AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Hematological Hemostatic Effects of Fractions of *Dialium guineense* Tree Bark in Ethanol-Induced Peptic Ulcer in Albino Rats

[1]Obidike, Ikechukwu Johnlouis, [1]Umeh, Virginus Agozie and [2]Hashimu, Dauda Anoh

[1]Department of Biochemistry, College of Natural Sciences,
Michael Okpara University of Agriculture, Umudike, Nigeria

[2]Department of Microbiology, Faculty of Sciences, Federal University of Lafia, Nasarawa State, Nigeria

ABSTRACT

Background and Objective: Peptic Ulcer Disease (PUD) has been linked to several complications including disrupted hematological stability. This study aims to determine the hematological hemostatic effects of fractions of *Dialium guineense* stem bark in ethanol-induced ulcers in albino rats. **Materials and Methods:** Thirty rats were divided into six groups (five rats each) to assess biochemical effects. Group 1 was the normal control, receiving only food and water, while Group 2 (negative control) received ethanol alone. Group 3 received ethanol plus omeprazole, while Groups 4, 5 and 6 received ethanol combined with ethanol, methanol and n-hexane extracts, respectively, at 250 mg/kg body weight. Statistical analysis was performed using one-way ANOVA in SPSS, with significance set at $p<0.05$. **Results:** The results showed a significant ($p<0.05$) increase in the bleeding time and clotting time in the negative control with a concomitant significant ($p<0.05$) decrease in the bleeding time and clotting time across the co-treated groups. The same trend was noticed in the osmotic fragility and then in urea and creatinine concentration of the rats across the co-treated groups. **Conclusion:** It can be said that the seeds of *Dialium guineense* could contain active compounds that could improve the membrane integrity of red blood cells, improve platelet aggregation, increase blood coagulation and possibly accelerate wound healing. The mechanism is possibly through increasing PDGF-BB which is a growth factor released during platelet aggregation to activate the healing process.

KEYWORDS

Dialium guineense, hematological stability, osmotic fragility, peptic ulcer disease, renal

INTRODUCTION

Medicinal plant parts and their constituents have been utilized as main therapeutic agents in the treatment of illnesses since the beginning of time[1]. Prepared and taken as concoctions or decoctions, herbal plant constituents have been used in the management of several ailments like hepatic injuries[2], hyperglycemia[3], renal injuries[4] and ulcers[5].

ISSN: xxxx-xxxx (Online)
ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2025.109.117

Received: 05 Nov. 2024
Accepted: 15 Jan. 2025
Published: 31 Mar. 2025

Dialium guineense (Wild), also known as "black velvet" or "velvet tamarind," is a member of the Fabaceae family[6]. It is a tree plant with smooth, greyish bark and dense, hairy leaves that grows to an average height of 30 m. Fruits are typically round and red and flowers are frequently pale[7]. Local users have shown that the African native plant *Dialium guineense* (DAG) is effective in treating peptic ulcers, especially when brewed as a tea[5,8]. Though initially attributed to the leaves[9], recent research shows that the tree bark could exhibit this behavior[5].

Peptic ulcer has been considered as one of the most prevalent of all digestive diseases in the recent century[10]. Peptic ulcers, which are usually chronic diseases, leaves single lesions which may occur at any point within the digestive tract[11]. In some countries, including the United States, the risk of developing peptic ulcer disease is about 10% over a lifetime[12]. It's possible that people in Nigeria use traditional methods of self-medication, making the prevalence of PUD unclear[13]. Nevertheless, PUD-related complications have resulted in high death rates; perforations account for roughly 30% of hemorrhage, while obstruction is the most common complication, accounting for roughly 10%[14]. The pathophysiology of peptic ulcers is multifactorial because it can be caused by, among others, the imbalance between acid and pepsin aggressive factors and the mucosal defense factors which are prostaglandins and blood flow[15].

