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Antiulcer activity of methanolic seed extract of *Citrullus lanatus* in albino rats

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Abstract
Citrullus lanatus is widely distributed in Africa where majority of people consume its fruits while discarding its seeds. Apart from its nutritional benefit, traditionally, its seeds are used in the treatment of various ailment including ulcer. This study aims at evaluating the antiulcer property of *Citrullus lanatus* seed in animal models. The antiulcer activity of *Citrullus lanatus* seed (CLS) was evaluated in ethanol-induced model of gastric ulcers in albino rats. CLS extract has showed a significant ($p<0.05$) anti-ulcer effect at both 200mg/kg and 400mg/kg dose level in a dose dependent manner as well as significant ($p<0.05$) reduction in the ulcer index when compared to control group. However, the antiulcer effect of CLS extract (400mg/kg) was greater compared to the standard drug Ranitidine (100mg/kg). The preliminary phytochemical screening showed the presence of flavonoids, Saponin, Alkaloids, Steroids and terpenoids. These findings suggest that the CLS extracts possess antiulcer potential which may contribute to its ethno medicinal use.

Keywords: antiulcer, ulcer index, ethanol-induced model, *Citrullus lanatus* seed

Introduction
Citrullus lanatus (family Cucurbitaceae) is a vine-like flowering plant originally from southern Africa and mostly grown for its sweet and juicy fruit in warm climates all over the world. The plant is traditionally used for centuries in the treatment of various health aliments [1]. Fruit of this plant (watermelon) contains about six percent sugar by weight, the rest being mostly water. It is mildly diuretic and as with many other fruits, it is a source of vitamin C and lycopene [2]. Most people spit out or throw away watermelon seeds while eating this fruit [3]. This is why seedless watermelons are so popular because they save people from the hassle of picking out seeds, spitting them out, and throwing them away. Furthermore, laughably false claims that swallowing the seeds will cause a watermelon to grow in the stomach makes most people unaware of the impressive nutritional value these seeds possess and their potential health benefits. Benefits acclaimed to watermelon seeds include their ability to boost hair health, support beautiful skin, increase energy, lower [2] blood pressure, stimulate digestion, regulate blood sugar, build strong bones and lower cholesterol levels.
Researchers have shown that watermelon seeds are highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals [2]. The seeds are also vermifuge and have hypotensive action [1]. Oil from the seeds are used in cooking and incorporated into the production of cosmetics [4]. Fatty oils in the seeds as well as in aqueous and alcoholic extracts paralyze tapeworms and roundworms [1]. The seeds are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for instance used to prepare snacks, milled into flour and used for sauces [3]. In Kebbi state, north-west Nigeria, *Citrullus lanatus* seed has been reported to have medicinal uses such as in the treatment of erectile dysfunction, hypertension, ulcer, diabetes and head ache. The present study reports on phytochemical constituents and antiulcer activity of *Citrullus lanatus* seed.

Material and Methods
Collection of the plant material and identification
Citrullus lanatus fruits were collected in March 2017 from Jega market, Jega Local Government Area, Kebbi State. Specimens were identified and authenticated by Dr Dharmendra Singh of Botany unit, Biological Sciences Department, Kebbi State University of Science and Technology Aleiro Kebbi State, Nigeria. A voucher specimen (No: 314 A) was deposited at the Herbarium of the same department.

Plants materials preparation
The seed content of the fruit were removed, wash, air dried for three weeks to dryness and pulverized in to a coarse powder using a motor and pestle.

Preparation of the plant extract
The powdered sample (200g) was extracted in one (1) liter of methanol for 72 hours. This was then filtered with the aid of sterile sieving cloth and evaporated in a drying cabinet at 45^{o}C to constant weight. The dried extract collected was weighed, labeled and stored in an air tight bottle container.

Qualitative phytochemical analysis
The methanolic seed extract of *Citrullus lanatus* was treated with various reagents to qualitatively test for the presents of phytoconstituent [5, 6, 7].

Experimental animals
Fifty (50) albino rats weighing (120-150g) of both sex were obtained from National Institute of Trypanosomiasis Research, Kaduna in march 2017 and were transported to the Animal house, Faculty of Science, Kebbi State University of Science and Technology, Aliero. They were kept in well-ventilated environment, had free access to rodent pellets (Vital Feeds Ltd, Nigeria) and water daily and allowed to acclimatize for two (2) weeks prior to the commencement of study.

