

Total Alkaloids, Tannin Content, and Antiulcer Assay of Four Selected Medicinal Plants in Nigeria

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ABSTRACT

The aim of this study was to determine the total alkaloids, total tannins content, and antiulcer activity of the extracts from these selected medicinal plants (*Acanthospermum hispidum*, *Pachypodanthium staudtii*, *Phaseolus lunatus*, and *Euphorbia deightonii*). The total alkaloids content (TAC) was evaluated according to the chloride colometric method in which atropine was used as standard. While the total tannins content (TTC) was also determined using Folin Ciocalteu assay in which gallic acid was used as a standard. The antiulcer activity of the extracts was investigated using the ethanol induced model in Wistar albino rats. Parameters such as gastric volume, pH, and ulcer index were used as indicators for the antiulcerogenic activity of the extracts.

The animals were orally treated with distilled water (Normal control group), Ranitidine 5 mg/kg (standard group), 0.5 mg/kg of ethanol (Negative control group) and 100, 200 and 400 mg/kg of the extracts, 1 h before oral administration of absolute ethanol to induce gastric mucosal injury.

The results showed that *E. deightonii* extract has the richest source of alkaloids and tannins (0.850 ± 0.001 mg AE/g and 0.133 ± 0.001 mg GAE/g respectively, while *A. hispidum* and *P. staudtii* has the least TAC and TTC (0.800 ± 0.001 mg AE/g) and (0.124 ± 0.001 mg GAE/g), respectively. The extract was considered safe with the LD₅₀ greater than 5000 mg/kg for *E. deightonii*, 2154, 3808 and 2154 mg/kg for *A. hispidum*, *P. staudtii* and *P. lunatus* respectively. The extracts at dose levels of 100, 200, and 400 exhibited significant decrease $*(P < 0.05$ at 95% CL) in the gastric volume, while the pH of the gastric juice was

significantly increased $*(P < 0.05$ at 95% CL) in the ethanol induced model. The extracts showed minimum inhibition of gastric acid ranging from 16- 90%. The results showed that the methanol extracts of the selected plants possess antiulcer as well as cytoprotective ability which could be attributed to the presence of secondary metabolites.

(Keywords: *Acanthospermum hispidum*, *Pachypodanthium staudtii*, *Phaseolus lunatus*, *Euphorbia deightonii*, antiulcer, cytoprotective ability)

INTRODUCTION

Peptic ulcers can be seen as open sores that develop on the inside mucosal lining of the digestive tract, specifically, the initial portion of the small intestine (duodenal ulcer), esophagus (esophageal ulcer), and stomach (gastric ulcer). Peptic ulcers develop when the balance between the digestive acids and the protective mucosal layer is disrupted (Gulia and Chondhary, 2011).

Peptic ulcer disease (PUD) is one of the disease conditions that affect many people around the world especially in the developing world (Zeeyauddin et al., 2011). Recent studies estimate that at least 70%, and possibly as high as 90%, of peptic ulcers are caused by *Helicobacter pylori* colonization. Other causes of peptic ulcer are; nonsteroidal anti-inflammatory drugs (NSAIDs) (Huang. Et al., 2002); such as aspirin and ibuprofen, ethanol, cigarette smoking, diet, and psychological factors (stress ulcers).

A number of drugs are available for the treatment of peptic ulcer disease. These include prostaglandins, proton pump inhibitors, histamine

receptor antagonists and mucoprotectives (Jothi et al., 2012). Adverse drug reactions from these drugs demanded for the use of alternative or herbal medicines. The use of herbal medicines has been reported in the treatment of PUD disease (Dhasa et al., 2010).

