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EFFECTS OF ETHANOIC EXTRACT OF BETA VULGARIS LEAVES ON INDOMETHACIN INDUCED GASTRIC ULCERATION IN FEMALE WISTAR RATS.

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ABSTRACT

Gastric ulcers are a prevalent gastrointestinal disorder characterized by erosions in the stomach lining, affecting a significant global population. The rise in gastric ulcer disease incidence underscores the need for effective interventions. Gastric ulcers often result from a disruption in the delicate balance between aggressive and defensive factors affecting the stomach mucosa. Various factors, including excessive gastric acid secretion and the influence of external agents like non-steroidal anti-inflammatory drugs (NSAIDs), contribute to ulceration. This study explores the potential therapeutic effects of Beta Vulgaris leaf extract on drug-induced gastric ulceration, focusing on its cytoprotective and anti-inflammatory properties. Thirty female Wistar rats were randomly divided into six groups. Group 1 (normal control) received distilled water, while Group 2 (ulcerated control) received indomethacin alone. Groups 3, 4, and 5 received graded doses of 200 mg/kg body weight of Beta Vulgaris leaf extract to assess potential negative or toxicological effects. Group 6 received pretreatment with cimetidine (20 mg/kg body weight) before indomethacin administration. Treatments, including reference drug and extract administrations, spanned 14 days prior to indomethacin dosing. All treatments were administered orally via an intubator, with ad libitum access to food (Pallets) and water throughout the study. Photomicrographs demonstrated that Beta Vulgaris leaf extract prevented ulceration in the stomach. Ulcer scores revealed a significant reduction in ulcer formation, particularly with the highest dose of Beta Vulgaris extract, compared to indomethacin-induced ulcers. Cimetidine also significantly inhibited ulcer formation. Beta Vulgaris leaf extract demonstrated substantial antiulcer activity, mucoprotection, and a reduction in gastric acid secretion, comparable to reference drugs. Its mechanism of action likely involves cytoprotective, antioxidant, immunoregulatory, and antisecretory properties. These findings suggest Beta Vulgaris as a potential preventive or complementary treatment for peptic ulcers.

INTRODUCTION

Gastric ulcers which is a condition characterized by erosions in the stomach lining that penetrate the mucosa and submucosa, are a prevalent gastrointestinal disorder affecting many across the globe (Ragheb *et al.*, 2023). The prevalence of Gastric ulcer disease has been recorded to increase by more than 25.8% since 1990 (Mahmoud *et al.*, 2023). It presently holds its position as the most prevalent digestive tract diseases. Greater than 2% of the western population suffers gastric ulcer and it presently affects about 10% to 15% of the world population. Hence, the need for the development of possible interventions (Khan, 2023).

This condition can be attributed to the disturbance of the homeostatic balance that exist between the aggressive and the defensive factors that interact with the mucosa layer of the stomach (Gao *et al.*, 2023). Influenced by both cholinergic stimulation and the increase in the level of the aggressive factors, the epithelial cells present in the mucosa layer of the stomach secrets an impermeable layer of mucous and bicarbonate that protects the mucosa from strong acid and enzyme interaction and when such delicate balance is destabilized, it results in gastric ulceration (Kuraganti, 2012).

The underlying etiology of gastric ulcers involves a complex interplay of factors, including excessive gastric

acid secretion, diminished mucosal defense mechanisms, and the impact of various external agents (Badr *et al.*, 2023). Aggressive factors that have been identified includes Alcohol, NSAIDs (such as indomethacin), acid, pepsin, bile salt among others while some of the defensive factors are bicarbonate, prostaglandin and epithelial renewal among others (Guzmán-Gómez *et al.*, 2023).

In an attempt to combat the ulceration that results from the destabilization of the delicate balance described above especially by drug agents, numerous studies have attempted to establish the therapeutic effect of various natural extracts in the mitigation of gastric ulcer (Awuchi *et al.*, 2023). This study attempts to also evaluate the ameliorative effect of beta vulgaris leaves on drug induced gastric ulceration.

Beta vulgaris is a plant majorly cultivated for its root and the major motivation is the economic and medicinal importance of the root (Amiri *et al.*, 2023). Previous studies have highlighted that beta vulgaris root is the second largest source of sugar which holds its position next to the popular sugar cane (Amiri *et al.*, 2023). The beta vulgaris plant contains a diverse array of bioactive compounds, including antioxidants, flavonoids, and polyphenols, which have demonstrated anti-inflammatory and cytoprotective potentials which is the reason for the medicinal importance (Michalak *et al.*, 2023). The phytochemical analysis of the Beta vulgaris leaf has been well documented in a study by Kousar *et al.* (2023).

The medicinal properties with which the leaves have been implicated includes antioxidant, anti-depressant, anti-microbial, antifungal, anti- inflammatory, diuretic, and expectorant properties (Amiri *et al.*, 2023). Beta vulgaris has in fact been ranked as the tenth plant with an abundant level of antioxidant (Al-Khafaji *et al.*, 2022).

This study holds significant promise not only in expanding our understanding of the gastroprotective properties of Beta Vulgaris root extract but also in shedding light on its potential mechanisms of action.

MATERIAL AND METHODS

Animal use and care

Thirty female Wistar rats weighing between 180g-200g were obtained from the animal holding unit, Babcock University, Ilishan-Remo and kept in a well-ventilated Wistar rats cage and were randomized into six groups of five rats each. Group 1 (normal control) animals received only distilled water. Rats in group 2 (ulcerated control) were treated with only indomethacin. Groups 3, 4 and 5 animals were administered respectively with 200 mg/kg b.w. of beta vulgaris extract (to monitor likely negative/toxicological effect of the extracts). Animals in group 6 underwent pretreatment with cimetidine at a dosage of 20 mg/kg body weight before receiving indomethacin. All treatments, including the administration of the reference drug and the Beta Vulgaris extract, spanned a period of 14 days preceding the administration of indomethacin. To ensure precision and consistency, all treatments were administered orally using a specialized oral intubator. Throughout the duration of the experiment, the rats had unrestricted access to food in the form of Pallets (Top Feeds) and water, provided ad libitum.

