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Investigation into the Gastro-protective Properties of *Alstoniaboonei* (De Wild) Leaves and Stem Bark

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ABSTRACT

Alstonia boonei (Apocynaceae) leaves and stem bark extracts have been described in folkloric medicine to be an effective analgesic for arthritis pain and also in the healing of wounds and ulcer. Occurrence of Gastro Intestinal Tract (GIT) I irritation and peptic ulcer disease are the major limitations in the use of Non-Steriodial anti-inflammatory drugs (NSAID) in the treatment of pain and inflammation..This study examined the ability of *Alstonia boonei* stem bark and leaf extracts and fractions to prevent gastroduodenal mucosal damage by NSAID as a better alternative to the conventional ulcer protective drugs given their various individual shortcomings. The chloroform fraction, ethyl acetate fraction, n-hexane fraction and aqueous fractions of the leaves and stem bark of the plant were obtained by solvent-solvent fractionation and subjected to phytochemical screening. The gastro-protective activity of the crude methanol extract, chloroform fraction, ethyl acetate fraction of the leaves and stem bark were evaluated using NSAID (Diclofenac) induced ulcer model in albino rats. The qualiitative phytochemical analysis revealed that the leaves and stem bark both contained alkaloids, saponins, terpenoids, flavonoids, tannins and carbohydrates while cardiac glycosides were present in the leaf but absent in the stem bark. The analyzed result revealed that all the extracts and fractions of *Alstonia boonei* stem bark and leaves possess significantly($p<0.00$) gastro-protective activity which justifies its use in ethno medicine to treat GIT disorders. Its potential can be harnessed to be a source of lead compound(s) that can be used to prevent NSAID induced ulcers and associated complications.

Keywords: *Alstonia boonei*, Phytochemical screening, Gastro-protective activity, Acute toxicity.

Introduction

Traditional medicine practice is supplied with therapeutic materials from natural sources such plants, animals and minerals from the earth. Planet earth is endowed with thousands of plant species which have been in use as medicinal remedies from mother nature.[1] These plants by the virtue of the metabolites that they manufacture/produce can have the ability to treat or prevent disease conditions.[2] It has long been recognized that the chemical structures of these metabolites from plants used in traditional medicine have the characteristics of high chemical diversity, biochemical specificity and other molecular properties.[3] The peculiar characteristics of metabolites from plants have provided important basis for the discovery and development of modern therapeutic drugs.[4] In fact, 25% of modern medicines currently in use worldwide are derived from plants that have been employed by traditional medical practitioners to treat diseases.[5] People now prefer traditional medicine to conventional medicine mainly because of the believe that natural products have less toxic adverse effects whereas only few herbs, extracts and herbal preparations have standard data concerning their safety, efficacy and toxicity available.[6] It has therefore become absolutely necessary to ascertain the safety, efficacy, toxicity and adverse effects of these medicines/drugs on the basis of suitable scientific evidence considering the ever-increasing popularity and extensive usage of phytotherapy all over the globe.[7]

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Gastritis and peptic ulcer disease (PUD) characterized by symptoms ranging from mild dyspeptic symptoms to life threatening complications such as deep gastro duodenal ulceration, perforation and hemorrhage are the major limitations in the use of non –steroidal anti-inflammatory drug (NSAIDs) in the treatment of pain and inflammation accompanying arthritis and rheumatic arthritis conditions especially in the elderly population. Conventional medications such as proton pump inhibitors (PPIs), prostaglandin analogues (PGA) or H_2 receptor antagonists (H_2RAs) are currently used in combination with NSAIDs to prevent a variety of upper gastrointestinal tract (GIT) toxicities.[8].