Acanthospermum hispidum (DC), (Family *Asteraceae*) is a medicinal plant commonly called “bristly starburr” and locally known as “ewe onitan meta” or “kaashinyaawo” in Western and Northern Nigeria, respectively (Mshana et al., 2010). The leaves are used locally for the treatment of jaundice, malaria, vomiting, cephalgias, headache, abdominal pain, convulsion, stomach-ache, constipation, eruptive fever, snake bite, epilepsy, blennorrhoea, hepato-binary disorder, microbial infection, and viral infection (Ramzi et al., 2009). The plant has been reported to contain phytochemicals such as carbohydrates, glycosides, flavonoids, terpenoids and saponins that may be useful adjuvant for antibiotic, antiviral, antitrypsomal, antiplasmodial, antimicrobial, antitumor and anthelmintic formulations (Edewor and Olajire 2011; Anup et al, 2012).

Pachypodanthium staudtii Engl & Diels, (Family *Annonaceae*) is a medicinal plant predominantly found in southern Nigeria. It is commonly called “Pepper tree” and locally called “Ntoko neto” in Eastern Nigeria. The stem bark and roots bark are used locally in the treatment of gastro-intestinal pains, headache, chest pains, tumors, toothache, bronchitis, and oedema (Aimé, et al., 2014)). The leaves, stems, and barks of *Pachypodanthium staudtii* plant contain phytochemicals such as anthocyanines, anthraquinones, flavonoids, phenols, sterols, triterpenes, and saponins (Agnaniet et al., 2004). The plant also possesses antibacterial activity, it was reported to be active against the following bacteria *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Providencia stuartii* and *Pseudomonas aeruginosa* (Aimé, et al., 2014; Agnaniet et al., 2004). *Pachypodanthium staudtii* plant has been proven to be biologically active, its bioactive components are as follows; *pachypodol*, *2,4,5-trimethoxystyrene*, *pachypophyllin*, *pachypostaudins A and B*, *pachypodanthine* (Fournier, et al., 1999), *pachysonto* (Mathouet, et al., 2007), *staudin* (Yapi, et al., 2012), *sabinene*, *β -elemene*, *E- β -caryophyllene*, *β -selinene*, *β -bisabolene*, *δ -cadinene*, and *2,4,5-trimethoxy-1-vinylbenzene* (Koonaa, and Koonaa., 2006).

Phaseolus lunatus, (Family *Fabaceae*) is commonly called “lima beans” and locally called “Awuje” or “Eree” in Southern and Eastern Nigeria respectively. *Phaseolus lunatus* like many other legumes is a good source of dietary fiber, a fat-free source of high-quality protein. It helps regulate blood sugar levels and lowers cholesterol, and insoluble fiber (Heuze et al., 2013). Lima beans like any other leguminous plant is rich in protein, thus it serves as a major source of food for man and livestock (Ibeawuchi, 2007). It also contains phytochemicals like saponins, oxalate and phytic acid.

Euphorbia deightonii Croizot, (Family *Euphorbiaceae*) is medicinal plant commonly called “Africa never dies” (because of its ability to survive in any weather condition) and locally called “oro agogo” in Northern Nigeria. The importance of *Euphorbia deightonii* Croizot is enormous it can be used for horticultural purposes in making hedges, markers, and ornamental flowers (Idu et al., 2011). In medicine, it is used in the treatment of leprosy, skin mucosae, infertility in women and as an aphrodisiac (Brian, 2013). It has been reported to contain resin, exudation-gum, and steroids (Brian, 2013), upon preliminary photochemical screening.

It is necessary to evaluate the potential of herbal medicine and therapy to prevent drug-herbal medicine interaction during patient management (Osazuwa et al., 2014). The aim of the present study was to determine the total alkaloids, total tannins content and antiulcer activity of the extracts from these selected medicinal plants (*Acanthospermum hispidum*, *Pachypodanthium staudtii*, *Phaseolus lunatus* and *Euphorbia deightonii*) in ethanol induced model.