Preparation of extracts

Leaves of beta vulgaris (BV) were collect from a local family farm in Ilishan Remo, Ogun State, Nigeria. The sample was air-dried at room temperature for 10 days to constant weight. The dried samples were then pulverized with an electric blender (model MS-223; Blender/Miller III, Taiwan, China), weighed and kept airtight prior to extraction. Powdered samples (500 g each) of the plant was extracted in 5 l of distilled water for 48 h with continuous shaking by orbital shaker maintained at 300 rpm. The solutions obtained were then filtered (with Whatman No. 1 filter paper) and the resulting filtrates lyophilized to give 15.5 g (SM) and 12.4 g (FE) residues, corresponding to yields of 3.1% and 2.48% respectively. The lyophilized samples were separately reconstituted in distilled water to give doses of 100 and 200 mg/kg body weight of each extract used in the study.

Ulcer induction

Ulceration was induced in the animal models with the use of Indomethacin following the methods described by previous literatures [4]. Animals were fasted for 24 hours prior to experimentation but had free access to water. The Control group received Indomethacin (40mg/kg bw,p.o).

Animal sacrifice and tissue collection

On the fourteenth day (4 h post ulcer induction), the female Wistar rats were euthanized by cervical dislocation. The abdomen was opened and the stomach excised. The stomach was thereafter opened along greater curvature and gastric content was drained into a centrifuge tube. The cleaned stomachs were scored for the degree of ulceration and then preserved in 0.1 M phosphate saline buffer (1:4 (w/v), pH 7.4) prior to macroscopic examination.

Quantification of ulceration

Degrees of ulceration in the indomethacin-treated animals were quantified using the procedure outlined by Szabo and Hollander (1989). Briefly, cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with square-grid eyepiece based on grading on a 0–2 scale (depicting severity of vascular congestions and lesions/hemorrhagic erosions) as presented in Table 1. Areas of mucosal damage were expressed as a percentage of the total surface area of the glandular stomach estimated in square millimeters.

Table 2: Ulcer scores and descriptive remark (planimetry method). Ulcer scoring- This is based on a modified method of scoring method.[6] Ulcers were independently assessed and scored by two observers using the following criteria

Score	Remark
0	Normal stomach mucosa
0.5	Dotted ulceration
1	Two ulceration connected together
2	Conspicuous ulceration

Histological Assessment

Profiling of the ulcerated gastric mucosa was based on the method using Periodic Acid Schiff staining (PAS) [7]. Stomachs fixed with 10% formalin and embedded in paraffin were sectioned at 5µm in an automated microtome. The gastric tissue integrity (mucosa-submucosa) was assessed for damage.

Statistical analysis

Results were expressed as mean of seven determinations ± standard error of mean. One-way analysis of variance (ANOVA) complemented with Student's *t*-test using SPSS software package for windows (Version 16) for differences between means was used to detect any significant difference ($p < 0.05$) between the treatment groups in this study.

RESULTS

Presentation of Findings

Photomicrographs

Figure 1a shows the pictomicrograph of the stomach of the Wistar rats in x40 magnification and figure 1b shows the pictomicrograph at x100 magnification. It is observed that the administration of beetroot extract to the animals prevented ulceration of the stomach.

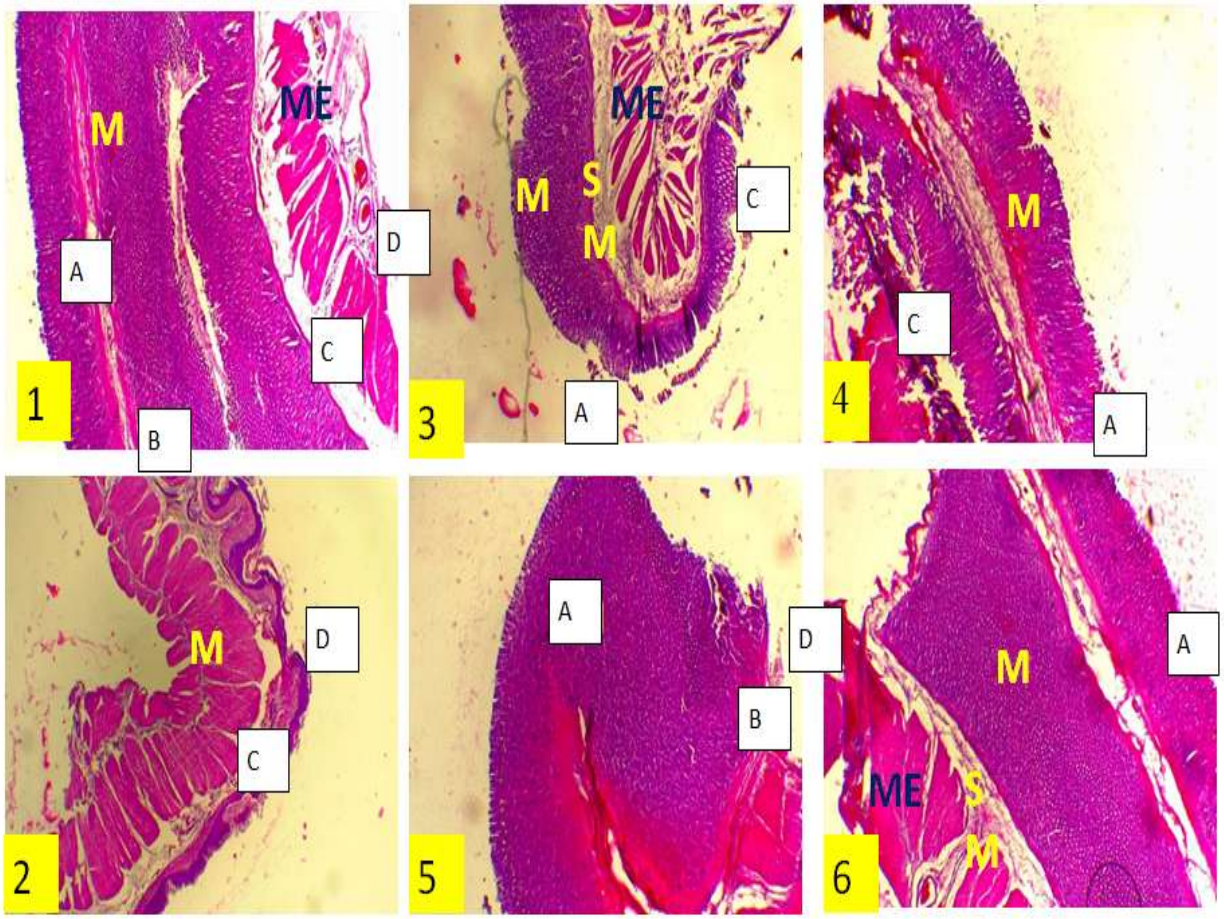

Figure 1a: Photomicrograph of the stomach of ulcerated rat (indomethacin-induced) treated with Beetroot extract (groups 3-5) and indomethacin (group 6) at lower magnification. M= mucosa; SM= submucosal; ME= muscular external; H & E; x40