Helicobacter pylori (*H. pylori*) is one of the main causative agents of Peptic Ulcer Disease (PUD). It creates an alkaline environment suitable for its survival by secreting urease and attaches itself to the gastric epithelium by expressing outer inflammatory protein adhesin (OipA)[16]. This bacterium expresses CagA and PicB which are its virulence factors that induce stomach mucosal inflammation[17]. The mechanism of causing peptic ulcers by another virulence factor called VacA is unclear although its gene is believed to encode for a vacuolating cytotoxin[18]. Such stomach mucosal inflammation can either be associated with an increase in the stomach acid secretion (hyperchlorhydria) or reduction of the stomach acid secretion (hypochlorhydria)[19].

Although the processes driving EtOH-induced gastric ulcer are not fully understood, evidence suggests that Pro-inflammatory cytokines, oxidative stress and apoptosis are critical in the progression of the condition. Neutrophil activation contributes to an enhanced inflammatory response, resulting in increased gastric expression of Nuclear Factor Kappa B (NF-kB). The NF-kB plays a central role in the production of pro-inflammatory cytokines such as TNF-α and IL-1β. These processes further intensify the inflammatory response through NF-kB-driven mechanisms[20,21]. This study therefore, seek to solidify notion for the use of *Dialium guineense* tree bark in the management of disruption in hematological hemostasis in ethanol-induced ulcer rats.

MATERIALS AND METHODS

Collection and identification of sample: The study was conducted between April and August, 2023 at the animal house and Laboratory Unit of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The stem bark of *Dialium guineense*, used in the study, was sourced from Ozubulu, located in Ekwusigo Local Government Area, Anambra State. The plant was identified and authenticated by Professor G.G.E. Osuagwu from the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike. A voucher specimen was preserved for reference in the herbarium of the Department of Physiology and Pharmacology at the College of Veterinary Medicine.

Preparation of extract: Tap water was used to thoroughly wash the *Dialium guineense* bark to prevent dirt from contaminating the samples. After being cleaned, the sample was allowed to air dry for eight weeks at room temperature. A hard metal grinder that was made locally was then used to grind the dried plant sample. The powdered sample was extracted into 50 g using a Soxhlet extractor (model SE-6P Infitek, China) and 96% ethanol. For 72 hrs, the extraction temperature was kept at 60°C. After that, the dried extract was oven-dried at 40°C (IEC60068-2-2 Dongguan, China) to produce

a 2.00 g dark yellow semi-solid sample. Until its use, the crude ethanol extract was kept at 10°C in a refrigerator (Thermocool, Nigeria). The percentage yield of the extract was calculated using the formula[1]:

$$\text{Yield}\,(\%) = \frac{X}{Q} \times 100$$

Where:

X = Weight of dried *Dialium guineense bark* extract after extraction (2.00 g)
Q = Weight of *Dialium guineense bark* extract powdered plant material before extraction

Study design study design for the biochemical estimation of *Dialium guineense* bark extract: Thirty male albino rats, obtained from the animal house of Michael Okpara University, Umudike, were randomly divided into six groups, housed in aluminum cages with five animals each and allowed to acclimate for twelve days. The animals were maintained in standard laboratory conditions, fed at room temperature, with a 12 hrs light and dark cycle and all rules related to the use and maintenance of laboratory animals were carefully adhered to.

The animals were grouped as such:

- **Group 1:** Normal control (received food and water only) *ad libitum*
- **Group 2:** Negative control (received 1 mL of ethanol only)
- **Group 3:** Drug control (received 1 mL of ethanol+omeprazole 20 mg)
- **Group 4:** Ethanol group (received 250 mg/b.wt., of ethanol fraction extract+1 mL of ethanol)
- **Group 5:** Methanol group (received 250 mg/b.wt., of methanol fraction extract+1 mL of ethanol)
- **Group 6:** n-hexane group (received 250 mg/b.wt., of n-hexane fraction extract+1 mL of ethanol)

Using an oral gavage, the plant extract was given orally to the animals according to their body weights and the ethanol was given every 2 hrs for 21 days. Following the administration, the animals were killed by cervical dislocation and cardiac punctures were used to obtain blood samples.

