Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Evaluation of antiulcer activity of the aerial parts of C*aralluma dalzielii* N. E. Brown in Wistar rats

Chinenye J. Ugwah-Oguejiofor[1]*, Firdausi S. Inuwa[1], Kabiru Abubakar[1], Oguejiofor M. Ugwah[2], Emmanuel H. Mshelia[3], Mohammed Umar[4]

[1]*Department of Pharmacology & Toxicology, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.*
[2]*Department of Pharmacy, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.*
[3]*Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.*
[4]*Department of Pathology, Usmanu Danfodiyo University Teaching hospital, Sokoto, Nigeria*.

ARTICLE INFO

Article history:
Received 24 August 2017
Revised 19 September 2017
Accepted 27 September 2017
Published online 09 October 2017

ABSTRACT

The aerial part of *Caralluma dalzielii* has been used by traditional healers in the North-west Nigeria for the treatment of stomach related problems including pain in the epigastrium. The study evaluated the antiulcer activity of aqueous extract of the aerial parts of *Caralluma dalzielii* (AECD) in Wistar rats. Phytochemical analysis of AECD was determined. The antiulcer activity of AECD (100, 200 and 400 mg/kg) was evaluated in absolute ethanol and indomethacin-induced ulcer models in Wistar rats following established methods. In the two models, ulcer indices and percentage gastroprotection were determined. Histopathological examination of the excised rats' stomachs was carried out. The result of the phytochemical analysis of AECD showed the presence of flavonoids, tannins, saponins, sterols, and glycosides. AECD caused a significant (p <0.05-0.001) decrease in mean ulcer indices in absolute ethanol (83.4-100%) and indomethacin-induced (31.6-81.6%) ulcer models compared with the control. The decrease in the ulceration in the two models were dose-dependent. Histological assessment of the stomachs showed an increase in repair of the mucosal membrane as the dose of AECD increased. There was a complete repair of the mucosal membrane at 400 mg/kg in the absolute ethanol-induced model. In conclusion, the results suggest that AECD has antiulcer effect in the absolute ethanol and indomethacin-induced ulcer models and this may be due to the presence of some bioactive compounds.

Keywords: *Caralluma dalzielii, Ethanol-induced, Indomethacin-induced, Antiulcer, Gastroprotection.*

Introduction

Peptic ulcer disease (PUD) is one of several disorders of the upper gastrointestinal tract (GIT).[1] Peptic ulcer is a range of diseases consisting of gastric and duodenal ulcers and gastritis.[1] Gastric and duodenal ulcers are breaks in the gastric and duodenal mucosa respectively. Both gastric and duodenal ulcers relate to the corrosive action of pepsin and hydrochloric acid on the mucosa of the upper GIT. The integrity of the GIT is dependent upon the balance between hostile factors such as gastric acid, *Helicobacter pylori*, Non-steroidal anti-inflammatory drugs (NSAIDs) and pepsin, and protective factors such as prostaglandins, mucus, bicarbonate, and blood flow to mucosa [2]. Injury to gastric and duodenal mucosa develops when deleterious effects of gastric acid overwhelm the defensive properties of the mucosa. Inhibition of endogenous prostaglandin synthesis leads to a decrease in epithelial mucus, bicarbonate secretion, mucosal blood flow, epithelial proliferation, and mucosal resistance to injury. Lower mucosal resistance increases the incidence of injury by endogenous factors such as acid, pepsin, and bile salts as well as exogenous factors such as NSAIDs, ethanol and other noxious agent.[2] Duodenal ulcers occur more frequently (about 80 % of PUDs) than gastric ulcers.[2, 3]

Proton pump inhibitors, H_2-receptor blockers among others have been used for the management of PUD. However, these drugs on clinical evaluation have been found to cause high incidence of relapse, side effects and development of tolerance [3]. Hence the scientific validation of the efficacy and proper utilisation of medicinal plants used in folk medicine for the treatment of peptic ulcer diseases may offer opportunity to overcome the limitations of conventional drugs for the management of ulcer. There are many medicinal plants which have been reported to possess anti-ulcerogenic activity.[4-10] however, several others have not been validated.[11]

