

Pharmacological Mechanism of Antiulcer Activity of Chromatographic Fraction B Obtained from *Acacia nilotica* Seedpods in Experimental Rats

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Abstract

Acacia nilotica (Lam) is an important multipurpose tree with many indigenous uses. It is most widely found in Northern Nigeria, tropical and sub-tropical countries. It is used therapeutically to alleviate and manage many illnesses. *Acacia nilotica* seedpod is used in local traditional medicine to treat gastric-related disorders. The present study was aimed at evaluating the possible mechanism of gastroprotection of chromatographic fraction B derived from *Acacia nilotica* n-butanol partitioned extract using ethanol for ulcer induction. Chromatographic fractionation of the n-butanol solvent partitioned extract yielded three different fractions (FA, FB, and FC). The possible mechanism of action of FB was assessed by investigating the involvement of endogenous nitric oxide, non-protein sulfhydryl group, and prostaglandin. The ulcer index of ulcerated rats pre-treated with N^G-nitro-L-arginine methyl ester (L-NAME) which is a nitric oxide synthase inhibitor and subsequently administered with Fraction B was not significantly higher ($p > 0.05$) from rats in the ulcerated group. Similarly, Pre-treatment with N-ethylmaleimide (NEM), a blocker of endogenous sulfhydryl to ethanol-induced ulcerated rats that were treated with chromatographic FB gave an ulcer index that was not significantly different ($p > 0.05$) from the ulcerated control group. However, prostaglandin synthesis inhibition by pretreatment with indomethacin did not affect the gastroprotective activity of FB. The study concludes that the observed anti-ulcerogenic activity exhibited by chromatographic Fraction B of *Acacia nilotica* n-butanol partitioned extract is possibly due to the increase in nitric oxide (NO) synthesis and endogenous sulfhydryl group.

Keywords: *Acacia nilotica*, Chromatographic fraction, Anti-ulcerogenic, N^G-nitro-L-arginine methyl ester, N-ethylmaleimide

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INTRODUCTION

The gastrointestinal (GI) tract is an epithelial-lined muscular tube extending from the oral cavity to the anus (Wallace, 2005). The stomach is constantly exposed to a wide range of substances that have the capacity to cause epithelial damage, such as hydrochloric acid and pepsin (Wallace, 2005). Despite continuous exposure to these injurious factors, under normal conditions a large number of mucosa defence mechanisms which include epithelial barrier, mucus secretion, prostaglandins, nitric oxide and continuous blood flow prevent local damage and maintain structural and functional mucosal integrity (Yandrapu & Sarosiek, 2015).

Peptic ulcer disease is a common benign ulceration of the epithelial lining of the stomach (gastric ulcer) or duodenum (duodenal ulcer) (Yaghoobi and Armstrong, 2022). Approximately 10% of the world's population has or may develop peptic ulcer (Xie *et al.*, 2022). Its incidence is slightly higher in men than women (1.3:1), and although it occurs at any age, duodenal ulcer occurs most often in the range of 30-55 years, whereas gastric ulcer occurs in the range of 50-70 years (Xie *et al.*, 2022). Yahya (2012) reported a decline in prevalence of duodenal ulcer and an increase in prevalence of gastric ulcer in a study covering a 15 year period between 1995 and 2010 in Nigeria.

Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including peptic ulcer disease (Shipa *et al.*, 2022). Although several plants have shown beneficial gastroprotective effects, earlier publications, and researchers from around the world, have pointed out that relatively little of the world's plant biodiversity has been extensively screened for bioactivity (Harvey, 2008). *Acacia nilotica* is a tropical and subtropical tree belonging to the family of Leguminosae and sub-family Mimosaceae. Its common names include Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia (Singh and Kumar, 2019). It is known as "*Bagaruwa*" in Hausa and "*Booni*" in Yoruba.

There are lots of orthodox drugs for the treatment of peptic ulcer. Although these drugs have brought about remarkable changes in ulcer therapy, their efficacy is still debatable. A report on clinical evaluation of these drugs shows that there are incidences of relapses, adverse effects like osteoporosis and danger of drug interactions during ulcer therapy (Panday *et al.*, 2014). Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs for new and novel molecules, which could offer better protection and reduce the incidence of relapse.