Renal parameters

Evaluation of creatinine concentration: Creatinine level was evaluated according to the Direct End Point Method as described by Obidike *et al*.[3].

The principle is that creatinine reacts with picric acid in alkaline conditions to form a colored complex, which has an absorption peak at 510 nm. The rate of formation of the color is proportional to the creatinine concentration in the sample. In this endpoint method, the difference in absorbances measured before and after color formation yields a creatinine value corrected for interfering substances.

Procedure: Test tubes were labeled test (reagent) blank, standard, control and sample tubes, respectively. Next, 3.0 mL of working reagent was added into each test tube. The 0.1 mL (100 µL) of sample was also added its respective tubes, but 0.1 mg distilled water was added to the reagent blank and mixed. The reaction mixture was placed in a water bath and heated for 15 min at 37°C. The absorbance of the reaction mixture was read on the spectrophotometer at 510 nm against the reagent blank. The creatinine value of the sample was determined by comparing the change in absorbance with that of the standard[4]:

$$\text{Creatinine}\,(\text{mg/dL}) = \frac{\text{OD of sample}}{\text{OD of standard}} \times \text{Concentration of standard}$$

Where:

OD = Optical density

Evaluation of urea concentration: Urea level was evaluated using the Urease Berthelot method as described by Obidike *et al.*[3]. The principle is that urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically as presented in the Berthelot reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \rightarrow \text{Indophenol (blue compound)}$$

Procedure: Test tubes were labeled as blank, standard and sample, respectively. Then 10 µL each of sample and standard reagent was added to the test tube labeled test and standard, respectively. Next, 100 µL of reagent 1 was added to all the test tubes, mixed and incubated at 37°C for 10 min. Then 2.50 mL of reagent 2 and 2.50 mL of reagent 3 were added to all the test tubes and the solutions mixed and immediately incubated at 37°C for 15 min.

The absorbances of the sample (A_{sample}) and standard ($A_{standard}$) were read against the blank at 578 nm:

$$\text{Urea (mg / dL)} = \frac{\text{OD of sample}}{\text{OD of standard}} \times \text{Concentration of standard}$$

Where:
OD = Optical density

Clothing time: Whole blood clotting time was measured by the capillary glass tube method described by Chen *et al.*[22]. Blood sample was collected via the retroorbital plexus with a glass capillary tube and kept on a slide to allow clotting. The blood was stirred with a dry needle every 30 sec until the needle wire provoked a fibrous protein, which was defined as clotting time.

Bleeding time assay: The bleeding time assay was estimated using the method described by Chen *et al.*[22]. Ninety minutes after the last administration, the rats' tails were marked with a tag approximately 5 mm long and then cut at the mark. Then, the tip of the tail was immersed in saline at 37°C and the time from cutting the tip of the tail to stopping the bleeding was recorded; this interval was defined as bleeding time.

Osmotic fragility: The estimation of osmotic fragility was done using the method described by de Freitas *et al.*[23]. About 10 µL of fresh blood was mixed with an equal volume of plant extract working solution containing 0.9% sodium chloride and allowed to stand for 15 min at room temperature. Solutions (2 mL) containing sodium chloride at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 were added to centrifuge tubes. Then 20 µL of blood samples were added and the sample was gently mixed and allowed to stand for 5 min at room temperature. The content was mixed again and centrifuged at 3000 rpm for 7 min. The absorbance of the supernatant was measured at 570 nm against 0.9% sodium chloride solution. The same procedure was used for standard samples.

Statistical analysis: Data were analyzed by One-way Analysis of Variance (ANOVA) using the Statistical Package of Social Sciences (SPSS) software version 22 for Windows. Results were expressed as Mean±Standard Deviation (SD) and difference in mean tested using *post hoc* LSD. A level of $p<0.05$ was considered significant. Table 1 shows the effects of *Dialium guineense* bark on bleeding time and clotting time of ethanol-intoxicated rats.

