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Anti-Ulcer activity of methanol extract of *Plantago rugelii* Decne. (Plantaginaceae)

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ABSTRACT

In spite of the increase in the use of herbal medicines, there are inadequate research on their effectiveness and toxicity. *Plantago rugelii* is commonly used in Nigeria folk medicine as an antimicrobial agent and topical agent for open wounds amongst others. This study investigated the phytochemical composition, quantitative proximate parameters, acute toxicity and the anti ulcer capabilities of the whole plant consisting of the roots, stem and leaves using various experimental animal models and established methods. Results confirmed the presence of alkaloids, saponins, carbohydrate, reducing sugars, deoxy sugars, phytosterols, protein and flavonoids. Moisture content (39.64 ± 1.09 %), total ash (17.22 ± 0.22 %), water insoluble ash (6.27 ± 0.18 %), acid insoluble ash (4.93 ± 0.36 %), alcohol soluble extractive (0.98 ± 0.04 %) and water soluble extractive (0.25 ± 0.06 %) values were obtained from the quantitative proximate analysis. An infusion of the whole plant (200 and 400 mg/kg) protected against gastric ulceration induced by aspirin and HCl in 60 % (v/v) ethanol in the animals. A higher dose of 400 mg/kg body weight from 200 mg/kg body weight of the plant infusion produce a correspondingly higher gastric ulcer protective activity. There were no mortality at 8 g/kg p.o after 24 hours and no sign of delayed toxicity or mortality after 14 days of observation. In conclusion, the present findings suggest that *P. rugelii* can protect against aspirin and ethanol-induced gastric ulcer in experimental animals.

Introduction

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [1]. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2].The medicinal value of these plants lies in their phytochemical component, especially the secondary metabolites such as alkaloids, tannins, anthocyanins, anthraquinone derivatives, flavonoids and other phenolic compounds [3,4]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect [5]. Natural compounds obtained from plants could be lead compounds allowing the design and rational planning of new drugs, bio metric synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds [6].

For over a century, peptic ulcer disease has been a major cause of morbidity and mortality [7]. The sharp rise in the incidence of peptic ulceration has been attributed to the increased use of non-steroidal anti-inflammatory drugs, alcoholic beverages, cigarettes, and infections with *Helicobacter pylori* [7]. The pathophysiology of the disease is as a result of the imbalance between aggressive and protective factors in the stomach such as increased acid–pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer [8]. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid plus pepsin, active oxidants, platelet aggravating factor, leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defense mechanisms (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide) [9]. In traditional medicines, various herbal preparations are used for treating ulcer [10] with the ideal aim being to relieve pain, heal the ulcer and delay its recurrence. To date, no drug meets all these goals of therapy.

Species of the genus *Plantago* (commonly known as plantain) have been used extensively for medicinal purposes [11]. *Plantago rugelii* (Plantaginaceae) is found all over the world including Asia, Australia, New Zealand, Africa and Europe. The leaves of *P. rugelii* have been utilized as topical for wounds, bites, stings, bronchial infection, hepatitis, and jaundice among others. Its seeds have been established to be used in treating urinary infections [12]. A search at literature reviews shows that the proximate and ulcer activity of this plant has not been evaluated. Thus, the aim of the study was to evaluate the quantitative proximate analysis and the protective role of *P. rugelii* on ulceration induced by aspirin and HCl in 60% (v/v) ethanol mixture on Wistar albino rats.

Materials and Methods

Collection and Preparation of Plant materials

Fresh *P. rugelii* whole plants with the roots, stem and leaves intact were collected in June 2012 from a forest in Owerre Olubor in Ika North East Local Government of Delta State Nigeria. The plant material was identified and authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan where a herbarium specimen was deposited and a specimen number FHI 109775 issued. The fresh whole plant consisting of the leaf,

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stem and root of the plants were carefully washed with water to remove earthy material and air-dried for a period of three weeks before they were reduced to fine powder with the aid of an electric milling machine. The powdered sample was stored in an air tight container until used.

Phytochemical analysis
Simple chemical tests to detect the presence of carbohydrates, proteins and secondary metabolites were done in accordance with standard methods [13,14,15].

Proximate analysis
The following quantitative parameters such as moisture content, total ash, water insoluble ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value of the powdered sample were carried out using standard methods [16,17].

