

Journal of Biological Research & Biotechnology

Bio-Research Vol. 18 No.1; pp. 1103-1110 (2020). ISSN (print): 1596-7409; eISSN (online):9876-5432

Antimicrobial and time-kill kinetics of the aqueous extract of *Citrullus lanatus* (Thunb.) seeds

^{§1}Babaiwa UF, ²Eraga SO and ¹Akerele JO

¹Department of Pharmaceutical Microbiology, ²Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Private Mail Bag 1154, Benin City, 300001, Nigeria

[§]Corresponding author: Babaiwa UF. Email/Phone: upe.babaiwa@uniben.edu, +234 80 35467818

Abstract

This study evaluated the antimicrobial property of the aqueous extract of *Citrullus lanatus* (watermelon) seeds and its concentration-effect relationship (time-kill studies) on typed bacterial and fungal strains. Crude powdered seeds of *Citrullus lanatus* were extracted by maceration with water. Antimicrobial assay of the aqueous extracts was determined against *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 24433) using standard microbiological methods. A total of 10⁶ CFU/mL of each test strain was used as a baseline to carry out the time-kill studies. Extract concentration at minimum inhibitory concentration (MIC), 2MIC and 4MIC were used over a period of 24 hours. Aqueous extract had an intermediate antibacterial activity with inhibition zone diameters (IZD) of 15 - 18 mm and MIC range of 2.5 - 20 mg/mL. Time-kill studies showed a bacteriostatic, non-concentration dependent mode of antimicrobial activity with characteristic regrowth for all test strains. *Citrullus lanatus* seeds aqueous extract exhibited antimicrobial activity with a bacteriostatic, non-concentration dependent mode of action against test bacterial strains. Further studies aimed at isolating and purifying the antimicrobial principle in the aqueous extract of *C. lanatus* seed is warranted as this could serve as a potential new antibiotic for treating microbial infections.

Keywords: *Citrullus lanatus*, antimicrobial, aqueous extract, pharmacodynamic studies, MIC

<https://dx.doi.org/10.4314/br.v18i1.5> Open Access article distributed under the terms of the Creative Commons License [CC BY-NC-ND 4.0] <http://creativecommons.org/licenses/by-nc-nd/4.0>.

Journal Homepage: <http://www.bioresearch.com.ng>.

Publisher: **Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.**

INTRODUCTION

The ability of an antimicrobial agent to inhibit or kill a microbe is often evaluated by a time - kill study also known as pharmacodynamics study. Pharmacodynamics is the study of the biochemical and physiological effects of drugs

(pharmaceutical or herbal). These effects can include those manifested within animals, humans, microorganisms or combination of organisms. It is often summarized as a dose-response relationship as seen by the relationships between drug concentration and its effects (Englehardt and Chiu, 2019).

Pharmacodynamic effect of an antimicrobial agent can be determined by studying the bacterial growth and death patterns following its administration *in vitro*. Ethical issues make it difficult to carry out this type of studies in humans and animal model. Thus, *in vitro* systems play a pivotal role in understanding the concentration-effect relationship of antimicrobial agents (Tonoyan *et al.*, 2017). The resultant time-kill curves show a clear relationship between the extent of antimicrobial activity and the concentration of the agent with respect to time (Li *et al.*, 2017). Thus time-kill studies can determine the bactericidal or bacteriostatic activity of an agent over time. A lethality effect of 90 % in six hours or 99.9 % in twenty-four hours is considered as a bactericidal mechanism of action (Konate *et al.*, 2012). Antimicrobial activity of an agent may also be considered bactericidal if the MBC/MIC ratio is equal four. In contrast, MBC of bacteriostatic agents is many folds greater than MIC (Keepers *et al.*, 2014). This lethality effect is said to be a concentration-dependent killing when cell death increases with increase in concentration of the agent. Antimicrobials of plant origin have enjoyed widespread application in the management of bacterial infections in Nigeria. However, optimization of dosage regimen has been a major challenge to the safe and efficacious use of these crude agents (Moore, 2018). Often times the minimum inhibitory concentration (MIC) is the only parameter used in the development of antimicrobial treatment regimen (Pohl *et al.*, 2018). Results obtained from the *in vitro* pharmacodynamic studies of an antimicrobial can provide better insight that could be used to optimize dosage regimen and help prevent treatment failures (Foster *et al.*, 2016). This assay provides descriptive information on the pharmacodynamics of an antimicrobial agent on susceptible bacteria strains. This study examined the pharmacodynamic relationship between varying concentrations of aqueous extract of *C. lanatus* seeds, microbial growth and death rate of four typed bacteria strain and a fungal strain, namely; *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 24433) using standard microbiological methods. This is with a view to optimize dosage regimen and prevent possible treatment failures associated with the use of herbal remedies. Watermelon (*Citrullus lanatus*) belong to the cucurbitaceae family. It is an annual fruit plant that is globular to oblong in shape and grown

