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Research Article

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Proximate, Phytochemicals and Antidiarrhoea Properties of Water Melon Seeds (Citrullus Lanatus)

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Abstract

Background: *Citrullus lanatus* commonly known as watermelon is used in the traditional medicine for the treatment various ailments. It is a vine-like flowering plant with thick exocap and fleshly mesocap grown in West Africa. This study investigated the proximate, phytochemical and antidiarrhoea properties of C. lanatus seed against castor oil induced diarrhoea in albino rats.

Materials and Methods: 200 mg/kg and 400 mg/kg of aqueous extract C. lanatus seed were used to pretreat the rats against diarrhoea, standard methods were used for proximate and phytochemical analysis.

Results: The result of proximate composition of Citrullus lanatus seed revealed high fat content followed by protein content while crude fiber had the least composition. The quantitative phytochemical analysis revealed the presence of some bioactive components-Saponins, alkaloids, flavonoids, phytates, oxalates and phenols. The result of the antidiarrhoea property of C. lanatus seed extract in comparison with loperamide hydrochloride (conventional drug), decreased the production of diarrhoea stool, reduced the frequency of defecation and delayed the onset of diarrhoea in albino rats.

Conclusion: The decrease in both the production of diarrhoea stool and frequency of defecation in the groups pre-treated with C. lanatus seed extract suggest that the plant could posses antidiarrhoea properties. Also this could be attributed to the presence of these phytochemicals.

Keywords: *Citrullus lanatus; Proximate; Phytochemicals; Antidiarrhoea; Loperamide; Hydrochloride*

Introduction

Diarrhoea is described as a condition of having at least three loose or liquid stools each day (Navaneethan, and Giamella 2008). It often last for a few days and can result in dehydration due to fluid loss. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decrease in responsiveness as it becomes more severe. Loose but non-watery stools in babies who are breast feed however, may be normal (Navaneethan, and Giamella, 2008). The most common cause of diarrhoea is an infection of the intestines due to a virus, bacteria, or parasite; a condition known as gastroenteritis. These infections are often acquired from food or water that has been contaminated.

Water melon (Citrullus lanatus) is commonly grown in West Africa. It belongs to the family of cucurbitaceae. It is a good source of lycopene which has been found to be protective against a growing cancer (Mandel., *et al*. 2005).

Lycopene is a red-pigment and a powerful antioxidant found in plant. It is also an open-chain unsaturated carotenoid that is responsible for the red color of watermelon, tomatoes, guava, and grape fruits. Watermelon is an excellent source of vitamin A. which is a powerful natural antioxidant. Citrulus lanatus is a good source of potassium: potassium is an important component of cell and body fluids that help in controlling heart rate and blood pressure. It thus, offers protection against stroke and coronary heart diseases. It is an excellent source of carotenoid. It is also rich in electrolytes and water. Also, it contains a good amount of vitamin – B6 (pyridoxine), thiamin of (vitamin B-1), vitamin–C, and manganese. Citrullus lanatus is popular in the indigenous system of folk medicine, which contains bioactive compounds such as sterol, alkaloids, vitamins and minerals. The seed is used in the treatment of urinary track infection, bed wetting, hypertension, diarrhoea, and gonorrhoea. (Ekene, and Erhirhie, 2013).

Materials and Methodology

MATERIAL: Dry seed of Citrullus lanatus

Equipment: Water bath (HH-2/England), Weighing balance (M – 100/England), Manual blender (Corona 0413)

Extraction: Citrullus lanatus plant extract was obtained using filtration method, 400g of the powered sample were weighed and put into a 1000ml beaker and 1.7 litre of warm water was added and mixed vigorously. The solution was allowed for 30 minutes, then filtered using filter paper and a milkly filtrate was obtained which was concentrated in a water bath at 50°C to remove water from the extract for 7 days, an aqueous concentrate of Citrullus lanatus was obtained and used for the administration.

Animal Collection: A total number of 15 albino rats with an average weight of 100g – 150g were used in this study. The rats were allowed to acclimatize for a period of two weeks under laboratory condition and were given food and water and were grouped into 5 groups of three animals each except the control which had four animals.