Studies have revealed that prolonged use of acid suppressing drugs (ASD) have the potential to contribute to alteration of important defense systems and immunity leading to fungal, protozoan and viral infections particularly in elderly population [9]. There is also evidence that long term gastric acid inhibitor use is significantly associated with the presence of vitamin B_{12} deficiency.[10] Considering these demerits of conventional medications in the prophylaxis of gastritis and PUD associated with NSAID use, this study seeks to explore the potentials of a medicinal plant *Alstonia boonei* to tackle this health problem.

Alstonia boonei (Apocynaceae) leaves and stem bark extracts have been described in folkloric medicine to be an effective analgesic used to manage arthritis pain and also in the healing of wounds and ulcer.[6] These claims have been supported by demonstration through scientific investigations,[11,12] showing significantly high analgesic and anti-inflammatory properties respectively which means that a combination of NSAID with the plant product may produce a better analgesic activity while preventing NSAID induced PUD and gastritis. However, there is little or no scientific data for the validation of the use of *A. boonei* as a gastro protective agent against NSAID induced PUD. This study is designed to evaluate the gastro-protective properties of the crude extracts and solvent fractions of *A.boonei* leaves and stem bark given their promising anti-inflammatory and antioxidant properties. This study was also motivated by the fact that

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flavonoids and other phenolic compounds which have been found to possess muco-protective and astringent properties are known to have high concentration in *A.boonei* fractions.[13]

Materials and Methods

The leaves and stem bark of a mature *Alstonia boonei* (De Wild tree) were collected in the morning hours of February 2020 from Agbani Nkanu, West Local Government Area of Enugu State. They were identified by Mr. Obi Patrick, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences Agbani, Enugu State. A voucher specimen with No PCG/474/B/012 was deposited at the herbarium of the department. The stem bark was cut into small pieces. The clean leaves and cut stem bark were shade dried under room temperature for 14 days. The dried materials were pulverized into a coarse powder using a mechanical grinder. The powdered materials were stored at room temperature (25-27 C), freshly prepared solutions of analytical grade chemicals and reagents were used in all the experiments

Extraction
About 308 g and 404 g of the powdered leaves and stem bark respectively of *A. boonei* were exhaustively extracted by cold maceration technique using 2 liters of 99.5 % methanol. Both extractions were accompanied by intermittent vigorous agitation at regular intervals. The mixture of powdered plant materials and methanol were filtered first through a muslin cloth then through a Whatman No. 1 filter paper fitted in a funnel plugged with cotton wool respectively. The filtrates were collected and concentrated using rotary evaporator to obtain the methanol extract (ME) of the leaves and stem bark respectively. Subsequently, the extracts were stored in the refrigerator.

Liquid-liquid fractionation of the extract
The crude methanol extract of each plant part was separated by liquid-liquid fractionation. The solvents namely n-hexane, chloroform, ethyl acetate and distilled water respectively were used to partition each of the crude methanol extract into different fractions. The solvent fractions were evaporated to dryness, weighed and their weights recorded. The fractions were stored in a refrigerator until further studies were carried out.

Phytochemical analysis of the extracts
The chemical constituents of the leaves and stem bark extracts and fractions of *Alstoniaboonei* were identified by performing phytochemical tests using standard method as described by Tiwari and others.[14]. The tests were for alkaloids, saponins, terpenoids, steroids, glycosides, flavonoids, tannins and carbohydrates.

Animals study
Adult male Wista rats (70-150 g) and adult female Swiss mice (20-23 g) were obtained from the University of Nigeria, Faculty of Veterinary medicine farm Nsukka Nigeria. The adult female Swiss mice were used for acute toxicity studies. The female animals were nulliparous and non-pregnant. The animals were fed with standard laboratory diet and had free access to water ad libitum. Each cage contained 4-8 rats or mice of the same sex. All animal experiments were conducted in compliance with NIH guide for the care and use of laboratory animals (Pub No 85-23 Revised 1985) and supervised by the Faculty animal care and Use Committee (UNN/FVM/ZOG/2021/036)

Preparation of extracts and fractions stock solutions
The extracts and fractions were dissolved in either tween 80 or dimethyl sulfoxide (DMSO) as a function of their solubility. The appropriate dose was calculated to obtain correct amount of extracts and fractions that were dissolved in the vehicles (stock solutions). All dilutions were done in distilled water.