Caralluma dalzielii N.E. Brown (Apocynaceae) is found in abundance in North-western Nigeria. It is a cactus-like shaped plant with 5-merous flowers which serve to easily identify this species of genus *Caralluma*. In Africa, this species is distributed across the Sahel,[12] but grows better in West Africa and Sudan [13] and can be up to 1 m high. The plant is perennial with small, leafless succulents and rarely, with a few fleshy leaves. Amongst the Hausa tribe of Nigeria, it is known as 'Karan Massallachi'. It is used to treat infertility, diabetes, leprosy, rheumatoid arthritis and severe pain on epigastrium [14]. The natives chew or sometimes make a decoction of the plant and use to prevent stomach pain especially during fasting and famine. Other reported ethnomedicinal uses for this plant are for faintness due to fasting, convulsion, emesis, paralysis [15] and otitis [16]. The chemical constituents of *C. dalzielii* have been investigated and five new pregnane glycosides, caradalzielosides (A-E) isolated from its aerial parts [17]. Also, cytotoxic activities of its various pregnane glycosides fractions have been documented.[18]

*Corresponding author. E mail: nenye789@yahoo.com
Tel: +234 80 36098241

https://doi.org/10.26538/tjnpr/v1i4.6

Although the aqueous extract of the aerial parts of *Caralluma dalzielii* (AECD) is popular in traditional medicine practice in North-west Nigeria as a remedy for stomach pain, there is no scientific report on the anti-ulcer potentials of the aqueous extract of the aerial parts of the plant. The study is designed to investigate the anti-ulcer activity of *Caralluma dalzielii* on absolute ethanol and indomethacin-induced ulcer in Wistar rats.

Materials and Methods

Plant collection and identification

The aerial parts of the plant were collected from Sokoto North local government area of Sokoto State, on 20th June, 2016. It was identified and authenticated by Dr. Halilu Mshelia of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto. The plant sample with voucher number Pcg/UDUS/Asdy/001 was deposited at the herbarium unit of same Department.

Extraction of plant material

The aerial parts of *C. dalzielii* was air dried under shade for a period of one month and pulverised. Three hundred grams (300 g) of the dried plant powder was macerated in 5 L of distilled water at room temperature for 48 h. It was then filtered and the filtrate evaporated to dryness over a water bath at 70°C.

Experimental Animals

Wistar rats of either sex weighing 200 - 250 g obtained from the animal facility centre of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the experiment. The animals were acclimatised in the experimental room for 2 weeks before the commencement of the study. Standard commercial chow and water were provided *ad libitium* for the animals. Housing conditions were maintained at 25^0C at 12 h day/ night light cycles. The study was approved by the Animal Research Ethical Committee, Usmanu Danfodiyo University, Sokoto. The care and handling of the animals were according to the established public health guidelines for care and use of laboratory animals.[19]

Preliminary phytochemical tests

Phytochemical analysis of AECD was carried out using standard procedures.[20, 21]

Evaluation of antiulcer activity

Ethanol-induced model

The procedures for ethanol-induced ulcers were an adaptation of the method of Hollander *et al.*[22] In this study, the rats were fasted for 24 h and were then divided into 5 groups of 5 animals each. Group 1 received distilled water (5 ml/kg) orally, groups 2, 3 and 4 received 100, 200 and 400 mg/kg oral doses of AECD respectively while group 5 received omeprazole (20 mg/kg) orally and served as positive control. After 1 h, all animals received 8 ml/kg oral dose of absolute ethanol. Two hours later, animals were euthanized by exposing them to overdose of chloroform in an enclosed chamber. Stomachs were then removed.

Indomethacin-induced ulcer model

The modified method of Kakub and Gulfraz[23] was employed. Rats were fasted for 24 h and afterwards grouped into 5 groups of 5 animals each. Distilled water (5 mL/kg), AECD (100, 200, 400 mg/kg) and Omeprazole (20 mg/kg) were administered orally to the animals in their respective groups prior to induction of gastric lesions. Two hours after the respective treatments, indomethacin at 100 mg/kg was administered orally to all the groups. Four hours later, the animals were euthanized and stomachs removed.

