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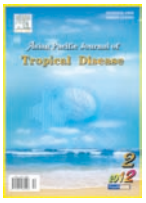

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# Quantitative determination, Metal analysis and Antiulcer evaluation of Methanol seeds extract of *Citrullus lanatus* Thunb (Cucurbitaceae) in Rats

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ABSTRACT

**Objective:** The use of herbs in treatment of diseases is gradually becoming universally accepted especially in non industrialized societies. *Citrullus lanatus* Thunb (Cucurbitaceae) commonly called water melon is widely consumed in this part of the world as food and medicine. This work was conducted to investigate the phytochemical composition, proximate and metal content analysis of the seed of *Citrullus lanatus* and to determine the antiulcer action of the methanol seed extract. **Methods:** Phytochemical screening, proximate and metal content analysis was done using the standard procedures and the antiulcer activity was evaluated against acetylsalicylic acid-induced ulcers. **Results:** The results revealed the presence of the following phytochemicals; flavonoids, saponins, tannins, alkaloids, glycosides. Proximate analysis indicated high concentration of carbohydrate, protein and fat while metal analysis showed the presence of sodium, calcium, zinc, magnesium at levels within the recommended dietary intake. Antiulcer potential of the extract against acetylsalicylic acid induced ulceration of gastric mucosa of Wister rats was evaluated at three doses (200mg/kg, 400mg/kg, and 800mg/kg). The ulcer parameters investigated included ulcer number, ulcer severity, ulcer index and percentage ulcer protection. The antiulcer activity was compared against ranitidine at 20mg/kg. The extract exhibited a dose related antiulcer activity with maximum activity at 800mg/kg ($P<0.001$). **Conclusions:** Proximate and metal content analysis of the seeds provides information that the consumption of the seeds of *Citrullus lanatus* is safe. This present study also provides preliminary data for the first time that the seeds of *Citrullus lanatus* possesses antiulcer activity in animal model.

## 1. Introduction

*Citrullus lanatus* Thunb. (family Cucurbitaceae) commonly called water melon is widely distributed in Africa and Asia, but originates from southern Africa occurring naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi. It is cultivated and thrives in warmer parts of the world. The growth is favoured in a dry climate and is mainly a dry season crop in monsoon areas, requiring only limited rainfall. From there followed the spread to the Mediterranean areas and in an eastern direction to India [1,2,3].

Synonyms include *Colocynthus citrullus* (Thunb.),*Cucurbita citrullus* Thunb.There are three species in the genus Citrullus, occurring naturally in Africa and Asia. *Citrullus colocynthis* ,*Citrullus ecirrhosus*, *Citrullus lanatus* [4].

*C. lanatus* is an annual herb with long (up to 10 m) stems lying or creeping on the ground, with curly tendrils. Leaves are 5-20 by 3-19 cm, and hairy, usually deeply palmate with 3-5 lobes, on 2-19 cm long petioles. Fruits vary considerably in morphology, size range from about 7cm in diameter to over 20cm. In addition, they vary in colour from pale yellow or light green (wild form) to dark green (cultivars), and with or without stripes; the pulp varies from yellow or green (wild forms) to dark red (cultivars). The flesh amounts to about 65% of the whole fruit, and of this 95% is water. The plant has become naturalized in many drier parts of West Africa [3,4].

Water melon fruit is a good source of vitamin A; vitamin C, the antioxidant lycopene and potassium. Cucurbitacin the bitter principle in some species has diuretic and purgative

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properties. The seeds have low fat and cholesterol [5]. The fruit has but few medicinal uses in West Africa; Bitter forms are used in Senegal as a drastic purge and are considered poisonous [6]. Some other ethno-medicinal uses of the fruit include diuretic, purgative, remedy for urinary conditions suggestive of gravel and stone in the bladder, gonorrhoea and leucorrhoea in women [7,8]. The seeds are chiefly used as a masticatory, and for medicine, food and oil and can be roasted and used as a coffee-substitute 8,9. The seeds are used as a vermifuge in Senegal, and juice squeezed from pulp roasted in fire-ash is drunk in Southern Nigeria as an anthelmintic [8,10]. They are used as a diuretic and for their strengthening properties in India and their beneficial use in acute cystitis and capacity to lower the blood pressure is recorded [9,11].

