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Antiulcerogenic effects of aqueous - methanol leaf extract of *Vernonia amygdalina* on aspirin-induced gastric ulcer in male albino rats

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ABSTRACT

Currently, there is increasing trend in the use of herb for the treatment of diseases and ailments. *Vernonia amygdalina* is a valuable shrub that is widely spread in the East and West Africa and its medicinal value has been reported in tradomedicine. The effects of aqueous - methanol leaf extract of *V. amygdalina* on aspirin-induced gastric ulcer in male albino rats was investigated using standard methods. A total of 43 rats were used for the study. Thirteen rats were used for the acute toxicity study while thirty rats randomly divided into six groups (n=5) were used for anti-ulcer study. The result of the phytochemical screening revealed the presence of alkaloids, saponins, terpenoids, flavonoids, tannins, resins and steroids. The result of the acute toxicity study revealed that the extract might have LD_{50} above 5000 mg/kg bt.w. The results of the gastrointestinal pH indicated insignificant ($p>0.05$) increase in all groups treated with the graded doses of the extract while the groups treated with omeprazole showed significant ($p<0.05$) increase in pH when compared to the negative control group. Ulcer index of the groups treated with omeprazole and graded dose of the extract showed a significant ($p<0.05$) dose response decrease when compared to the negative control group. The result of the anti-oxidant assay showed significant ($p<0.05$) increase in the catalse activity of the group treated with omeprazole and the group treated with 600mg/kg of the extract but showed no significance ($p<0.05$) increase in the enzyme activities at lower doses of the extract when compared to the normal group. The study has shown that the extract elicited ulcer curative efficacies and perhaps justifies its use in traditional medicine for ulcer management and cure.

Keywords: *Vernonia amygdalina*, phytochemicals, peptic ulcer, aspirin, omeprazole

INTRODUCTION

Peptic ulcer remains one of the gastrointestinal disorders that have affected many people worldwide over the centuries with over 5-10% of the world population generally affected [1]. It is a chronic disease that occurs when there is a fracture in the mucosa lining of the stomach. When a physiological balance exists between aggressive factors and mucosa defense, this balance is compromised in favour of aggressive factors which lead to injury in the gastric mucosa [2]. Some of the aggressive factors are stressful lifestyle, smoking, socio-economic status, *Helicobacter pylori*, steroidal and non-steroidal anti-inflammatory drugs, ethanol and family history [3,4]. Drugs have a devastating effect on gastric mucosa as a result they has been used to induce gastric ulcer in animal models. Report has shown that drugs compromise the integrity of gastric mucosa by aiding acid reflux into the subluminal layer of the mucosa and submucosa [5]. It may also act through a general mechanism affecting the release of hormones and the regulation of nerve function involved in acid secretion [6]. Approximately 500,000 new cases of ulcer are reported annually, with 5 million cases in the United States alone [7]. Although, ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding,

perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction [8]. Several orthodox drugs have been manufactured to combat this disorder. Most of these drugs are costly and have intolerable side effects when taken. Some orthodox drugs employed in the treatment of this disorder include histamine (H_2) receptor antagonists, proton pump inhibitors, cytoprotectants, antacids, and prostaglandin analogues [1]. Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics, antacids and proton pump inhibitors [8]. Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are costly and associated with unwanted side effects [1]. To this effect, there is need to find alternative solutions to this global life threatening problem. In this context, this research aims to investigate the antiulcerogenic potential of the aqueous - methanol leaf extract of *Vernonia amygdalina* in animal models following aspirin-induced gastric ulcers to add to current knowledge on alternative treatment of gastric ulcers using plant extracts. Several prospective medicinal plants for peptic ulcer treatment have been studied and reported in literature globally [9,10,11,12,13,14]. [15] reported on some medicinal plants viz; *Allophylus serratus* Kurz, *Cissus quadrangularis*, *Ocimum sanctum* Linn, *Mangifera indica* L., *Zingiber officinale* Roscoe, *Butea frondosa* Roxb. L., *Glycyrrhiza glabra* L., *Solanum nigrum* L., and *Terminalia chebula* Retz. Similarly, medicinal plants such as *Cynodon dactylon*, *Azadirachta indica*, *Glycyrrhiza glabra*, *Swietenia mahagoni*, *Bauhinia purpurea* L., *Ficus religiosa*, *Melastoma malabathricum*, *Ocimum sanctum*, *Spondias mombin* L., *Eruca sativa*, and *Osyris quadripartite* have been reported as possessing some form of antiulcer activity upon investigation in antiulcer testing models [16,17,18,19,20,21,22,23]. A triple herbal therapy comprised of *Enterica*, *Dyspepsia* decoctions, and *Natural Pain Killer* (NPK 500) capsules manufactured by the Centre for Plant Medicine Research (CPMR) is currently used in the management of peptic ulcer disease at the CPMR out-patient clinic in Ghana [24]. These three products contain medicinal plants such as *Carapa procera*, *Trichilia monadelpha*, *Persea americana*, *Trema orientalis*, *Momordica charantia*, *Vernonia amygdalina*, *Cassia siebieriana*, *Citrus aurantifolia*, *Bidens pilosa*, *Morinda lucida*, *Maytenus senegalensis*, *Psidium guajava*, *Cnestis ferruguinea*, *Spondias mombin*, and *Latana camara.* The observed activity is these plants are attributed with the presence of flavonoids, alkaloids, terpenoids, tannins, saponins, and phenolic acids. *Vernonia amygdalina* commonly known as "bitter leaf" is a valuable shrub that is widely spread in the East and West Africa. It is use in tradomedicine in treatment of many ailments viz; laxative, pile (hemorrhoids) and gastrointestinal troubles [25,26,27,28,29,30], relief pain and lower body temperature, diabetes [31] etc.