MATERIALS AND METHODS

The plants were collected from Orba in Enugu State Nigeria, in the month of September and authenticated by Mr. Alfred O. Ozioko of International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Nigeria. The parts of the medicinal plants selected for this study were washed, air dried under shade, and pulverized to fine powder using a hand blender. 20.0 g of each pulverized plant parts was weighed and macerated 200 mL of methanol and extracted at room temperature after 48 h with agitation. The filtrates were concentrated *in*

vacuo at reduced pressure and temperature (40 °C) to obtain extracts. The extracts were stored in an air-tight plastic container in the refrigerator and used for the study.

Phytochemical Analysis

The qualitative test for alkaloids and tannins in the extracts was done according to the procedure reported (Mythili et al., 2014). The total alkaloids content of the plant extracts was determined by colometrically using Atropine as a standard and the total tannins content was determined by Folin-Ciocalteu method using Gallic acid as a standard (Mythili et al., 2014).

Animals

Wistar of albino rats of both sexes (85-300 g), obtained from the animal house of the Department of Zoology, University of Nigeria Nsukka. The animals were housed in groups of 6 rats per cage in a 12 h light/dark cycle at room temperature and were fasted for 24 h with free access to water before the experiment. All the animals were used according to the guidelines for the care and use of laboratory animal's procedure.

Chemicals

The solvent used are: Dimethyl sulphoxide (DMSO), Methanol, Hydrochloric acid, Chloroform and Phosphoric acid, were purchased from Sigma Aldrich. The reagents are: Sodium carbonate (M&B England), Bromocresol green (Sigma Aldrich), Phosphate buffer (Sigma Aldrich), Atropine sulphate (Sigma Aldrich), Sodium hydroxide (BDH England), Sodium tungstate (Sigma Aldrich), Sodium molybdate (Sigma Aldrich), Sodium phosphate (BDH, India) Gallic acid (Qualikems), Ferric chloride (Merck Darmstadt, Germany), Citric acid (BDH England). The control drug: Ranitidine (Evans Medical LTD. Nigeria).

Acute Toxicity Test (LD₅₀) of the Extracts

The acute toxicity test of the plant extracts was done to determine the safe dose for the extracts according to method previously described by Lorke (Enegide et al., 2013). The acute toxicity

(LD₅₀) of the extracts was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = highest dose that gives no mortality.

D₁₀₀ = lowest dose that produced mortality.

Antiulcer Assay

Ulcer was induced in the rats by oral administration of ethanol. The animals were fasted for 36 h before administration of ethanol. (Except for animals in group I which served as the normal control). Seventy-five animals were used in this study, the animals were divided 6 groups of 15 rats each. (Except for group I, group II and group III with 5 animals each). The animals in group I were given 10 mL/100 g of distilled water (Normal control). After 1h of ethanol administration, the rats in Group II received 0.5 mL/100 g of ethanol orally (Negative control). Rats in Group III received ranitidine 5 mL/100 g and were served as (Standard control). Rats in Groups IV, V and VI received the already prepared plant extracts at doses 100, 200, and 400 mg/kg, respectively (Gupta et al; 2012; Boligon et al., 2014).

The peptic ulcers were induced in rats by administering 0.5 mL /100g of 90 % ethanol orally after 1h of treatment with the plant extract and ranitidine. The animals were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with chloroform inhalation and the stomach was incised and ulceration scored (Patel et al., 2012).

Macroscopical Evaluation of Stomach

The stomachs of the rats were opened along the greater curvature and rinsed with 0.9 % of saline to remove gastric contents and blood clots and then were examined for lesions in the glandular part under 10 times magnified lens to access the formation of ulcer. The number of ulcers was estimated using the ulcer index as previously reported (Patel et al, (2012)²⁸, using the following scale.

Normal colored stomach.....(0)
Red coloration.....(0.5)

- Spot ulcer.....(1.0)
- Hemorrhagic streak.....(1.5)
- Deep ulcer..... (2.0)
- Perforation..... (3.0)

The mean score of each animal was expressed index. Percentage inhibition of ulceration and estimation of ulcer index was estimated using the following formula:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where U_N is the average number of ulcers per animal; U_S is the average number of severity; U_P refers to the percentage of animals with ulcers.