Previous studies have shown that *Citrullus lanatus* seeds possesess antimicrobial activities [12], it has also been shown to be useful in the treatment of prostatic hyperplasia [13] and have antioxidant, anti-inflammatory and analgesic properties [14]. This forms the basis of this present study which is to explore the antioxidant property of the seeds for antiulcerative actions. It was also important to estimate the proximate and metal content analysis to know the safety profile of consumed seeds.

## 2. Materials and method

### 2.1 Plant collection and preparation

Ripe watermelon pods were obtained from the local market in Benin City, Edo State, Nigeria. (September, 2011). The seeds extracted from the pods after allowed rotting manually by washing, only healthy looking seeds (brown in colour, not floating on water, without mechanical damage or sign of infection) were collected. The seeds were identified and authenticated by Dr Bamidele A, of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria.

The collected seeds were oven-dried at 35 degrees Celsius, until a constant weight was obtained. The dried seeds were reduced into fine powder using a Laboratory mill. The powder was weighed and kept away from light before extraction.

### 2.2 Extraction and Concentration

Extraction was by maceration over a 72 hour period. 500g of the powdered seeds material was extracted with 1.5 litres of methanol in three successive extractions (500ml every 24 hours). The jar was tightly closed and thoroughly shaken intermittently.

After 72 hours, the different portions were combined and filtered; the filtrate was collected in a glass jar as a brown coloured liquid. The extract was concentrated using a Rotary Evaporator. The concentrated Methanolic Extract of *Citrullus lanatus* seeds, (MECLS) was then quantitatively transferred into amber coloured bottles covered with aluminium foil and kept in a refrigerator before use.

### 2.3 Phytochemical screening

Phytochemical screening was carried out on the powdered sample to detect the presence secondary metabolites using standard procedures [15,16].

### 2.4 Proximate and metal content analysis

Proximate analysis of the powdered crude drug was carried out using standard procedure [17,18]. The parameters determined were its ash content, moisture content, protein content, lipid content, fibre content, carbohydrate content and gross energy value. Each parameter was determined for three replicates. Analysis of the sample for copper, iron, manganese, nickel, lead, and zinc content was carried out in triplicate on the Atomic absorption spectrum (Buck Scientific VGP 210) [19].

### 2.5 Antiulcer screening

Wister rats of either sex, weighing 195-220g were obtained and kept in the animal house of the Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin city, Nigeria. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Ethical approval was obtained from the Animals Use and Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The animals were divided into five groups of five animals each. The first three groups received 200 mg/kg, 400 mg/kg and 800 mg/kg of methanolic extract of *Citrullus lanatus* seeds (MECLS), suspended in normal saline, respectively. The forth group served as the positive control and received 20 mg/kg of ranitidine while the fifth group served as negative control and received 2 ml/kg normal saline [20]. The extract and controls were administered orally for 7 days by gastric gavage. After 7 days of treatment, the animals were fasted for 24 hours prior to induction. Ulcer was induced by oral administration of Acetylsalicylic acid, ASA (200 mg/kg) suspended in 10% aqueous acacia on the day of sacrifice [21]. The animals were sacrificed 4 hours later, dissected, the stomach removed, cut open along the greater curvature and examined. By means of a magnifying lens, the ulcer wounds were observed, the number of ulcer spots counted, the ulcer index was determined and the percentage inhibition calculated [21] using the following equation.

$$\text{Percentage inhibition} = \frac{\text{UIC} - \text{UIT}}{\text{UIC}} \times 100$$

Where:

UIC = ulcer index of control group, UIT = ulcer index of treatment group.

The ulcer index (UI) was calculated using the following equation:UI = UN + US + UP × 10-9 1 12

Where, UN = mean number of ulcers per animal, US = mean ulcer severity (obtained as the cumulative diameter of all ulcers in millimetres) 22, UP = percentage of animals with ulcers in each group.

### 2.6 Statistical analysis

All results are expressed as Mean±S.E.M. The result was analyzed by one way ANOVA followed by t-test.

## 3. Results

The result of phytochemical screening is as presented in the table below.