A= mucosa B= submucosa C= Muscularis layer D= Serosa

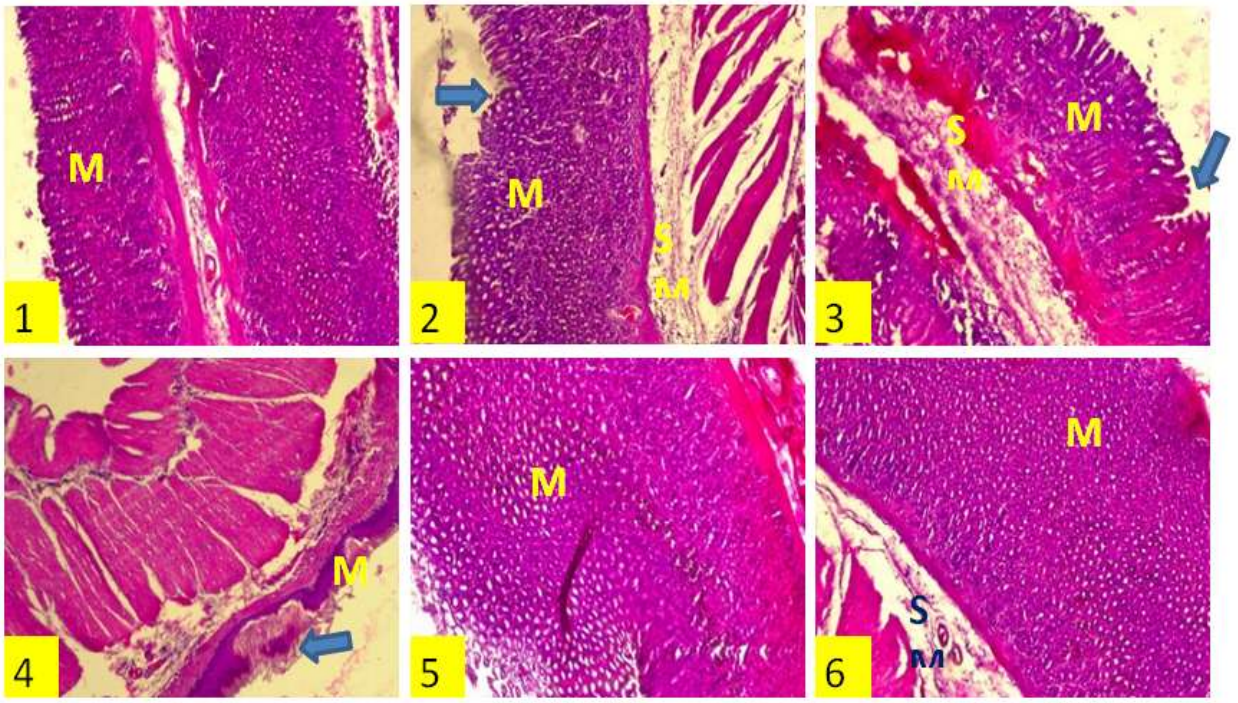

Figure 1b: Photomicrograph of the stomach of ulcerated rat (cimentidine-induced) treated with Beetroot extract (groups 3-5) and indomethacin (group 6). M= mucosa; SM= sub mucosal; ME= muscular external; H & E; x100

A= mucosa B= submucosa C= Muscularis layer D= Serosa

Ulcer Scoring

Figure 2 below shows the ulcer scores accessed across all groups. Indomethacin caused an increase in ulcer score in Grp 2, when compared with grp 1 (control) #p<0.05. However, the effect of graded dose BVE in indomethacin induced ulceration, irrespective of its dose, has very significant effect following pretreatment with BV extract. p<0.0001 when compared to Indomethacin

Consequently, the effect of cimetidine in indomethacin induced ulceration, caused a very significant decrease compared to indomethacin.

The extract and reference group significantly inhibited ulcer formation. However, the highest dose of BVE did better compared to cimetidine.

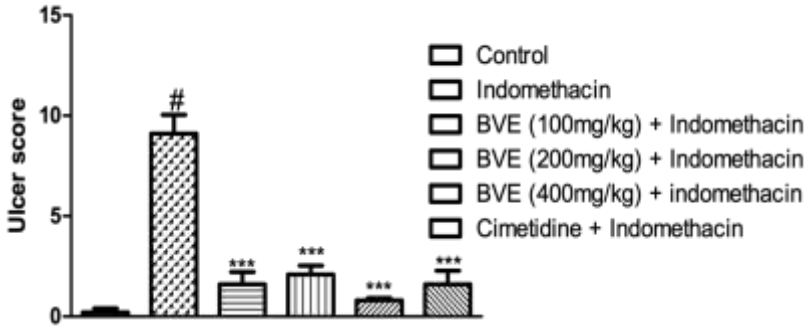

Figure 2: Showing the ulcer score in each of the groups.

#p<0.05 when compared to control
*p<0.01, **p<0.001, ***p<0.0001 when compared to Indomethacin

DISSCUSSIONS

It is generally accepted that medicinal plants play a vital role against various diseases. Beta vulgaris plants extracts have significant antiulcer activity in Wistar rats. It has mucoprotective activity and gastric anti-secreta effects when compared with that of reference drugs. The extract has been shown to be non-toxic at the experimented dosages (Baião D, *et al.*, 2017). The antiulcer activity is probably due to the presence of flavonoids through multiple mechanisms of action such as cytoprotectors (increased mucus), antioxidants (increased activity of SOD and CAT enzymes and GSH

levels), immunoregulatory (reduction in proinflammatory cytokines and increase in anti-inflammatory cytokines), antisecretory (reduction in H^+) (Pawar *et al.*, 2010). Nitric oxide, an endogenous substance known to improve microcirculation around ulcers. and anti-*H. pylori*. (Ajiboye and Nkwopara, 2019) Thus, they can potentially be used as preventive and complementary drugs or as dietary supplements to prevent the development of peptic ulcer and its episodes of recurrence and/or assist in the traditional treatment of ulcerative lesions. Many products of natural origin, especially composed of plant foods and plants, often referred to as complementary and alternative medicines, such as nutraceuticals and herbal medicines, respectively, have stood out for their therapeutic properties, which can assist in the management of many diseases. Results showed that beet root plants prevented ulcer in rats in a dose-dependent manner. A variety of botanical products have been reported to possess antiulcer activity; finally, it should be noted that substances such as flavonoids, and tannins that possess antiulcer activity are of particular therapeutic importance. The results of this study indicate that extracts of leaves and plants extracts of some medicinal plant have good potentials for use in peptic ulcer disease. Results showed that these medicinal plants could prevent ulcer in rats in a dose-dependent manner. The present study was designed to explore the mechanism of action of beetroot plant against experimentally induced gastric ulcers. The beta vulgaris extracts were tested against cimetidine and indomethacin induced gastric ulcer models in female Wistar rats. It is concluded from this study that the drug possesses antiulcer activity in different gastric ulcer models. The antiulcer activity of the drug can be attributed to free-radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosal barrier.

