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Phytochemical Screening, Proximate Analysis and Anti-Ulcer Activity of Methanolic Seed Extract of *Citrullus lanatus*

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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. In this study, phytochemical screening, proximate analysis and anti-ulcer activity of the methanolic extracts of *Citrullus lanatus (*Lam) seeds was evaluated using crude extraction. The results of proximate analysis in methanolic *Citrullus lanatus* seeds extract shows relatively low moisture content (7.36%) and low protein content (6.99%), however, it shows high carbohydrate content (45.60%) and high fibre content (21.28%). The preliminary phytochemical screening revealed the presence of flavonoids, Saponin, Alkaloids, Steroids and terpenoids. The anti-ulcer activity of the methanolic extract of *Citrullus lanatus* seeds was evaluated in swiss albino mice against aspirin induced peptic ulcer. After the administration of the methanolic extract of *Citrullus lanatus,* the acute toxicity was determined by oral administering a single dose of 2,000 mg/kg orally to swiss albino mice which was observed within an hour post-dosing and once daily for a period of 2 weeks. *Citrullus lanatus* seeds shows a significant ($p<0.01$) anti-ulcer activity in a dose dependent manner as well as significant ($p<0.01$) reduction in the ulcer index when compared to control group, the anti-ulcer activity results was relatively comparable to the positive control. This dose did not produce mortality or acute signs of toxicity throughout the observation period. Our study shows that *Citrullus lanatus* seeds has considerable anti-ulcer activity**.** These findings suggest that *Citrullus lanatus* seeds possess anti-ulcer potential which may contribute to its ethno-medicinal uses.

Keywords: Citrullus Lanatus Seed, Anti-Ulcer, Ulcer Index, Methanol-Induced Model.

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 (Deshmukh *et al.*, 2015). 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 (Olamide *et al.*, 2011). Most people spit out or throw away watermelon seeds while eating this fruit (Tabiri *et al.*, 2016). 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 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. The seeds are also vermifuge and have hypotensive action. Oil from the seeds is used in cooking and incorporated into the production of cosmetics (Tabiri *et al.,* 2016). Fatty oils in the seeds as well as in aqueous and alcoholic extracts paralyze tapeworms and roundworms. The seeds are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are for example used to prepare snacks, milled into flour and used for sauces. *Citrullus lanatus* seed has been reported to have medicinal uses such as in the treatment of erectile dysfunction, hypertension, ulcer, diabetes and headache in Jigawa state, north-west Nigeria. The present study reports on phytochemical constituents and antiulcer activity of *Citrullus lanatus* seeds. Peptic ulcers are sores that develop on the inside mucosal lining of the digestive tract, specifically the initial portion of the small intestine (duodenum), stomach and less commonly the oesophagus. It is defined as mucosal erosion equal or greater than 5 mm. An acid peptic disorder occurs when the injurious effects of acid and pepsin overwhelm the mucosal barrier. Peptic ulcers occur worldwide with a lifetime prevalence of 5 to 10% and equal prevalence among men and women. The incidence of ulcer increases with age because of excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs) and the reduction in tissue prostaglandins. Other risk factors for the development of peptic ulcer disease (PUD) include *Helicobacter pylori (H. pylori)*, genetics, smoking, emotional stress and excessive alcohol consumption. In Nigeria, the true prevalence rate of peptic ulcer among the populace is not certain, although Nigeria had been listed as an area of high incidence. Also, the prevalence of duodenal ulcers has declined while that of gastric ulcer has increased. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, promote gastro-protection, block apoptosis and stimulate epithelial cell proliferation for effective healing. This is achieved using antacids, proton pump inhibitors, H2-receptor antagonists and mucosal protective agents. The high cost of treatment and limitations of conventional anti-ulcer drugs present a clear need for newer agents. The plant kingdom may provide a useful source of new anti-ulcer compounds for development as pharmaceutical entities or simple dietary adjuncts to existing therapies. The leaves and seeds of *Citrullus lanatus* have been extensively studied for their anti-ulcer activities. However, despite the medicinal usefulness of the seeds, they fall among the lesser known and highly under-utilized part of the plant. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric micro circulation, prostagland in E2 (PGE2) content.