Determination of ulcer index

Immediately after the animals were sacrificed, the stomachs were excised and dissected out along the greater curvature and the mucosa was rinsed with cold normal saline (0.9 % NaCl w/v) to remove contaminants and then stretched out as much as possible. The ulcerated surface in each stomach was measured with a transparent millimetre (mm) scale rule.[24] The sum of the lengths of all lesions for each stomach were used as the ulcer index (UI) [25] and the inhibition percentage were calculated.[25] Gastroprotection/Inhibition (%) for all groups were calculated based on the formula below:

$$\% \text{ gastroprotection/inhibition} = \frac{UIC - UIT}{UIC} \times 100$$

UIC (Ulcer index control) and UIT (Ulcer index in test rats)

Histopathological evaluation

The freshly excised stomachs were fixed in Bouin's solution for histological studies. The tissue sections stained with haematoxylin and eosin were examined microscopically for histopathomorphological changes.[26]

Statistical analysis

The results of the experiment were presented as mean ± standard deviation. Comparison between means was made using one-way analysis of variance (ANOVA) and Student's t-test. Significant differences were considered at $p < 0.05$.

Results and Discussion

The percentage yield of the AECD was 18.7% w/w. The Phytochemical screening gave positive test result for saponins, flavonoids, alkaloids, sterols, terpenes, glycosides and tannins. It results were negative for the presence of phenolic constituents. In the phytochemical analysis, the AECD was found to possess flavonoids, tannins, saponins and sterols as phytochemical constituents. Tannins are known to affect the integrity of mucosa membrane. Tannins being astringent can precipitate micro-proteins in the site of ulcer thereby preventing absorption of toxic substances by forming a protective pellicle and resisting the mucous layer against the attack of proteolytic enzymes.[27, 28] Saponins protect stomach mucosa from acid by selectively inhibiting prostaglandin F_2, which causes vasoconstriction of mucosal blood vessels.[29] Flavonoids act by increasing the mucosal prostaglandin content, decrease of histamine secretion from mast cells by inhibition of histidine decarboxylase and inhibition of *Helicobacter pylori* growth. Free radical scavenging ability of flavonoids has also been reported to protect the gastrointestinal tract from ulcerative and erosion lesions.[30] The presence of these phytochemical components may also contribute to its protective effect by maintaining an effective microvascular supply of gastric mucosa.

Oral administration of ethanol produced severe ulcerations in the untreated animals (Figure 2E) with ulcer index of 68.25± 4.08 (Table 1). However, pre-treatment with AECD caused a significant ($p < 0.001$) decrease in the ulceration in a dose dependent manner (Table 1). At a dose of 400 mg/kg there was no ulceration noted (Figure 2C) and the gastroprotection was 100% (Figure 1). The extract caused more protection than the positive control at all dose levels (Figure 1). Ethanol-induced gastric ulcer was employed to study the cytoprotective effect of the extract. Ethanol-induced gastric lesion formation occurs probably due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury.[31] It produces mucosal damage by direct

Table 1: Effect of AECD on ethanol-induced ulceration in rats.

Treatment	Dose (mg/kg)	Ulcer index
Control	-	68.25 ± 4.08
AECD	100	11.36 ± 0.62**
	200	3.34 ± 0.25**
	400	0 ± 0**
Omeprazole	20	43.32 ± 0.97*

Values are expressed as mean ± SD (n = 5). Significance difference compared to control group represented as *($p < 0.01$), **($p < 0.001$).

Table 2: Effect of AECD on indomethacin-induced ulceration in rats.

Treatment	Dose (mg/kg)	Ulcer index
Control	-	30.40 ± 1.31
AECD	100	20.8 ± 1.90*
	200	10.40 ± 0.69*
	400	5.6 ± 0.78**
Omeprazole	20	2.40 ± 0.32**

Values are expressed as mean ± SD (n = 5). Significance difference compared to control group represented as *($p < 0.05$), ** ($p < 0.01$).