Gastric Volume and pH Measurement

The abdomen of each animal was carefully opened, the cardiac end of the stomach was dissected out and gastric contents were collected. The volume of the gastric content was measured using a measuring cylinder and the pH of the gastric contents measured using a pH meter as previously described by (Ezealisyi *et. Al.*, 2014).

Statistical Analysis

The results were expressed as the mean \pm SEM of at least five determinations (n=5). Statistical comparison was performed by one-way analysis of variance using Graph pad prism 5 software followed by Dunnett's *post hoc* least significant difference (LSD) test. Comparing with normal control group, $P < 0.05$ was considered to be significantly different.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis of the Plant Extract

The result of the phytochemical analysis carried out on the different plant extracts showed the presence of alkaloids and tannins in abundance (Table 1). The total alkaloids and total tannin content of the various plant extracts were calculated using the regression equation $y = 0.000x + 0.792$, $R^2 = 0.973$ and $y = 0.000x + 0.12$, $R^2 = 0.982$, respectively, for TAC and TTC presented in mg AE/g and mg GAC/g of extract values respectively. The calibration curves are shown in Figures 1-3.

Table 1: Total Alkaloids and Total Tannins Content of the Extracts.

Plants	Family	Parts investigated	TAC (mg AE/g)	TTC (mg GAE/g)
<i>A. hispidum</i>	<i>Asteraceae</i>	Leaf	0.800 \pm 0.001	0.125 \pm 0.001
<i>P. staudtii</i>	<i>Annonaceae</i>	Stem bark	0.820 \pm 0.001	0.124 \pm 0.001
<i>P. lunatus</i>	<i>Fabaceae</i>	Seed	0.840 \pm 0.001	0.131 \pm 0.001
<i>E. deightonii</i>	<i>Euphorbiaceae</i>	Leaf	0.850 \pm 0.001	0.133 \pm 0.001

Values are expressed as mean \pm SD (n=3). The absorbance against the reagent blank was determined at 470 nm and 760nm with the UV/Visible spectrophotometer for alkaloids and tannins respectively. Total alkaloids content was expressed as mg atropine equivalent (AE) and total tannin content was expressed as mg gallic acid equivalent (GAE).