CONCLUSION

It is generally accepted that medicinal plants play a vital role against various diseases. Beta vulgaris plants extracts have significant antiulcer activity in Wistar rats. It has mucoprotective activity and gastric anti-secretary effects when compared with that of reference drugs. The extract has been shown to be non-toxic at the experimented dosages (Baião D *et al.*, 2017).

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REFERENCES

1. Abdullahi, A. L.; Agbo, M. O.; Garmaniel, K. S. and Wambebe, C. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennioides* Roots, Phytotherapy *Research*, 19:431and, 2001; 19: 431-434.
2. Adedapo, A. A.; Dina, O. A.; Saba, A. B. and Oladipo, O. D. Evaluation of *Telfaria occidentalis* and *Sorghum bicolor* extracts as potent haematinics in domestic rabbits. *Nigerian Journal of Animal Production*, 2002; *29(1)*: 8893.
3. Al-Khafaji, A. M., Al-Amri, N. J. K., & Al-Dulaimi, N. H. A. Growth, yield, and antioxidant traits of different parts of beetroot as affected by vermicompost and glutathione. Iraqi Journal of Agricultural Sciences, 2022; 53(5): 1107-1114.
4. Alphin, R. S., & Ward, J. W. Actions of hexopyrronium bromide on gastric secretion in dogs and on gastric secretion and ulceration in rats. Archives internationales de pharmacodynamie et de therapie, 1967; 168(1): 82-100.
5. Amiri, N., Aberoumand, A., & Ziaei-nejad, S. Effect of Beta vulgaris L. as feed ingredient on muscle growth, nutritional factors, and quality of common carp, Cyprinus carpio. Food Science & Nutrition, 2023.
6. Anonymous. 2011. http://allonhealth.com/beetroot-juice/index.htm, 2011.
7. Asuzu, I. U. and Anaga, A. O. Pharmacological Screening of the aqueous extract of *Alstonia boonei* bark. *Fitoterapia*, 1991; LXII(5): 411-417.
8. Asuzu, I. U. and Chineme, C. N. Effect of *Morinda incida* leaf extract on *Trypanosoma brucei* infection in mice. *Journal of Ethnopharmacology*, 1990; *30*:301-313
9. Awuchi, C. G., Saha, P., Amle, V. S., Nyarko, R. O., Kumar, R., Boateng, E. A., & Asum, C. A Study of Various Medicinal Plants used in Ulcer Treatment: A Review. Journal for Research in Applied Sciences and Biotechnology, 2023; 2(1): 234-246.
10. Badr, A. M., El-Orabi, N. F., Mahran, Y. F., Badr, A. M., Bayoumy, N. M., Hagar, H., & Atawia, R. T. In vivo and In silico evidence of the protective properties of carvacrol against experimentally-induced gastric ulcer: Implication of antioxidant, anti-inflammatory, and antiapoptotic mechanisms. Chemico-Biological Interactions, 2023; 382: 110649.
11. Baião D, Silva D, Mere Del Aguila E, Paschoalin V. Nutritional, Bioactive and Physicochemical Characteristics of Different Beetroot Formulations, 2017.
12. Baião D, Silva D, Mere Del Aguila E, Paschoalin V. Nutritional, Bioactive and Physicochemical Characteristics of Different Beetroot Formulations, 2017.
13. Ballinger, A. and Smith, G., COX-2 inhibitors vs. NSAIDs in gastrointestinal damage and prevention. *Expert Opinion on Pharmacotherapy*, 2001; 2(1): 31-40.
14. Brandstaeter, S., Fuchs, S., Aydin, R. and Cyron, C., Mechanics of the stomach: A review of an