Acacia nilotica pod is used locally in the North East, North West and North Central regions of Nigeria to treat peptic ulcer (Abubakar *et al.*, 2022). It is boiled as a decoction and taken with or without milk. Also, several acute toxicity studies have demonstrated the relatively low toxicity of *Acacia nilotica* extracts or fractions, providing initial evidence of its safety. For instance, in a study by Ganie *et al.* (2018), acute oral toxicity tests on an *Acacia nilotica* bark extract in mice showed no mortalities or significant signs of toxicity at the highest tested dose, suggesting a wide margin of safety. Similar findings were reported in acute toxicity studies conducted on *Acacia nilotica* leaf extracts by Umar *et al.* (2015) and on gum extracts by Gupta *et al.* (2017). However, this pharmacological claim has not been scientifically substantiated. Thus, there is the need to investigate both its protective and healing effect on ulcer.

MATERIALS AND METHODS

Plant Material and Authentication

Acacia nilotica pods were obtained from Chenchena Market, Kaduna, Nigeria. It was identified and authenticated in the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen with the voucher number (UILH/002/1174) was prepared and deposited in the Herbarium of the same Department.

Experimental Rats

Male and female Wistar rats (*Rattus norvegicus*) weighing 180.6 ± 20 g were obtained from the Animal Holdings of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were housed in plastic cages under standard laboratory conditions (12 hr light/dark cycles), with free access to standard animal feed (Top Feed, Nigeria) and tap water *ad libitum*.

Drugs, Chemicals and Assay Kits

Indomethacin was a product of Greenfield Pharmaceuticals, China. Ranitidine was obtained from Brawn Laboratories, India. Alcian blue, pepsin and bovine albumin were obtained from Kemlight Laboratories, Mumbai. Myeloperoxidase assay kit was obtained from Alvis Biotechnology Ltd, UK. TNF- α ELISA kit, Interleukin 1 β ELISA kit and prostaglandin E1 ELISA kit were obtained from Elabscience Biotechnology, USA. Quercetins, gallic acid, L-NAME (N-G-nitro-L-arginine), NEM (N-ethylmaleimide), folin ciocalteu phenol were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Assay kits for albumin, bilirubin, uric acid, aminotransferases, and urea were products of Randox Laboratories, UK. All other chemicals and reagents used were of analytical grade.

Ethical Clearance

This study was carried out after ethical approval from the University of Ilorin Ethical Clearance Committee with the approval number UERC/ASN/2015/118.

Solvent-Partitioning of Aqueous Extract Of *Acacia nilotica* Pods

Aqueous extract of *Acacia nilotica* pod was partitioned by using solvents of increasing polarities. This was done using the protocol designed by Kupchan *et al.* (1973). Briefly, 60 g of crude aqueous extract of *Acacia nilotica* pod was solubilized using 30 mL of distilled water. This was successively fractionated using n-hexane, ethyl acetate and n-butanol using a separating funnel. The resulting solvent-partitioned fractions were evaporated to dryness using rotary evaporator. The n-butanol partitioned fraction of aqueous extract of *Acacia nilotica* pod was subjected to column chromatography as described by Jayaprakasha *et al.* (1998).