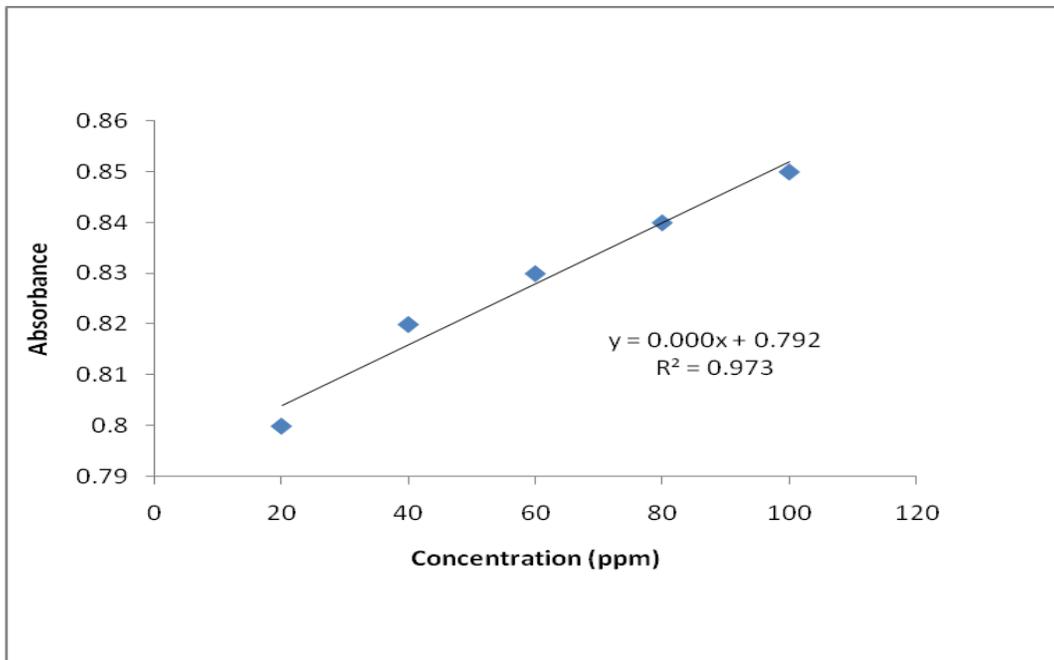


Figure 1: Calibration Curve for Atropine.

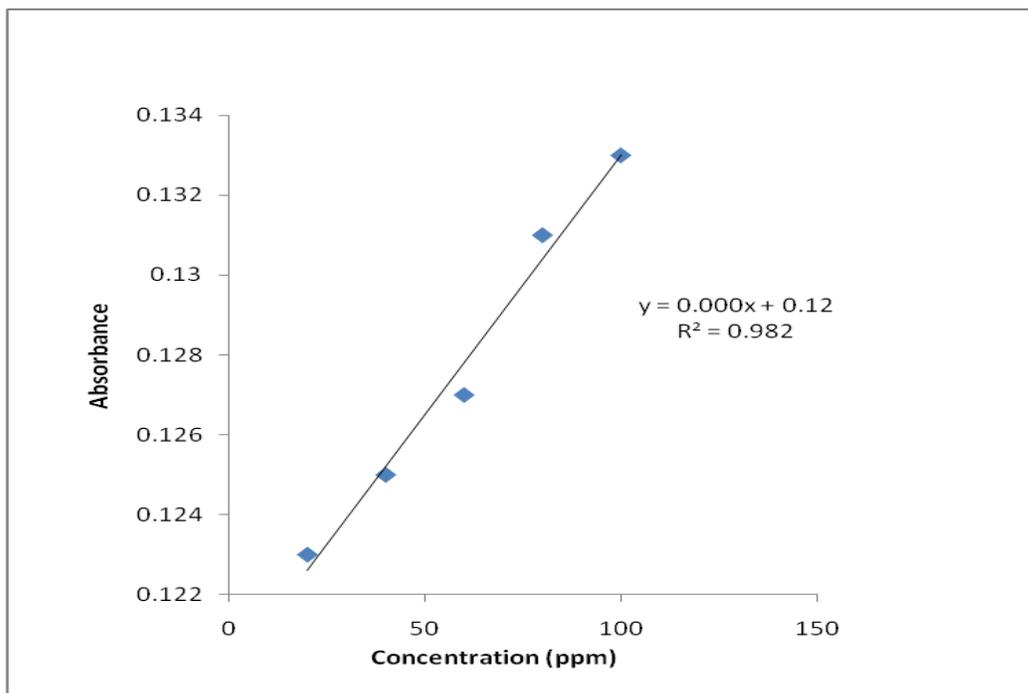


Figure 2: Calibration Curve for Gallic Acid.