Statement of the Problem

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. 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. The seeds are also vermifuge and have hypotensive action. Oil from the seeds is used in cooking and incorporated into the production of cosmetics. Fatty oils in the seeds as well as in aqueous and alcoholic extracts paralyze tapeworms and roundworms. 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. 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 headache. The vast medicinal history of watermelon aroused the interest in this study.

Objective of the Study

The objectives of the present study are to evaluate the phytochemical screening, proximate analysis and anti-ulcer activity of the methanolic seed extract of watermelon.

Materials and Methods

Sample Collection

Seeds from an uninfected and healthy *Citrullus lanatus* tree was collected from Ringim Local Government Area, Jigawa State. The seeds were dried for one week at room temperature after which the dried leaves will be blended into powdery form and stored in a sealed container prior to use. Swiss albino mice of both sexes weighing 120 - 150 g were housed in cages and they were maintained at a temperature of approximately 25°C. They were fed with standard dry pellets and tap water. The mice were allowed to acclimatize to the environmental conditions for 14 days before the experiments commenced.

Methanolic Extraction of *Citrullus lanatus* Seeds

The method of Debela 2002 wass employed. The aqueous and ethanol extract of *Citrullus lanatus seeds* was prepared by soaking 50g of the powdered sample in a conical flask containing 100 ml of methanol respectively placed on a shaker for 24 h. The extract was then be filtered using sterile Whattman filter paper. The extract was be concentrated using rotavapor and stored in an airtight container.

Phytochemical Analysis of *Citrullus lanatus* Seeds

Test for tannins: The method as described by Debela 2002 was employed. About 0.5 g of the sample was mixed with 10 ml of distilled water and filtered. Few drops of 1% ferric

chloride solution were then added to 2 ml of the filtrate. The occurrence of blue-black, green or blue green precipitate indicates the presence of tannins.

Test for steroids: Salkowski test as described by Debela 2002 was employed. The crude extract was mixed with chloroform and a few drops of concentrated H_2SO_4 was added. The mixture was agitated vigorously and allowed to stand for 5 mins. A red coloration at the lower layer indicates the presence of steroid.

Test for cardiac glycosides: The method as described by Debela 2002 was employed. 0.5% (w/v) extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride was then mixed followed by the addition of 1 ml of concentrated sulphuric acid. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for saponins: The method as described by Debela 2002 was employed. 1 g of each sample extract was boiled with 5 ml of distilled water and filtered. About 3ml of distilled water was added to the filtrate and shaken vigorously for about 5 mins. Persistent frothing indicates the presence of saponins.

Test for phenol: The method as described by Debela 2002 was employed. 1% (w/ v) of the extract was mixed with 2 ml of distilled water followed by the addition of few drops of 10% ferric chloride. The formation of a blue or green color indicates the presence of phenols.

Test for alkaloids: The method as described by Debela 2002 was employed. 0.5% (w/v) of the extract was mixed with 5 ml of 1% aqueous HCl on water bath with continuous stirring for few minutes and then filtered. 1 ml of the filtrate was pipetted individually into 3 test tubes. To each 1 ml in each test tube; Mayer, Wenger and Dragendroffs reagents was then added respectively. The formation of precipitate indicated the presence of alkaloids. Mayer's gives a white precipitate; Wenger's gives a reddish-brown precipitate while Dragendroff's gives orange, brown precipitate the three reagents can be used to ascertain the presence of alkaloids.

Test for terpenoides: The method as described by Debela 2002 was employed. 5% (w/v) of each sample extract was mixed with 2 ml of chloroform ($CHCl_3$) in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with reddish brown coloration indicates a positive result.