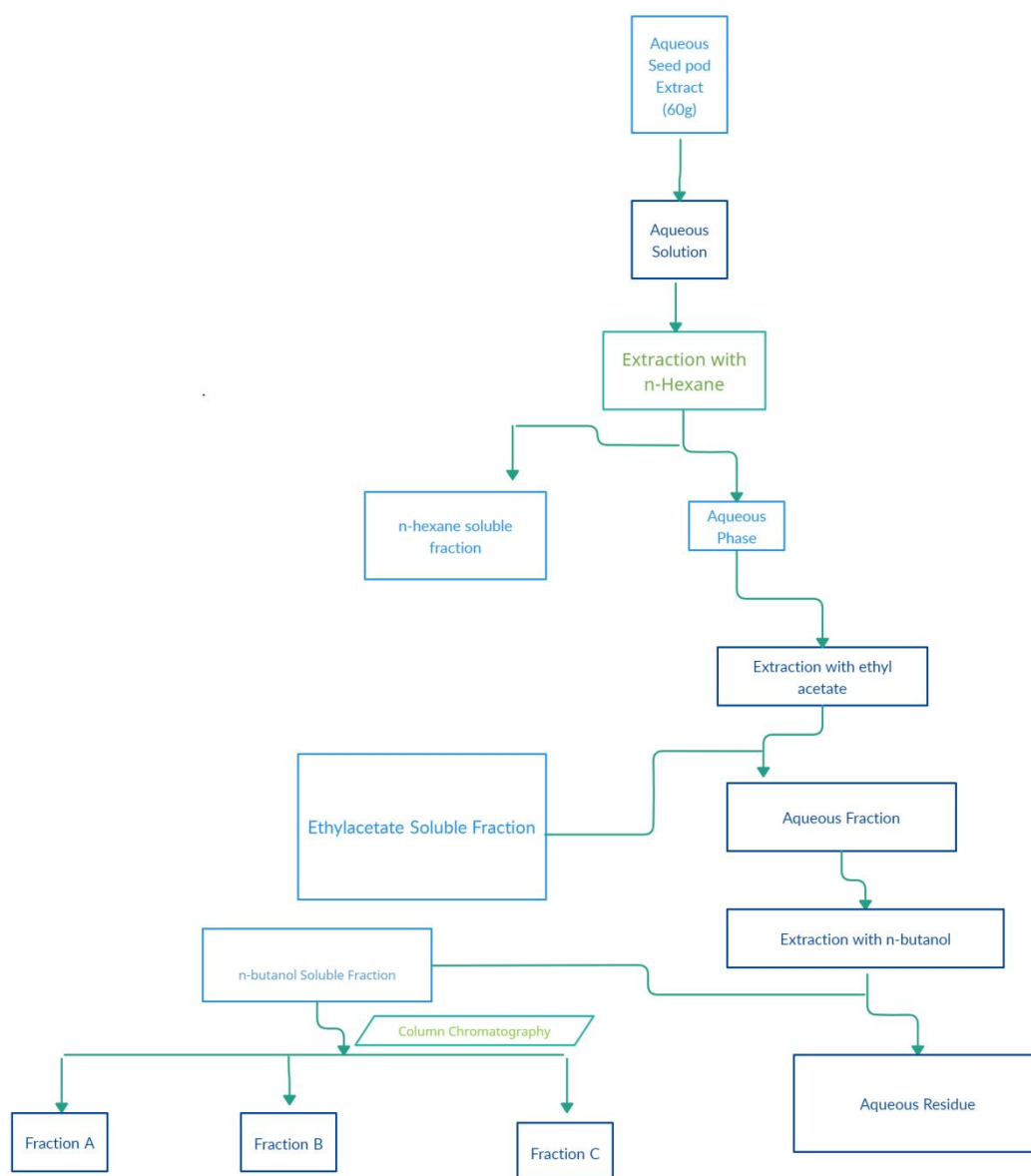


Figure 1: Schematic representation of the partitioning of aqueous extract of *Acacia nilotica* pods

Column Chromatography n- Butanol Fraction of Aqueous Extract of *Acacia nilotica* Pods

Silica gel slurry was prepared by suspending 70 g of silica gel (60 - 200 μm) in 100 mL of the mobile phase; Toluene: Ethyl acetate: Formic acid (5: 4: 1). This was packed into a column (60 cm \times 18 mm). N-butanol fraction (7 g) was dissolved in a little quantity of n-butanol and then impregnated onto silica gel. This was dried in an oven at 100 $^{\circ}\text{C}$ for 40 minutes. It was then loaded onto the column. The mobile phase was used for elution. The eluates (fractions) were collected into beakers. Twenty three (23) fractions were collected. Few drops of each fraction was spotted on an activated TLC plate and allowed to dry up. The plate was developed in a TLC chamber containing Toluene: Ethyl acetate: Formic acid (5: 4: 1) solvent system. The plate was removed and air dried. Separation was detected using iodine crystals. Fractions with similar R_f values were pooled together.