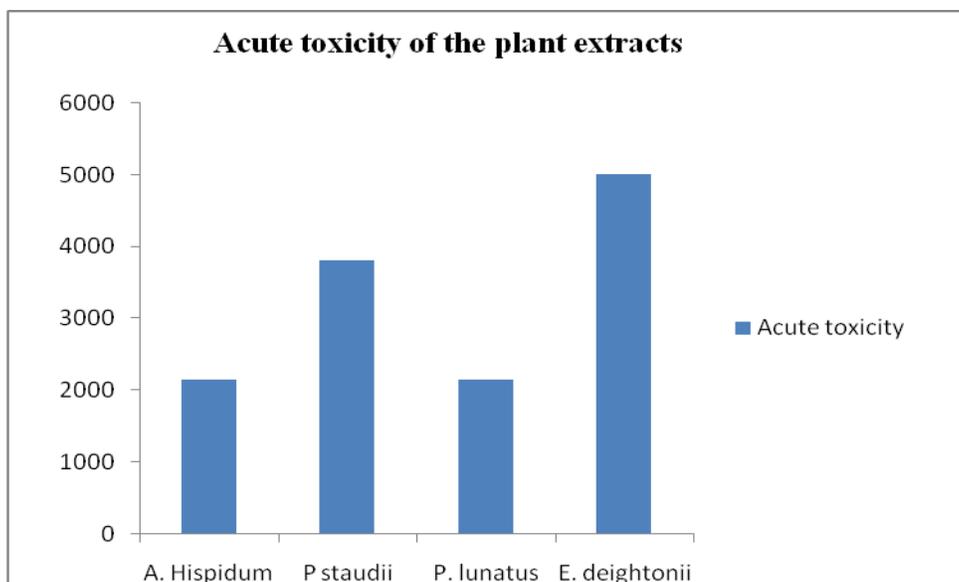


Figure 3: Acute Toxicity Test (LD₅₀) on Plant Extracts.

Antiulcer Activity of the Plant Extracts

The effects of the extract on the gastric lesion induced by ethanol (0.5 mL/ 100 g body weight) are shown in Tables 2 and 3. Pretreatment with ethanol showed presence of superficial and deep ulcers and no case of perforations in all the animals.

The extracts showed a reduction in the ulcer index in all the test doses: *A. hispidum* at doses 100, 200, and 400 mg/kg (11.39±3.96, 6.41±1.48, and 6.30±0.98, respectively) with (16%, 53%, and 54% ulcer inhibition, respectively), *P. staudtii* at doses 100, 200, and 400 mg/kg (9.81±4.95, 8.49±2.28, and 8.43±2.05, respectively) with (27%, 37%, and 38% ulcer inhibition, respectively), *P. lunatus* at doses 100, 200, and 400 mg/kg (5.85±0.14, 6.55±0.16, and 7.65±0.34, respectively) with (44%, 52%, and 57% ulcer inhibition, respectively), *E. deightonii* at similar doses has an ulcer index of (3.85±0.15, 2.61±0.28, and 1.42±0.39, respectively) with the highest ulcer inhibition (70%, 80%, and 90%, respectively) compared to the control groups.

The animals that received ranitidine (5 mL/100 g) showed significant ($P < 0.05$) decrease in gastric lesion with (10.84±2.61) ulcer index and 20 % ulcer inhibition compared to control. Treatment with the plant extracts significantly ($P < 0.05$) reduced the volume of gastric content to

3.18±0.63, 3.58±0.34, and 3.32±0.57 at doses 100, 200, and 400 mg/kg, respectively for *A. hispidum*. 1.88±0.21, 3.06±0.36, and 2.44±0.64 at doses 100, 200, and 400 mg/kg, respectively for *P. staudtii*. 2.32±0.65, 1.86±0.45, and 1.36±0.35 at doses 100, 200, and 400 mg/kg, respectively for *P. lunatus*. 2.39±0.89, 0.98±0.48 and 1.10±0.25 at the same doses for *E. deightonii*. The pH was significantly ($P < 0.05$) increased to (6.03±0.24, 6.58±0.16, and 6.53±0.18) at the tested dose levels for *A. hispidum*, (6.06±0.25, 6.00±0.59, and 6.32±0.26) at the tested dose levels for *P. staudtii*, (5.65±0.14, 6.09±0.16, and 6.39±0.15) at the tested dose for *P. lunatus*, (6.18±0.15, 6.25±0.28 and 5.65±0.29) for *E. deightonii* at the same tested dose levels.