MATERIALS AND METHODS

EQUIPMENT: All the equipment used in this study were of laboratory working standard. REAGENTS: All the chemicals used in this research were of analytical grade, commercially available and were obtained from Merck, England; BDH, England; and Fluke chemicals, Buchs, Switzerland.

SOURCES OF EXPERIMENTAL MATERIALS

Wistar male albino rats were purchased from the Animal House of ChrisKing, Mgbakwu, Anambra State *Vernonia amygdalina* leaves were collected from Ogboji Ezzagu, Ebonyi State and was identified and authenticated by a taxonomist, Mr P. Ugwuozor of Department of Biotany, UNIZIK.

METHODS

PREPARATION AND EXTRACTION OF PLANT EXTRACT

The leaves were washed, cleaned and air-dried at room temperature for 3 weeks. The dried leaves were ground into powder using a manual grinding machine. Afterwards, extraction was done by cold maceration of 1000g of ground leaves in aqueous- methanol for 48 hours. This was proceeded by filtration using whatman filter paper and the filtrate was concentrated/lyophilized by freeze-dried.

DRUG AND EXTRACT PREPARATION

Aspirin was used to induce ulcer while omeprazole was used as standard anti-ulcer drug. All were purchased from Joez Pharmacy; Zik Avenue Awka, Anambra State. The drugs were dissolved in 5% 5ml tween 80 and were administered to the rats in concentrations according to their body weight as recommended by [32]. The extract was dissolved in 5% 5ml; tween 80 and administered in concentrations of 200, 400, and 600mg/kg body weight according to the recommendation of [33].

TEST FOR TANNINS

Bromine water test

Exactly 1.0ml of bromine water and 1.0ml of extract was pipetted into a test tube and observed for colour change. The appearance of red coloration indicates the presence of tannins.

Acid test

Exactly 3.0ml of extract was added to 2.0ml of HCl in a test tube. The mixture was observed for colour change or formation of precipitates. The formation of red precipitate confirms the presence of tannins

Lead Acetate Test

Exactly 3 drops of 5% lead acetate solution were added into a test tube containing 2.0ml of extract. It was then observed for presence of precipitate.

TEST FOR FLAVONOIDS

Ferric chloride test for phenolic Nucleus

Test tube containing 1.0ml of extract, 1.0ml of 10% ferric chloride was pipette. It was mixed properly and observed carefully for colour change to greenish brown or black. Exactly 5.0ml of distilled water was pipetted into a conical flask containing 1.0ml ofthe extract and boiled. The soluble fraction of the mixture was decanted into a test tube while hot. Then 2 drops of olive oil were added into this test tube. The mixture was gentle agitated and observed for presence of emulsion.

Frothing Test

To a conical flask containing 1.0 ml of the extract, 5.0ml of distilled water was added and then boiled. The soluble fraction of the mixture was decanted into a test tube while hot; to 1.0ml of this, 3.0ml of distilled water was added. The solution was agitated vigorously and observed for froth.

TEST FOR RESIN

Exactly 0.2ml of the extract was mixed with 20 ml of distilled water in a beaker. The formation of precipitates indicates the presence of resins.

TEST FOR STEROID

To a test tube containing 0 .5ml of the extract was added 1ml H_2SO_4. The mixture was agitated gently and the colour at the interface was observed and recorded.

MEDIAN LETHAL DOSE (LD50) OF THE AQUEOUS METHANOL EXTRACT OF VERNONIA AMYGDALINA

The Median Lethal Dose (LD50) was determined by modified [25] method using Wistar Albino Rats. Thirteen male rats were used in the study. The studies were carried out in two phases. In phase one, a total of nine rats were used. The rats were divided into three groups of three rats per group. Group one was administered with 10mg/kg body weight of the extract, group two was treated with

100mg/kg body weight of the extract while group three was administered with 1000mg/kg body weight of the extract. All the administrations were done by oral intubation. The animals were monitored for twenty-four hours for mortality and general behaviour. From the result of the phase one, phase two was carried out. In this phase, a total of four rats were used, and they were divided into four groups of one rat per group. Group one was treated with 2000mg/kg body weight (bt.w), group two was treated with 3000mg/kg bt.w; group three was treated with 4000mg/kg bt.w while group four was treated with 5000mg/kg bt.w of the extract.

EXPERIMENTAL DESIGN

Thirty (30) Wistar male albino rats weighing (190-245 g) were used for this study. The rats were housed in silver-coated metal cages in Department of Pharmacology,Nnamdi Azikiwe University, Agulu. The cages were cleaned on daily basis to prevent coprohagy and maintained on sterile pellet diet and water *ad libitum*. The animals were exposed to warm rays of the morning Sun for about 10 minutes while their cages were being clean to keep them warm and dried daily. Before the experiments were conducted, the animals were acclimatized for one week. The rats were fasted for 24 hours before the experiment but were allowed free access to water up to 2 hours before the experiment according to the method of [26]. The animals were grouped into six (6) groups of five (5) each. Group One (1) was given served as normal control, group two (2) served as negative controls, group three (3) served as positive control group while groups four (4), five (5) and six (6) were used for anti-ulcer studies.