Preparation of standard stock solutions
Omeprazole (0.4 % w/v) and diclofenac sodium (1 % w/v) respectively were prepared in DMSO. Dilutions were made using distilled water.

Acute toxicity study
The acute toxicity (LD50) of the extracts and fractions of *Alstonia boonei* leaves and stem bark was tested in mice using the sequential method described by Organization for Economic Co-operation and Development Guidelines[15]. A single dose (2000 mg/kg) of each of the extracts and fractions was administered to 3 female mice. The control group received the vehicle. After 24 hours, each of the extracts and fractions was administered at the same dose of 2000 mg/kg to 3 additional female mice. Thereafter, all the animals were observed carefully for 14 days during which mortality, body weight, and gross behavioral changes were noted daily.

Gastro-protective activity evaluation
Sixty male Wistar rats were randomly selected into 15 groups of 4 animals each. The calculated doses of reference standards and plant extracts and fractions were administered as prescribed per body weight. The rats were pretreated for 7 days with the plant extracts, fractions, omeprazole (positive control) or vehicle (negative control). On the 7th day all the animals excluding the negative control group (Group 1) were orally administered 80 mg/kg body weight of diclofenac to induce gastric ulceration 1 h after the last dose of test sample or standard drug. However, 12 h prior to the day of ulcer induction, the animals' were fasted but received water *ad libitum*. The administration of the extracts and fractions was performed once a day in all the experimental groups. After 7 h of the last treatment, the treated rats were sacrificed by cervical dislocation, their abdomen were opened through midline incision using a dissecting kit on a dissecting board. The stomachs were incised along the greater curvatures. The animals with four (4) in each group were treated as follows:

Group 1: (normal) no drug or extracts administered, ulcer was not induced.
Group 2: (negative control); 80mg/kg of diclofenac sodium was administered to induce ulcer.
Group 3: (positive control); 80 mg/kg of diclofenac sodium + 20 mg/kg of omeprazole.
Group 4: 80 mg/kg of diclofenac sodium + 50 mg/kg of methanol extract of the stem bark
Group 5: 80 mg/kg of diclofenac sodium + 100 mg/kg of methanol extract of the stem bark.
Group 6: 80 mg/kg of diclofenac sodium + 50 mg/kg of chloroform fraction of the stem bark.
Group 7: 80 mg/kg of diclofenac sodium + 100 mg/kg chloroform fraction of the stem bark.
Group 8: 80 mg/kg of diclofenac sodium +50 mg/kg of the ethyl acetate fraction of the stem bark.
Group 9: 80 mg/kg of diclofenac sodium + 100 mg/kg of the ethyl acetate fraction of stem bark.
Group 10: 80 mg/kg of diclofenac sodium + 50 mg/kg of the methanol extract of the leaves.
Group 11: 80 mg/kg of diclofenac sodium + 100 mg/kg of the methanol extract of the leaves.
Group 12: 80 mg/kg of diclofenac sodium + 50 mg/kg of the chloroform fraction of the leaves.
Group 13: 80 mg/kg of diclofenac sodium + 100 mg/kg of the chloroform fraction of the leaves.
Group 14: 80 mg/kg of diclofenac sodium + 50 mg/kg of the ethyl acetate fraction of the leaves.
Group 15: 80 mg/kg of diclofenac sodium + 100 mg/kg of the ethyl acetate fraction of the leaves.