Assessment of Endogenous Nitric Oxide

In order to assess the involvement of endogenous nitric oxide (NO) in the anti-ulcerogenic effect of chromatographic fraction B, an inhibitor of NO synthase, N-G-nitro-L-arginine methyl ester (L-NAME) was used (Arrieta *et al.*, 2003). Twenty four rats of both sexes were randomly grouped into four groups of six experimental rats each and treated as follows:

Group 1: Control

Group 2: L-NAME (70 mg/kg bw)

Group 3: Fraction B (5 mg/kg bw)

Group 4: L-NAME + Fraction B

N-G-nitro-L-arginine methyl ester (L-NAME) was dissolved in normal saline and given intraperitoneally 30 minutes before the fraction, control group received normal saline. Absolute ethanol (1 mL) was given to all the animals 30 minutes after treatment with the fraction; experimental rats were sacrificed after an hour. Stomach was removed and the intensity of gastric ulcer was assessed as ulcer index. A portion of the stomach was homogenized, centrifuged and the supernatant was used to measure the level of nitric oxide (Grisham *et al.*, 1996), Tumor Necrosis Factor- α (Kamel *et al.*, 2018) and Interleukin- 1 β (Karamese *et al.*, 2016).

Assessment of Non-protein Sulfhydryl Group

In order to assess the involvement of endogenous non protein sulfhydryl group in the anti-ulcerogenic effect of chromatographic fraction B, a blocker of endogenous sulfhydryl, N-ethylmaleimide (NEM) was used (Arrieta *et al.*, 2003). Twenty four rats of both sexes were randomly grouped into four groups of six experimental rats each and treated as follows:

Group 1: Control

Group 2: NEM (10 mg/kg bw)

Group 3: Fraction B (5 mg/kg bw)

Group 4: NEM + Fraction B

N-ethylmaleimide (NEM) was dissolved in normal saline and given subcutaneously 30 minutes before the fraction; control group received normal saline. Absolute ethanol (1 mL) was given to all the animals 30 minutes after treatment with the fraction; experimental rats were sacrificed after an hour. Stomach was removed and the intensity of gastric ulcer was assessed as ulcer index. A portion of the stomach was homogenized, centrifuged and the supernatant was used to measure the level of reduced glutathione, Tumor Necrosis Factor- α (Kamel *et al.*, 2018) and Interleukin- 1 β (Karamese, 2016).

Assessment of Prostaglandin

In order to assess the involvement of endogenous prostaglandin in the anti-ulcerogenic effect of chromatographic fraction B, a blocker of prostaglandin synthesis; indomethacin (10 mg/kg) was used (Arrieta *et al.*, 2003). Twenty four rats of both sexes were randomly grouped into four groups of six animals each and treated as follows:

Group 1: Control (5 mM NaHCO₃)

Group 2: Indomethacin (10 mg/kg bw)

Group 3: Fraction B (5 mg/kg bw)

Group 4: Indomethacin + Fraction B

Indomethacin was dissolved in 5 mM NaHCO₃ and given subcutaneously 30 minutes before the fraction; control group received normal saline. Absolute ethanol (1 mL) was given to all the animals 30 minutes after treatment with the fraction; animals were sacrificed after an hour. Stomach was removed and the intensity of gastric ulcer was assessed as ulcer index. A portion of the stomach was homogenized, centrifuged and the supernatant was used to measure the level of mucosal prostaglandin (Contreras *et al.*, 2020).

Data Analysis

The data were expressed as the mean \pm SEM of six replicates. Means were analyzed using one-way analysis of variance and complemented with the Duncan multiple range tests. The Statistical Package for Social Sciences, Version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, while differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of N^G-nitro-L-arginine methyl ester on Anti-ulcerogenic Activity of Fraction B of *Acacia nilotica* Pods

The ulcer index of rats pre-treated with L-NAME and experimental rats subsequently treated with fraction B were not significantly different ($P > 0.05$) from rats in the ulcerated group (Table 1) although there was a significant difference ($P < 0.05$) in ulcer index of animals treated with only fraction B when compared to the ulcerated rats. The levels of pro inflammatory cytokines (TNF- α and IL- β) as shown in Table 2 were significantly increased in groups pretreated with L-NAME when compared with the control. This increase was significantly reduced in the group that was subsequently treated with fraction B. Mucosal nitrate level in animals pretreated with L-NAME was significantly reduced when compared with ulcerated groups. Post treatment with fraction B led to an increase in nitrate level.