ANTI-ULCER EFFECTS OF THE EXTRACT

Group II, III, IV, V and VI were all administered 200mg, 5ml/kg Aspirin and after two (2) hours of this pre-treatment, (5%, 5ml) tween 80 was administered to II (Ulcer-negative control), 20mg, 5ml/kg omeprazole was administered to group III (positive control) while group IV, V and VI were administered with 200, 400 and 600mg/kg body weight of the extract respectively.

ULCER EVALUATION

Ulcers of the gastric mucosa appeared as elongated-undulating bands of hemorrhagic lesions. The gastric mucosa of each rat was thus examined for damage. The ulceration was scored as follow;

Colour		Ulcer Score
Normal coloration	=	0
Red coloration	=	0.5
Spot ulcer	=	1.0
Haemorrhagic stress	=	1.5
Deep stress	=	2.0
Perforation	=	3.0

Then ulcer index was calculated using the formula $U_I = (U_N + U_S + U_P) \times 10^{-1}$.
U_I = ulcer index, U_N = average number of ulcer animal, U_S = average severity of ulcer, U_P = percentage of animals having ulcer.

MEASUREMENT OF GASTRIC SECRETION AND pH

The stomach of all the animals were carefully exercised while keeping the oesophagus closed at the greater curvature and the luminal contents were collected and measured using a calibrated cylinder. The gastric juice was collected and centrifuged at 300 rpm for 10 minutes and the pH measured using digital pH meter.

MEASUREMENT OF THE TOTAL ACIDITY

The total acidity of the luminal content of each animal was estimated using the formula described by [10].

$$\text{Total acidity} = \frac{0.02M\ NaOH \times X}{Y}$$

where X = vol. of NaOH, Y vol of total secretion obtained from the stomach

ASSAY OF ANTI-OXIDANT ENZYME (CATALASE)

The anti-oxidant, catalase test was done using the method of [5]. 0.1ml of the serum was mixed with 0.4 ml of hydrogen peroxide (H_2O_2) and 1 ml of PO_4 buffer (pH 7) and was incubated for 1 minute. Then 2 ml of 5% $K_2Cr_2O_7$ was added to stop the reaction. It was mixed and boiled for 10 minutes, cooled and read at 570 nm wavelengths. One unit of activity was defined as an enzyme activity (u/ml) degrading one micromole of H_2O_2 in 1 minute under standard condition.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 10 and the group means were compared by Duncan's Multiple Range Test. Differences between means was regarded significant

RESULTS

Qualitative Phytochemical Analyses of *V. amygdalina* Leaf Extract.The results of the qualitative phytochemical screening of the aqueous - methanol leaf extract of the *V. amygdalina* are shown in Table 1.

Table 2: Phytochemical Test for the Aqueous Methanolic Leaf Extract

S/N	Phytochemical constituents	Observation
1.	Resin	+
2.	Saponin	++
3.	Tannins	++
4.	Flavonoids	++
5.	Terpenoids	++
6.	Alkaloids	++
7.	Steroids	++

Guide: – = Not present, + = Present, ++ = Fairly present , +++ = Appreciable amount

Mean lethal dose (LD_{50}).

Acute toxicity screening (LD50) showed that the extract had an oral LD50 > 5000mg/kg in wistar albino rats are shown in tables 3 and 4 below.

Table 3: Results of mean lethal dose (LD_{50})

Phase 1	Dose (mg/kg)	Death recorded
I	10	0/3
II	100	0/3
III	1000	0/3

LD_{50} >1000mg/kg

Table 4: Results of mean lethal dose (LD_{50})

Phase II	Dose (mg/kg)	Death recorded
I	2000	0/1
II	3000	0/1
III	4000	0/1
IV	5000	0/1