Table 1: Effects of *Dialium guineense* bark on bleeding time and clotting time in ethanol-induced peptic ulcer in albino rats

Groups	Bleeding time (sec)	Cloting time (sec)
Group 1 (Normal group)	104.71±5.11*	93.55±2.35*
Group 2 (Negative control)	165.37±2.18	150.44±3.41
Group 3 (Drug control)	134.04±3.01*	123.33±3.51*
Group 4 (Ethanol 250 mg/kg extract+1 mL ethanol)	120.40±3.62*	120.52±2.11*
Group 5 (Methanol 250 mg/kg extract+1 mL methanol)	127.71±2.52*	115.10±2.14*
Group 6 (n-hexane 250 mg/kg extract+1 mL ethanol)	127.51±5.22*	114.94±2.16*

Values are displayed as Mean±Standard Deviation and values marked (*) are statistically significant ($p<0.05$) compared to group 2

Table 2: Effects of *Dualium guineense* bark extract on plasma fragility in ethanol-induced peptic ulcer in albino rats

Group	F (0%)	F (0.1%)	F (0.2%)	F (0.3%)	F (0.4%)	F (0.5%)	F (0.6%)	F (0.7%)	F (0.8%)	F (0.85%)
Group 1 (Normal group)	97.11±0.13*	97.44±0.10*	93.23±0.55*	73.55±0.31*	64.70±0.55*	53.33±0.10*	37.88±0.93*	21.21±0.32*	7.19±0.11*	2.59±0.97*
Group 2 (Negative control)	99.40±0.12	99.62±0.33	98.33±0.86	89.12±0.11	80.55±0.65	74.12±1.91	52.23±1.74	36.12±0.32	20.11±1.33	14.29±1.98
Group 3 (Drug control)	97.12±0.13*	97.23±0.15*	93.29±0.48*	75.35±0.44*	68.23±0.24*	60.57±1.56*	45.33±1.29*	26.12±1.57*	12.20±0.59*	9.33±0.18*
Group 4 (Ethanol 250 mg/kg extract+1 mL ethanol)	97.00±0.67*	98.33±0.17*	92.35±0.37*	73.59±0.83*	61.62±1.63*	60.23±1.10*	43.43±0.54*	25.13±1.12*	13.33±0.12*	9.90±0.10*
Group 5 (Methanol 250 mg/kg extract+1 mL methanol)	97.99±0.36*	97.64±0.31*	94.33±0.52*	75.11±0.97*	64.85±1.22*	60.52±0.67*	44.35±0.35*	25.33±0.34*	11.30±0.10*	5.39±0.88*
Group 6 (n-hexane 250 mg/kg extract+1 mL ethanol)	97.20±0.36*	97.55±0.25*	93.33±0.49*	74.13±0.74*	65.24±1.55*	59.17±0.25*	45.55±0.15*	26.20±0.22*	13.33±0.13*	7.67±0.23*

Values are displayed as Mean±Standard Deviation and values marked (*) are statistically significant ($p<0.05$) compared to group 2 and F: Fragility

Table 3: Effects of different fractions of *Dialium guineense* stem bark on serum urea, creatinine and sodium concentration in ethanol-induced ulcer in albino rats

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mEq/L)
Group 1 (Normal group)	16.76±0.54	0.71±0.08	132.16±0.56
Group 2 (Negative control)	17.70±0.69	1.13±0.05	129.54±1.06
Group 3 (Drug control)	15.74±0.60*	0.94±0.03*	133.40±2.18*
Group 4 (Ethanol 250 mg/kg extract+1 mL ethanol)	15.04±0.39*	0.95±0.04*	135.86±0.76*
Group 5 (Methanol 250 mg/kg extract+1 mL methanol)	15.82±0.82*	0.89±0.04*	136.64±0.77*
Group 6 (N-hexane 250 mg/kg extract+1 mL ethanol)	15.98±0.43*	0.86±0.05*	136.64±1.49*

Values are Mean±Standard Deviations, *Statistically significant ($p<0.05$) compared to negative control (group 2)

https://doi.org/10.3923/ajbs.2025.109.117 | Page 113

RESULTS

The bleeding time results indicated a significant ($p<0.05$) prolongation in the negative control group when compared to the normal control group. In contrast, groups co-treated with various fractions of *Dialium guineense* exhibited a significant ($p<0.05$) reduction in bleeding time compared to the negative control group.