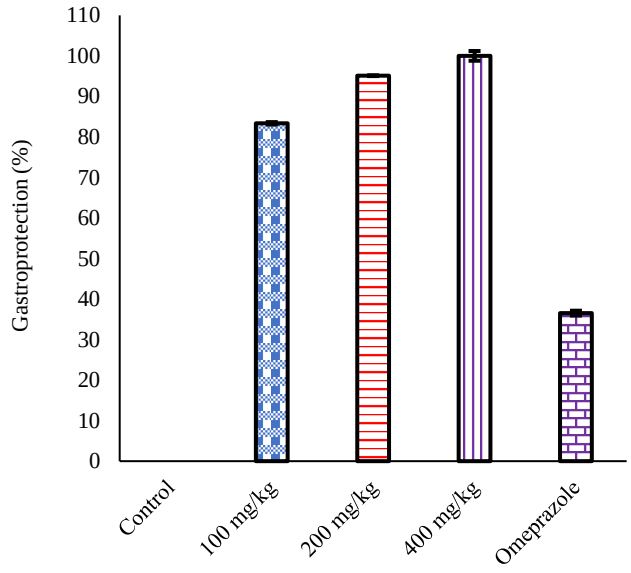

Figure 1: Effect of AECD on percentage ulcer inhibition in ethanol-induced ulcers.

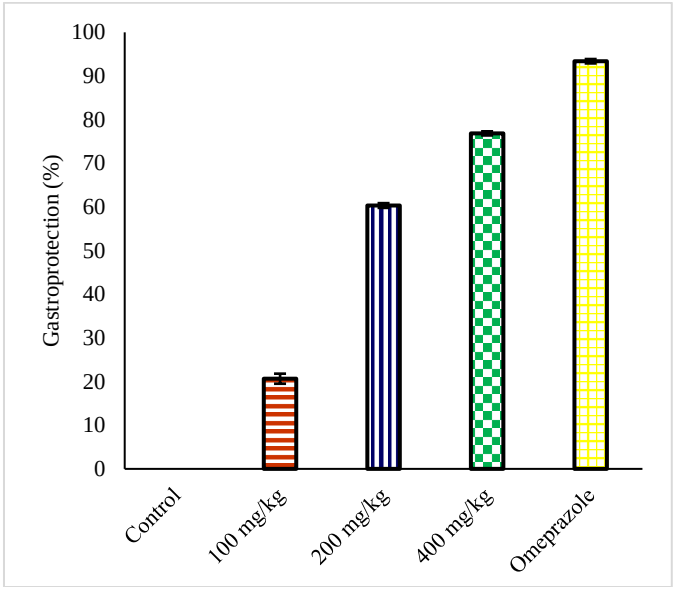

Figure 3: Effect of AECD on percentage ulcer inhibition in indomethacin-induced ulcers.