Histology of the Stomach of the Experimental Animals

The photographs of the stomachs of the animals were taken using a digital camera. The various degrees of ulceration in the stomach of the animals were seen using a magnifying glass. Severe ulcerations ranging from spot ulcer, (hemorrhagic) blood streaks, deep ulcer (erosion) and pin-hole spots were present in the stomach (Figure 4).

Table 2: The Ulcer Index and Percentage Inhibition of the Plant Extract on Ethanol Induced Wistar Albino Rats.

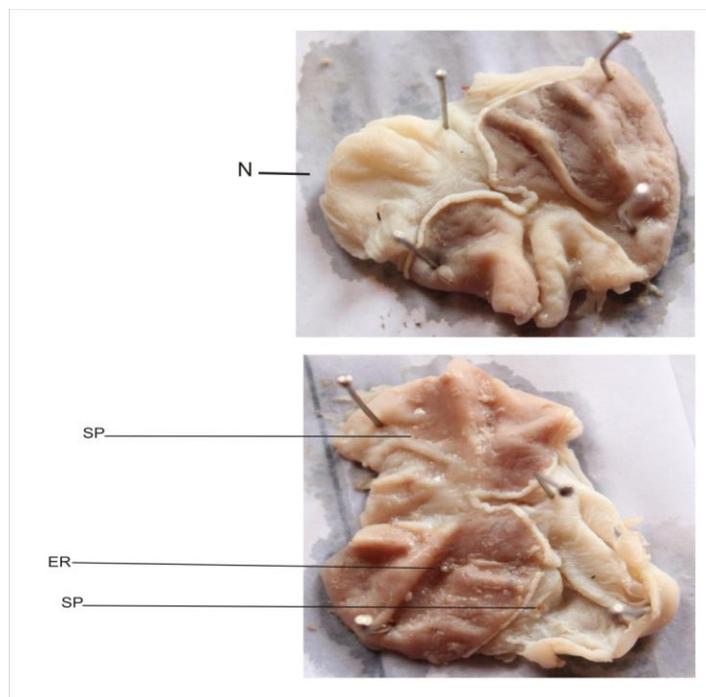
Treatment	Doses (mg/kg)	Ulcer index	Protection (%)
Control (D. water)	10 mL/100 g	0	-
Control (ethanol)	0.5 mL/100 g	13.61±7.24	-
Ranitidine	5 mL/100 g	10.84±2.61	20
<i>A. hispidum</i>	100	11.39±3.96	16
	200	6.41±1.48	53
	400	6.41±1.48	54
<i>P. staudtii</i>	100	9.81±4.95	28
	200	8.49±2.28	37
	400	8.43±2.05	38
<i>P. lunatus</i>	100	5.85±0.14	44
	200	6.55±0.16*	52
	400	7.65±0.34*	57
<i>E. deightonii</i>	100	3.85±0.15	70
	200	2.61±0.28*	80
	400	1.42±0.39*	90

Values are expressed as mean±SEM (n=5). *P<0.05; (shown significant difference and ANOVA followed by Dunnett's post hoc LSD test compared with control group (normal control))

Table 3: The Gastric Volume of the Plant Extract on the Ethanol Induced Wistar Albino Rats.

Treatment	Doses (mg/kg)	Gastric Juice	
		pH	Vol. (mL)
Control (D. water)	10 mL/100 g	3.54±0.20	13.00±0.26
Control (ethanol)	0.5 mL/100 g	7.86±0.64	4.56±0.40
Ranitidine	5 mL/100 g	6.22±0.28	2.60±0.09
<i>A. hispidum</i>	100	6.03±0.24	3.18±0.63
	200	6.58±0.16	3.58±0.34
	400	6.53±0.18	3.32±0.57
<i>P. staudtii</i>	100	6.06±0.25	1.88±0.21
	200	6.00±0.59	3.06±0.36
	400	6.32±0.26	2.44±0.64
<i>P. lunatus</i>	100	5.65±0.14	2.32±0.65
	200	6.09±0.16	1.86±0.45*
	400	6.39±0.15	1.36±0.35*
<i>E. deightonii</i>	100	6.18±0.15	2.39±0.89
	200	6.25±0.28	0.98±0.48
	400	5.65±0.29*	1.10±0.25*

Values are expressed as mean±SEM (n=5). *P<0.05 (shown significant difference and ANOVA followed by Dunnett's post hoc LSD test compared with control group (normal control))



Keys: N = Normal stomach colouration, ER = Erosion, SP = Ulcer spots.