on trailing vines of about 4.6 m in length. A longitudinal section of the fruit shows an outer rind, a layer of white pulp and an interior edible pulp containing seeds, a few varieties are seedless. The crushed seeds made into an emulsion has been used to treat catarrh, bowel disorders, fever and as an anti-helminthic agent (Adedeji, 2018). In Nigeria, the seeds are used to treat gastrointestinal and urinary tract infections and the rind is used in treatment of diabetes, hypertension and to conceal blemishes from acne (Erhirhie and Ekene, 2013). Apart from the medicinal uses of watermelon, it is also a source of food and few reports exist on its potential as a source of phytochemicals and nutrients (Maoto *et al.*, 2019).

MATERIALS AND METHODS

Materials

Ciprofloxacin powder (Sigma Aldrich, Taufkirchen, Germany), ketoconazole (Biochemika, Mumbai, India), dimethyl sulphoxide (DMSO) (JHD Science-Tech Co., China) were used in the study. Fresh fruits of *Citrullus lanatus* used for this study were obtained from Tamboga market in Benin City, Edo State, Nigeria and identified by a taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. A voucher specimen was deposited in the Departmental Herbarium.

Methods

Preparation of crude extracts

The fruits were cleaned with water, seeds were extracted, rinsed and air dried for five days. The seeds were pulverized using a kitchen blender (Kenwood BL460, England) and about 1.0 kg of the powdered seeds was macerated with distilled water for 48 hours. The resultant solution was clarified using Whatman filter paper No. 1. The clarified filtrate was concentrated in a hot air oven at 45 °C for 72 hours to obtain a dried aqueous extract which was weighed and kept at 4 °C in an airtight container until required for use (Babaiwa *et al.*, 2017).

Antimicrobial assay

Specimen collection

Microorganisms used for this study were originally from American Type Culture

Collection (ATCC) United States of America. They were obtained from stock culture of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria. Reference strains used in this study were *Bacillus subtilis* (NCTC 8236)), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 10145) and *Candida albicans* (ATCC 24433).

Preparation of test microorganisms

Test bacteria were sub-cultured from stock into sterile nutrient agar plates and incubated at 37 °C for twenty-four hours. Sabouraud dextrose agar was used to subculture test fungus which was incubated at 35 °C for forty-eight hours. Following incubation, pure colonies were harvested and sub-cultured into sterile broth and incubated for twelve hours. This was standardized to 0.5 McFarland to give an inoculum size of approximately 10⁸ CFU/mL. The standard inoculum was diluted 1:100 to give inoculum size of 10⁶ CFU/mL using the M07-A10 approved guideline (CLSI, 2015).

Preparation of stock solutions

Ciprofloxacin stock solution was prepared by dissolving 5.0 mg of ciprofloxacin powder in 10 % DMSO solvent and making up the volume to 10.0 mL, giving a final concentration of 0.5 mg/mL while ketoconazole stock solution was prepared with 10.0 mg ketoconazole powder in 10 % DMSO solvent to give a 1.0 mg/mL final concentration solution. A final concentrations of 1000 mg/mL stock solution of the seed extract was prepared by dissolving 1.0 g of extract in 1.0 mL of solvent (Babaiwa *et al.*, 2017).