Proximate Analysis

Moisture Content [AOAC, 1990]

Procedure: A clean flat dish was dried for 15mins at 105°C. It was allowed to cool in the desiccator for 15mins and weighed (W_1). 2g of well mixed sample was transferred to the dish (W_2). The dish was cleaned and placed in the oven at 105°C for 4hrs. Later, the dish was removed, covered and placed in the dessicator and allowed to cool for 15mins, weighed as quickly as possible (W_3).

$$\% \text{ Moisture} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Ash and mineral content [AOAC, 1990]

Procedure: Crucibles are placed in a muffle furnace for 15mins or more. Then, they are removed and placed in a dessicator for 30mins to cool off and weighed. 2g of each sample is weighed into different dishes (crucibles). The dishes are placed on a hot plate under a fume chamber and slowly the temperature is increased until smoking ceases and the sample becomes thoroughly charred. Then, the dishes are placed at the centre of the muffle furnace and ash until fully ashed (when there is notice of grey colour of ash). After which the dishes with the ash were placed in dessicators and allowed to cool and weighed.

Let weight of the sample = W_1

Let weight of the ask = W_2

$$\% \text{ Ash} = \frac{W_2}{W_1} = 100$$

Fat/Oil content [AOAC, 1990]

Method A: soxhlet extraction method (used for solid products)

Procedure: 5g of dry samples were weighed after moisture determination and transferred into the thimble. 250ml of a round bottom flask washed and dried at 105°C. For 30mins and cooled at room temperature in a dessicator and later weighed. Each thimble containing each sample was placed in an extractor, 150ml of ethyl ether was measured into the round bottom flask and the extractor was set up. The condenser was connected to a water tap and the extractor was set up. The condenser was connected to a water tap and the heating mantel was turned on and then fats were extracted for 6hrs, and the solvent (ethyl ether) recovered. The round bottom flask were placed in an oven at 105°C for 30mins and later placed in the dessicator to cool and the weight noted Fat:

$$(\%) \quad \frac{W_t \text{ of fat}}{W_t \text{ of fat sample}} \quad x\ 100$$

Crude protein/Nitrogen

Procedure

Digestion: 0.5g of the prepared samples were weighed onto a quarter size filter paper and transferred to a kjedahl digestion flask. 1% tablet of the catalyst was added and 15ml of concentrated sulhuric acid was also added into the flask. The flask was heated gently in an inclined position under a fume until frothing ceases, it is boiled briskly until the digest is clear under a fume cupboard, it is then allowed to cool and made up to 100ml volume using distilled water.

Distillation: 10ml of the digest is taken into the distillation flask, 10ml of 45% sodium hydroxide solution is added. The flask is then connected to a distillation apparatus before making alkaline. Ammonia was steam distilled into 5ml boric acid indicator in a 100ml conical flask, 50ml of the distillate is then collected.

Titration: Standard acid (0.05N) H_2SO_4 was titrated against the distillate. % crude protein = % N x protein factor.

Nitrogen factor (N) = 1.4, protein factor = 6.25

$$\% N = \frac{\text{Sample Titre} - \text{Blank Titre x N of acid x 1.4}}{\text{Weight of Sample (in 10ml)}}$$

Phytochemical Quantification: [Herbone, 1973; AOAC, 1990] Method was adopted for preparation/extraction of sample for GCms quantitative analysis.

Twenty grams (20g) of the homogenized sample was mix with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture, the homogenate was placed into a 500ml beaker and 300ml of n-hexane was added for extraction. Crude extract obtained was evaporated with a rotary vacuum evaporator at 40°C, residue was then transferred with n-hexane into a 5ml florisil column for clean up.

Florisil Clean Up

Transferred residue was heated in an oven at 130°C overnight (15h) and transferred to a 250ml size beaker and placed in a desiccator. 0.5g anhydrous $NaSO_4$ was added to 1.0g of activated florisil (magnesium silicate) (60–1000 mn mesh) on an 8ml column plugged with glass wool. The packed column was filled with 5ml n-hexane for conditioning, the stopcock was open to allow n-hexane run out until it just reaches top of sodium sulphate into a receiving vessel whilst tapping gently the top of the column till the florisil settled well in the column. The extract was transfered on to the column with a disposable Pasteur pipette from an evaporating flask. A 1ml portion of n-hexane was added to the column to elute. Dry eluate dissolved in 1ml n-hexane for gas chromatographic analysis.