LD_{50} >5000mg/kg

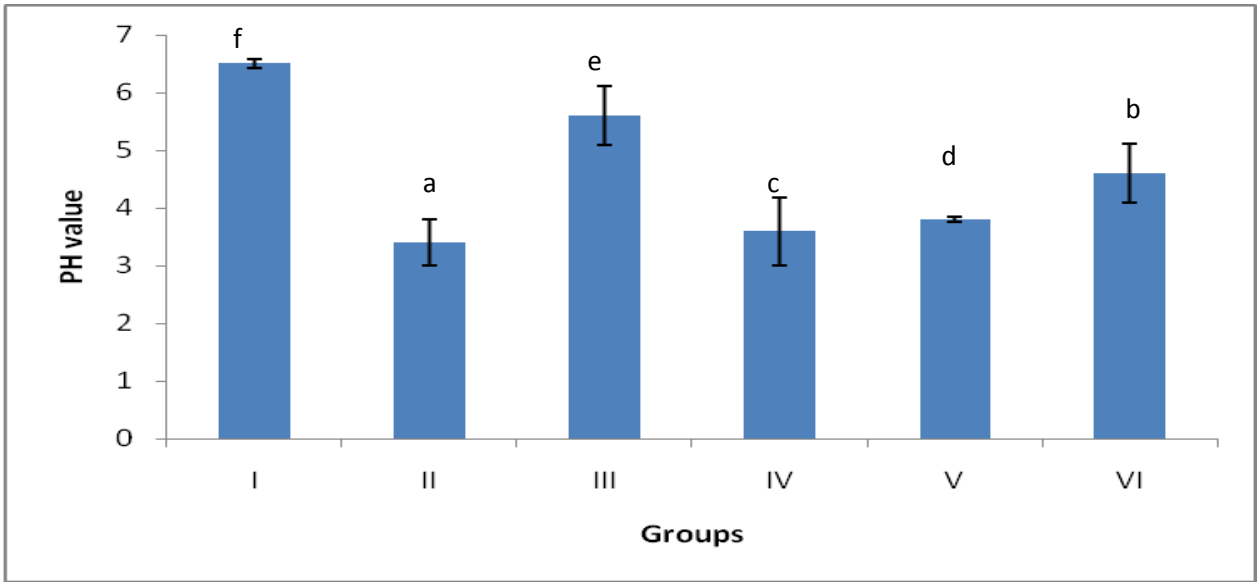

Figure 1: Effect of aqueous – methanol leaf extract of *V. amygdalina* on PH of the Gastric contents of the Animals. Bars with different letters showed significant value at P<0.05. The results of the pH screening showed a significant increase at p<0.05 in all the treated groups compared to ulcer group (group 2). The increase is higher in group treated with omeprazole (group 3). The effects of the plant extract are dose dependent. Though, omeprazole is more potent in reducing the gastric pH of the stomach.

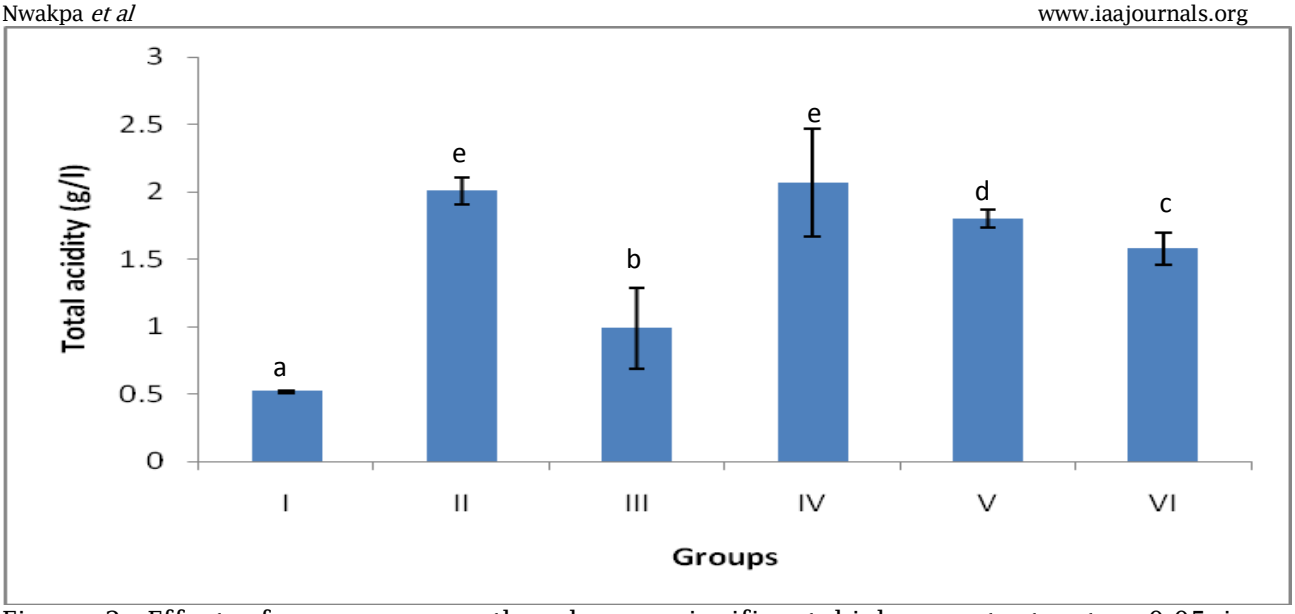

Figure 2: Effect of aqueous – methanol leaf extract of *V. amygdalina* on total acidity (g/l) of the luminal contents of the Animals. Bars with different letters showed significant value at P<0.05. The results of total acidity showed a significant higher contents at p<0.05 in group 2 compared to treatment groups except group 4. The decrease is more in group 2 when compared to others. The effects of the plant extract is dose dependent