emerging field of biomechanics. *GAMM-Mitteilungen*, 2019; 42(3).
15. Brown Etris, M., Pribble, J., LaBrecque, J. Evaluation of two wound measurement methods in a multi-center, controlled study. Ostomy Wound Manage, 1994; 40(7): 44-48.
16. Brune B, Dimmeler S, Molina y Vedia L, Lapetina EG. Nitric oxide: a signal for ADP-ribosylation of proteins. *Life Sci.*, 1994; 54: 61–70.
17. Brune B, Dimmeler S, Molina y Vedia L, Lapetina EG. Nitric oxide: a signal for ADP-ribosylation of proteins. *Life Sci.* 1994; 54: 61–70.
18. Bulstrode, C.J.K., Goode,A.W., Scott, P.J. Stereophotogrammetry for measuring rates of cutaneous healing: a comparison with conventional techniques. Clin Sci, 1986; 71: 4, 437-443.
19. Burkill, H. M. *The useful plants of West Tropical Africa 2nd edition volume 1*. Royal Botanical Gardens, Kew, London, 1985; 305.
20. Carroll, R. W. Status of lowland gorilla and other wildlife in Dzangah region of SouthWest Central African Republic. *Primate Conservation*, 1980; 7: 38-41.
21. Chan, F. and Graham, D. Prevention of non-steroidal anti-inflammatory drug gastrointestinal complications - review and recommendations based on risk assessment. *Alimentary Pharmacology & Therapeutics*, 2004; 19(10): 1051-1061.
22. Charles, H. Wound assessment: measuring the area of a leg ulcer. Br J Nursing, 1998; 7(13): 765-768.
23. Clifford, T., Howatson, G., West, D. and Stevenson, E., The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Nutrients*, 2015; 7(4): 2801-2822.
24. Cole, G.W.The measurement of the volume of leg ulcers. J Dermatol Surg Oncol, 1988; 14(4): 421-423.
25. Cooper, D.M. Clinical assessment/measurement of healing: evolution and status. Clin Mat, 1991; 8: 263-271.
26. Coleridge Smith, P.D., Scurr, J.H. Direct method of measuring venous ulcers. Br J Surg, 1989; 76(7): 689.
27. Cousin, D. and Huffman, M. A. Medicinal properties in the diet of gorillas: An ethnopharmacological evaluation. *African Study Monographs*, 2002; 23(2): 65-89.
28. Dilhuydy, J. M. Patients' attraction to CAM a reality which physicians can neither ignore nor deny *Bulletin of Cancer*, 2003; *90*: 623-628.
29. Dimaline, R. and Varro, A., Attack and defence in the gastric epithelium - a delicate balance. *Experimental Physiology*, 2007; 92(4): 591-601.
30. Duffin, R., Shaw, C. A., & Rossi, A. G. Sildenafil reduces alcohol-induced gastric damage: just say 'NO'. British journal of pharmacology, 2008; 153(4): 623-624.
31. Dzenda, T. Modulatory role of crude methanolic extract of *Tephrosia vogelli* leaves on contractile activity of smooth muscles. *M.Sc. Thesis*, Ahmadu Bello, University, Zaria, 2004; 129 – 138.
32. Ekpendu, T. O. E.; Obande, O. D. and Anyogo, P. U. Studies on Nigerian *Tephrosia* species Part 1. *Journal of Pharmaceutical Research and Development*, 1998; 3(1): 13 – 19.
33. Ekpendu, T. O. E.; Obande, O. D.; Anyogo, P. U. and Attah, A. D. Nigerian ethnomedicine and medicinal plant flora-the Benue experience part 1. *Journal of Pharmaceutical Research and Development*, 1998; 3(1): 37-46.
34. Elegbe, R. A. Comparative studies on starvation- and indomethacin-induced ulcerations in albino rats. Biochemistry and experimental biology, 1978; 14(2): 158-166.
35. Elliott, S., McKnight, W., Cirino, G. and Wallace, J. A nitric oxide-releasing nonsteroidal anti-inflammatory drug accelerates gastric ulcer healing in rats. *Gastroenterology*, 1995; 109(2): 524-530.
36. Eno, A. E. and Azah, N. Effect of ethanolic extract from *Elaeophorbia drupifera* leaves on the gastrointestinal smooth muscles of rabbit. *Nigerian Journal of Physiological Sciences*, 2004; 19(1-2): 60-68.
37. Eruvbetine, D.; Dipeolu, M. A. and Kazzim, M. M. Preliminary investigation into the adoption ethnoveterinary practices for the treatment of non-ruminant diseases. *Tropical Veterinarian*, 1998; 16: 91-97.
38. Familluyi, K. A.; Adeyamo, S. O. and Ozore, O. Mosquito Repellence of *Ocimmum gratissimum* and *Citrus sinensis* peel. *Journal of Chemical Society of Nigeria*, 2001; *26(1)*: 28-29.
39. Ferrua, M. and Singh, R. Modeling the Fluid Dynamics in a Human Stomach to Gain Insight of Food Digestion. *Journal of Food Science*, 2010; 75(7): R151-R162.
40. Förstermann U, Mülsch A, Böhme E, Busse R. Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from rabbit and canine arteries. *Circ Res.*, 1986; 58: 531–538.
41. Förstermann U, Mülsch A, Böhme E, Busse R. Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from rabbit and canine arteries. *Circ Res.*, 1986; 58: 531–538.
42. Förstermann, U., & Sessa, W. C. Nitric oxide synthases: regulation and function. European heart journal, 2012; 33(7): 829–837d. https://doi.org/10.1093/eurheartj/ehr304. Nitric oxide synthases: regulation and function. *European heart journal*, *33*(7), 829–837d. https://doi.org/10.1093/eurheartj/ehr304.
43. Franks, P.J., Moffatt, C.J., Connolly, M. et al. Factors associated with healing leg ulceration with high compression.Age & Ageing, 1995; 24(5): 407-410.
44. G. J. Kapadia, M. A. Azuine, G. S. Rao, T. Arai, A. Iida, and H. Tokuda, "Cytotoxic effect of the red

beetroot (Beta vulgaris L.) extract compared to doxorubicin (adriamycin) in the human prostate (PC-3) and breast (MCF-7) cancer cell lines," AntiCancer Agents in Medicinal Chemistry, 2011; 11(3): 280– 284.