Preparation of extract
Powdered plant material (2.66 kg) was extracted with 7.0 L of methanol by maceration at room temperature for 72 hours [18]. The extract was concentrated to dryness using a rotary evaporator at 40 ^{o}C under reduced pressure. The concentrated extract was air dried, weighed and stored in an air-tight container.

Animal treatment
Adult Swiss albino mice (19 – 30 g) and Wistar albino rats (190 – 220 g) of both sexes were obtained from the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were fed with standard laboratory animal food pellets (Ewu floor mill). Animals were exposed to natural lighting conditions and were handled in accordance with international principles guiding the use and handling of experimental animals

Acute Toxicity Studies
Overnight-fasted Swiss albino mice were used for this study. The animals were randomly divided into five groups of 5 animals each and the extract was administered orally at doses of 1, 2, 4 and 8 g/kg to groups I, II, III and IV respectively while the control group V, received distilled water by same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 hours were observed for another 14 days (no extract administration) for any sign of delayed toxicity [19].

Ulcer Induced by Aspirin
Adult Wistar albino rats were randomly selected into 5 groups each of 5 animals per group. Animals in group I (normal group) received 3 ml/kg of 5 % gum acacia while groups II and III received omeprazole (20 mg/kg) and ranitidine (150 mg/kg) respectively. Groups IV and V received 200 and 400 mg/kg of the extract for a 7 day duration.
After 7 days of administration of the extract and drugs, animals were fasted for 24 h but were allowed free access drinking water up to 2 h before the experiment. Following 1 hr of the administration of the last dose of the test and control compounds, the rats were fasted for 24 h and were administered aspirin 200 mg/kg) p.o. Four hours after aspirin induction, the animals were sacrificed and their stomachs were dissected out and gently rinsed with 0.9 % saline solution. Ulceration in the glandular part of the stomach was assessed. The area of the gastric lesions and hemorrhage was measured [20].

Ulcer induced by 0.3 M solution of HCl in 60 % (v/v) ethanol
The rats were divided into five groups of five each like the aspirin-induced ulcer models, but the control and drug-treated animals received 0.3 M solution of HCl in 60 % (v/v) ethanol instead of aspirin. The rats were fasted for 24 hr with free access to water, the standard and test drugs were administered orally 1 hr before the induction of ulcer [21]. The animals were sacrificed after 1 h of ulcerogen administration, and their stomachs were excised, then the procedure followed was similar to that for aspirin-induced ulcer model

Determination of Ulcer Index and Inhibition
On macroscopic examination, the presence of an ulcer (or ulcers) and the occurrence of gastric hemorrhage were scored [22]. The scores were used to determine the ulcer index using the expression Ulcer Index = {Ulcer Number + Ulcer Score + Ulcer Area} × 10^{-1}. The percentage ulcer inhibition was also determined using the expression [22].

$$\text{Percentage (\%)Ulcer Inhibition (UI)} = \frac{\text{UI control} - \text{UI test}}{\text{UI control}} \text{ X } 100$$

Statistical analysis
Obtained data were analyzed using Statistical Package for Social Sciences (SPSS 17.0, Chicago, IL) at 0.05 level of significance. Results were presented as mean and standard deviation (Mean ± SD). The statistical significance between the control and each of the treated groups was determined by Dennett's post hoc test after one-way ANOVA. The level of significance was set at $P < 0.05$.