Antiulcer study
The antiulcer activity of *Citrullus lanatus* seed (CLS) extract were evaluated using ethanol induced ulceration model (8). The rats were fasted for 48 hours but allowed free access to water *ad libitum*. They were randomly selected and divided into five (5) groups of five (5) rats each. Group I (control) received normal saline 10ml/kg body weight, Group II (untreated control) received no treatment, Group III received the standard drug (Raniidine100mg/kg p.o.) while Groups IV - V received 200 - 400mg/kg CLS extract respectively. Thirty minutes later, ulceration was induced by gastric instillation of 1ml of 99% absolute ethanol and one hour after ethanol administration, rats were anaesthetized using chloroform and the stomach were removed, opened along the greater curvature to macroscopically examine any ulcerative lesions (elongated black-red lines parallel to the long axis of the stomach). The number, length and severity of ulcers were noted and scored on an arbitrary 0 – 3 point scale [9]. The scores were as below;
0 = Normal colored stomach.
0.5 = Red coloration.
1 = Spot ulcers.
1.5 = Hemorrhagic streak.
2 = Ulcers.
3 = Perforation.
Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows;

$$\% \text{ Inhibition of ulcer index} = \frac{\text{Mean Ulcer Index (Control Group)}}{\text{Mean Ulcer Index (Test Group)}} \times \frac{}{\text{Mean Ulcer Index (Control Group)}} \times 100$$

Statistical analysis
All the results will be expresses as the mean ± S.E.M. The data were analyzed for statistical significance by one-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered statistically significant.

Results

Extraction and phytochemistry
Methanolic CLS extract percent yielded was 6.3%. Upon drying, the extract was very powdery in texture and brownish in colour. Preliminary qualitative phytochemical analysis of CLS extract revealed the presence of some secondary metabolites (Table 1).

Table 1: Phytochemicals constituents of *Citrullus lanatus* seed

Phytochemicals	Results
Phenol	ND
Flavonoids	+
Tannins	+
Alkaloids	+
Terpenoids	+
Steroids	+
Saponins	+
Antraquinones	ND
Cardiac glycosides	ND
Phlobatannis	ND

+ = Present ND = Not present

Anti-ulcer activity
The animals pre-treated with CLS extract groups showed significant ($p<0.01$) as well as dose dependent inhibition of ulcer index as compared to control group (Table 2). CLS extract showed 30.95 and 73.81 % ulceration inhibition at 200 and 400 mg/kg respectively whereas ranitidine (100mg/kg) showed 53.52% ulceration inhibition.

Table 2: Antiulcer effect of *Citrullus lanatus*methalonic seed extract on ethanol induced Albino rats.

Group	Dose	Ulcer Index (Mean±Sem)	% Ulcer Protection
Normal control normal saline	(5ml/kg)	0.0 0±0.00	-
Untreated control Ethanol	1.5ml/kg	10.50±0.50	-
CLS extract	200mg/kg	7.25±0.14*	30.95%
CLS extract	400mg/kg	2.75±0.32*	73.81%
Ranitidine	100mg/kg	4.88±0.72*	53.52%

Values are expressed as mean ± SEM * = significantly different from control ($p<0.05$) by using analysis of variance (n=4), using SPSS Values was considered significantly different.

Discussion

Bioactive compounds from plants belong to diverse chemical groups such as tannins, alkaloid, glycosides, terpinoids etc. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [10]. In the present study, the choice of solvent was methanol a polar solvent capable of dissolving polar and non-polar compounds from plant material. Phytochemicals detected in methanolic CLS extract includes; flavonoids, terpernoids, tannins, saponins, alkaloids, steroids and saponnins.
Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance [11, 12]. Different experimental models such as cold restraint stress-induced ulcer model, Diclofenac-induced ulcer model, ethanol-induced ulcer model and water immersion stress-induced ulcer model have been used by researcher to study antiulcer activity of medicinal plants in albino rats. In this study ethanol-induced model was adopted and the result showed that ethanol was capable of inducing ulcer in rats of this study. Ethanol induced gastric ulceration may be due to stasis in gastric blood flow which

contributes to the development of the haemorrhage and necrotic tissue injuries [13]. Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting [14].

The pathogenesis of ulcer remains controversial but it causes is known to be aggregated by an imbalance between the aggressive factors (i.e acids, pepsin, and *H.pylori*) and factors that maintain the mucosal integrity (i.e mucus, bicarbonate and prostaglandins) [15]. CLS extract exhibited a dose-dependent protection against ethanol-induced gastric mucosa damage in rats. The antiulcer activity of CLS extract may be attributed to free-radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosal barrier [11]. It is possible that the extracts contain bioactive compounds that could enhance protective factors (against aggressive factors) thereby protecting the gastric mocusa from injury as well as maintaining its integrity. Active principles such as flavonoids, alkaloids, tannins, soponnins and terpenoids have been reported in previous studies to possess antiulcer property [16, 11, 17, 18]. Flavonoids are polyphenolic compounds with known antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion [19]. Tannins are known to 'tar' the outer most layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant [20].

Ranitidine, the standard drug produced 53.52% inhibition of ulcer in this study. It belongs to the class of H2-receptors antagonists commonly used in the treatment of peptic ulcer and gastro-oesophageal reflux disease. Serious side effects attributed to the use of H_2 antagonists is the precipitating cause of impotence, headache, skin rash and arrhythmias [21]. The current shift from conventional treatment to the use of herbal therapy is justified as it is believed that herbal formulations enjoy patronage because they are more compatible with the body, less toxic with little or no adverse effect, easily available and affordable.

Conclusion

The data obtained in this study indicates that the methanolic extract of *Citrullus lanatus* seeds exhibited antiulcer properties. These findings justify the ethno medicinal use of this seed in the treatment of ulcer and thus validate its traditional claim.

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