Similarly, clotting time results showed a significant ($p<0.05$) increase in the negative control group compared to the normal control group. However, groups co-treated with different fractions of *Dialium guineense* demonstrated a significant ($p<0.05$) decrease in clotting time relative to the negative control group.

Table 2 shows that the result of the osmotic fragility test showed significantly ($p<0.05$) increased osmotic fragility in the negative control group compared to that of the normal control group. Meanwhile, the osmotic fragility of the groups that co-treated different fractions of *Dialium guineense* showed a significant ($p<0.05$) decrease which occurred across all fractions compared to that of the untreated group (group 2).

Table 3 shows the effects of different fractions of *Dialium guineense* stem bark on serum urea, creatinine and sodium concentration in ethanol-induced ulcers in albino rats. The result of the creatinine analysis indicated that the creatinine concentration of group 2 (negative control) was increased. However, the concentration of the co-treated groups was significantly ($p<0.05$) reduced. Meanwhile, the change in the urea concentration was within a comparable range across all groups. However, for the sodium concentration, the concentration of group 2 (negative control) was reduced while the concentration of all other groups was significantly ($p<0.05$) increased compared to the negative control.

DISCUSSION

The result of this study showed that *Dialium guineense* stem bark reduced the clotting time, bleeding time and osmotic fragility of the red blood cell while concomitantly reducing the urea and creatinine concentration in ethanol-induced ulcer rats. Any membrane stabilizing agent's effectiveness rests on its capacity to lessen adverse effects or return the damaged cell membrane's normal physiology to its pre-damaged state[24]. Due to the high concentration of oxygen and hemoglobin within the cell, as well as the membrane's high polyunsaturated fatty acid content, all of which are potent oxidative process promoters, erythrocytes are vulnerable to oxidative damage[24]. The increase in the ethanol-induced group may be a sign of toxicity and be linked to the intoxicant's induction of oxidative stress. The decrease in the groups that co-treated different fractions of *Dialium guineense* could be suggestive of improved membrane integrity associated with the plant extract. This could be indicative that *Dialium guineense* could be used among a combination of plants used in the treatment of various diseases characterized by anemia, jaundice and fatigue[25]. The ability of *Dialium guineense* bark extract to reverse the increased fragility caused by high ethanol might be attributed to its high antioxidant properties. Antioxidants have been utilized to shield erythrocyte membrane integrity from oxidative stress[26] and antioxidant-rich herbal preparations have also been shown to stabilize red blood cell membranes[27]. Flavonoids, which have been shown to have antioxidant properties, may be the cause of this extract's capacity to protect erythrocyte membranes[26]. According to reports, flavonoids have strong effects that can stabilize the erythrocyte membrane and scavenge free radicals[28].

Certain substances have already been identified as anti-oxidants that can stop the oxidation of susceptible substrate, such as glutathione, tocopherol and proactive enzyme[29]. According to Ahur *et al.*[25], toxicants (ethanol) is known to damage the liver by producing an excess of highly reactive metabolites that deplete glutathione, weaken the erythrocyte membrane through oxidative stress and ultimately cause erythrocyte lysis. This is why the negative control group had a higher fragiligram.

Activated platelet adhesion, aggregation, secretion functions and activation of intrinsic and extrinsic coagulation systems, which result in blood coagulation and fibrin formation, are all closely linked to thrombosis, a major cause of morbidity and mortality[30]. The two stages of hemostasis are coagulation cascade and platelet aggregation. Accordingly, coagulation and platelet factors contribute to blood hemostasis[22]. Two highly validated targets that play important roles in the coagulation cascade are thrombin and FXa[31].