**Table 1**
Result of phytochemical screening

| PHYTOCHEMICALS | INFERENCE |
|---|---|
| Carbohydrate | Present |
| Reducing sugar | Absent |
| Tannins | Present |
| Saponins | Present |
| Alkaloids | Present |
| Terpenoids | Present |
| Glycosides | Present |
| Flavonoids | Present |
| Volatile oil | Absent |
| Phenolics | Present |

The result of phytochemical analysis (Table 1) showed that *Citrullus lanatus* seeds contain the following secondary metabolites: tannins, saponins, flavoniods, terpenes, alkaloid, glycosides and phenolics.
The result of proximate analysis are summarised on table 2:

**Table 2**
Result of proximate analysis

| ANALYSIS | RESULT (%) |
|---|---|
| Moisture | 4.80±0.05 |
| Ash | 3.4±0.80 |
| Crude fat | 35.40±1.00 |
| Crude protein | 21.50±0.03 |
| Crude fibre | 2.50±0.10 |
| Carbohydrate | 32.40±0.70 |

All values were measured in triplicate. Result is presented as Mean±S.E.M

The proximate analysis of powdered sample of *Citrullus lanatus* seeds showed relatively low moisture content (4.8%) and a low ash value (3.4%) but high crude fat (35.50%) and carbohydrate (32.40%).

**Table 3**
The results of metal analysis are presented below:

| METALS | RESULT (mg/kg) |
|---|---|
| Zinc (Zn) | 1.20±0.10 |
| Lead (Pb) | 0.08±0.00 |
| Calcium (Ca) | 16.80±1.20 |
| Magnesium (Mg) | 11.40±1.00 |
| Potassium (K) | 7.80±0.00 |
| Sodium (Na) | 5.70±0.10 |

Note: all values were measured in triplicate. Result is presented as Mean±S.E.M

The metal analysis of powdered sample of *Citrullus lanatus* seeds showed the presence of calcium, magnesium, zinc, lead, potassium and sodium, calcium and magnesium shows the highest quantity while zinc and lead showed the least quantity

**Table 4**
The result of antiulcer screening showed the following percentage ulcer inhibition

| Treatment | No. of ulcer spots(UN) | Ulcer severity in mm (US) | %Ulceration/group | Ulcer index (UI) | % Ulcer inhibition |
|---|---|---|---|---|---|
| Control | 13.0±2.86 | 224 | 100 | 33.60 | – |
| Ranitidine(20mg/kg) | 0.6±0.60 | 3 | 20 | 2.36 | 92.97** |
| 200mg/kgMECLS | 6.2±0.97 | 102.5 | 100 | 21.17 | 36.99 |
| 400mg/kgMECLS | 3.2±0.86 | 37 | 100 | 14.02 | 58.27* |
| 800mg/kgMECLS | 1.0±0.32 | 12 | 100 | 9.30 | 72.32** |

Values are Mean±S.E.M, * indicates $P<0.05$, ** indicates $P<0.01$ when compared to control

**Table 5**
Comparison between percentage content of watermelon seed and the recommended daily allowance.

| Content | Result (%) | Dietary Recommended Allowances in a male aged 40-50 years old [24] | Dietary Recommended Allowances in a female aged 40-50 years old [24] |
|---|---|---|---|
| Moisture content | 4.80±0.05 | | |
| Ash | 3.40±0.80 | | |
| Crude Protein | 21.50±0.03 | 56g/day | 46g/day |
| Crude Fibre | 2.50±0.10 | 38g/day | 25g/day |
| Crude Fat | 35.40±1.00 | 20-35% of calories | 20-35% of calories |
| Carbohydrate | 32.40±0.70 | 130g/day | 130g/day |

The percentage content of the seeds of *Citrullus lanatus* is within the recommended levels.

**Table 6**

Comparison between metal content of *Citrullus lanatus* seeds and the recommended daily allowance

| Metal | Result (mg/kg) | Dietary Recommended Allowances in a healthy 25-year old male[24] | Tolerable Upper Intake level [24] |
|---|---|---|---|
| Calcium | 16.80±1.20 | 1000mg | 2500mg |
| Magnesium | 11.40±1.00 | 400mg | 350mg |
| Zinc | 1.20±0.10 | 11mg | 40mg |
| Lead | 0.08±0.00 | 0.10mg | – |
| Potassium | 7.80±0.00 | 4700mg | – |
| Sodium | 5.70±0.00 | 1500mg | 2300mg |

The metal content of *Citrullus lanatus* seeds when compared with the recommended daily allowance falls below the tolerable upper intake level

The result of the antiulcer activity of the methanol extract of *Citrullus lanatus* seeds shows that the highest activity was at 800mg/kg (72%) followed by 400mg/kg (58%) which were significant $P<0.01$ and $P<0.05$ respectively