45. Galura G.M., Chavez L.O., Robles A., McCallum R. Gastroduodenal Injury: Role of Protective Factors. *Curr. Gastroenterol. Rep*, 2019; 21: 34. doi: 10.1007/s11894-019-0701-x.
46. Gao, J., Cao, B., Zhao, R., Li, H., Xu, Q., & Wei, B. Critical Signaling Transduction Pathways and Intestinal Barrier: Implications for Pathophysiology and Therapeutics. Pharmaceuticals, 2023; 16(9): 1216.
47. Githiori, J. B.; Hoglund, J.; Waller, P. J. and Baker, R. L. Evaluation of anthelmintic properties of extracts of some plants used in livestock deworming by pastoralists and smallholder farmers in Kenya against *Heligosmoides polygyrus* infections in mice. *Veterinary Parasitology*, 2003; 118: 215-226.
48. Githiori, J. B.; Hoglund, J.; Waller, P. J. and Baker, R. L. Evaluation of anthelmintic properties of extracts of some plants used in livestock deworming by pastoralists and smallholder farmers in Kenya against *Heligosmoides polygyrus* infections in mice. *Veterinary Parasitology*, 2003; 118: 215-226.
49. Gudi T, Hong GK, Vaandrager AB, Lohmann SM, Pilz RB. Nitric oxide and cGMP regulate gene expression in neuronal and glial cells by activating type II cGMP-dependent protein kinase. *FASEB J.*, 1999; 13: 2143–2152.
50. Gudi T, Hong GK, Vaandrager AB, Lohmann SM, Pilz RB. Nitric oxide and cGMP regulate gene expression in neuronal and glial cells by activating type II cGMP-dependent protein kinase. *FASEB J.*, 1999; 13: 2143–2152.
51. Guzmán-Gómez, O., García-Rodríguez, R. V., Pérez-Gutierrez, S., Rivero-Ramírez, N. L., García-Martínez, Y., Pablo-Pérez, S. S., & Chamorro-Cevallos, G. Protective effect of the phycobiliproteins from arthrospira maxima on indomethacin-induced gastric ulcer in a Rat model. Plants, 2023; 12(8): 1586.
52. Hawkins, G.W. Hanks, The gastroduodenal toxicity of nonsteroidal anti-inflammatory drugs. A review of the literature, J Pain Symptom Manage, 2000; 20(2): 140–151.
53. Hirose, H., Takeuchi, K., & Okabe, S. Effect of indomethacin on gastric mucosal blood flow around acetic acid-induced gastric ulcers in rats. Gastroenterology, 1991; 100(5): 1259-1265.
54. Holmes RP, Assimos DG, The impact of dietary oxalate on kidney stone formation Urological Research, 2004; 32(5): 311-316.
55. Huang, J., Sridhar, S. and Hunt, R., Role of Helicobacter pylori infection and non steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *The Lancet*, 2002; 359(9300): 14-22.
56. Ibrahim, M. A. Ethnotoxicology among Nigerian agropastoralists. In: Ethnoveterinary Research and Development (Eds.) McCorkle, C. M.; Mathias, E. and Schill Van Vean, T. W. *Intermediate Technology* Publications, London, 1996; 54-59.
57. Ibrahim, M. A. and Abdu, P. A. Ethnoagroveterinary perspectives of poultry management and production among Hausa/Fulani of rural Nigeria. *Proceedings of the 29th Annual Conference of NVMA, Kaduna*, 27th – 30th October, 1992.
58. Kanaizumi T., Nakano H., Matsui T., Tatsumi H., Ishikawa H., Kuramoto H., Shimizu R., Shiratori T. Gastric emptying in patients with gastric and duodenal ulcer. *Tohoku J. Exp. Med*, 1989; 158: 133–140. doi: 10.1620/tjem.158.133.
59. Kazimierczak R, Siłakiewicz A, Hallmann E, Srednicka-Tober D, Rembiałkowska E. Chemical composition of selected beetroot juices in relation to beetroot production system and processing technology. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2016; 44(2): 491–8.
60. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci USA*, 1996; 93: 9114–9119.
61. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci USA*, 1996; 93: 9114–9119.
62. Khan, W. The Study the Protective Effect of Milk on the Stomach. World of Medicine: Journal of Biomedical Sciences, 2023; 1(1): 1-12.
63. Klausner, E., Lavy, E., Friedman, M. and Hoffman, A., Expandable gastroretentive dosage forms. *Journal of Controlled Release*, 2003; 90(2): 143-162.
64. Kolawole I. AJIBOYE, Francis S. OLUWOLE, Oyebimpe F. AJIBOYE Oral Administration of L-Arginine Ameliorate Nsaid-Induced Gastric Injury in Wistar Rats, 2019.
65. Kolawole, I. and Francis, S., Effects of a Type V Phosphodiesterase Inhibitor (Tadalafil) on Indomethacin-Induced Gastric Ulceration in Rats. *International Journal of Tropical Medicine*, 2012; 7(3): 111-116.
66. Kousar, F., Khanem, A., Ullah, I., & Younas, F. Phytochemical analysis and synergistic antimicrobial potential of extracts from Carica papaya and Beta vulgaris. Kuwait Journal of Science, 2023.
67. Kuraganti, R. P. Evaluation of Anti-Ulcer Activity of Aqueous and Alcoholic Extracts of Nelumbo Nucifera in Rats (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)), 2012.

68. L. Vali, ´ E. Stefanovits-B ´ anyai, K. Szentmih ´ alyi et al., "Liver- ´ protecting effects of table beet (Beta vulgaris var. rubra) during ischemia-reperfusion," Nutrition, 2007; 23(2): 172–178.
69. Liu XB, Hill P, Haile DJ. Role of the ferroportin iron-responsive element in iron and nitric oxide dependent gene regulation. *Blood Cells Mol Dis.*, 2002; 29: 315–326.
70. M. J. Ormsbee, C. W. Bach, and D. A. Baur, "Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance," Nutrients, 2014; 6(5): 1782–1808.
71. M. J. Ormsbee, J. Lox, and P. J. Arciero, "Beetroot juice and exercise performance," Nutrition and Dietary Supplements, 2013; 5: 27–35.
72. M. K. Reddy, R. L. Alexander-Lindo, and M. G. Nair, "Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors," Journal of Agricultural and Food Chemistry, 2005; 53(23): 9268– 9273.
73. M. Khazaei, H. Salehi, Protective effect of Falcaria vulgaris extract on ethanol induced gastric ulcer in rat, Iranian J Pharmacol Ther, 2006; 5: 1–4.
74. M.R. Griffin, J.M. Scheiman, Prospects for changing the burden of nonsteroidal anti inflammatory drug toxicity, Am J Med, 2001; 110: 33S–37S.
75. Macdonald, G., Harrison's Internal Medicine, 17th edition. - by A. S. Fauci, D. L. Kasper, D. L. Longo, E. Braunwald, S. L. Hauser, J. L. Jameson and J. Loscalzo. *Internal Medicine Journal*, 2008; 38(12): 932-932.
76. MacNaughton, W., Cirino, G. and Wallace, J., Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sciences*, 1989; 45(20): 1869-1876.
77. Mahmoud, M. F., Abdo, W., Nabil, M., Drissi, B., El-Shazly, A. M., Abdelfattah, M. A., & Sobeh, M. Apple (Malus domestica Borkh) leaves attenuate indomethacin-induced gastric ulcer in rats. Biomedicine & Pharmacotherapy, 2023; 160: 114331.
78. Mbi, C. N. and Bilikha, J. B. Conventional drug production from medicinal plants with contribution from the Cameroon Pharmacopoeia. *Herbal 98 Abstracts*, Ibadan, Nigeria, 1998; 13-14.
79. McCorkle, C. M. and Mathias-Muddy, E. Ethnoveterinary Medicine in Africa. *Africa*, 1992; 62(1): 59-93.
80. Medeiros, J., Gadelha, G., Lima, S., Garcia, J., Soares, P., Santos, A., Brito, G., Ribeiro, R. and Souza, M., Role of the NO/cGMP/KATP pathway in the protective effects of sildenafil against ethanol-induced gastric damage in rats. *British Journal of Pharmacology*, 2008; 153(4): 721-727.
81. Michalak, M., Zagórska-Dziok, M., Klimek-Szczykutowicz, M., & Szopa, A. Phenolic Profile and Comparison of the Antioxidant, Anti-Ageing, Anti-Inflammatory, and Protective Activities of Borago officinalis Extracts on Skin Cells. Molecules, 2023; 28(2): 868.
82. Min C., Hesheng L., Jihong C., Qiaoyun T., Xianzhen L., Chireyeth S. Effects and Mechanism of Changes of Local Neurotransmitters in Rats' Pylorus and Bile Reflux to the Stomach with Stress Ulcer. *Dig. Dis. Sci.*, 2005; 50: 1898–1903. doi: 10.1007/s10620-005-2958-1.
83. Minga, M. M. K. Collection of Tanzanian Medical Plants for biological activity studies. *Proceedings of the 7th Tanzanian Veterinary Association Scientific Conference*, Arusha-Tanzania, 1989; 20-23.
84. N. Sharma, B. S. Tanwer, and R. Vijayvergia, "Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles," International Journal of PharmTech Research, 2011; 3(1): 110–113.
85. Noach, L., Bosma, N., Jansen, J., Hoek, F., Van Deventer, S. and Tytgat, G., Mucosal Tumor Necrosis Factor-or, Interleukin-1/3, and Interleukin-8 Production in Patients with Helicobacter pylori Infection. *Scandinavian Journal of Gastroenterology*, 1994; 29(5): 425-429.
86. Nwude, N. Ethnoveterinary pharmacology and ethnoveterinary practices in Nigeria: an overview. *Tropical Veterinarian*, 1997; 15: 117-123.
87. Nwude, N. Ethnoveterinary pharmacology and ethnoveterinary practices in Nigeria: an overview. *Tropical Veterinarian*, 1997; 15: 117-123.
88. Nwude, N. and Ibrahim, M. A. Some plants used in traditional medical practice in Nigeria. *Paper Presented at the 17th Annual conference of NVMA, Zaria*, October 28th-30th, 1980.
89. O'Dell TJ, Hawkins RD, Kandel ER, Arancio O. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc Natl Acad Sci USA.*, 1991; 88: 11285–11289.
90. O'Dell TJ, Hawkins RD, Kandel ER, Arancio O. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc Natl Acad Sci USA*, 1991; 88: 11285–11289.
91. Odeola, H. A. Welcome address to the participants of the 1st International Workshop on Herbal Medicinal Products, *Herbal 98 Abstracts*, Ibadan, Nigeria, 1998; 12.
92. Okpara, J. O.; Okpala, E. J.; Ayo, J. O. and Mamman, M. Evaluation of the antidiarrhoeal activity of *Adansonia digitata* leaf ethanolic extract. In: *Proceedings of the 31st Annual Conference of Nigerian Society for Animal Production*, Kano, 12th-15th March, 2006; 54 – 56.
93. Olaniyi, A. A. Basic requirements and Strategies for Chemical Standardization and evaluation of herbal medicines. *Herbal as Abstracts*, Ibadan, Nigeria, 1998; 11-12.