Antimicrobial susceptibility tests

Using the agar well diffusion method of Murray *et al.* (1999) with some modifications, antimicrobial susceptibility tests were carried out. Standardized inoculum of each microorganism (200 µL) was mixed thoroughly with 30.0 mL of sterile Mueller Hinton agar (45 - 50 °C) poured into sterile Petri dishes and allowed to set. A sterile cork borer (10.0 mm) was used to bore six wells in each agar plate. The agar disks were removed and each of the sealed wells filled with 200 µL (equivalent to 200 mg) of aqueous extracts of *C. lanatus* seeds. The same procedure was carried out with ciprofloxacin (0.5 mg/mL) and ketoconazole (1.0 mg/mL) where 200 µL was emptied into each well to give 0.1 mg (100 µg)

and 0.2 mg (200 µg) per well, respectively. Negative control (10 % DMSO solution) and positive control (viability test for used organisms) were carried out for each set of experiment. Plates were incubated at 37 °C for 18 - 24 hours for bacteria and at 35 °C for 48 hours for *Candida albicans*. Diameters of zones of inhibition were measured in millimeters (mm) and used as an index of the inhibitory or killing action of the test agent against a given organism. Experiments were done in triplicates.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of the extract and test agents (ciprofloxacin and ketoconazole) on susceptible microorganisms was determined using the agar well dilution method of Afolayan and Meyer (1997). Following the manufacturer's instructions, nutrient and Sabouraud agars were prepared separately by placing them in a hot water bath at 50 °C. Different volumes of the extract stock solution were introduced into the molten agar to obtain a concentrations range of 6.25 - 400 mg/mL. Ciprofloxacin stock solution (0.5 mg/mL) was serially diluted two folds, giving a concentration range of 5.0 - 0.015625 µg/mL. The concentration ranges for ketoconazole stock solution (1.0 mg/mL) were 200 - 0.2 µg/mL. The nutrient agar-extract and nutrient agar-ciprofloxacin as well as the Sabouraud agar-ketoconazole mixtures were introduced separately into sterile plates and allowed to set and then dried at 40 °C. Test bacteria were streaked onto the solidified agar-extract and agar-ciprofloxacin plates while test fungus was streaked onto the agar-extract and agar-ketoconazole plates. Each set of experiments had a negative (10 % DMSO solution) and a positive (viability test for used organisms) control. The plates were incubated at 37 °C for 18 - 24 hours while plates for *C. albicans* were incubated at 35 °C for 48 hours. Experiments were done in triplicates. The lowest concentration of the extract or ciprofloxacin or ketoconazole that inhibited growth of the test microorganisms was taken as the MIC.

Minimum bactericidal/fungicidal concentration (MBC/MFC) determination

Following the MIC determination, plates showing no visible growth were swabbed and streaked onto freshly prepared Mueller Hinton agar plates already containing the same predetermined concentrations of extract or ciprofloxacin or ketoconazole. The plates for

bacteria were incubated at 37 °C for 18 - 24 hours and those for fungus at 35 °C for 48 hours. The lowest concentration that inhibited growth of test organisms for the extract and ciprofloxacin was taken as the MBC while the lowest concentration of ketoconazole that inhibited growth of the test fungus was recorded as the MFC.

Time-kill studies of extract on susceptible microorganisms

The time-kill studies were carried out using the broth macro dilution method of Okoli and Iroegbu (2005) with some modifications. Kill kinetics were done at MIC, 2MIC, and 4MIC (previously determined) of test microorganisms. These concentrations were developed in 2.0 mL tubes containing double strength nutrient broth. This was inoculated with 200 µL of test microorganism prepared by the colony suspension method previously described. The final inoculum density was approximately 10⁵ CFU/mL. Positive control (viability test for used organisms) was carried out. The tubes were incubated in a shaker water bath at 37 °C for bacteria and at 35 °C for *C. albicans* for twenty-four hours. At various time intervals ranging from 0, 1, 2, 3, 4, 5, 6 and 24 hours, approximately 100 µL from each sample was withdrawn and diluted tenfold in sterile normal saline (0.9 %w/v NaCl). About 100 µL of each dilution was plated in duplicate in sterile nutrient agar using the pour plate method. The plates were incubated for 24 hours at 37 °C for bacteria

and 35 °C for *C. albicans*. Plates with 30 - 300 colonies were counted with results expressed as log₁₀ CFU/mL and then used to plot the time-kill curves.