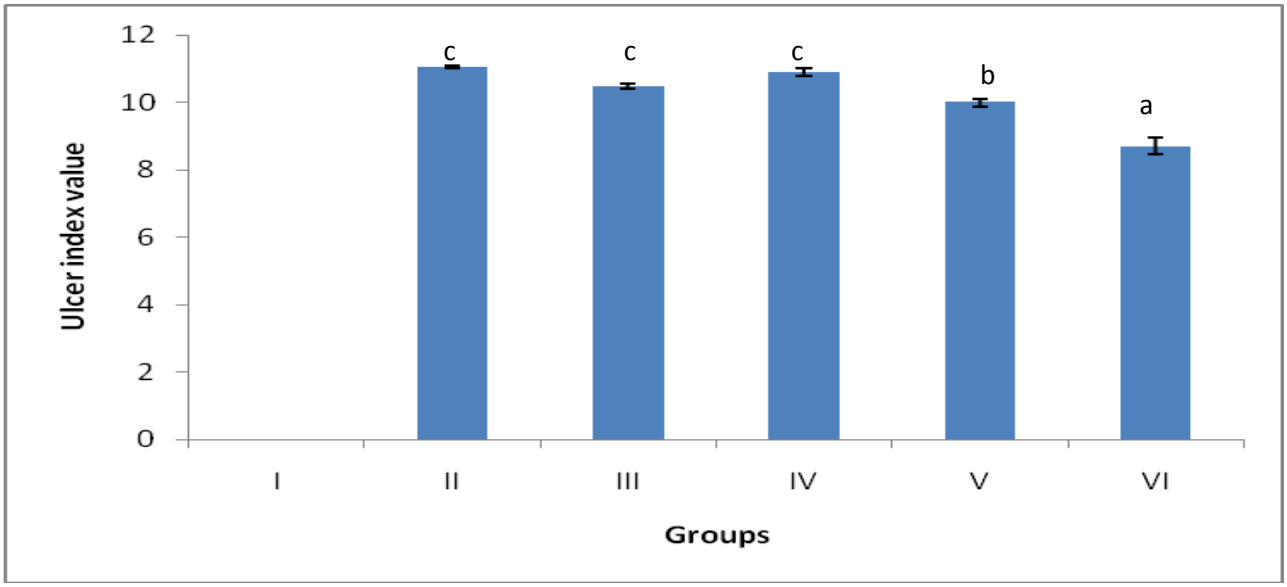

Figure 3: Effect of aqueous – methanol leaf extract of *V. amygdalina* on Ulcer Index value of the Animals. Bars with different letters showed significant value at P<0.05. The ulcer index study showed a general decrease in the treatment group; 3, 4, 5 and 6. However, the decrease is not significant (p>0.05) in group 3 and 4 but significant at p<0.05 in group 5 and 6 when compared to the negative control group after 2 hours of treatment.

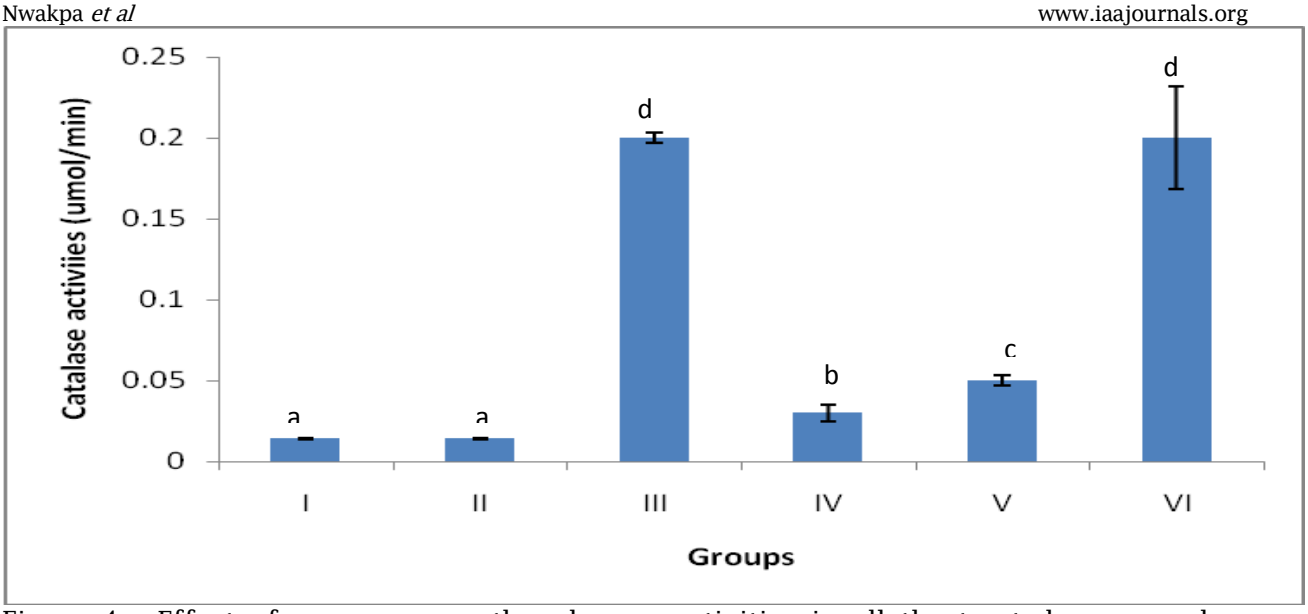

Figure 4: Effect of aqueous – methanol leaf extract of *V. amygdalina* on catalase activities of the animals. Bars with different letters showed significant value at P<0.05.The result of the anti-oxidant (catalase tests) assay showed significant ($p < 0.05$) increase in the enzyme activities in all the treated groups when compared to group 2. However, there is no significant at p<0.05 change in group 3 when compared to group 4. The effects of the plant extract is dose dependent