Thrombin inhibitors and FXa inhibitors (like rivaroxaban) make up a large number of clinically prescribed anticoagulants. Thrombin interacts with most zymogens and their cofactors, is essential for preserving hemostasis and functions as both an anticoagulant and a procoagulant in blood coagulation[32].

One possible sign of impaired hemostasis is the increase in bleeding and clotting times observed in the ethanol-induced group (group 2). This is consistent with the discovery made by Chen *et al*.[22] that increased bleeding and clotting times indicate a malfunction or inhibition of the blood coagulation or platelet aggregation pathways. The noteworthy ($p<0.05$) decrease observed in the co-treated groups may suggest enhanced platelet aggregation, leading to decreased bleeding and clotting time and ultimately, better hemostasis. Numerous cells, including platelets that are activated by collagen exposure, are involved in this process. Thus, von Willebrand factor (vWF) mediates the adhesion of the glycoprotein on the cell surface to the epithelium[33]. Alpha granule membranes are activated by adherence to the epithelial surface, releasing TXA2 and ADP, two important components of the platelet aggregation process that shorten the bleeding time from wounds or burst vessels. Following aggregation, an intrinsic and extrinsic factor-driven coagulation cascade is triggered[34].

Increase in serum creatinine and urea has been correlated not only to renal assault[4] but also peptic ulcer disease[35]. Renal dysfunction induces inflammation and oxidative stress which damages or further exacerbate stomach or intestinal mucosa[36]. The decrease in the creatinine concentration of the co-treated groups could be indicative of reduced inflammation and reversed oxidative stress and reversal of exacerbation of the stomach mucosa by the different fractions of *Dialium guineense* stem bark.

SIGNIFICANCE STATEMENT

This study evaluates the effects of fractions of *Dialium guineense* tree bark on hematological stability in ethanol-induced peptic ulcer in albino rats. The result indicated that the fractions reduced osmotic stability, bleeding time, clotting time with concomitant reduced urea and creatinine. This result will open a new chapter into research of the specific phytochemical composition of the extract which could exhibit the hematological hemostasis noticed coupled with possible isolation and identification of the bioactive component of *Dialium guineense* which propelled the result expressed.

CONCLUSION

This study indicates that *Dialium guineense* stem bark extracts may protect against blood-related disruptions in peptic ulcer disease. Co-administration with ethanol improved hemostatic function by reducing bleeding and clotting times, enhancing platelet aggregation and stabilizing red blood cell membrane integrity. Additionally, the extracts supported renal health, suggesting a therapeutic potential in aiding hemostasis and tissue repair via mechanisms involving Platelet-Derived Growth Factor BB (PDGF-BB).

REFERENCES

1. Chita, E.I. and I.J. Obidike, 2020. Nutraceutical, antioxidant and hepatic histomorphological effects of *Tetrapleura tetraptera* leaves in monosodium glutamate-intoxicated rats. Asian J. Emerging Res., 2: 223-238.
2. Johnlouis, O.I., N.C. Ifeanyi and O.P. Oluchukwu, 2022. Consequence of *Tetrapleura tetraptera* leaves on pro-oxidants, hepatic functions and histomorphology in monosodium glutamate-intoxicated rats. Res. J. Med. Plants, 16: 37-48.

