

In Vitro Antibacterial Effects of Citrullus Lanatus Fruit Extract On Staphylococcus Aureus and Escherichia Coli

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Abstract

Investigation was carried out on the effects of back and endocarp of *Citrullus lanatus* fruits on two bacteria, *Staphylococcus aureus* and *Escherichia coli*, after determining the phytochemical composition of the fruit. Aqueous and ethanolic extracts of the plant were tested using agar well diffusion method at Concentrations of 50 mg/ml, 100 mg/ml and 150 mg/ml. the fruit were found to contain phytochemicals like tannins, saponins, flavonoids and glycosides, some at high occurrences, while some occurring moderately. It was observed that the ethanolic extracts showed high activity on *E. coli* (8.15 mm), but moderate activity on *S. aureus* (2.35mm).aqueous extracts were however less inhibitory active at all the test concentrations. The study showed that the fruit is useful in combating the activities of the test bacteria, and as a possible source of alternative useful drugs.

Key Words: Extracts; *Citrullus lanatus* fruit; *E. coli*; *S. aureus*; Inhibition

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I. Introduction

It has been proved for a long time that efforts have been directed towards the provision of empirical proofs to back the use of plant species in trade and medicinal practices (1). However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated. In some advanced countries, about 70 – 80% of the population has used some forms of alternative or complimentary medicine such as acupuncture, while in most African countries 80% of the population depends traditional medicine for primary healthcare (2).

Citrullus lanatus (Thunb.) Matsum & Nakai is a slender, sprawling slightly hairy monoecious and annual plant. The stem runners may extend to 0.3 – 10m. in its natural environment, it grows in grassland or bushland, often along watercourses at altitudes of 50 – 1400m (3). It grows in well drained soils and can withstand drought better than other melons. The fruit is consumed in different parts of the world, as the juice quenches thirst of water. It contains powerful antioxidants such as lycopene and carotenoids which travel through the body, neutralizing free radicals that can lead to stroke or heart attack. (3). The rind can be used in jam, jelly, pickle and wine production while the seeds are used in oil production. *C. lanatus* also contains arginine which has been shown to improve insulin sensitivity in diabetic patients with insulin resistance (4). Lycopene has also been linked to anti high blood pressure and cancer control (5). The notable varieties of watermelon include; Carolina cross, Yellow crimson, Orangetlo, and Cream Saskatchewan. The fruit takes between 65 – 100 days from the date of planting to harvest (5; 6).

Staphylococcus aureus Staphylococci are Gram-positive, nonspore forming, facultatively anaerobic, nonmotile, catalase-positive or negative, small, spherical bacteria from pairs to, grape-like clusters, from where the name *Staphylococcus* comes from (staphyle, meaning a bunch of grapes, and kokkos, meaning berry). They are spherical cocci, about 0.8 – 1.0µm in diameter, characteristically in grape like clusters formed due to cell division occurring in more than one plane with daughter cells remaining close together (2). They are non motile, non sporing and with the exception of some rare strains, also non capsulated. They are aerobes and facultative anaerobes, with optimum growth temperature of 37°C and pH of 7.5. they grow on media such as nutrient, blood mac-conkey, milk, selective salt and enrichment salt medium agars (7).

Escherichia coli are gram negative, capsulated or non-capsulated, non acid fast, non sporing bacilli that are either motile by means of peritrichous flagella or no motile. They are catalase positive, and reduce nitrates to nitrites. They are aerobes and facultative anaerobes that grow rapidly on ordinary media including mac conkey agar. They break down glucose and other carbohydrates both fermentatively under anaerobic conditions and oxidatively under aerobic condition (8). *Escherichia coli* (*E. coli*) is a bacteria that is commonly found in the lower intestine of warm-blooded organisms. Most *E.coli* strains are harmless, but some can cause serious food

poisoning (2). *E. coli* can cause four main types of clinical syndromes, which include; Urinary tract infection; Diarrhoea and dysentery; pathogenic infection and septicemia (7).

II. Materials And Methods

The watermelon fruit endocarp and back were separated and air dried a room temperature for two weeks, then grounded into fine powder using laboratory mortar and pestle. 50g of the powder were used to create the different concentrations using water and ethanol, and stored below ambient temperature. Concentrations of 50 mg/ml, 100 mg/ml and 150 mg/ml were used for the antibacterial investigation.

Phytochemical analysis was according to Marvic, (2002) (9). The tests were conducted for alkaloid, tannins, flavonoids saponins and glycosides. The seed analysis of *Citrullus lanatus* using standard analytical procedures, by Aisha and Malami (2013) (10) was also adopted.

The test organisms were obtained from Usmanu Danfodiyo University city campus, faculty of veterinary medicine, Sokoto. The isolates were sub-cultured on slant bottles and stored at 4° C prior to being used. A pure culture of the test organism was inoculated into 10ml of sterile peptone water and incubated at 37° C for 24hrs. Sterile cork borer was used to make wells in the plates already ceded with the test organisms. Thereafter, sterile micropipette was used to fill the wells, taking care not to have the extract running over the plate. The plates were covered and incubated at 37° C for 24hrs before observing the inhibition. The measurement was carried out using a meter rule, according to Bauer, *et al.*, (2000) (11).