DISCUSSION

Ulcers are reported as the most common cause of hospitalization for upper gastrointestinal (GI) bleeding and are often a clinical concern due to the widespread use of aspirin and nonsteroidal anti-inflammatory drugs, both of which have been shown to induce ulcer. Drug consumption has been considered as one of the leading causes of ulcer in humans; hence, researchers used the animal model of gastric injury induced by aspirin to simulate conditions that humans may be exposed to study the antiulcer efficacy of natural products or new therapeutics intended to be used for gastric protection [34]. In this study, ulcer was induced with Aspirin at 200mg/kg body weight for 2 hours as shown by the ulcer index of the negative control compared to the normal. The reduction of gastric acid production as well as the protection of gastric mucosa has been the major approach for the treatment and management of peptic ulcer [35], hence the use of *V. amygdalina* as an anti- ulcer agent in this study. The phytochemical screening of the aqueous – methanol leaf extract of *V. amygdalina* revealed the presence of saponins, tannins, flavonoids, alkaloids, terpenoid, resins and phenols. This study is in conformity with the reports of [36,37] which previously reported the presence of these phytochemicals in *V. amygdalina*. The presence of these phytochemicals may be responsible for either gastric acid reduce or protection of the gastric mucosa or both. The high margin of safety was specified by the fact that in the LD50 determination, no death occurred even after an oral dose of 5000mg/kg in rats. This showed that the extract of *V. amygdalina* may have LD50 above 5000 mg/kg body weight of the extract.

In the curative studies, there was a a significant increase at p<0.05 in the stomach pH in all the groups treated with graded doses of *V. amygdalina* when compared to the negative control group especially at a dose 600mg/kg body weight in dose dependent manner. Though, omeprazole is more potent in

reducing the gastric pH of the stomach. This reduction in pH is in line with the objectives of ulcer treatment and management [38]. As shown in Fig.3, oral administration of aspirin induced gross lesions in the gastric lumen of rats with markedly high ulceration index. The ulcer index study showed an extreme significant ($p<0.05$) decrease in the ulcer index of the stomach at 600 and 400mg/kg body weight of the aqueous methanol leaf extract of *V. amygdalina* and a slight decrease at 200mg/kg body weight when compared to the negative control group after 2hours of treatment. This shows that the curative effect of *V. amygdalina* extract could be more potent on the protection of gastric mucosa rather than pH reduction. The report of this study is accordance with the result of [39]. Hydrolysable tannins contain glucose moiety and have been used internally as astringent and as heavy metal antidote [40]. Tannins being astringent may precipitate microproteins on the site of ulcer thereby forming an impervious protective pellicle on the lining to resist the attack of proteolytic enzymes [41]. This could be likened to the effect of drugs which act by providing a cytoprotective defense against acid peptic digestion. [42] reported the biological properties of tannins and observed that tannins have anti-cancer activity and can be use in cancer prevention, thus suggesting that *V. amygdalina* has potential as a source of important bioactive molecules for the treatment of cancer. The presence of tannins in *V. amygdalina* supports the traditional medicinal use of this plant in treatment of inflamed tissues, Studies have revealed the inhibitory effect of saponins on inflamed cells and to acts as gastro protective agents [43]. Saponins in *V. amygdalina* supported the usefulness of this plant in managing inflammation. Flavonoid which is another important constituent of *V. amygdalina* exhibited a wide range of biological activities like antiulcerogenicandantiulcer,antimicrobial ,anti-inflammatory, analgesic, anti-allergic, cytostatic and anti-oxidant properties [44]. Furthermore, alkaloids are substances known to affect the integrity of the mucous membrane [45]. Alkaloids have been shown to suppress acid secretion [46]. It is likely that the protective activity of the extract against aspirin induced ulceration is as a result of suppression of acid secretion. Steroids and terpenoids are also present in *V. amygdalina* and these substances have been shown to possess anti-ulcer activities. From the result of the anti-oxidant assay, there is a significant ($p < 0.05$) increase in activities of the enzyme in all the treated groups. However, there is no significant at $p<0.05$ change in group 3 when compared to group 4. Anti-oxidants play a protective role against cellular damage by scavenging free radicals [47]. Reduction in the concentration of free radicals enhances cyclooxygenase activity thereby increasing prostaglandin synthesis [48]. Ulcer induction by aspirin might results from inhibition of synthesis of prostaglandins which lead to overproduction of leucotrienes and other products of the 5-lipoxygenase pathway. These agents break mucosal barrier, stimulate an increase in gastric mucosal permeability to H^+ and Na^+ ions, consequently reducing the transmucosal potential difference and induce formation of erosions and ulcers [48]. However, there are multiple aetiologic factors in ulcer pathogenesis and the ability of the extracts to protect against aspirin - induced ulceration may indicate their ability to inhibit one or more multiple inciting stimuli in ulcerogenesis.

CONCLUSION

The *V. amygdalina* contained vase array of phytochemicals in variable proportion. The aqueous - methanol leaf extract of *V. amygdalina poses* anti-ulcer effect. This effect might be due to the synergic effect of these phytochemicals.

