be more suitable for curing ulcer while the plant extract may be better utilized to protect against ulcer. Also the plant extract antiulcer activity may still improve if further purified.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage (Itopa, *et al.,* 2019). The increase in the liver enzyme activity in the negative control agrees with the report of Roland *et al.*, (2009) who stated that induced stress of any kind can lead to a release of liver enzymes into blood circulation. Serum ALT and AST activities were not significantly different for curative test animals at lower dose when compared with those in the normal control and that of ranitidine. This is an indication that the integrity of liver has not been compromised at this dose level. Alkaline phosphatase was particularly elevated suggesting a possible liver toxicity by ethanol causing a likely damage to the liver cell membranes resulting in the release of ALP. ALP is a membrane bound enzyme (Akanji *et al.*, 2013).

Conclusion

Methanol extract of *D. mespiliformis* leaf have both curative and prophylactic effect against ethano-induced gastric ulcer. The prophylactic effect displayed by the plant extract against the induced ulcer was significantly higher than that of the curative effects. Therefore,

methanol extract *D. mespiliformis* leaf if further screened and purified could be a novel source of antiulcer remedy.

References

Adamu, S.O., & Johnson, T. L. (1997). *Statistics for Beginners, Book 1.* Ibadan: SAAL. Publications, 184-199.

Adefolalu, F. S., Jigam, A. A., Akanya, H. O., & Luka, S. (2017). Quantitative phytochemical determination and antioxidant study of *Diospyros mespiliformis* leaves extract on ethanol induced gastric ulcer in rats). In book of abstract: 30th Annual International Conference of Biotechnology Society of Nigeria (BSN) held at Federal University of Technology, Minna from 27th – 30th August, 2017. pp 100.

Adzu, B., Chindo, B. A., Tarfa, F. D., Salawu, O. A., & Igoli, O. J. (2015). Isolation and analgesic property of lupeol from Diospyros mespiliformis stem bark. *Journal of Medicinal Plants Research*, *9*(30), 813-819.

Akanji, M. A., Salau, A. K., & Yakubu M. T. (2013).Safety Evaluation of Aqueous Extract of *C rateva adansonii* Leaves on Selected Tissues of Rats. *FountainJournal of Natural and Applied Sciences*, 2(1), 17 – 28

Bagalkotkar, G., Chuan, T. S., Khalivulla, S. I., Hamzah, A. S., Shaari, K., Lajis, N. H., ... & Stanslas, J. (2011). Isolation and cytotoxicity of triterpenes from the roots of Phyllanthus pulcher Wall. ex Mll. Arg.(Euphorbiaceae). *African Journal of Pharmacy and Pharmacology*, *5*(2), 183-188.

Belemtougri, R. G., Constantin, B., Cognard, C., Raymond, G., & Sawadogo, L. (2006). Effects of two medicinal plants Psidium guajava L.(Myrtaceae) and Diospyros mespiliformis L.(Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. *Journal of Zhejiang University-Science B*, *7*(1), 56-63.

Bhowmik, D., Chiranjib, T. K., & Pankaj, K. S. (2010). Recent trends of treatment and medication peptic ulcerative disorder. *Int J Pharm Tech Research*, *2*(1), 970-80.

Chan, F. K. L., & Graham, D. Y. (2004). Prevention of non-steroidal anti-inflammatory drug gastrointestinal complications–review and recommendations based on risk assessment. *Alimentary Pharmacology & Therapeutics*, *19*(10), 1051-1061.

Ezike, A. C., Akah, P. A., Nnamani, E. M., Okoli, C. O., Ojike, F. U., Eze, F. S., ... & Azosiri, I. J. (2011). Studies on the Antiulcer and Gastrointestinal Effects of Stem Bark Extract of Bridelia ferruginea. *Journal of Complementary and Integrative Medicine*, *8*(1).

Gokhale, A. B., Damre, A. S., & Saraf, M. N. (2003). Investigations into the immunomodulatory activity of Argyreia speciosa. *Journal of Ethnopharmacology*, *84*(1), 109-114.

