Academic Research Publishing Group

Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 2, No. 6, pp: 39-43, 2016
URL: http://arpgweb.com/?ic=journal&journal=16&info=aims

Antimicrobial Activity of *Citrullus Lanatus* (Watermelon) Seeds on Some Selected Bacteria

Bello, H. S.*
Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

Ismail, H. Y.
Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

Goje, M. H.
Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

Mangga, H. K.
Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

Abstract: Plants are reservoirs of different kinds of phytochemicals used for treatment of infections and other diseases. This study was aimed at evaluating the phytochemical and antimicrobial activity of *Citrullus lanatus* (watermelon) seeds against some selected bacteria. Water and ethanol were used to obtain the extracts and they were analyzed for the presence of alkaloids, flavonoids, glycosides, tannins and saponins. Out of the phytochemicals screened, only saponins were not detected in both aqueous and ethanolic extract. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were selected for antibiotic susceptibility testing. Antibacterial activity revealed that *S. aureus, E. coli,* and *P. aeruginosa* were most susceptible to the extracts at concentrations ranging from 6.25mg/ml to 50mg/ml. *Klebsiella pneumoniae* was observed to be resistant especially to the ethanolic extract. Largest zone of inhibition (8 mm) was produced against *P. aeruginosa* using aqueous extract at 50mg/ml. Higher concentrations were generally efficacious than the lower concentration whereas, ciprofloxacin (control) exert better activity than either of the extracts. Results for the MIC and MBC suggest that the constituents of the extract could be bactericidal as they were in close range. The findings of this study showed that seeds of *C. lanatus* contained bioactive compounds with potent antibacterial activity and thus could be used as herbal preparations just as the other parts of the plant.
Keywords: *Citrulus lanatus* seeds; Antimicrobial; Extract; Phytochemicals.

1. Introduction

Emerging and reemerging infectious diseases are one of the most important challenges confronting health care systems in many developing countries in the 21st century. An estimate of over 50% of all deaths was attributed to infectious diseases worldwide [1]. This is more or less connected to poor healthcare facilities in developing countries and most importantly, defying treatment by microorganisms to antibiotics that were hitherto efficacious. These have led to renewed interest in search for compounds with novel structures that could play role in effective healthcare delivery and curb the menace of resistance [2]. Hence, many studies have shown that plant-derived compounds are promising.

The use of plants' parts for medication has a very long history [3]. Different communities in the world have used plants in their folk medicine and the trend has been maintained up to the 21st century. In 2001 alone, an investigation revealed that about 122 compounds used in modern medicine were isolated and identified from ethno-medicinal plants [4]. In most of the developing countries, treatment using herbs have been a common practice and it was thought that, effective health care cannot be achieved in such countries unless orthodox medicine is complemented with traditional one [5]. The World Health Organization (WHO) estimates that 80 percent of the populations in Asia and Africa use herbal medicines for some aspect of primary healthcare. As such for many years, WHO have put-in-place many policies on quality, efficacy and safety of the products [6]. If this could point to anything, it might be its global acceptability even among authorities. Plants have proven to be the most useful in the treatment of diseases and they provide an important source of the world's pharmaceutical raw materials [7]. This is due to the fact that plants contain highly bioactive compounds or phytochemicals which include but not limited to alkaloids, carotenoids, flavanoids, glycosides, steroids, terpenoids, and tannins; that can inhibit microbial growth or cure ailments [8]. They are fund to be antibacterial, antifungal, anti-inflammatory, antihypertensive, anticancer and exhibit hemolytic and foaming activity [8].

*Corresponding Author

Citrullus lanatus (watermelon) is a spreading, hairy, tendril-bearing and annual vine plant reaching a length of several meters. Its leaves are long-stalked, oblong-ovate, 8 to 20 centimeters long, deeply 3 to 7 lobed, pinnatifid with usually narrowed segments. Watermelon leaves are dark green with prominent veins [9]. The watermelon fruit contains about 6% sugar and 91% water by weight. Watermelon is a good source of vitamin C and contains about 250 millligrams of citrulline per cup. In addition, B vitamins, manganese, potassium, carotenoid pigment, lycosides, flavonoids, tannins and polyphenols are also present in different proportions [10]. Being a member of curcubitaceae, the presence of curcubitacin, triterpenes, sterols and alkaloids cannot be ruled out [11]. [12] reported a number of trado-medical uses of different parts of water melon including febrifuge, diuretic, diabetes, demulcent, pectoral, tonic, vermifuge, hypotensive and in the treatment of nocturnal enuresis. Although most studies on medicinal use of this plant were using leaves, fruits, roots or flowers, it is anticipated that the seeds could also be effective as the other parts of the plant due to large number of chemical compounds present in the seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids [13]. Although the chemicals are believed to be for plant's protection, they can also play role as antimicrobial [13, 14]. It is against this backdrop that this research was designed with a view to evaluating the antimicrobial efficacy of the seeds extract against some selected bacterial species.