REFERENCES

1. Boakye-Yiadom, M., Kumadoh, D., Adase, E and Woode, E (2021). Medicinal Plants with Prospective Benefits in the Management of Peptic Ulcer Diseases in Ghana. *Hindawi BioMed Research International*, 5574041, 14.
2. Wasman S, Mahmood A, Salehhuddin H, Zahra A, Salmah I (2010). Cytoprotectiveactivities of Polygonum minus aqueous leaf extract on ethanol-induced gastriculcer in rats. J Med Plants Res., 4: 2658e2665.
3. Ofusori, AE., Moodley, R., Jonnalagadda, SB (2021). Antiulcerogenic effects of Celosia trigynaplant extracts on ethanol-induced gastric ulcer in adult Wistar rats. *Journal of Traditional and Complementary Medicine,* 10: 586e593
4. Noemi D. Paguigan Darryl Hannah B. Castillo Christine L. Chichioco-Hernandez(2014).Anti-ulcer activity of leguminosae plants. Arq. Gastroenterol. 51 (1): 64-68
5. Oh TY, Yeo M, Han SU, et al. Synergism of Helicobacter pylori infection andstress on the augmentation of gastric mucosal damage and its prevention with-tocopherol.Free Radic Biol Med. 2005;38:1447e1457..
6. Varadharaj V, Muniyappan J. Phytochemicaland phytotherapeutic propertiesof Celosia species-A review.Int J Pharm Phytochem Res. 2017;9:820e825.
7. Zhu, k., Yang, X., Yang, C., Ye, X., Zhang, H (2020). Gastro-protective actions of Aloe barbadensis Miller mitigate ethanol-induced gastric injury in rats. Tropical Journal of Pharmaceutical Research, 19 (12): 2645-2650
8. Saleh MM, Qader SW, Thaker AA. Gastroprotective activity of Eruca sativa leafextract on ethanol-induced gastric mucosal injury in Rattus norvegicus.Jordan JBiol Sci. 2016;9:47e52
9. Borrelli F, Izzo AA (2000). The Plant Kingdom as a Source of Anti-ulcer. Phytother Res. 14: 581-91.
10. Kumar A, Dewan B, Rama T (2011). Evaluation of anti-ulcerogenic properties from the root of *Flemingia strobilifera* J Basic Clin Pharm. 2(1):
11. Patil PH, Patil JY, Mahale JN, Patel JB, Surana SJ (2010). Evaluation of antiulcer activity of the terpenoid fraction from the leaves of *Thespesia populnea* (L) (Malvaceae) in albino rats. Res J Pharm Bio Che Sci. 4:495-513.
12. Boakye-Yiadom, M., Kumadoh, D., Adase, E and Woode, E (2021). Medicinal Plants with Prospective Benefits in the Management of Peptic Ulcer Diseases in Ghana. *Hindawi BioMed Research International*, 5574041, 14.
13. Lakshmi,TS.,Lakshmi,SM., Neelufar, SS., Koteswara, GR and. Prasanna, GR (2013). Medicinal plants as anti-ulceragents,"*JournalofPharmacognosyandPhytochemistry*,. 2(4): 91–97.
14. Kaur, D., Rana, AC., Sharma, NN and Kumar, SS (2012). Herbal drugs with anti-ulcer activity. *Journal of Applied Pharmaceutical Science*, 2(3):160–165.
15. Al-Radahe, S., K. A. Ahmed, KA., Salama, S et al.,(2012). Anti-ulcer activity of Swietenia mahagoni leaf extract in ethanol-induced gastric mucosal damage in rats," *Journal of Medicinal Plants Research*, 6(12): 988–997, 2012.
16. Zakaria, ZA., Abdul Hisam,EE., Norhafizah, CM et al.(2012). Methanol extract of Bauhinia purpurea leaf possesses anti-ulcer activity. *Medical Principles and Practice*, 21(5), 476–482, 2012.
17. Zabidi,Z.,Zainulddin,WNW.,. Mamat, SS et al.,(2012). Antiulcer activity of methanol extract of Melastoma malabathricum leaves in

rats,"*Medical Principles and Practice*, 21(5): 501–503.

18. P. S. Bhajoni, G. G. Meshram, and M. Lahkar, "Evaluation of the antiulcer activity of the leaves of Azadirachta indica: an experimental study," *Integrative Medicine International*, vol. 3, no. 1-2, pp. 10–16, 2016.
19. S. A. Brito, C. L. F. de Almeida, T. I. de Santana et al., "Antiulcer activity and potential mechanism of action of the leaves of Spondias mombin L," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 1731, 20 pages, 2018.
20. M. Abebaw, B. Mishra, and D. A. Gelayee, "Evaluation of anti-ulcer activity of the leaf extract of Osyris quadripartita Decne. Santalaceae in rats," *Journal of Experimental Pharmacology*, vol. Volume 9, pp. 1–11, 2017.
21. Kupchan, S.M., Hemmnigway, R.J., Karim, A. and Werner, D. (1969). Tumor inhibitors XLVII Vernodalin and Vernomygdin. Two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina*. *Del. J. Org. Chem.* 34: 3908-3911.
22. Huffman, M.A. and Seifu, M. (1989). Observations on the illness and consumption of a possibly medicinal plant, *Vernonia amygdalina* (Del.), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. *Primates,* 30: 51 – 63.
23. Izevbigie, E.B. (2003). Discovery of water-soluble anticancer Agents (Edotides) from a vegetable found in Benin City. *Nigeria Experimental Biology.* 228: 293-298., E.B. (2003). Discovery of water-soluble anticancer Agents (Edotides) from a vegetable found in Benin City. *Nigeria Experimental Biology.* 228: 293-298.
24. Ojiko, O.A. and Nwanjo, H. (2006). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Afr. J. Biotech.* 5(18): 1648-1651
25. Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxocology.* 54(4): 275-287
26. Pederneral, A.M., Guardia, T/. Cal;eron, C.g/. Rotellim, A/F., Dela Rocha, N.F., Di Genaro, S. and Pelze, L.F. (2006). 'Antiulcerogenic and anti-inflammatory activity of the methanolic extract of *Larrea divaricate* Cav. In rat'. *J. Ethnopharmacol.* 105: 415-420.
27. Qadir, M. (2013). Researchgate government college university Faisalabad.
28. Tanawski, A., Szabo, I.L., Hussain, S.S. and Soreghan, B. (2001). Regeneration of gastric mucosa during ulcer healing is triggered by growth factors and signal transduction pathways. *J. Physiol. Paris.* 95: 337-344.
29. Hoogerwerf, W.A. and Pasricha, P.J. (2001). Pharmacologic therapy in treating achalasia. *Gastrointest. Endosc Clinic N. Am.* 11: 311 – 324.
30. Dharmani, P., Kuchibhotla, V.K., Maurya, R., Srivastava, S., Sharam, S. and Palit, G. (2004). Evaluation of anti-ulcerogenic and ulcer-healing properties of *ocimum sanctum* Linn. *J. Ethopharmacol.* 96: 197 – 206.
31. Hodek, P., Trefil, P. and Stiborova, M. (2002). 'Flavonoids – Potent and versatile biologically active compounds interacting with cytochrome. P 450. *Chemico- Biol. Intern.* 139(1): 1-21.
32. Ebong, MPE and Umoh, IB (2010). Phytochemical screening of activity directed extracts of *Vernonia amygdalina* leaves. Global Journal of Pure and Applied Sciences,16 (1): 151-154
33. Ali, M., Diso, SU.,. Waiya, SA., Abdallah, MS (2019). Phytochemical Screening and Antibacterial Activity of Bitter Leaf (Vernonia amygdalina. Annals of Microbiology and Infectious Diseases, 2(4): 01-07