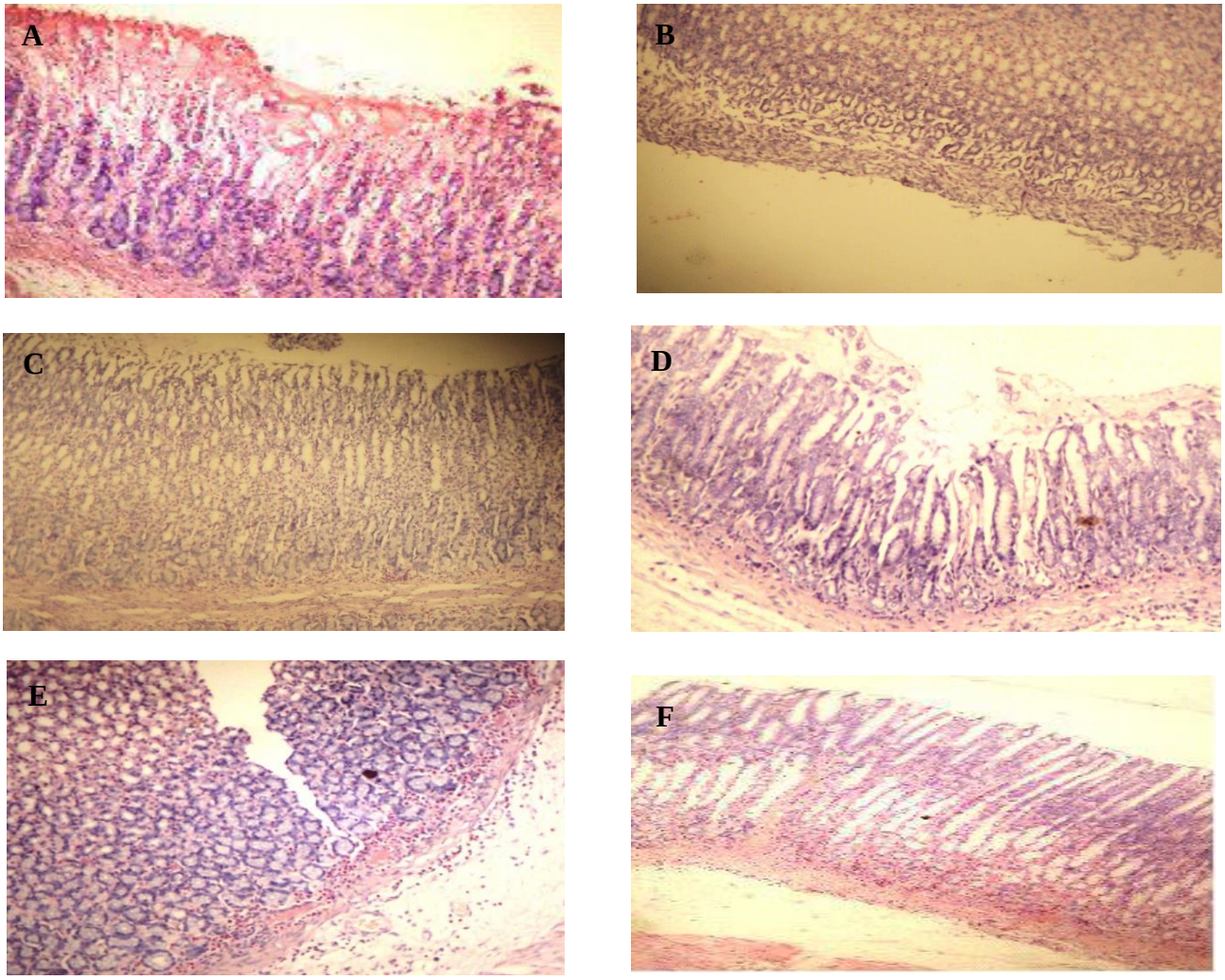

Figure 2: Effect of AECD pre-treatment on ethanol-induced gastric ulcer in rat Stomach tissue stained with haematoxylin and eosin (200X). **(A)** stomach treated with AECD-100 mg/kg plus ethanol, **(B)** stomach treated with AECD-200 mg/kg plus ethanol, **(C)** stomach treated with AECD-400 mg/kg plus ethanol, **(D)** stomach treated with omeprazole-20 mg/kg plus ethanol, **(E)** stomach after ethanol treatment and (F) normal rat stomach.

necrotizing action which in turn reduces defensive factors, secretion of bicarbonate and production of mucus [32]. When this happens, gastric mucosal injury becomes evident. This leads to cell death and exfoliation in the surface epithelium.

The AECD perhaps stimulated the growth of gastric mucosa epithelial cells resulting in the observed cytoprotective effects in rats. Histopathological studies further confirmed the extract's mucosal protective effect.

Oral administration of indomethacin produced ulcerations in the untreated animals with ulcer index of 30.40 ± 1.31 (Table 2). Pre-treatment with AECD caused a significant ($p < 0.05$) decrease in the ulceration in a dose dependent manner (Table 2). At 400 mg/kg mean ulcer index was 5.6±0.78 (Table 2) while the gastroprotection was 76.85% (Figure 3). In this case, the positive control, omeprazole, conferred greater protection to the animals than all the AECD treated groups (Figure 3). Indomethacin, an NSAID, acts in a non-selective manner, principally by inhibiting activity of cyclooxygenase enzyme, leading to a decrease in the production of prostaglandins.[33] The inhibition of cyclooxygenase pathway increases the level of leukotrienes in gastric mucosa. Leukotrienes exerts potent action on mucosal vasculature leading to inflammation and pain.[34] Inhibition of cyclooxygenase also potentiates the gastric acid secretion effects of histamine.[35] Prostaglandin E_2 produced by the gastric mucosa, inhibits secretion of hydrochloric acid and stimulates secretion of mucus and bicarbonate conferring cytoprotective effect on the mucosal layer. The impairment of mucosal defence by indomethacin allows gastric acid to elicit direct erosion of mucosal layer. The extract in a dose-dependent manner alleviated this condition. The extract may therefore be acting through prostaglandin-mediated pathway since non-prostanoid protects gastric mucosa through the mobilization of endogenous prostaglandins.[36] However, the gastroprotective effect in the absolute ethanol-induced model was more potent than that of the indomethacin-induced model. This may be because the extract could be acting more through prevention of stasis in gastric blood flow than through prostaglandin pathway.