94. Owen, D., Normal Histology of the Stomach. *The American Journal of Surgical Pathology*, 1986; 10(1): 48-61.
95. Oyewole, J. A. O. Evaluation of the anti-inflammatory properties of *Sclerocarya birrea* (Anacardiaceae) stem-bark extracts in rats. *Journal of Ethnopharmacology*, 2003; 85: 217-220.
96. Oyewole, J. A. O. Evaluation of analgesic, anti-inflammatory and antidiabetic properties of *Sclerocarya birrea*. (A. Rich). *Phytotherapy Research*, 2004; 18: 601-608.
97. P. Ninfali and D. Angelino, "Nutritional and functional potential of Beta vulgaris cicla and rubra," Fitoterapia, 2013; 89: 188–199.
98. P.A. Akah, O.E. Orisakwe, K.S. Gamanies, A. Shittu, Evaluation of Nigerian traditional medicines: 11. Effects of some Nigerian folk remedies on peptic ulcer, J Ethnopharmacol, 1998; 62(2): 123–127.
99. Pal, A., Indireshkumar, K., Schwizer, W., Abrahamsson, B., Fried, M. and Brasseur, J., Gastric flow and mixing studied using computer simulation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 2004; 271(1557): 2587-2594.
100. Pantopoulos K, Hentze MW. Nitric oxide signaling to iron-regulatory protein: direct control of ferritin mRNA translation and transferrin receptor mRNA stability in transfected fibroblasts. *Proc Natl Acad Sci USA.*, 1995; 92: 1267–1271.
101. Liu XB, Hill P, Haile DJ. Role of the ferroportin iron-responsive element in iron and nitric oxide dependent gene regulation. *Blood Cells Mol Dis.*, 2002; 29: 315–326.
102. Pawar, R., Patil, U., Gadekar, R., Singour, P. and Chaurasiya, P., A potential of some medicinal plants as an antiulcer agents. *Pharmacognosy Reviews*, 2010; 4(8): 136.
103. Plassmann, P. Measuring wounds. J Wound Care, 1995; 4(6): 269-272.
104. Pozdnyakov N, Lloyd A, Reddy VN, Sitaramayya A. Nitric oxide-regulated endogenousADP-ribosylation of rod outer segment proteins. *Biochem Biophys Res Commun*, 1993; 192: 610–615.
105. Pozdnyakov N, Lloyd A, Reddy VN, Sitaramayya A. Nitric oxide-regulated endogenous ADP-ribosylation of rod outer segment proteins. *Biochem Biophys Res Commun*, 1993; 192: 610–615.
106. Prabhu, V. and Shivani, A., An overview of history, pathogenesis and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. *Annals of Medical and Health Sciences Research*, 2014; 4(1): 22.
107. R. Chakole, S. Zade, and M. Charde, "Antioxidant and antiinflammatory activity of ethanolic extract of Beta vulgaris Linn. roots," International Journal of Biomedical and Advance Research, 2011; 2: 124–130.
108. Ragheb, A. Y., Masoud, M. A., El Shabrawy, M. O., Farid, M. M., Hegazi, N. M., Mohammed, R. S., ... & Aboutabl, M. E. MS/MS-based molecular networking for mapping the chemical diversity of the pulp and peel extracts from Citrus japonica Thunb.; in vivo evaluation of their anti-inflammatory and anti-ulcer potential. Scientific African, 2023; 20: e01672.
109. Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature*, 1983; 306: 174–176.
110. Reddy KM, Ruby L, Lindo A, Nair GM. Relative inhibition of lipid peroxidation cyclooxygenase enzymes and human tumor cells prolifieration by natural food color, Journal of Agricultural and Food Chemistry, 2003; 53: 9268-9273.
111. S. G. V. Jain and P. K. Sharma, "Anti-inflammatory activity of aqueous extract of Beta vulgaris L," Journal of Basic and Clinical Pharmacy, 2011; 2: 83–86.
112. Santos, C. L., Souza, M. H., Gomes, A. S., Lemos, H. P., Santos, A. A., Cunha, F. Q., & Wallace, J. L. Sildenafil prevents indomethacin-induced gastropathy in rats: role of leukocyte adherence and gastric blood flow. British journal of pharmacology, 2005; 146(4): 481-486.
113. Satoh, K.; Hagakawa, T; Kase, Y.; Ishige, A.; Sasaki, H.; Nishikawa, S.; Kurosawa, S.; Yakabi, K. and Nakamura, Y. Mechanism for contractile effect of Diakenehuto in isolated guinea pig ileum. *Digestive Diseases and Science,* 2001; 46(2): 250-256.
114. Schuman EM, Madison DV. A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science*, 1991; 254: 1503–1506.
115. Sheehan, D., & Hrapchak, B. Theory and Practice of Histotechnology; Columbus. OH- Battelle Memorial Institute, 1987.
116. Shristi, J. Neha, B.P. Indu, G. Rajesh, A review on some Indian medicinal plants for antiulcer activity, J Sci Res Pharm, 2012; 1: 6–9.
117. Singh B, Hathan B. Optimization of osmotically dehydrated beetroot candy using response surface methodology. Journal Food & Nutritional Sciences, 2013; 2: 2320-7876.
118. Singh B, Hathan BS. Chemical composition, functional properties and processing of beetroot—a review. Int J Sci Eng Res., 2014; 5(1): 679–84.
119. Singh, V. K. Garg, P. K. Sharma, and S. Gupta, "Wound healing activity of ethanolic extract of Beta vulgaris," Pharmacologyonline, 2011; 1: 1031–1038.
120. Skene,A.I., Smith, J.M., Doré, C.J. et al.Venous leg ulcers: a prognostic index to predict time to healing. BMJ, 1992; 305: 6862, 1119-1121.
121. Solomon, C., Munro,A.R., van Rij,A.M., Christie, R. The use of video image analysis for the