Determination of ulcer score and ulcer index
A ventral midline incision was made on each animal and the stomach exteriorized and opened through the greater curvature, rinsed, laid out on a flat surface and examined for the presence of mucosal lesions. The gastric damage in the glandular regions was located in the gastric mucosa elongated black red lines parallel to the long axis of stomach using a 10x magnifying lens to locate and score the lesions. Severity of the gastric mucosal damage was graded as grade 0, (no lesions); grade 0.5, (hemorrhagic erosion, <5 nm); Grade 1, (hemorrhagic

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erosion, more than 5 nm); Grade 2, (many small linear ulcers shorter than 2 mm).
The ulcer scores/ratings for calculating ulcer index as well as protective ratios for the ulcers seen in each group as described by Takagi and Okabe in Salami work, [16] were used to evaluate the severity of the gastric lesions. The grading is summarized below:
0 = no lesion.
0.5 = hemorrhagic erosion, <5 nm
1 = mucosal oedema.
2 = 1-5 small lesions
3 = more than 5 small lesions or 1 intermediate lesion.
4 = 2 or more intermediate lesions or 1 gross lesion
5 = Perforation ulcer
The ulcer index (UI) and percent protective ratio were further calculated using the formula below:
UI = Total ulcer score / no of animals treated
% Protective ratio = UI of ulcerogen treated group – UI of drug treated group / UI of ulcerogen treated group x 100. The results were recorded.

Statistical analysis
The data obtained were analyzed and the significance of difference between the control and treated groups determined using one-way analysis of variance (ANOVA) followed by Dunnett's t-test (comparing all the test groups against the positive control). P-values less than 0.05 were considered to be statistically significant

Results and Discussion

Percentage yield of crude extract and fractions
The results of percentage yields of methanol crude extracts and fractions of the leaves and stem back of the plant are given in Table 1 and Table 2, respectively. The results showed that the leaves yielded more crude extract (14.74%) than the stem bark (5.62%). It was also noted that with the leaves and stem back, the n-hexane gave the highest fraction yield than the others. The yields of crude extract (14.74%) chloroform fraction (11.35%) and Ethyl acetate fraction (1.39%) of the leaves is similar with 9.4%, 13% and 3.1% respectively reported in previous studies done with another medicinal plant[17]. The low percentage yields which although did not limit the experimental studies could be attributed to time and season of harvest.

Phytochemical tests
The results of the qualitative phytochemical analysis of *Alstonia boonei* are presented in Table 3. It was found out that alkaloids, saponins, terpenoids, glycosides, flavonoids, tannins and carbohydrates were present in both the leaves and stem bark. Steroids were not present in the stem bark. The plant parts contained a substantial amount of secondary metabolites. Thus, gastro-protective potentials of these medicinal plants may be attributed to the combined action of the various antioxidant components of these secondary metabolites present in the leaves extract as discussed in other studies[18]

Acute toxicity study
All the mice survived throughout the14 days study period. During observation, the animals at the limit dose of 2000 mg/kg did not exhibit any toxic signs. No behavioral changes such as tremor, convulsion, self-mutilation, salivation, lethargy or sleep were observed during the first four hours after *A. boonei* extract administration. No changes in faeces and body coat condition and reactivity to noise and touch were observed. There were no significant differences in body and organ weights of mice treated with the extract compared with the controls. Macroscopic examination did not reveal any changes in organ appearance. The results in this study as presented in table 4, clearly demonstrated that the plant parts are safe and supports the work done on rats on other studies[12, 17,]

Protective effect of Alstonia boonei leaves and stems bark against diclofenac-induced gastric ulcers
The gastric damage was characterized by marked mucosal lesion including large size hemorrhagic spots and petechial lesions.

Table 1: Percent yield of *Alstoniaboonei* leaves extracts and fractions

Extraction and Fractions	Initial weight (g)	Final weight (g)	% yield
Methanol extract	30	45.39	14.74
Chloroform fraction	30	5.15	11.35
Ethyi acetate fraction	30	0.72	1.59
n-Hexane fraction	30	8.34	18.37

Note: 30 g of the crude extract was used in the fractionation.