Conclusion

The aqueous extract of the aerial parts of *C. dalzielii* possesses antiulcer activities in absolute ethanol and indomethacin-induced ulcer models in Wistar rats justifying its use in the treatment of ulcer by traditional healers.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the findings presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors are grateful to the laboratory and animal facility centre staff of Department of Pharmacology and Toxicology Usmanu Danfodiyo University, Sokoto.

References

1. Akah PA, Onyirioha CA, Nworu CC, Ndu OO. Gastro-protective effects of the leaf extract and fractions of *Fleurya aestuans* l (*urticaceae*). Int J Health Res. 2009; 2(1):65-73.
2. Kurata JH, Nogawa AN. Meta-analysis of risk factors for peptic ulcer: nonsteroidal antiinflammatory drugs, *Helicobacter pylori*, and smoking. J Clin Gastroenterol. 1997; 24(1):2-17.
3. Arun M, Asha VV. Gastroprotective effect of *Dodonaea viscosa* on various experimental ulcer models. J Ethnopharmacol. 2008; 118(3):460-465.
4. Akah PA, Nwafor SV, Okoli CO, Orji I. Evaluation of anti-ulcer properties of *Pseudocedrela kotschyi* stems bark extract. Disc Inno. 2001; 13: 132-135.
5. Antonio MA, Souza-Brito ARM. Oral antiinflammatory anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (*Turneracea*). J Ethnopharmacol. 1998; 61: 215-228.
6. Akah PA, John-Africa L, Nworu CS. Gastroprotective properties of *Ocimum gratissimum L*. against experimental ulcers in rat. Int.J Pharmacol. 2007; 3(5): 461- 467.
7. Ugwah MO, Etuk EU, Bello SO, Aliero AA, Ugwah-Oguejiofor CJ. Comparative studies of anti-ulcerogenic activities of three Nigerian medicinal plants: A preliminary evaluation. J Med Plants Res. 2013; 7(9):490-495.
8. Al-Harbi MM, Raza M, Ahmed MM, Afzal M, Shah AH. Gastric antiulcer and cytoprotective effect of *Commiphora molmol* in rats. J Ethnopharmacol. 1997; 55: 141-150.
9. Nwafor SV, Okoye CF. Antiulcer properties of ethanol root extract of *Cissampelos mucronata*. Pharm Biol. 2005; 43 (5):396-403.
10. Austin A, Jegadeesan M. Gastric and duodenal anti-ulcer and cytoprotective effects of *Cissus quadrangularis L.*variant in rats. Nig J Natural Prod Med. 2000; 6:10-14.
11. Oluranti AC, Michael UO, Jane UC, Ayembe NA. Ethno botanical studies of medicinal plants used in the management of Peptic ulcer disease in Sokoto State, North Western Nigeria. Int Res J Pharm Pharmacol. 2012; 2(9):225-230.
12. Plowes DCH. The Stapeliads of Senegal. Catcus world 2008; 26:151-158.
13. de Kock D, Meve U. A Checklist of Brachystelma, Ceropegia and the genera of the Stapeliad. International Asclepiad Society, UK; 2007. 125p.
14. Ugwah-Oguejiofor CJ, Abubakar K, Ugwah MO, Njan AA. Evaluation of the antinociceptive and anti-inflammatory effect of *Caralluma dalzielii*. J Ethnopharmacol. 2013; 150(3):967-972.
15. Burkill HM. The useful plants of west tropical Africa, ed 2. Vol. 1: families AD. Kew, Royal Botanic Gardens. 1985.
16. Inngjerdingen K, Nergård CS, Diallo D, Mounkoro PP, Paulsen BS. An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. J Ethnopharmacol. 2004; 92(2):233-244.
17. Oyama M, Iliya I, Tanaka T, Iinuma M. Five new steroidal glycosides from *Caralluma dalzielii*. Helv Chim Acta. 2007; 90(1):63-71.
18. De Leo M, De Tommasi N, Sanogo R, Autore G, Marzocco S, Pizza C, Morelli I, Braca A. New pregnane glycosides from *Caralluma dalzielii*. Steroids. 2005; 70(9):573-585.
19. National Institutes of Health. Guide for the care and use of laboratory animals. 1985.
20. Trease GE, Evans WC. A Text Book of Pharmacognosy. Oxford, UK: ELSB Baillere Tindal; 1987.
21. Harbone J B. Phytochemical methods. London: Chapman and Hall; 1993. 68p.
22. Hollander D, Taranawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. Gastroenterol. 1985; 88: 366-374.
23. Kakub G, Gulfraz M. Cytoprotective effects of *Bergenia ciliata Sternb*, extract on gastric ulcer in rats. Phytother Res. 2007; 21(12):1217-1220.
24. Magaji RA, Okasha MA, Abubakar MS, Fatihu MY. Anti-ulcerogenic and anti-secretory activity of the n-butanol portion of *Syzygium aromaticum* in rat. Nig J Pharm Sci. 2007; 6:119-126.
25. Navarrete A, Trejo-Miranda JL, Reyes-Trejo L. Principles of root bark of *Hippocratea excelsa (Hippocrataceae)* with gastroprotective activity. J Ethnopharmacol. 2002; 79(3):383-388.