3. Obidike, I.J. and M.E. Ngozi, 2024. Effects of carrot (*Daucus carota*) stalk on blood glucose level, biochemical functions and serum antioxidant activity in alloxan-induced diabetic rats. Asian Sci. Bull., 2: 46-59.
4. Johnlouis, O.I., C.E. ije, N.C. Ifeanyi and O.Q. Chiamaka, 2023. Effects of *Tetrapleura tetraptera* leaves on renal architecture and haematological indices in monosodium glutamate-intoxicated rats. Asian Sci. Bull., 1: 8-17.
5. Johnlouis, O.I., A.G. Sunday, O.C. Adanma and O.J. Ngozi, 2024. Evaluation of anti-ulcerogenic effects of the crude extract and fractions of *Dialium guineense* tree bark in ethanol-induced peptic ulcer in albino rats. Asian J. Biol. Sci., 17: 254-273.
6. Besong, E.E., M.E. Balogun, S.F.A. Djobissie, D.C. Obu and J.N. Obimma, 2016. Medicinal and economic value of *Dialium guineense*. Afr. J. Biomed. Res., 19: 163-170.
7. Assiki, T., A. Diallo, E. Badjabaïssi, M. Assih and T. Kpatcha *et al*., 2022. Antidiarrheal activity of *Dialium guineense* willd fruit pulp in Wistar rats. BioMed Res. Int., Vol. 2022. 10.1155/2022/4161714.
8. Agbaje, E.O. and Y.P. Doe, 2015. Gastric and duodenal antiulcer effects of aqueous bark extract of *Dialium guineense* Wild. (Fabaceae) and the possible mechanisms in laboratory models. J. Phytopharmacology., 4: 268-275.
9. Ezeja, M.I., Y.S. Omeh, I.I. Ezeigbo and A. Ekechukwu, 2011. Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). Ann. Med. Health Sci. Res., 1: 55-62.
10. de Lira Mota, K.S., G.E.N. Dias, M.E.F. Pinto, Â. Luiz-Ferreira and A.R.M. Souza-Brito *et al*., 2009. Flavonoids with gastroprotective activity. Molecules, 14: 979-1012.
11. Singh, A.K., S.K. Singh, P.P. Singh, A.K. Srivastava, K.D. Pandey, A. Kumar and H. Yadav, 2018. Biotechnological aspects of plants metabolites in the treatment of ulcer: A new prospective. Biotechnol. Rep., Vol. 18. 10.1016/j.btre.2018.e00256.
12. Lanas, A. and F.K.L. Chan, 2017. Peptic ulcer disease. Lancet, 390: 613-624.
13. Ray-Offor, E. and K.A. Opusunju, 2020. Current status of peptic ulcer disease in Port Harcourt metropolis, Nigeria. Afr. Health Sci., 20: 1446-1451.
14. Irabor, D.O., 2005. An audit of peptic ulcer surgery in Ibadan, Nigeria. West Afr. J. Med., 24: 242-245.
15. Shams, S.G.E. and R.G. Eissa, 2022. Amelioration of ethanol-induced gastric ulcer in rats by quercetin: Implication of Nrf2/HO1 and HMGB1/TLR4/NF-κB pathways. Heliyon, Vol. 8. 10.1016/j.heliyon.2022.e11159.
16. Malfertheiner, P., F. Megraud, C.A. O'Morain, J.P. Gisbert and E.J. Kuipers *et al*., 2017. Management of *Helicobacter pylori* infection-The maastricht V/Florence consensus report. Gut, 66: 6-30.
17. Rugge, M., E. Savarino, M. Sbaraglia, L. Bricca and P. Malfertheiner, 2021. Gastritis: The clinico-pathological spectrum. Digestive Liver Dis., 53: 1237-1246.
18. Sugano, K., J. Tack, E.J. Kuipers, D.Y. Graham and E.M. El-Omar *et al*., 2015. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut, 64: 1353-1367.
19. Simões, S., R. Lopes, M.C.D. Campos, M.J. Marruz, M.E.M. da Cruz and L. Corvo, 2019. Animal models of acute gastric mucosal injury: Macroscopic and microscopic evaluation. Anim. Models Exp. Med., 2: 121-126.
20. Arab, H.H., S.A. Salama, H.A. Omar, E.S.A. Arafa and I.A. Maghrabi, 2015. Diosmin protects against ethanol-induced gastric injury in rats: Novel anti-ulcer actions. PLoS ONE, Vol. 10. 10.1371/journal.pone.0122417.
21. Chauhan, I., S. Agrawal and R.K. Goel, 2018. Status of inflammatory markers and growth factor in gastric ulcer protective effects of *Punica granatum* L. peel extract in rat. Nat. J. Physiol. Pharm. Pharmacol., 8: 12-17.
22. Chen, H., M. Jin, Y.F. Wang, Y.Q. Wang and L. Meng *et al*., 2014. Effect of *Toona microcarpa* harms leaf extract on the coagulation system. BioMed Res. Int., Vol. 2014. 10.1155/2014/615363.
23. de Freitas, M.V., R. de Cássia M. Netto, J.C. da Costa Huss, T.M.T. de Souza, J.O. Costa, C.B. Firmino and N. Penha-Silva, 2008. Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. Toxicol. *in vitro*, 22: 219-224.