Results and Discussion

The percentage yield of the crude methanol extract was found to be 4.73%. The phytochemical screening revealed the presence of alkaloids, saponins, carbohydrate, reducing sugars, deoxy sugars, phytosterols, protein and flavonoids. Tannin, anthraquinones and phenolic compounds were revealed to be absent. The result is presented in Table 2. The presence of secondary metabolites may impart characteristic odour, taste, colour, medicinal or poisonous properties on the plant [23]. They also aid in the biological actions for which they are identified in folk medicine. The geographical distribution of plants, affect both the morphology and expression of phytochemicals [24, 25], this may be the case with *P. rugelii which* lacked tannin, anthraquinone and phenolic compounds. The results of the phytochemical composition of the whole plant powder consisting of the roots, stem and leaves showed that the plant has an appreciable number of phytochemicals.
The quantitative proximate analysis gave a moisture content of 39.64 ± 1.09 %, total ash of 17.22 ± 0.20 %, acid insoluble ash of 4.93 ± 0.36 %, water insoluble ash of 6.27 ± 0.18%, alcohol soluble extractive value of 0.98 ± 0.04 % and water soluble extractive value of 0.25 ± 0.18 % as seen in Table 3. Moisture content in plants assists in maintaining the protoplasmic contents of cells but make herbs perishable and susceptible to microbial degradation during storage [26]. The moisture content is higher than the value set by Africa Pharmacopoeia, hence suggests that *P. rugelli* will be prone to microbial attack and hence could have a short shelf life. The total ash value indicates the level of mineral elements content preserved in a plant and it was recommended that plants with ash content above 8.8% are useful health wise [27]. The total ash value of the powdered drug is high when compared to standard and this suggests that it has a high deposit of mineral elements, which may be nourishing and suitable for consumption. The level of contamination or adulteration by sand (silicate) can be detected by the level of acid insoluble ash. Acid insoluble ash of 4.93 % was obtained and this gives an indication of the level of insoluble mineral like silicate in the crude plant. The water and alcohol soluble extractive values aid in the detection of exhausted and already used drugs which could be used as adulterants [28]. The alcohol extractive value was observed to be higher than the water extractive value indicating that water could be the best solvent for its extraction.
Table 4 shows that the LD_{50} was above 8000 mg/kg in the treated mice. The administered graded doses of the methanol extract of *P. rugelli* did not result in mortality over the 24 hr period. No death or delayed toxicity was observed in the animals after 14 days. Hence, the acute toxicological results showed that the plant is relatively safe but further toxicity evaluations using mammalian tissues and organs will be necessary.
In both animal models of ulcer, the comparison of the relative area (%) of gastric erosion and hemorrhage in the control, omeprazole, ranitidine and *P. rugelii* extract treated rats showed that the plant possesses gastroprotective effects (Figures 1 and 2). Figure 3 shows the stomach of the negative control rats (no treatment), those treated with both ranitidine and omeprazole (positive controls) and the different doses of *P. rugelii* extract.
Ulcer index parameter was used for the evaluation of anti-ulcer activity in the study because ulcer formation is directly related to factors such as gastric volume, free and total acidity. Ulcerogenic agents are known to increase the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting into increase in ulcer index [29]. Aspirin is an anti-inflammatory drug known for its gastric toxicity frequently characterized by gastric ulcers and hemorrhage [30]. Hence, the drug is frequently used as a model in studies on *in vivo* cytoprotective activity of new substances or compounds [31]. The results as presented in Figures 1 and 2 showed that the *P. rugelii* plant extract and the reference drugs (ranitidine and omeprazole) groups produced a lower total ulcer number, ulcer score, ulcer area, ulcer index and percentage ulcer inhibition in a dose dependent manner in comparison with the negative

Table 1: Ulcer Score [22]

Lesions	Length of Ulceration	Score
Ulcer	Length > 10 cm	4
	Length 2 – 10 cm	2
	Length 1 – 2 cm	1
	Length < 1 cm	0.5
Bleeding		2

Table 2: Phytochemical screening of the powder plant of *P. rugelii*

Phytochmicals/constituents	Observation	Inference
Alkaloids	Yellow precipitate formed	+
Saponins	Yellow emulsion formed	+
Carbohydrate	Violet ring formed	+
Reducing sugars	Brick red precipitate formed	+
Deoxy sugars	Violet ring formed	+
Phytosterols	Gradual change in colour from violet to blue and to green	+
Protein	Yellow precipitate	+
Flavonoids	Yellow colour persist	+
Tannin	Green black colour not seen	-
Anthraquinones	Green colour observed	-
Phenolic compounds	No coloration observed	-

+ indicates presence of components - indicates absence of components

Table 3: Proximate composition *of P. rugelii* on dry matter basis

Parameter	Percentage values (%)
Moisture content	12.46 ± 0.08
Total ash	17.22 ± 0.20
Acid insoluble ash	4.93 ± 0.36
Water soluble ash	6.27 ± 0.18
Alcohol extractive index	0.98 ± 0.04
Water extractive index	0.25 ± 0.06

Values expressed as % mean ± SD (Standard deviation), n=3

Table 4: Acute toxicity profile of *P. rugelii* plant extract

Dose (mg/kg)	Mortality Ratio	% Mortality
1000	0/5	0
2000	0/5	0
4000	0/5	0
8000	0/5	0