Castor oil induced diarrhoea in rats and faecal count

Castor oil-induced diarrhoea was determined by the method of Awoutes., et al. (1978). Rats weighing between 100 – 150 g fasted for 18 hours were randomly distributed into five groups. Group 1 (untreated) received feed and water, group 2 (control) received 10ml/kg of distilled water, group 3 and 4 received 200 and 400 mg/kg of extract while group 5 received 5mg/kg loperamide. After 1 hour of treatment with extract, distilled water and standard drug, diarrhoea was induced by administration of 1ml of castor oil orally to each of the animals. The faecal droppings were recorded. Percentage inhibition was calculated (Izzo et al., 1992; Mukherjee et al., 1995; Karim et al., 2010). % inhibition: (control – test)/control x 100.

Results and Discussions

Nutrient Composition	Percentage (%)
Moisture content	4.46
Ash content	3.14
Fat content	41.82
Protein content	39.97
Fibre content	0.19
Carbohydrate content	10.42

Table 1: *Proximate analysis of (Citrullus lanatus)*

The above table showed the result of the Quantitative phytochemical screening carried out on Citrullus lanatus seed.

Values are mean ± standard deviations of n=3 determinations. Values in each column with different superscript letter (a,b) differ significantly when comparing Group 1 and other groups at 5% level $p<0.05$. Values with different superscript letter (c,d) differ significantly when comparing Group 2 with other groups at 5% level $p<0.05$.

Component	Sub-Class	Concentration µg/g
Phenol		2.18
Flavonoids	Rutin	5.85
	Anthocyanin	3.80
	Catechin	27.45
	Kaempferol	9.80
	Epicatechin	1.05
	Total	47.95
Alkaloids	Ribalindine	11.96
	Lunamarine	20.49
	Aspartein	0.0001
	Total	32.45
Saponin	Sapogenin	58.52
Oxalate		1.55
Phytate		0.25

Table 2: *Quantitative phytochemical analysis of Citrullus lanatus seed.*

Groups	Treatment	Mean Wet Faeces	Persentage Inhibition (% 1)	K^+ (mmol/L)	Na^+ (mmol/L)
1	Untreated	0.0 ± 0.0000	-	6.400 ± 3.316a	96.50 ± 5.75a
2	Control	0.8 ± 0.8367	-	7.133 ± 0.513ac	173.33 ± 23.86ac
3.	200 mg/kg extract	0.2 ± 0.4472	75%	8.433 ± 3.372ac	88.00 ± 21.17ad
4.	400 mg/kg extract	0.2 ± 0.4472	75%	6.500 ± 1.007ac	82.00 ± 29.86ad
5.	5 mg/kg Loperamide hydrochloride	0.4 ± 0.5477	50%	5.867 ± 0.577ac	84.67 ± 4.62ad

Table 3: *Effect of Citrullus lanatus seeds extract on castor oil induced diarrhoea and serum electrolytes.*

Discussion

Citrullus lanatus which is generally known as watermelon belong to the Cucurbitaceae family. The extract of the seed of watermelon is said to be medicinal because it can relieve inflammation/irritation, which leads to an increase in the passage of urine (Oknurobo., *et al*. 2012).

The result of the proximate analysis showed high percentage fat and protein, while crude fiber had the least value. Oyeleke., *et al*. 2012, reported that the seed of watermelon has high fat content, followed by protein, moisture and ash.

The phytochemical screening carried out on the dried watermelon seeds revealed the constituent presented in Table 2. The Table showed the concentration of the quantitative phytochemicals of Citrullus lanatus in this order saponin > flavonoids > alkaloid > phenol > oxalate > phytate. The result revealed epicatechin, rutin, kaempferol and anthrocyamin and catechin as subclass of flavonoid, Ribalinidine, lunamarine, aspartein as subclass of alkanoid and sapogenin as subclass of saponin. This result showed high concentration of saponins, flavonoids and alkaloids. These three components possessed antidiarrhoea activity (Phytochemical.info.com, 2012; Nwachoko and Jack, 2015; Nwachoko, 2016).

Table 3, showed the inhibitory effect of aqueous extract of Citrullus lanatus on the wet faecal count of castor oil induced diarrhoea in albino rats. The percentage inhibition of castor oil induced diarrhoea in groups treated with the extract of Citrullus lanatus was higher compare to that treated with standard drugs (group 5), the plant extract significantly inhibit diarrhoea. The high inhibition rate on the wet faecal count may be attributed to the chemicals present in the plant.

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