26. Shah AH, Khan ZA, Baig MZ, Qureshi S, Al-Bekairi AM. Gastroprotective effects of pretreatment with *Zizyphus sativa* fruits against toxic damage in rats. Fitoterapia. 1997; 68(3):226-234.
27. Bigoniya P, Rana AC, Agrawal GP. Evaluation of the antiulcer activity of *Wrightia tinctoria* bark extract in induced acute gastric ulcers in rat. Nig J Nat Prod Med. 2006; 10(1):36-40.
28. Nwafor PA, Effraim KD, Jacks TW. Gastroprotectective effects of aqueous extract of *Khaya senegalensis* bark in indomethacin induced ulceration in rats. W Afr J Pharmacol Drug Res. 1996; 12:46-50.
29. Aguwa CN, Okunji CO. Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. J Ethnopharmacol. 1986; 15(1):45-55.
30. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phytother Res. 2000; 14(8):581-591.
31. Guth PH, Paulsen G, Nagata H. Histologic and microcirculatory changes in alcohol-induced gastric lesions in the rat: effect of prostaglandin cytoprotection. Gastroenterol. 1984; 87(5):1083-1090.
32. Rujjanawate C, Kanjanapothi D, Amornlerdpison D, Pojanagaroon S. Anti-gastric ulcer effect of *Kaempferia parviflora*. J Ethnopharmacol. 2005; 102(1):120-122.
33. Hiruma-Lima CA, Calvo TR, Rodrigues CM, Andrade FD, Vilegas W, Brito AR. Antiulcerogenic activity of *Alchornea castaneaefolia*: effects on somatostatin, gastrin and prostaglandin. J Ethnopharmacol. 2006; 104(1):215-224.
34. Kapui Z, Boer K, Rozsa I, Blasko GY, Hermecz I. Investigations of indomethacin-induced gastric ulcer in rats. Arzneim-Forsch. 1993; 43(7):767-771.
35. Reeves JJ, Stables R. Effects of indomethacin, piroxicam and selected prostanoids on gastric acid secretion by the rat isolated gastric mucosa. Br J Pharmacol. 1985; 86(3):677-684.
36. Konturek SJ, Radecki T, Piastucki I, Brzozowski T, Drozdowicz D. Gastrocytoprotection by colloidal bismuth subcitrate (De-Nol) and sucralfate. Role of endogenous prostaglandins. Gut. 1987; 28(2):201-205.