RESULTS

The extract obtained from the extractive process was brownish in colour with a percentage yield of 3.963 %. Results from the antimicrobial assay of the extract shown in Table 1 revealed that the test strains were susceptible to the inhibitory effect of the aqueous extract, standard ciprofloxacin and ketoconazole. An intermediate antimicrobial activity was observed for the extract against all test strains; with MIC range of 2.5 - 20.0 mg. This inhibitory effect was observed to be bacteriostatic against all test strains and the MIC doses produced the highest inhibitory activity when compared to other concentrations (2MIC and 4MIC). The extract induced a more rapid kill on the Gram positive bacteria (*Staphylococcus* and *Bacillus* species) with a characteristic steep pharmacodynamic time kill curve at 4MIC (Figures 1 and 2) while a reduced inhibitory response was observed for Gram negative *E. coli*, with periods of regrowth associated with *P. aeruginosa* (Figures 3 and 4). The extract at 4MIC was unable to reduce the number of *C. albicans* and Gram-negative survivors to zero in 24 hours of assay (Figures 3, 4 and 5). This is suggestive of a bacteriostatic mechanism of action against these microbes.

Table 1: Susceptibility of the extract and standard drugs against test microorganisms

Test	Microorganism	Aqueous extract	Ciprofloxacin	Ketoconazole
IZD (mm)	<i>Pseudomonas aeruginosa</i> (ATCC 10145)	17.0 ± 0.13	33.0 ± 0.14	NA
	<i>Escherichia coli</i> (ATCC 25922)	16.0 ± 0.11	38.0 ± 0.03	NA
	<i>Bacillus subtilis</i> (NCTC 8236)	16.0 ± 0.01	35.0 ± 0.23	NA
	<i>Staphylococcus aureus</i> (ATCC 25923)	15.0 ± 0.21	30.0 ± 0.21	NA
	<i>Candida albicans</i> (ATCC 24433)	18.0 ± 0.23	NA	32.0 ± 0.31
MIC (µg)	<i>Pseudomonas aeruginosa</i> (ATCC 10145)	2.0 × 10 ⁴ ± 0.003	0.25 ± 0.001	NA
	<i>Escherichia coli</i> (ATCC 25922)	2.5 × 10 ⁴ ± 0.002	0.0625 ± 0.002	NA
	<i>Bacillus subtilis</i> (NCTC 8236)	2.5 × 10 ⁴ ± 0.004	0.125 ± 0.010	NA
	<i>Staphylococcus aureus</i> (ATCC 25923)	2.0 × 10 ⁴ ± 0.001	0.25 ± 0.001	NA
	<i>Candida albicans</i> (ATCC 24433)	2.0 × 10 ⁴ ± 0.001	NA	10.0 ± 0.02
MBC/MFC (µg)	<i>Pseudomonas aeruginosa</i> (ATCC 10145)	> 2.0 × 10 ⁴	0.25 ± 0.001	NA
	<i>Escherichia coli</i> (ATCC 25922)	5.0 × 10 ⁴ ± 0.002	0.0625 ± 0.002	NA
	<i>Bacillus subtilis</i> (NCTC 8236)	5.0 × 10 ⁴ ± 0.004	0.125 ± 0.010	NA
	<i>Staphylococcus aureus</i> (ATCC 25923)	> 2.0 × 10 ⁴	0.25 ± 0.001	NA
	<i>Candida albicans</i> (ATCC 24433)	> 2.0 × 10 ⁴	NA	10.0 ± 0.02

Key: IZD (Inhibition zone diameter), MIC (Minimum inhibitory concentration), MBC/MFC (Minimum bactericidal/fungicidal concentration), NA (Not applicable), Values ± SEM

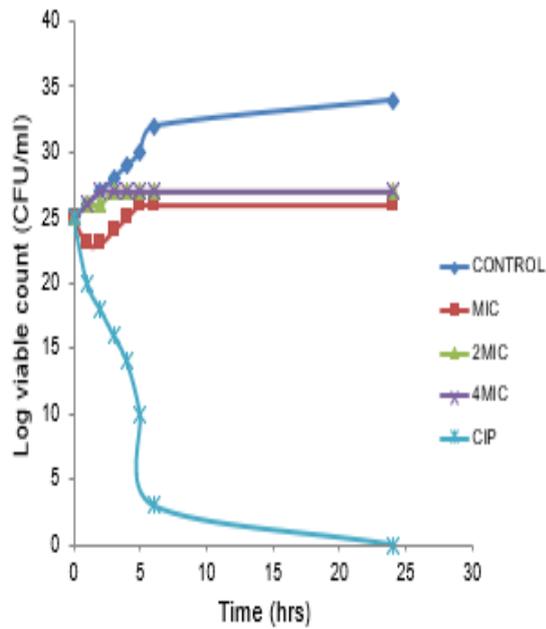


Figure 1: Time-kill curve of different concentrations of aqueous extract and ciprofloxacin (CIP) against *Staphylococcus aureus*