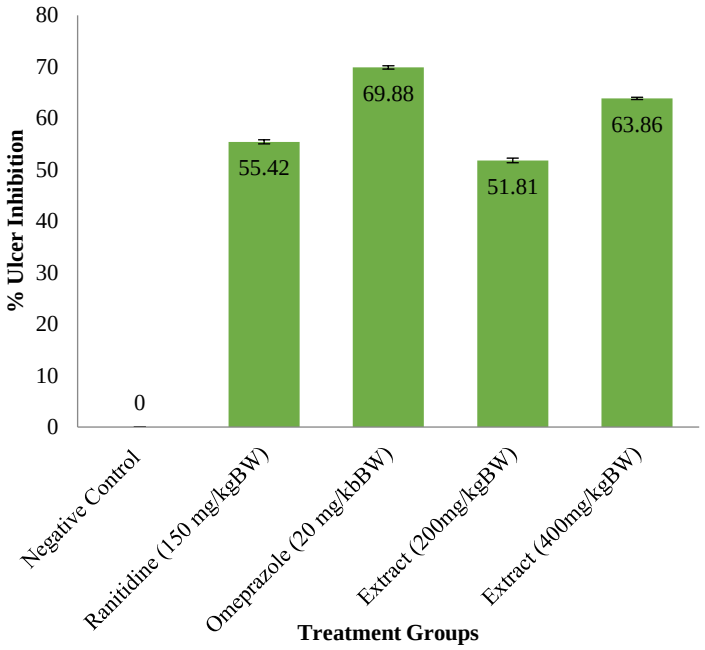


Figure 1: Percentage ulcer inhibition using the Aspirin induced model.

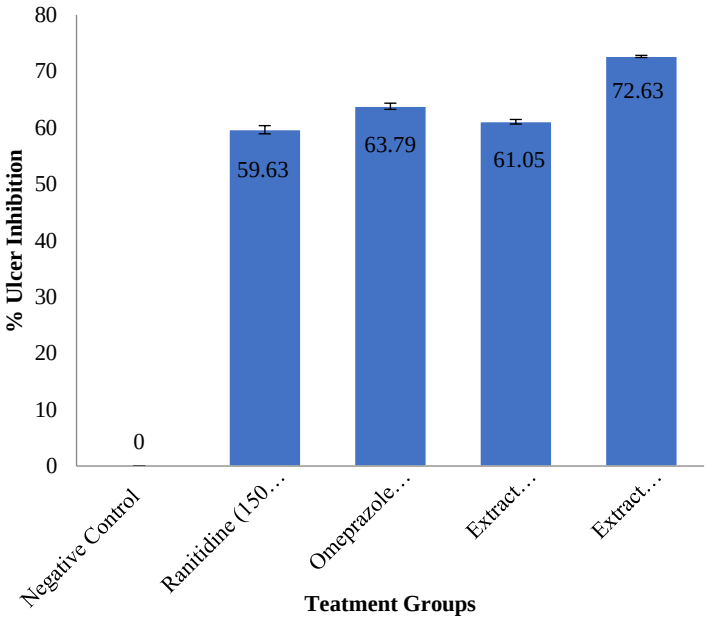
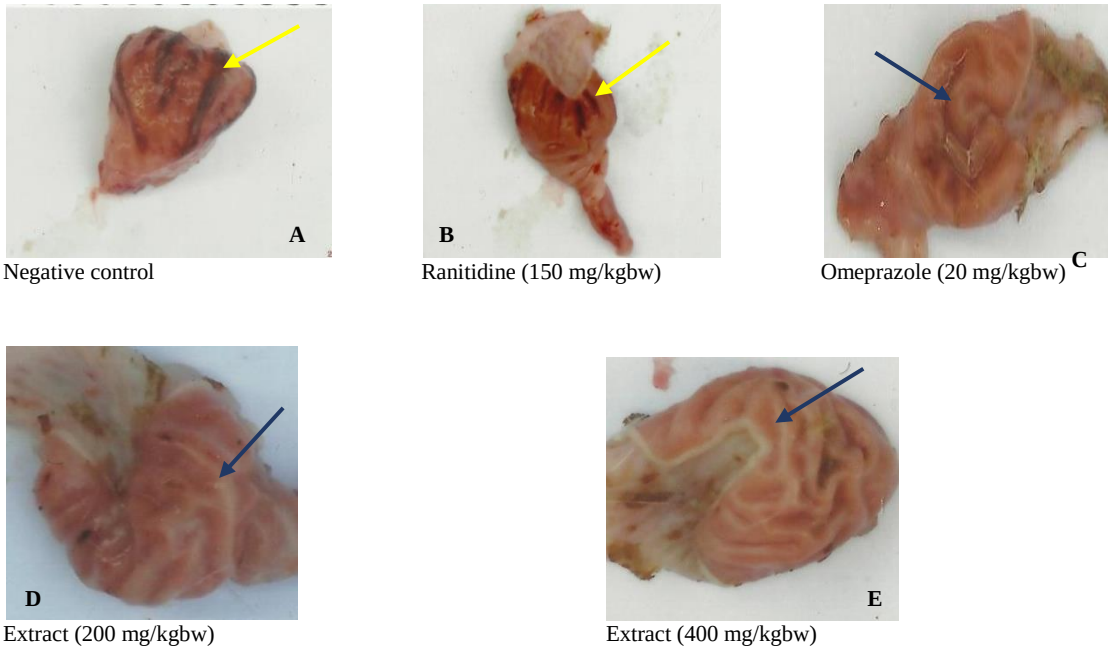