34. Raimi, C.O., Oyelade A.R. and Adesola, O.R. (2020). Phytochemical screening and in-vitro antioxidant activity on Vernonia amygdalina (ewuro-bitter leaf). European Journal of Agriculture and Forestry Research,.8(2):12-17,
35. Gul, H., Abbas, K., Qadir, MI (2015). Gastro-protective effect of ethanol extract of *Mentha longifolia* in alcohol- and aspirin-induced gastric ulcer models Bangladesh J Pharmacol. 10: 241-245
36. Jimmy E O. and Ebong A. U. (2017). Raised dosage of cashew leaves extract (anacardium occidentale) more potent than omeprazole in gastric secretion ulcer treatment. European Journal of Biomedical and Pharmaceutical Sciences. 4(6): 132-136.
37. Awaad, AS., El-Meligy, RM., Soliman, GA (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. J Saudi Chem Soc., 17:101-24. 22
38. Benchikh F: Pharmacological Effects 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: 2018.23
39. Bhalke, RD, Giri, MA, Anarthe, SJ, Pal, (2010), SC:Antiulcer activity of the ethanol extract of leaves of Sesbania grandiflora(linn.).Int J Pharm Pharm Sci., 2(4): 206-08.
40. Divyapraba, M., Chitra, P., Rameshwari, KS (2021). Anti-ulcer activity of Psidium guajava on pylorus ligation induced gastric ulcer in albino rats. IJPSR, 12(1): 443-449.
41. Okwuosa, CN., Nwachukwu, DC., Achukwu, PUO., Ezeorah, CG., Eze AA (2009). Anti-ulcer activity of the leaf extracts of Borreria ocymoides in rats. *Bio-Research,* 7(1): 451 – 455
42. Iwamoto, J.,Murakami, M., Monma, T., Ueda, MH., Tamamushi, M., Konishi, N., Yara, S., Hirayama, T., Ikegami, T., Honda, A and Mizokami, Y. (2020). Current states of prevention of drug-induced gastroduodenal ulcer in real clinical practice: a cross-sectional study. J Clin Biochem Nutr. 66(2): 158–162.
43. Mousa, AM., El-Sammad, NM., Hassan, SK., Madboli, ANA., Hashim, AN.,. Moustafa, ES., Bakry, SM and Elsayed, EA (2019). Antiulcerogenic effect of Cuphea igneaextract against ethanol-induced gastriculcer in rats. BMC Complementary and Alternative Medicine (2019) 19:345
44. Al-Mofleh, I.A., Alhadier, A.A., Mossa, J.S., Al-Sohaibani, M.O., Al-Yahya, M.A., Rafatullah, S. and Shaik, S.A. (2008). Gastroprotective effect of an aqueous suspension of Black Cumin Nigella sativa on necrotizing agent induced gastric injury in experimental animals. The Saudi Journal of Gastroenterology, 14 (3): 128-134.
45. British National Formulary (1996). A joint publication of the British Medical Association and the Royal Pharmaceutical Society of GreatBritain, 3(39): 33-34,
46. John, T.A. and Onabanjo, A.O. (1990).Gastroprotective effect of the bark extractsEntandrophragma utile in experimental ethanol inducedulceration.J.Ethnopharmacol., 29: 87-93
47. Merck Index (1989). An encyclopedia of chemicals,drugs, andbiologicals11thedition(Budavaris), Merck and Co. Inc, NJ, USA
48. Uraku, AJ., Uraku, OH., Edwin, N., Ezeali, C., Ezeani, N.N., Obasi, OU., Ogoh, NV., Dingwoke, EJ., Orinya, OF., Nweke, FN., Ozioma, PE., Enemchukwu, BN (2020) Protection by methanol and flavonoid-rich leaf extracts of *Talinum triangulae* against lead-induced toxicity in rats. *Research Journal of Medicinal Plant.* 14 (3): 125-132