## 4. Discussion

Phytochemical screening of chemical constituents of different parts of *Citrullus lanatus* showed that the leaves contained mainly flavonoids, tannins and simple phenols. Stem revealed the highest content in saponins. Fruits exerted the highest contents of alkaloids and simple phenols whereas seeds were rich in terpenes and steroids as well as flavonoids. All organs were devoid of anthraquinones[12]. Several studies show that alkaloids and terpenes are widely spread in the genus Citrullus [22].These secondary metabolites are responsible for the pharmacological activities such as antiulcer, antimicrobial, antioxidant, analgesic, aphrodisiac and many other ethno-medicinal uses [23].

The low moisture content indicates that the powdered sample is less liable to spoilage by microbial contamination if properly stored. The ash value indicates the quantity of inorganic components, hence a low value indicates that the powdered sample contain more of organic components [18]. It is a good source of protein, carbohydrate and fat as these are present in large amounts and within the dietary recommended allowance. The seeds are therefore highly consumable and should not be discarded as some people do when they eat watermelon.

The presence in food and drugs, of metals such as cadmium, mercury, zinc and lead, which are injurious to health, is regulated by law. These metals are known as heavy metals and are only allowed in trace amounts in foods and drugs. Heavy metals can increase acidity of the blood25. Long term use of excess level of zinc, above 40mg/kg, can cause abdominal pain, dyspepsia, nausea, vomiting, diarrhoea, gastric irritation, and gastritis and much more complications on prolonged use [25]. Cholestatic liver disease and possible changes in the basal ganglia have been reported to be associated with hypermanganesaemia (long term intake of manganese above 11mg) in children [25]. The United State of America's Department of Health and Human Services (DHHS) has determined that nickel and certain nickel compounds may reasonably be anticipated to be carcinogens [26]. Long term exposure to lower levels of cadmium in air, food, or water leads to a build up of cadmium in the kidneys and possible kidney disease. Other potential long term effects are lung damage and fragile bones, abdominal pain, choking and tenesmus [27].

Permitted daily exposure has been employed as a key indicator for maximum safe intake limit of individual elements. Permitted daily exposure (PDE) is defined as the maximum patient exposure (expressed in μg/Kg/day) to an element, possibly on a chronic basis, that is unlikely to produce any adverse health effect [28]. This value represents the upper limit, for consumption quantities over prolonged periods, still to be regarded as tolerable. Short-term excess consumption is accepted if average value for consumption over a long period is not exceeded. The percentage content of a metal in a plant would partly depend on the industrial activity and metallic content of the soil in the area where the plant is grown.

The metal analysis of powdered sample of *Citrullus lanatus* seeds showed the presence of calcium, magnesium, zinc, lead, potassium and sodium. The quantity of these metals in the powdered sample revealed that they are well below tolerable upper intake level and within the recommended daily intake in healthy individuals established by the Dietary Reference Intakes (DRIs).

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms [29]. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [23].

The result of the anti-ulcer screening of methanolic extract of *Citrullus lanatus* seeds, as presented in table 4 showed a significant ($P<0.001$) dose dependent anti-ulcer effect at the various doses used, comparable to that of the standard drug (Ranitidine). Highest anti-ulcer activity is seen with 800mg/kg dose. The extract may not have reduced the incidence of ulceration in the test animals at the dose levels employed but it significantly ($P<0.001$) reduced the number of ulcer spots in a dose-dependent manner.

The gastroprotective effect exhibited by MECLS could be speculated to be attributed to its antioxidant property, which

in turn could be linked to the presence of flavonoids and polyphenolic compounds, saponins and tannins [23]. These compounds most likely inhibit gastric mucosal injury by preventing or inhibiting aspirin-induced oxidative damage of gastric mucosa [23].

## 5. Conclusion

This study has confirmed the presence of secondary metabolites such as flavonoids, tannins, saponins, terpenoids claimed to present in the most members of the Cucurbitaceae family. Proximate and metal content analysis of the seeds provides information that the consumption of the seeds of *Citrullus lanatus* is safe. This study also provides preliminary data for the first time that the seeds of *Citrullus lanatus* possesses significant anti-ulcer activity in animal model. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavanoids, saponin and tannins. Further studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of the extract and to identify the chemical constituents responsible for it.

## Conflict of interest statement

We declare that we have no conflict of interest.

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