measurement of venous ulcers. Br J Dermatol, 1995; 133: 4: 565-570.
122. Sonnenberg, Geographic andtemporal variations inthe occurrence of peptic ulcer disease, Scand J Gastroenterol Suppl, 1996; 110: 11.
123. Stacey, M.C., Burnand, K.G., Layer, G.T. et al. Measurement of the healing of venous ulcers. Aust N Z J Surg, 1991; 61: 11: 844-848.
124. Stenger S, Thuring H, Rollinghoff M, Bogdan C. Tissue expression of inducible nitric oxide synthase is closely associated with resistance to *Leishmania major*. *J Exp Med*, 1994; 180: 783–793.
125. Szabo S., Yoshida M., Filakovszky J., Juhasz G. "Stress" is 80 Years Old: From Hans Selye Original Paper in 1936 to Recent Advances in GI Ulceration. *Curr. Pharm. Des.*, 2017; 23: 4029–4041. doi: 10.2174/1381612823666170622110046.
126. Szabo, S., & Hollander, D. Pathways of gastrointestinal protection and repair: mechanisms of action of sucralfate. The American journal of medicine, 1989; 86(6): 23-31.
127. T. S. Kujala, J. M. Loponen, K. D. Klika, and K. Pihlaja, "Phenolics and betacyanins in red beetroot (Beta vulgaris) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds," Journal of Agricultural and Food Chemistry, 2000; 48(11): 5338–5342.
128. Tallman, P., Muscare, E., Carson, P. et al. Initial rate of healing predicts complete healing of venous ulcers.Arch Dermatol, 1997; 133(10): 1231-1234.
129. Tytgat G.N. Etiopathogenetic principles and peptic ulcer disease classification. *Dig. Dis.*, 2011; 29: 454–458. doi: 10.1159/000331520.
130. Viana, G. S. B.; Bandeira, M. A. M.; Moura, L. C.; Souza-Filho, M. V. P.; Matos, F. J. A. and Ribeiro, P. A. Analgesic and anti-inflammatory effects of tannin fraction from *Myracrodrum urundava Allemao*. *Phytotherapy Research*, 1997; 11: 118-122.
131. Vimala, G. and Gricilda Shoba, F., A Review on Antiulcer Activity of Few Indian Medicinal Plants. *International Journal of Microbiology*, 2014: 1-14.
132. Vinson JA, Hao Y, Su X, Zubik L. Phenol antioxidant Quantity and quality in foods: Vegetables, Journal of Agricultural and Food Chemistry, 1998; 46: 3630-363.
133. Vomero N.D., Colpo E. Nutritional care in peptic ulcer. *Arq. Bras. Cir. Dig*, 2014; 27: 298–302. doi: 10.1590/S0102-67202014000400017.
134. Vowden, K. Common problems in wound care: wound and ulcer measurement. Br J Nurs, 1995; 4: 13: 775-779.
135. W. Christiana, Winkler, K. Schroecksnadel, H. Schennach, and D. Fuchs, "In vitro effects of beet root juice on stimulated and unstimulated peripheral blood mononuclear cells," The American Journal of Biochemistry and Biotechnology, 2005; 1: 180–185.
136. Wallace, J. L., McKnight, W., Reuter, B. K., & Vergnolle, N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology, 2000; 119(3): 706-714.
137. Wanyama, J. B. Confidently used ethnoveterinary knowledge among pastoralists of Samburu, Kenya. *Intermediate Teachnology*, Nairobi, Kenya, 1997; 109.
138. Wruss J, Waldenberger G, Huemer S, Uygun P, Lanzerstorfer P, Müller U, et al. Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. J Food Compos Anal, 2015; 42: 46–55.
139. Yandrapu H., Sarosiek J. Protective Factors of the Gastric and Duodenal Mucosa: An Overview. *Curr. Gastroenterol. Rep.*, 2015; 17: 24. doi: 10.1007/s11894-015-0452-2.
140. Yegen B.C. Lifestyle and Peptic Ulcer Disease. *Curr. Pharm. Des.*, 2018; 24: 2034–2040. doi: 10.2174/1381612824666180510092303
141. Zandifar, E., Sohrabi Beheshti, S., Zandifar, A., & Haghjooy Javanmard, S. The effect of captopril on impaired wound healing in experimental diabetes. International journal of endocrinology, 2012.
142. Žitňanová I, Ranostajová S, Sobotová H, Demelová D, Pecháň I, Ďuračková Z. Antioxidative activity of selected fruits and vegetables, Biologia, 2006; 61: 279-284.