Test for flavonoids: The method as described by Debela 2002 was employed. A small quantity of each test extract was dissolved separately in dilute NaOH. A yellow solution that turns colorless on addition of concentrated HCl indicates the presence of flavonoids.

Test for quinones: The method as described by Debela 2002 will be employed. 1% (w/v) of extract will be mixed with 1 ml of concentrated H_2SO_4. The formation of a red color indicates the presence of quinones.

Test for antraquinones: Borntrager's test was used as described by Debela 2002 was be employed. About 0.2% (w/v) of the sample extract was shaken with 10 ml of benzene and then filtered. 0.5 ml of 1% ammonia solution was added to the filtrate and thereafter shaken. Appearance of pink, red or violet color indicates the presence of free anthraquinones.

Proximate Analysis of *Citrullus lanatus* Seeds

Proximate analysis of the powdered *Moringa oleifera* was carried out using standard procedure (Parmar *et al.*, 1993). The parameters to be determined were ash content, moisture content, protein content, lipid content, fibre content and carbohydrate.

Mean Ulcer Index

Treatment with MO methanolic seed extract for consecutive fourteen days (14) reduced the severity of ulcer intensity. In this study significant increase in the extent of ulceration mainly in glandular part of gastric mucosa was observed after aspirin treatment, as evidenced by increased mean ulcer index ($p < 0.01$).

Mucosal Thickness

Methanolic seed extract treatment for 14 days increased mucosal thickness in group 4 while indomethacin treated group was not found to have increased the thickness as evidenced by the results ($P < 0.01$).

Measurement of Ulcer Index (UI)

The dissected rats were examined for ulceration under a 3-fold magnifier. The number of ulcers was recorded, and the severity scored as follows:

0 = no ulcer; 1 = superficial ulcers; 2 = deep ulcers; and 3 = perforated or penetrated ulcer. Ulcer index (UI) was calculated using the formula:

UI = UN + US + UP x 10-1

Where UN = average number of ulcers per animal, US = average of severity score, and UP = percentage of animals with ulcer (Vogel, 2002). The percentage protection was calculated using the following formula:

$$\% \text{ Protection} = \frac{\text{Mean ulcer index of control} - \text{Mean ulcer index of test}}{\text{Mean ulcer index of control}} \times 100$$

Determination of Total Acidity

Gastric juice was collected from the ligated rats, the volume and pH were measured. For the determination of total acidity, 0.5 mL of the supernatant fluid was pipetted into a 100 mL beaker. Two drops of phenolphthalein solution were added, and the solution titrated with 0.1N NaOH until a pink colour appears (Vogel, 2002). The titration was repeated where the volume of gastric juice was adequate. The total volume of alkali added was noted for each titration. Total/titratable acidity was calculated and expressed as micro Eq/L per 100 g of body weight. Acidity was calculated using the formula:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality of NaOH} \times 100 \text{ mEq}}{0.1 \text{ L}}$$

Indomethacin Induced Ulcers in Swiss Albino Mice

Swiss albino mice of either sex were fasted overnight. The animals were divided into 5 groups of 6 animals each. Group I was administered distilled water in 0.1% Tween 80 orally. The animals in Groups II, III and IV were pre-treated orally with 200-800 mg/kg of MO extract in 0.1% Tween 80 respectively while Group V was pre-treated with the standard drug cimetidine also dissolved in 0.1% Tween 80. After 30 min, 20 mg/kg indomethacin dissolved in 0.1% Tween 80 solution was administered orally to the animals. Six hours later, the mice were sacrificed in chloroform chamber and their stomachs removed. Formol-saline (2% v/v) was injected into the totally ligated stomachs and stored in plain tubes filled with formol-saline overnight. The next day, the stomachs were opened along the greater curvature, washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal. The mean count for each group was then calculated. Inhibition (protection) of the lesion production is expressed as percentage value (Vogel, 2002).