Figure 4: Stomach of Experimental Wistar Albino Rats showing their Various Ulcerations.

DISCUSSION

Nature has been a source of medicinal agents for thousands of years. Thus it is important that the usefulness of the medicinal plant based industry should be harnessed. The crude extracts of *Ancanthospermum hispidum* DC, *Pachypodanthium staudtii* Engl and Diels, *Phaseolus lunatus* and *Euphorbia deightonii* Croizot plants studied were found to contain alkaloids and tannins using Colometric and Folin Ciocalteu assay in which atropine and gallic acid were used as standards, respectively. This method measured the amount of the extract needed to inhibit the oxidation of the reagents used (Ezealisyi et al., 2014). The total alkaloids and tannins content (TAC) and (TTC) of the extract was determined from the regression equation for the calibration curve ($y=0.000X+0.792$; $R^2=0.973$) and ($y=0.000X+0.12$; $R^2=0.982$) shown in Figures 1 and 2, respectively. The result showed that *Euphorbia deightonii* extract is the richest source of alkaloids and tannins (0.850 ± 0.001 mg AE/g and 0.133 ± 0.001 mg GAE/g respectively). While *Ancanthospermum hispidum* and *Pachypodanthium staudtii* has the least TAC and

TTC content (0.800 ± 0.001 mg AE/g) and (0.124 ± 0.001 mg GAE/g), respectively.

Peptic ulcers are one of the major ailments affecting humans and it develops because of imbalance between aggressive factors (acid, pepsin, *H. pylori*, bile salts) and defensive factors (mucous, bicarbonate blood flow, epithelial cell restoration and prostaglandins) (Perumalla. and Nayeem, 2012). The treatment of peptic ulcer is mainly aimed at reducing the hydrochloric acid secretion, increasing gastric cytoprotection, eradication of *H. pylori* or curing Zollinger Ellison syndrome. The discovery of potential antiulcer agent from plants is a developing area. So far, several plants have been screened for antiulcer activity and many formulations have been developed by combining extracts of these plants.

In this study, ethanol induced gastric ulcers were employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane

damage leading to increased intracellular permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Soll, 1990; Surendra, 1990).

The plant extracts showed a marked reduction in the ulcer parameters studied on the test animals in a dose dependent manner with a significant increase in both the pH and percentage ulcer inhibition. Phytochemical analysis of the extract showed that it contains trace amounts of alkaloids and tannins. It should be stated that the ability of the plant extract to reduce acidity might be due to the presence of phytochemical compounds (alkaloids and tannins). Recent reports and extensive literature review indicate that tannins shows cytoprotective action by increasing mucosal content of prostaglandins and mucous in gastric mucosa (Dandagi, et al., 2008).

CONCLUSION

The methanol extract of *Acanthospermum hispidum* leaf, *Pachypodanthium staudtii* stem bark, *Phaseolus lunatus* seed and *Euphorbia deightonii* leaf plant showed significant antiulcer activity which is evident by the data obtained. These extracts having a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its safety profile and can be a potent natural and safe alternative to conventional antiulcer treatment. However, there is a shortage of clinical trial regarding its potency and efficacy. This antiulcer activity could be attributed to the cytoprotective property of the plant extracts and inhibition of the gastric acid.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest in relation to this study. This research did not receive any specific grant from funding agencies in the public, commercial, of not-for-profit-sector.

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