Effect of N-ethylmaleimide on anti-ulcerogenic Activity of Fraction B of *Acacia nilotica* Pod

Pre-treatment with NEM in ethanol-induced ulcerated rats gave an ulcer index that is significantly different ($P < 0.05$) from ulcerated control group (Table 3). Ulcer index of experimental rats that was subsequently treated with chromatographic fraction B in NEM pre-treated group was not significantly different from the ulcerated control. The level of pro-inflammatory cytokines (TNF- α and IL- β) as shown in Table 4 was significantly ($P < 0.05$) increased in groups pretreated with NEM when compared with the ulcerated control. This increase was significantly reduced in group that was subsequently treated with fraction B. Mucosal total glutathione level in experimental rats pretreated with NEM was significantly ($P < 0.05$) different compared to that in ulcerated control group. Post treatment with Fraction B led to a non-significance increment in total glutathione level.

Role of Prostaglandin on Anti-ulcerogenic Activity of Fraction B from *Acacia nilotica* Pods

The ulcer index of rats pretreated with 10 mg/kg indomethacin as shown in Table 5 was significantly different ($P < 0.05$) from the ulcerated control. Group that was later treated with fraction B had a reduced ulcer index that was however not significantly different ($P > 0.05$) from the ulcerated control group. Mucosal prostaglandin levels of all treatment groups were not significantly different ($P > 0.05$) from each other.

Table 1: Ulcer Index of Animals with Ethanol-Induced Ulceration Pre-Treated with N^G-nitro-L-arginine methyl ester.

Group	Ulcer index (mm)
Ulcerated Control	25.00 ± 3.296 ^a
L-NAME (70 mg/kg bw)	32.83 ± 5.34 ^b
Fraction B (5 mg/kg bw)	15.00 ± 1.92 ^c
L-NAME + Fraction B	33.50 ± 2.01 ^{bd}

Values are mean of six replicates ± SEM. Values with different superscript are significantly different from each other ($P < 0.05$) L-NAME: N^G-nitro-L-arginine methyl ester

Table 2: Concentration of Some Mucosal Inflammatory Cytokines and Nitrate in Animals with Ethanol-induced Ulceration Pre-treated with N^G-nitro-L-arginine methyl ester

Group	Tumor necrosis factor- α (pg/mL)	Interleukin-1 β (pg/mL)	Nitrate (μ M)
Ulcerated Control	128.80 ± 10.98 ^a	141.60 ± 7.21 ^a	12.42 ± 0.46 ^a
L-NAME (70 mg/kg bw)	295.00 ± 27.41 ^b	261.30 ± 13.26 ^b	9.58 ± 0.21 ^b
Fraction B (5 mg/kg bw)	67.27 ± 7.72 ^c	37.69 ± 3.71 ^c	17.97 ± 0.49 ^c
L-NAME + Fraction B	160.50 ± 12.85 ^a	165.50 ± 7.79 ^a	19.02 ± 0.15 ^d

Values are mean of six replicates ± SEM. Values with different superscript are significantly different from each other ($P < 0.05$) L-NAME: N^G-nitro-L-arginine methyl ester

Table 3: Ulcer Index of Animals With Ethanol-induced Ulceration Pre-treated with N-ethylmaleimide

Group	Ulcer index (mm)
Ulcerated Control	25.00 ± 3.30 ^a
L-NEM (10 mg/kg bw)	38.33 ± 1.65 ^b
Fraction B (5 mg/kg bw)	15.00 ± 1.92 ^c
L-NEM + Fraction B	23.50 ± 3.56 ^a

Values are mean of six replicates ± SEM. Values with different superscript are significantly different from each other ($P < 0.05$) NEM: N-ethylmaleimide

Table 4: Concentration of Mucosal Inflammatory Cytokines and Glutathione in Animals with Ethanol-induced Ulceration Pre-treated with N-ethylmaleimide