24. Leite, D.O.D., C.J. Camilo, J.W.G. Castro, A.R. Dantas and F.F.G. Rodrigues *et al*., 2023. Influence of methanolic extracts from seeds and pulp of *Annona squamosa* L. on osmotic and morphological fragility in human erythrocytes. Curr. Res. Toxicol., Vol. 4. 10.1016/j.crtox.2023.100103.
25. Ahur, V.M., Y.A. Adenkola, S.A. Saganuwan and J.T. Ikye-Tor, 2013. Ameliorative properties of aqueous extract of *Ficus thonningii* on erythrocyte osmotic fragility induced by acetaminophen in *Rattus norvegicus*. Vet. Res. Forum, 4: 207-212.
26. Podsiedlik, M., M. Markowicz-Piasecka and J. Sikora, 2020. Erythrocytes as model cells for biocompatibility assessment, cytotoxicity screening of xenobiotics and drug delivery. Chem. Biol. Interact., Vol. 332. 10.1016/j.cbi.2020.109305.
27. Rodrigues, F.F.G., A.A. Boligon, I.R.A. Menezes, F.F. Galvão-Rodrigues and G.J.T. Salazas *et al*., 2021. HPLC/DAD, antibacterial and antioxidant activities of *Plectranthus* species (Lamiaceae) combined with the chemometric calculations. Molecules, Vol. 26. 10.3390/molecules26247665.
28. Panghal, A., K.B. Sathua and S.J.S. Flora, 2020. Gallic acid and MiADMSA reversed arsenic induced oxidative/nitrosative damage in rat red blood cells. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e03431.
29. Suwalsky, M., J. Colina, M.J. Gallardo, M. Jemiola-Rzeminska, K. Strzalka, M. Manrique-Moreno and B. Sepúlveda, 2016. Antioxidant capacity of gallic acid *in vitro* assayed on human erythrocytes. J. Membr. Biol., 249: 769-779.
30. Gong, G., Y. Qin and W. Huang, 2011. Anti-thrombosis effect of diosgenin extract from *Dioscorea zingiberensis* C.H. Wright *in vitro* and *in vivo*. Phytomedicine, 18: 458-463.
31. Nar, H., 2012. The role of structural information in the discovery of direct thrombin and factor Xa inhibitors. Trends Pharmacol. Sci., 33: 279-288.
32. Adams, T.E. and J.A. Huntington, 2006. Thrombin-cofactor interactions: Structural insights into regulatory mechanisms. Arterioscler. Thromb. Vasc. Biol., 26: 1738-1745.
33. Budi, H.S., D.E. Ramadan, S. Anitasari and E.W. Pangestika, 2022. Estimation of platelet count and bleeding time of mice treated with *Musa paradisiaca* var. sapientum (L.) *Kuntze* extract. J. Exp. Pharmacol., 14: 301-308.
34. Periayah, M.H., A.S. Halim and A.Z.M. Saad, 2017. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. Int. J. Hematol.-Oncol. Stem Cell Res., 11: 319-327.
35. Kim, M., C.S. Kim, E.H. Bae, S.K. Ma and S.W. Kim, 2019. Risk factors for peptic ulcer disease in patients with end-stage renal disease receiving dialysis. Kidney Res. Clin. Pract., 38: 81-89.
36. De Mattos, A.M., L.V. Marino, P.P. Ovidio, A.A. Jordao, C.C. Almeida and P.G. Chiarello, 2012. Protein oxidative stress and dyslipidemia in dialysis patients. Ther. Apheresis Dialysis, 16: 68-74.