Results and Discussion

The results of the phytochemical screening were presented in Table 1. This study has confirmed the presence of secondary metabolites such as flavonoids, phenols, tannins, saponins, alkaloid and glycosides which are claimed to be found in *Citrullus lanatus.* The proximate analysis of *Citrullus lanatus* seeds provides an information that its consumption is safe and beneficial to human health. The results of the nutrional content in *Citrullus lanatus* seeds (Table 2) shows relatively low moisture content (5.60%). ash content (4.99%), low protein content (16.21%), high carbohydrate content (58.33%), low fibre content (1.66%) and lipid (0.30%) respectively. The low moisture content indicates that the powered sample in less liable spoilage by microbial contamination of properly stored. The ash value indicate that the powdered sample contain more of organic components. It is a good source of protein, carbohydrate and fat as these are present in large amount and within the dietary recommended allowance. According to the work conducted by Mansur *et al*., 2018), it was revealed that *Citrullus lanatus* seeds contains carbohydrate (54.4%), protein (14.6%), lipid (0.26%), crude fibre (1.52%), moisture (4.3%) and ash (3.6%), and nitrogen (2.3%), which was in good agreement with our findings and similar work was conducted by (Gladwin *et al.*, 2020; Irabor *et al*., 2020; Cletus *et al*., 2022; Athanasiadis *et al*., 2023).

Table 1: Results of phytochemical of *Citrullus lanatus* seeds

PHYTOCHEMICALS	INFERENCE
Flavonoids	Positive
Alkaloids	Positive
Phenols	Positive
Tannins	Positive
Saponins	Positive
Glycosides	Positive

Table 2: Results of phytochemical of *Citrullus lanatus* seeds

COMPOUNDS	% COMPOSITION
Fibre	1/66
Moisture	5.60
Ash content	4.99
Carbohydrate	58.33
Protein	16.21
Lipids	0.30

Table 3: Effect of methanolic seeds extract of *Citrullus lanatus* on indomethacin-induced ulcers in rats

Experiment	Ulcer Index	PH gastric Juice	Acidity (Eq/L)	Mucosal thickness (µm)	Protection (%)
Distilled water 10 mL/kg	8.22±2.11	4.88	6.04	22.88	.....
MCLS extract 200mg/kg	3.44±2.21	9.66	6.10	44.90	46.44
MCLS extract 400mg/kg	8.66±1.54	4.44	3.33	49.95	69.44
MCLS extract 600mg/kg	7..99±0.33	4.77	8.09	76.73	58.22
MCLS extract 800mg/kg	8.44±3.44	2.99	7.99	83.22	97.99

Ulcer index and gastric juice volume are expressed as mean ± SEM; (n = 6); ns (not statistically significant) when compared with distilled water (control) alone using one-way ANOVA, MCLS = Methanolic *Citrullus lanatus* seeds

Discussion

Researchers have revealed that 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 (Pandey *et al.*, 2014). In this study, the choice of solvent was methanol a polar solvent capable of dissolving polar and non-polar compounds from plant material this make it to high extraction yield. The phytochemicals detected in MCLS 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 (Vinela *et al.*, 2014). Active principles such as flavonoids, alkaloids, tannins, soponnins and terpenoids have been reported in previous studies to possess anti-ulcer property (Nethaji *et al.*, 2013). The flavonoids are polyphenolic compounds with known antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion (Salmah *et al.*, 2012). 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 (Martin *et al.*, 1994; Azuzu *et al.*, 1990). 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 present study of proximate and phytochemical screening revealed that *Citrullus lanatus* seeds are safer to be used. The study showed that administration of the aqueous seed extract of *Citrullus lanatus* at 200-800 mg/kg decreases gastric volume, total acidity while gastric pH was increased. In indomethacin-induced ulcer model, the extract also produced a significant ($p < 0.01$) and dose-dependent reduction in ulcer index. This dose did not produce mortality or acute signs of toxicity throughout the observation period. Our study shows that *Citrullus lanatus* seeds has considerable anti-ulcer activity. These findings suggest that *Citrullus lanatus* seeds possess anti-ulcer potential which may contribute to its ethno-medicinal uses.

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