Table 2: Percent yield of *Alstoniaboonei* stem bark extracts and fractions

Extraction and Fractions	Initial weight (g)	Final weight (g)	% yield
Methanol extract	404	22.71	5.62
Chloroform fraction	10	0.90	3.96
Ethyi acetate fraction	10	0.28	1.23
n-Hexane fraction	10	2.12	9.33

Note: 10 g of the crude extract was used in the fractionation.

Table 3: Phytochemical screening of methanol extracts of leaves and stem bark of *Alstonia boonei*

Phytochemical test	Leaves extract	Stem bark extract
Alkaloids	+	+
Carbohydrates	+	+
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Steroids	+	-
Tannins	+	+
Terpenoids	+	+

+ = Present - = Absent

On further examination, there were hemorrhagic spots of different sizes along the longitudinal axis of the glandular part of the rat stomach. The animals pretreated with *A.boonei* extracts and fractions showed very mild lesions with interstitial hemorrhage and no lesions at all in some animals on morphometric evaluation. Pretreatment results with all the extracts and fractions of *Aboonei* showed that they were capable of providing a level of gastro mucosal protection that is significantly.($p<0.00$) greater than that obtained by pretreatment with omeprazole against diclofenac sodium induced gastric injury.($p<0.05$) The crude extract of the leaves of *Alstonia bonnie* offered the highest protection of the gastric mucosa of the test animals against diclofenac sodium induced damage at 80 mg/kg single dose. This is evident in figure 1, which shows the percentage preventive index/ratio of 90.99% (ulcer index = 0.25) and 90.99% (ulcer index=0.25) produced at the doses of 50 mg/kg and 100 mg/kg respectively when compared with that provided by the positive control group pretreated with 20 mg/kg dose of omeprazole with 36% preventive index. ($p<0.05$). Pretreatment with the chloroform fraction of the leaves of *A.boonei* provided the second highest protection with preventive index of 72.73% (Ulcer index = 0.75) and 81.82% (Ulcer index = 0.5) at the doses of 50 mg/kg and 100 mg/kg respectively compared to the control group ($p<0.05$). Ranking third is the group pretreated with the chloroform fraction of the stem bark of *A.boonei* with Percentage Preventive index of 72.7% (Ulcer index = 0.75) and 63.64% (Ulcer

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index=1) at the doses of 50 mg/kg and 100 mg/kg respectively compared to the control group ($p<0.05$). Although the gastro protective activity of some of the extracts and fraction were not dose dependent, it is remarkable that these doses of the extract and fractions of leaves and stem bark of *Alstonia boonei* produced significantly greater protection of the gastric mucosa than the omeprazole at 20 mg/kg dose against diclofenac sodium induced gastric ulcer. This level of significance ($p<0.00$) of difference between the positive control (rats given Diclofenac + 20mg Omeprazole) and the group given extract and fractions having highest gastro-protective activity is supported by findings in other previous studies[19,20,21]. Non-steroidal anti-inflammatory drugs including diclofenac sodium are known to cause gastric ulcers especially when abused or chronically used. This method was used because of the fact that NSAID induced peptic ulcers are the second most common etiology of peptic ulcer disease beside those caused by *Helicobacter pylori* infection.[6] NSAIDs are known to induce ulcers through several mechanisms including; inhibition prostaglandin synthesi,[22]oxidative mucosal damage as a result of increased production of reactive oxygen species, lipid peroxidation and neutrophil infiltration,[23] disruption of surface active phospholipids on the mucosal surface and direct killing of the epithelial cells particularly by acidic NSAIDs.[24] Therefore, the significantly high ulcer score, ulcer index following oral administration of diclofenac in the ulcerated rats may be attributed to any of the above mechanisms or a combination of them.