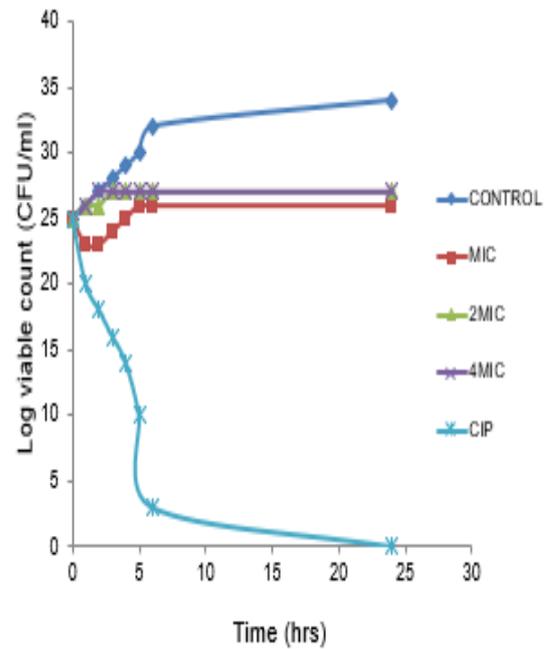


Figure 2: Time-kill curve of different concentrations of aqueous extract and ciprofloxacin (CIP) against *Bacillus subtilis*

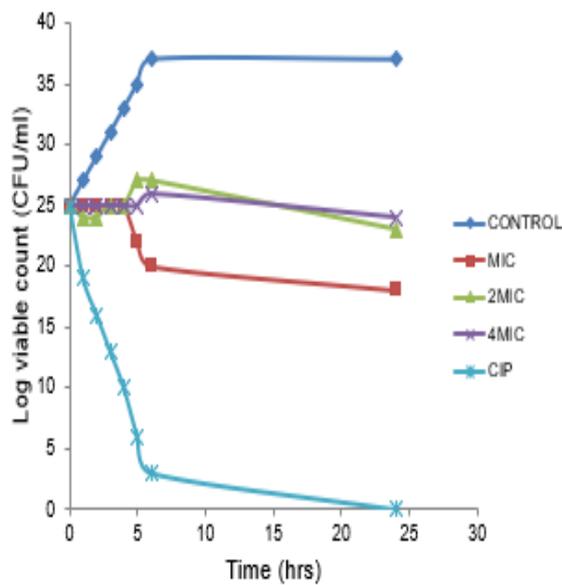


Figure 3: Time-kill curve of different concentrations of aqueous extract and ciprofloxacin (CIP) against *Pseudomonas aeruginosa*

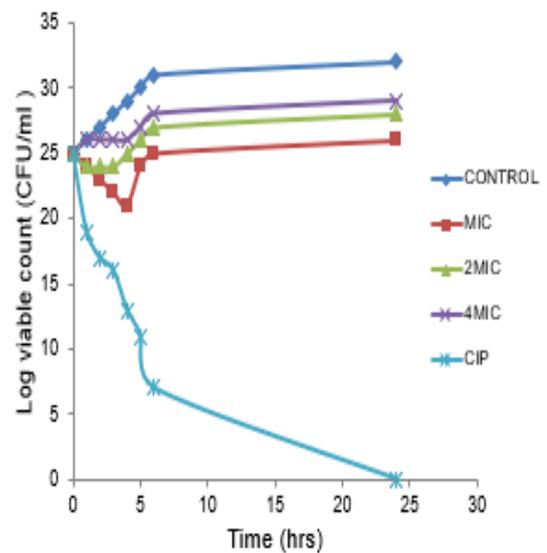


Figure 4: Time-kill curve of different concentrations of the aqueous extract and ciprofloxacin (CIP) against *Escherichia coli*

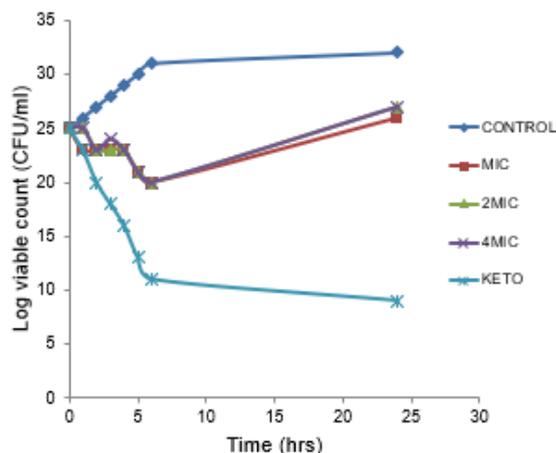


Figure 5: Time-kill curve of different concentrations of aqueous extract and ketoconazole (KETO) against *Candida albicans*