Group	Tumor necrosis factor- α (pg/mL)	Interleukin-1 β (pg/mL)	GSH (μ mol/mL)
Ulcerated Control	128.80 ± 10.98 ^a	141.60 ± 7.21 ^a	20.20 ± 2.71 ^a
L-NEM (70 mg/kg bw)	280.40 ± 36.03 ^b	191.00 ± 14.66 ^b	11.73 ± 0.46 ^b
Fraction B (5 mg/kg bw)	67.27 ± 7.72 ^c	37.69 ± 3.71 ^c	26.15 ± 2.61 ^a
L-NEM + Fraction B	119.30 ± 6.96 ^a	158.60 ± 18.24 ^a	15.48 ± 1.14 ^a

Values are mean of six replicates ± SEM. Values with different superscript are significantly different from each other ($P < 0.05$)

NEM: N-ethylmaleimide

Table 5: Ulcer Index and Prostaglandin Concentration of Animals with Ethanol-induced Ulceration Pre-treated with Prostaglandin

Group	Ulcer index (mm)	Prostaglandin (pg/mL)
Ulcerated Control	22.17 ± 0.40 ^a	30.99 ± 1.80 ^a
Indomethacin (10 mg/kg bw)	30.00 ± 1.67 ^b	27.22 ± 0.84 ^a
Fraction B (5 mg/kg bw)	13.33 ± 0.76 ^c	29.0 ± 0.73 ^a
Indomethacin + Fraction B	22.0 ± 2.05 ^a	34.28 ± 2.02 ^a

Values are mean of six replicates ± SEM. Values with different superscript are significantly different from each other (P < 0.05)

DISCUSSION

Ulcer index is a visualized indicator used to reflect the gastric ulcer model injury or evaluate the extent of ulcer, which is commonly used in the anti-ulcer studies (Sani *et al.*, 2022). The contribution of endogenous NO, sulfhydryl compounds and prostaglandins to the anti-ulcerogenic action of chromatographic fraction B of *Acacia nilotica* pod were examined in this study. NO is a ubiquitous mediator that plays an important role as an endogenous modulator of numerous physiological functions (Zakaria *et al.*, 2014). NO maintains normal functions of the gastrointestinal mucosa and has cytoprotective role by regulating gastric mucosa blood flow, epithelial secretion and barrier function (Lin *et al.*, 2022). Results obtained in this study show that the ulcer index of experimental rats pretreated with L-NAME (an inhibitor of NO synthase) significantly increased when compared to the control, level of mucosal NO in form of nitrate was also significantly reduced. NO level significantly increased in group that was subsequently treated with fraction B of the extract. The concentration of cytokines was also significantly reduced in groups post treated with fraction B of the extract. This suggests that chromatographic fraction B probably exerted its anti-ulcerogenic effect by up regulation of the synthesis of NO.

Endogenous sulfhydryl compounds help to maintain the integrity of the mucus membrane by joining its subunits through disulfide bridges (Jia *et al.*, 2015). In this study, experimental rats pre-treated with NEM (a glutathione depletor) significantly increased the ulcer index when compared to the ulcerated control group. Administration of fraction B gave a significant reduction, the reduced ulcer index was however not significantly different from the ulcerated control group. Mucosal glutathione level was also not significantly different in group administered both NEM and Fraction B when compared with the ulcerated control. This result suggests that chromatographic Fraction B may partly depend on sulfhydryl group to exert its anti-ulcerogenic effect.

Prostaglandin is required for gastric mucosal integrity (Sánchez-Mendoza *et al.*, 2014), indomethacin given at a lower dosage (10 mg/kg s.c) will only inhibit prostaglandin synthesis (Arrieta *et al.*, 2003) but will not induce ulceration. In this study, administration of indomethacin gave a significant increase in ulcer index and a reduction in mucosal prostaglandin concentration. Subsequent administration of fraction B gave a significant reduction in ulcer index when compared to the prostaglandin pre-treated groups showing that indomethacin did not inhibit its anti-ulcerogenic effect. These result show that prostaglandin does not play role in *Acacia nilotica* pod anti-ulcerogenic effect.

CONCLUSION

This research put forward the scientific veracity for the local use of *Acacia nilotica* seedpod for the treatment of ulcer in the North East, North West and North Central regions of Nigeria. The findings from our research suggests that the observed anti-ulcerogenic activity exhibited

by chromatographic Fraction B of *Acacia nilotica* n-butanol partitioned extract is possibly due to the increase in NO synthesis and endogenous sulfhydryl group.

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