2. Materials and Methods

2.1. Collection and Preparation of Seeds

Watermelon (*Citrullus lanatus*) seeds used for this study were obtained from fresh retailed watermelon fruit from Monday Market, Maiduguri (*11°50′N 13°09′E*). The seeds were washed and dried for one week and properly stored until use. The seeds were finely grinded into powder with pestle and mortar in preparation for extraction.

2.1.1. Preparation of Extracts

The extraction was performed by cold maceration method as described by Tiwari, *et al.* [15] using water and ethanol as solvents. In the aqueous extraction, 50g of the seed powder was weighed and soaked in 400ml distilled water in a conical flask. The mixture was kept at room temperature for 24 hours with intermittent agitation and then filtered into a clean beaker. The filtrate was concentrated to dryness by evaporation at 45°C in an oven.

Similarly, for the ethanolic extract, 50g of the powder was weighed and soaked in 400ml of ethanol in a conical flask. It was subjected to treatment similar to that of the aqueous extract.

2.1.2. Phytochemical Analysis

Phytochemical analysis of the extracts was carried out qualitatively using accepted laboratory techniques as described by [15, 16]. Tests on the presence of alkaloid, flavonoids, glycosides, saponins and tannins were conducted accordingly.

2.2. Screening of Test Organisms

Clinical bacterial isolates were obtained from the Microbiology unit of the University of Maiduguri Teaching Hospital (UMTH) Maiduguri, Borno State, Nigeria. The isolates include *Esherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.* The stock cultures were inoculated into freshly prepared nutrient agar to test for their viability. They were stored at controlled temperature for subsequent antibacterial testing.

2.3. Antimicrobial Susceptibility Testing

Antibacterial activity of the extracts was investigated using modified Kirby-Bauer disc diffusion method. The discs were prepared from Whatman No. 1 filter paper using a 6mm-sized perforator. The discs were placed into petri dish and sterilized in hot air oven. The discs were impregnated with appropriate extract concentration following the methods of Lalitha [17]. A total of 0.5g of the concentrated extracts was weighed and dissolved into appropriate volumes of water to obtain a concentration of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Ciprofloxacin was used as control at 30mg/ml concentration. The bacterial inocula were suspended in sterile distilled water to obtain defined bacterial cells (McFarland standards No. 5). Swab sticks were soaked in the standardized inocula and pressed against the test tube walls to drain-off the excess water. The swab sticks were immediately used to inoculate the bacteria on dried surfaces of nutrient agar. It was allowed to stand for 10 to 15 minutes. The discs were then applied using sterile forceps and pressed gently to attach to agar surfaces and were incubated at 35°C for 20 hours. Zones of inhibition were measured using meter rule. The concentrations were used to determine minimum inhibitory and minimum bactericidal concentrations as described by Benson [18].

3. Results

Phytochemical screening of the aqueous and ethanolic extracts shows that, flavonoids, alkaloids, glycosides and tannins were present in both extracts. Saponins were however not detected as shown in Table 1.

Table-1. Phytochemicals of *Citrullus lanatus*

Phytochemical	Extract	
	Aqueous	Ethanolic
Saponin	-	-
Flavonoid	+	+
Alkaloid	+	+
Glycoside	+	+
Tannin	+	+

Antibacterial activity of the extracts against the selected isolates is presented in Tables 2 and 3. In the aqueous extract higher concentrations were associated with wider zones of inhibition with the exception of *K. pneumoniae* in which no inhibition was recorded at 50mg/ml. Similar pattern was observed in the ethanolic extract but with more activity than the former ranging between 7mm and 3mm (Table 3). In all the organisms tested, 50mg/ml was observed to be most efficacious while *E. coli* (ethanolic) and *P. aeruginosa* (aqueous) being the most susceptible. However, among all the organisms tested, the control (ciprofloxacin) was shown to have more effect than the extracts.

Results for the minimum inhibitory concentration (MIC) showed that 6.25mg/ml of the extract was inhibitory to *S. aureus* (aqueous and ethanolic), *E. coli* (ethanolic) and *P. aeruginosa* (aqueous) whereas 25mg/ml was inhibitory to *E. coli* (aqueous) and *P. aeruginosa* (ethanolic). *K. pneumoniae* however, was resistant to the ethanolic extract but inhibited by aqueous extract at 12.5mg/ml. Table 4 shows the minimum bactericidal concentration (MBC) whereby 6.25mg/ml was shown to be the MBC for the test organisms with the exception of *E. coli* where 12.5mg/ml was recorded.