Figure 2: Percentage ulcer inhibition induced by administration of 0.3 M HCl in 60% (v/v) ethanol mixture

control group. There was statistically significant difference ($p < 0.05$) of the extract treated groups and the positive control groups when compared with the negative control group. There was no statistically significant difference ($p > 0.05$) between the extract treated groups when compared with the reference drugs treated groups.

Although this study was able to provide evidence that the *P. rugelii* extract possessed protective activity against gastric ulcer, the active compounds involved in exerting this protective effect are not yet known with certainty. One of the suggestions relates to the antioxidant effects of the flavonoids and saponins that are known to be present in *P. rugelii*. Antioxidant compounds have been demonstrated to have cytoproteetive effects, thus being capable of protecting cells from various kinds of damage and possibility of functioning as anti- ulcer drugs [32,33,34]. Antioxidants act by inhibiting lipid peroxidation and by scavenging free radicals, thereby preventing the occurrence of gastric ulcer [35].

Several studies demonstrated that flavonoids from various plants are reportedly capable of preventing the occurrence of gastric ulcer. This may take place through an increase in the amounts of neutral glycoprotein's and in prostaglandin concentrations and inhibition of histamine secretion from mast cells by inhibition of histidine decarboxylase [33] thus reducing stimulation of 1-12 receptors [36] or by secretion of prostaglandin-like compounds [37]. In addition to flavonoids, other compounds playing a protective role against ulceration are the saponins, which have hemolytic, expectorant, immune-stimulant, and anti- inflammatory properties [38]. It is thought that the anti-inflammatory effects of saponins reduces the risk of ulcers, by increasing defensive factors of gastric mucosa and stopping the inflammatory process resulting from induction by aspirin (indicated by



Negative control | Ranitidine (150 mg/kgbw) | Omeprazole (20 mg/kgbw)

Extract (200 mg/kgbw) | Extract (400 mg/kgbw)

Figure 3: Pictorial representation of the stomach of untreated (negative control), standard (Ranitidine and Omeprazole) and the extract treated groups. The yellow and blue structures indicate hemorrhagic/ erosive areas and mucosal build up respectively.

absence of edema in the gastric mucosa of rats receiving *P. rugelii* infusion). The protective effect of saponins against gastric ulcer may also be mediated by the formation of a protective mucus layer on the gastric mucosa and by selective inhibition of PGF2α[33]. The plant has been shown to possess these phytochemicals (Table 2).

Though flavonoids and saponins present in the plant may be the prime agents responsible for the anti-ulcer properties, other phytoconstituents may also be responsible. The isolation of the active anti-ulcer constituents and the mechanism(s) by which they exert such activity are subjects for further studies.

Conclusion

The roots, stem and leaves of *P. rugelii* contains several phytochemicals which may be responsible for its acclaimed ethnomedicinal use. Oral administrations of the plant extract at 200 mg/kg and 400 mg/kg have protective activity against the occurrence of gastric ulceration in rats and comparable to ranitidine and omeprazole in preventing experimental gastric ulcer. Higher dosages of the plant extract infusion are correlated with a correspondingly higher gastric ulcer protective activity. The study also showed that the oral administered extract of *P. rugelii* is relatively safe at the highest tested dose of 8 g/kg in mice.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work 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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