III. Results And Discussions

The results for phytochemical screening and antibacterial inhibition activity of the different extracts compared to the positive and negative controls are presented in Tables below. Phytochemical analysis (Table no 1), showed moderate presence of tanins and flavonoids in endocarp but the absence of saponins, while glycosides and alkaloids were slightly present. Aqueous extract from the bark showed no presence of saponins, which were moderately present in the bark. there were however no alkaloids in the bark, while tannins, glycosides and flavonoids occurred slightly.

Table no 1: Phytochemical Analysis of Aqueous extract

Phytochemical	Endocarp	Peel
Saponin	--	++
Tannin	++	+
Glycosides	+	+
Alkaloids	+	--
Flavonoids	++	+

Key; -- Absent + slightly present ++ moderately present +++ highly present

TABLE no 2: Phytochemical Analysis of Ethanolic extract

Phytochemical	Endocarp	Peel
Saponins	--	+++
Tannins	++	+
Glycosides	+	+
Alkaloids	+	--
Flavonoids	+++	++

Key; -- Absent + slightly present ++ moderately present +++ highly present

Table no 2 also reported absence of saponins in endocarp, but were highly present in the bark. Moderate occurrence was observed in tannins from endocarp and flavonoids from bark, while slight glycosides were recorded in both extracts, slight alkaloids occurred in endocarp, just like the tannins in peel. From table 3, it shows the inhibition of growth by highest concentration was impressive, at 2.11 for *s. aureus*, very close to that by the positive control which was 2.13. The inhibition of *E. coli* was however far from that of the control at 2.56, compared with 4.38. 100mg/ml recorded 1.75 and 1.77 for *E. coli* and *S. aureus* respectively. The ethanolic extract, however had better inhibition than the control, at 8.15 and 2.35, compared with 5.75 and 2.24 on *E. coli* and *S aureus*, respectively. These are in accordance with the findings of Godwin and Mercer (1983) (12).

Table no 3 shows the antimicrobial inhibition by the peel extract was found to be effective especially in the ethanol extraction. The highest 150 mg/l treatment showed a remarkable control ability against both the *E. coli* and *S. aureus* recording an inhibition zone of 8.15 and 11.39 mm respectively. The aqueous extract also showed high control activity in the highest concentration with 10.56 and 9.11 mm inhibition zones against *E. coli* and *S. aureus* respectively. The inhibitions by these concentrations were however, not as good as the control drug. The lowest inhibition was observed from the 50 mg/l concentration of both the aqueous and ethanolic

extracts. The aqueous extract recorded 1.63 and 0.74 mm against *E. coli* and *S. aureus* respectively, while the ethanolic extract recorded 0.43 and 1.41 mm against *E. coli* and *S. aureus*, respectively

TABLE no 3: Antibacterial activity of *C. lanatus* fruit peel

Extract	Concentration (mg/ml)	zone of inhibition (mean square)	
		<i>E. coli</i>	<i>S. aureus</i>
Aqueous	150	10.56±2.34	9.11±4.21
	100	5.75±3.12	5.77±2.04
	50	1.63±2.80	0.74±0.89
Ethanolic	150	8.15±0.98	11.35±0.34
	100	7.00±1.09	8.93±0.73
	50	0.43±1.57	1.41±2.90
Negative Control		14.33±3.02	13.13±3.43
Positive Control		15.61±2.05	13.01±1.02

TABLE no 4: Antibacterial activity of *C. lanatus* fruit Endocarp

Extract	Concentration (mg/ml)	zone of inhibition (mean square)	
		<i>E. coli</i>	<i>S. aureus</i>
Aqueous	150	9.61±1.09	9.83±0.56
	100	6.54±2.80	5.56±3.02
	50	1.75±3.76	1.09±1.89
Ethanolic	150	11.38±1.70	10.33±3.42
	100	8.71±3.12	7.32±0.69
	50	2.21±1.56	1.84±2.50
Negative Control		14.25±1.12	13.80±0.42
Positive Control		15.61±2.02	13.01±3.19

Inhibitory effects of the endocarp extract as revealed in Table no. 4 showed an appreciable control activity although it was not as effective as the control drug. The highest inhibition was also observed in the highest concentration of 150 mg/ml in both the aqueous and ethanolic extracts. The highest inhibition zone was recorded by the 150 mg/ml ethanolic extract *E. coli*, followed by the same concentration of ethanolic extract on *S. aureus* with 10.33 mm. The 150 mg/ml aqueous extract had inhibition zones of 9.61 mm and 9.83 mm against *E. coli* and *S. aureus* respectively. The lowest activity was observed from the lowest concentrations of 50 mg/ml of both the aqueous and ethanolic extracts. This shows that the inhibitory activity of the endocarp is concentration dependant.

The observed results are in conformity with the findings of Marvic (2001) (13) and Mathew, *et al* (2014) (14) on the phytochemical composition which they report to protect cells from damage stimulate anti-cancer enzymes and help remove cancer causing substances from the body.

IV. Conclusion And Recommendations

The research revealed that ethanolic extracts of the fruit peel of *Citrullus lanatus* had the best antibacterial activity against the test organisms than the ethanolic extract of the endocarp. It was also found to be more effective than the aqueous extract of both the peel and the endocarp.

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