Table 4: Protective effect of *Alstonia boonei* leaves and stem bark against diclofenac sodium-induced gastric ulcers

Group	Treatment	No of rats used	Dosage	Ulcer Index (mg/kg)	% Preventive Index
1	DIC	4	-	2.75	0
2	DIC + Omeprazole	4	-	1.75	36.36
3	DIC + MSB	4	50	1.50	45.45
4	DIC + MSB	4	100	1.25	54.55
5	DIC + CSB	4	50	0.75	72.73
6	DIC + CSB	4	100	1.00	63.64
7	DIC + MEL	4	50	0.25	90.99
8	DIC + MEL	4	100	0.25	90.99
9	DIC + CFL	4	50	0.75	90.99
10	DIC + CFL	4	100	0.50	81.82
11	DIC + EASB	4	50	0.75	72.73
12	DIC + EASB	4	100	1.00	63.64
13	DIC + EAL	4	50	1.25	54.55
14	DIC + EAL	4	100	1.00	63.64

DIC = Diclofenac sodium, MSB = Methanol extract of stembark, CSB = Chloroform fraction of stembark, MEL = Methanol extract of leaves, CFL = Chloroform fraction of leaves, EASB = Ethyl acetate fraction of stem bark, EAL = Ethyl acetate fraction of leaves

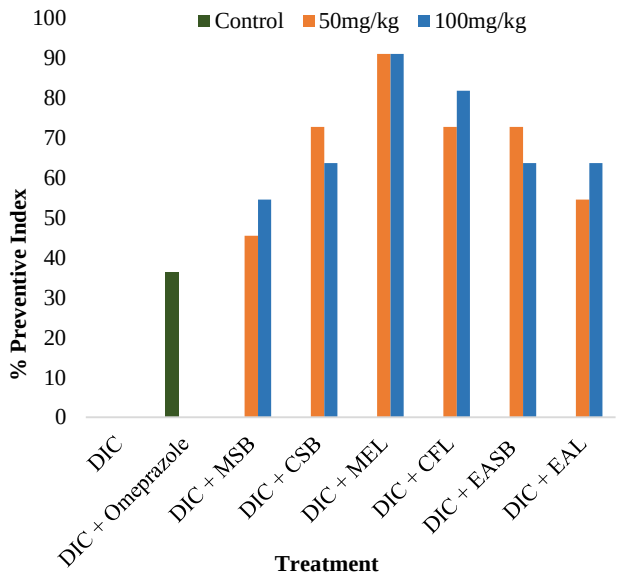

Figure 1: Percentage Preventive Index Compared to treatments of different doses of *A. boonei* extracts and solvent fractions.
DIC = Diclofenac sodium, MSB = Methanol extract of stembark, CSB = Chloroform fraction of stembark, MEL = Methanol extract of leaves, CFL =Chloroform fraction of leaves, EASB =Ethyl acetate fraction of stem bark, EAL =Ethyl acetate fraction of leaves

In an attempt to correlate the gastro-protective effects of the extract and fractions of *A.boonei* observed in this study with chemical compound or phytochemicals present in *Alstonia boonei*, literature review revealed that the detected phytochemicals such as phenolic and flavonoid constituents possess anti-inflammatory[25] and astringent properties.[13] Researchers propose that these activities could be partly due to the presence of polar functional groups in the polyphenolic structures which have the capacity to perhaps scavenge free radicals. Also, A.boonei leaves have been confirmed to contain tannins, saponins, and alkaloids which have been found by previous studies to be immune modulatory agents that could hasten wound healing and inflamed mucus membrane.[12]

Conclusion

The study determined the pretreatment with methanol extracts and solvent fractions of *Alstonia boonei* gastro protection against diclofenac sodium induced damage to the gastric mucosa of rats. The findings of the present study revealed that the methanol extracts, chloroform and ethyl acetate fractions of *Alstonia boonei* leaves and stem bark respectively have high significant ($p<0.00$) gastro-protective properties against diclofenac induced gastrointestinal damage.

Conflict of Interest

Authors declare no conflict of interest.

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Authors' Declaration

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

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