Table-2. Antibacterial effect of aqueous extract of *Citrullus lanatus* on selected bacteria

Bacteria	Zone of inhibition (mm)				CPX (30 mg/ml)	MIC (mg/ml)
	50 mg/ml	25mg/ml	12.5 mg/ml	6.25 mg/ml		
E. coli	5	4	0	0	6	25
S. aureus	6	4	4	3	33	6.25
P. aeruginosa	8	6	3	3	23	6.25
K. pneumoniae	4	3	3	0	8	12.5

Table-3. Antibacterial effect of ethanolic extract of *Citrullus lanatus* on selected bacteria

Bacteria	Zone of inhibition (mm)				CPX (30 mg/ml)	MIC (mg/ml)
	50 mg/ml	25mg/ml	12.5 mg/ml	6.25 mg/ml		
E. coli	7	6	6	3	8	6.25
S. aureus	6	6	4	3	25	6.25
P. aeruginosa	5	4	0	0	26	25
K. pneumoniae	0	0	0	0	0	0

Table-4. Minimum bactericidal concentration of *Citrullus lanatus* extracts on the test organisms

Bacteria	Extract concentration (mg/ml)	
	Aqueous	Ethanolic
E. coli	12.5	12.5
S. aureus	6.25	6.25
P. aeruginosa	6.25	6.25
K. pneumoniae	6.25	>50

4. Discussion

In the present study, results showed that watermelon seeds contain phytochemicals including alkaloids, flavonoids, glycosides and tannins. No saponins were observed in both the aqueous and ethanolic extracts. The role of these phytochemicals as antimicrobial has been reported by many investigators [8, 12, 19]. Their presence in watermelon seeds was also reported by many authors including [1, 14, 20]. The absence of saponins in this study was in contrast to the works reported by [12, 14] but corresponds to that of [1, 20]. The disparity observed might be attributed to differences in geographical location as it can affect plant's active constituents, which may be induced by many factors like climate, soil and propagation method [21]. Presence of these phytochemicals in the extract was a clear indication of antimicrobial potentials of the watermelon seeds.

Antibacterial activity of the seed extracts showed that all the organisms tested were susceptible. The ethanolic extract exhibited more potency on *S. aureus* and *E. coli* in contrast to the aqueous extract which produced more effect on *P. aeruginosa*. Comparatively, different researchers have reported contradictory observations in this regards. Braide, *et al.* [14] observed that water extracts presents better response to the antibacterial activities than the ethanol whereas [20] reported the contrary. This contradiction might be a function of methodological differences and strain variability. *K. pneumoniae* showed the highest resistance especially to the ethanolic extract. This organism has been reported to be resistant to seeds extract of watermelon at concentrations lower than 125mg/ml [20].

Susceptibility of *S. aureus* and *E. coli* observed in this study is in agreement with that of Godwin, *et al.* [1] who made similar observations. In a recent study, susceptibility of *S. aureus* was attributed to presence of saponins [22]. Our findings therefore, support the view that other phytochemicals besides saponins are active against Gram positive bacteria also.

Higher extract concentrations were shown to be more potent than the lower concentrations. Nwankwo, *et al.* [20] have made similar observations although activity even at low concentration indicates high potency of the extract against the organism. The potency of the extract was further buttressed by the results of MIC and MBC in which most of the concentrations were in close range. The ciprofloxacin (control) used however, showed more effect than the extract which may reflect variation in the concentration of the active compounds in the antimicrobials. Isolation and purification of phytochemicals in the extract may likely exert more effect like the commercial antibiotic.

5. Conclusion

Results from this study showed the antibacterial potentials of seed extract of *C. lanatus* due to the presence of some phytochemicals. Most of the bacteria tested were observed to be susceptible to the extract. The activity observed is a clear indication of therapeutic property possessed by the seeds just like other parts of the plant. Therefore, the seeds of watermelon could be a good source of antibacterial agents and thus, should be harnessed.

References

[1] Godwin, O. O., Williams, A. U., Andrew, N. A., Atoyebi, B., Ezeh, P. A., and Udosen, I. J., 2015. "An Assessment of the Phytochemicals and Antibacterial Activity of Seed Extract of Citrullus Lanatus (Watermelon)." *International Journal of Research & Review,* vol. 2, pp. 148–156.

[2] Wangensteen, H., Samuelsen, A. B., and Malterud, K. E., 2004. "Antioxidant activity in extracts from coriander." *Food Chemistry,* vol. 88, pp. 293-297.