Itopa, U. S., Aliyu, N. M., Jigam, A., Adefolalu, F S., Ibikunle, G. F., & Lawal, B. (2019). Anti-plasmodial, Anti-inflammatory, anti-nociceptive and safety profile of Maytenus senegalensis root bark extract on hepato-renal integrity in experimental animals. *Nature Springer*: *Comparative Clinical Pathology* https://doi.org/10.1007/s00580-019-02965-4

Kalra, P., Sharma, S., & Suman, S. K. (2011). Antiulcer effect of the methanolic extract of Tamarindus indica seeds in different experimental models. *Journal of Pharmacy and Bioallied Sciences*, *3*(2), 236.

Kasper, D. L., Braunwald, E., Fauci, A. S., Hauser, S. L., Longo, D. L., & Jameson, J. L. (2005). *Harrison's principles of internal medicine.* New York, Jonhn Wiley, 1467-1468.

Kumar, M. R., Niyas, M. K., Mani, T. T., Rahiman, O. M. F., & Kumar, S. B. (2011). A review on medicinal plants for peptic ulcer. *Der Pharmacia Lettre*, *3*(3), 414-420.

Lam, E. K., Tai, E. K., Koo, M. W., Wong, H. P., Wu, W. K., Yu, L., ... & Cho, C. H. (2007). Enhancement of gastric mucosal integrity by Lactobacillus rhamnosus GG. *Life sciences*, *80*(23), 2128-2136.

Lau, J. Y., Sung, J., Hill, C., Henderson, C., Howden, C. W., & Metz, D. C. (2011). Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. *Digestion*, *84*(2), 102-113.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, *54*(4), 275-287.

Malfertheiner, P., Chan, F. K., & McColl, K. E. (2009). Peptic ulcer disease. *The Lancet*, *374*(9699), 1449-1461.

Mann, A., Gbate, M., & Umar, A. N. (2003*). Medicinal and economic plant of Nupe land*. Minna: Jude Evans Books and Publication: pp 203.

Mard, S. A., Bahari, Z., Eshaghi, N., & Farbood, Y. (2008). Antiulcerogenic effect of Securigera securidaca L. seed extract on various experimental gastric ulcer models in rats. *Pakistan Journal of Biological Sciences*, *11*(23), 2619.

Meyer-Rosberg, K. N., Scott, D. R., Rex, D., Melchers, K., & Sachs, G. (1996). The effect of environmental pH on the proton motive force of *Helicobacter pylori*. *Gastroenterology*, *111*(4), 886-900.

Mohamed, I. E., El Bushra, E., Choudhary, M. I., & Khan, S. N. (2009). Bioactive natural products from two Sudanese medicinal plants Diospyros mespiliformis and Croton zambesicus. *Records of Natural Products*, *3*(4), 198.

Nordmann, R. (1994). Alcohol and antioxidant systems. *Alcohol and Alcoholism,* 29(5)513-522.

Normandin, S. (2007). Claude Bernard and an introduction to the study of experimental medicine: Physical vitalism, dialectic, and epistemology. *Journal of the History of Medicine and Allied Sciences*, *62*(4), 495-528.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology,* 28,56–63.

Singh, R., Madan, J., & Rao, H. (2008). Antiulcer activity of black pepper against absolute ethanol induced gastric mucosal damage in mice. *Pharmacognosy Magazine*, *4*(15), 232.

Suleyman, H., Demirezer, L. O., & Kuruuzum-Uz, A. (2014). Effects of Rumex patientia root fractions on indomethacin and ethanol induced gastric damage in rats. *Pharmazie*, *5*(9),147-149.

Tarek, M. K., Hamed, M. A., Hashem, R. M., Shabana, M. H., & Ahmed, Y. R. (2012). Protective and therapeutic effects of Argyreia speciosa against ethanol-induced gastric ulcer in rats. *Zeitschrift für Naturforschung C*, *67*(2), 47-57.

Vasudeva, N., Sethi, P., Sharma, S. K., Kumar, S., & Sharma, S. (2012). Antiulcer potential of the ethanolic extract of Aerva persica Merrill root in rats. *Journal of acupuncture and meridian studies*, *5*(2), 80-86.

Vyawahare, N. S., Deshmukh, V. V., Gadkari, M. R., & Kagathara, V. G. (2009). Plants with antiulcer activity. *Pharmacognosy Reviews*, *3*(5), 108-115.

Wright, P., Leathwood P., & Plummer, D. (1972). Enzymes in rat urine: Alkaline phosphatase. *Enzymologia,* 42(4), 317–327.