[3] Schulz, V., Hänsel, R., and Tyler, V. E., 2001. *Rational phytotherapy. A physician's guide to herbal medicine*. 4th ed. Berlin: Springer-Verlag.

[4] Rahman, B., 2014. "Phytochemical investigation of Citrullus lanatus (Watermelon) rind." A thesis report, submitted to the Department of Pharmacy, East West University, Bangladesh.

[5] Elujoba, A. A., Odeleye, O. M., and Ogunyemi, C. M., 2005. "Traditional Medical Development for medical and dental primary healthcare delivery system in Africa." *Africa Journal of Traditional, Complementary and Alternative Medicine,* vol. 2, pp. 46-61.

[6] World Health Organization (WHO), 2005. *National policy on traditional medicine and regulation of herbal medicines*. Geneva: Report of a WHO global survey.

[7] Ajayi, I. E., Ajibade, O., and Oderinde, R. A., 2011. "Preliminary phytochemical analysis of some plant seeds." *Research Journal of Chemical Sciences,* vol. 1, pp. 58-62.

[8] Saxena, M., Saxena, J., Rajeev, N., Dharmendra, S., and Gupta, A., 2013. "Phytochemistry of medicinal plants." *Journal of Pharmacognosy and Phytochemistry,* vol. 1, pp. 168–182.

[9] Njoroge, G. N., Gemmill, B., Bussmann, R., Newton, L. E., and Ngumi, V. W., 2007. "Pollination ecology of citrullus lanatus at yatta, Kenya." *International Journal of Tropical Insect Science. ICIPE,* vol. 24, pp. 73-77.

[10] Iakshmi, A. J. and Kaul, P., 2011. "Nutritional potential, bioaccessibility of minerals and functionality of watermelon (Citrullus vulgaris) seeds." *LWT - Food Science and Technology,* vol. 44, pp. 1821-1826.

[11] Yuan, G., Wahlqvist, M. L., He, G., Yang, M., and Li, D., 2006. "Natural products and anti-inflammatory activity." *Asia Pacific. Journal of Clinical Nutrition,* vol. 15, pp. 15-143.

[12] Hassan, L. E. A., Sirat, H. M., Yagi, S. M. A., Koko, W. S., and Abdelwahab, S. I., 2011. "In vitro antimicrobial activities of chloroformic, hexane and ethanolic extracts of Citrullus lanatus var. citroides (watermelon)." *Journal of Medicinal Plant Research,* vol. 5, pp. 1338-1344.

[13] Borchardt, W. F., Wyse, D. L., and Biesboer, D. D., 2008. "Antioxidant and antimicrobial activity of seed from plants of mississipi river basin." *Journal of Medicinal Plant Research,* vol. 2, pp. 81-90.

[14] Braide, W., Odiong, I. J., and Oranusi, S., 2012. "Phytochemical and antibacterial properties of the seed of watermelon (Citrulus lanatus)." *Prime Journal of Microbiology Research,* vol. 2, pp. 99-104.

[15] Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H., 2011. "Phytochemical screening and extraction: A review." *Internationale Pharmaceutica Sciencia,* vol. 1, pp. 98-106.

[16] Association of Official Analytical Chemists (AOAC), 1984. *Official Methods of Analysis*. Washington D.C. USA.

[17] Lalitha, M. K., 2014. *Manual on antimicrobial susceptibility testing*. Tamil Nadu: Under the Auspices of Indian Association of Medical Microbiologist.

[18] Benson, M., 2001. *Microbiological applications laboratory manual*. 8th ed.

[19] Okorondu, S. I., Sokeri, T. G., Akujobi, C. O., and Braide, W., 2010. "Phytochemical and antimicrobial properties of musa paradisiacal stalk plant." *International Journal of Biological Sciences,* vol. 2, pp. 128-132.

[20] Nwankwo, I. U., Onwuakor, C. E., and Nwosu, V. C., 2014. "Phytochemical analysis and antibacterial activities of citrullus lanatus seed against some pathogenic microorganisms." *Global Journal of Medical research*, vol. 14, pp. 17– 22.

[21] Adoum, O. A., Akinniyi, J. A., and Omar, T., 1997. "The effect of geographical location on the antimicrobial activities and trace element concentrationsin the root of." *Calotropis Procera (Ait.) R. Br, Annals of Borno*, vol. 13, pp. 199-207.

[22] Adunola, A. T., Chidimma, A. L., Olatunde, D. S., and Peter, O. A., 2015. "Antibacterial activity of watermelon (Citrullus lanatus) seed against selected microorganisms." *African Journal of Biotechnology*, vol. 14, pp. 1224-1229.