DISCUSSION

The water maceration process of extraction gave a low percentage yield of the extract. The low extractive capacity of water observed may probably be due to the low solubility of the major components of the seeds in water. It has been reported that the seed contains about 41.84 % of fat, 28.05 % of carbohydrate and 21.46 % of proteins (Egbuonu, 2015).

The extract had an intermediate antimicrobial activity with inhibition zone diameters (IZD) of 15 - 18 mm. Existing literature has reported the inhibitory activity of *C. lanatus* seed extract against some Gram positive and Gram-negative bacteria (Braide *et al.*, 2012). Results from our study is also in line with the study of Hassan *et al.*, (2011), who demonstrated the inhibitory activity of this seed against *Aspergillus niger* and yeast. Inhibition zone diameters of this extract against test strains were observed to be small. This may be partly due to interference from impurities commonly associated with crude extracts. Ciprofloxacin and ketoconazole had larger inhibition zone diameters against test bacteria and fungus respectively. This is consistent with pure compounds. Time-kill curve of the aqueous extract against *Staphylococcus aureus*, showed an initial decrease in cell number in the first hour of the experiment. This may be due to the rapid death of susceptible cells in the population. As previous reports have documented that colonies of bacterium exist as mixed population with varied susceptibilities (Shao *et al.*, 2017). This observed regrowth during the third hour of the experiment may be due to rapid kill of susceptible subpopulation coupled with amplification of resistant cells

(Drusano *et al.*, 2014). Rate of kill of *Staphylococcus aureus* by this extract showed no linear relationship with concentration over time; as there was no significant difference in the kill rate of 2MIC and 4MIC. Suggesting that an increase in concentration of the extract may not translate to increase in its kill rate

The kill pattern of the aqueous extract on *Bacillus subtilis* cells from the sixth hour (Figure 2) to the 24th hour of the experiment was bacteriostatic. This inhibitory effect may be due to reversible inhibition of enzyme systems, uncoupling of transport systems and possibly permeability changes within the affected cells. There was no significant difference in the antibacterial effect produced at MIC, 2MIC and 4MIC ($p > 0.05$). The standard drug, ciprofloxacin exhibited high bactericidal activity against *Bacillus subtilis* from the beginning of the experiment reducing viable cell numbers to zero at the twenty fourth hour. This may be due to the fact that ciprofloxacin is in its pure form and contains no extraneous substances that could interfere with its antibacterial action.

Steady reduction in viable cell number of *Pseudomonas aeruginosa* (Figure 3), from the fourth to the twenty fifth hour of the experiment at MIC is considered to be bacteriostatic. This may be due to the irreversible damage to cellular organelles and/or cellular pathways that are essential for the survival and growth of *Pseudomonas aeruginosa*. This extract has shown a concentration independent bactericidal activity against the test bacterium. Viable number of *Escherichia coli* and *Candida albicans* cells were observed to decrease slowly in the first five hours of the experiment (Figures 4 and 5) with a percentage death of 25.0 % each. This percentage lethality is however less than 90.0 % lethality required for bactericidal effect (Konate *et al.*, 2012). Thus, the extract investigated in this study has shown a bacteriostatic activity against these strains. A steady regrowth was seen beyond the fifth up to the twenty fourth hour. This initial decrease in viable number may be due to reversible damage caused by the extract to cellular components and also interference with some cellular pathways. The observed regrowth could be due to the ability of the organism to effect repairs of its damage component and possibly by pass cellular pathways that the extract may have interfered with. There was no significant difference ($p = 0.275$) between the rate of kill observed at MIC, 2MIC and 4MIC.

Standard drug (ketoconazole) exhibited fungicidal activity from the beginning of the experiment reducing viable cell number

significantly by the twenty fourth hour of the experiment. This high antifungal activity is consistent with pure compounds.

CONCLUSION

The outcome of time-kill studies based on MIC, 2MIC and 4MIC values have shown that the aqueous extract of *C. lanatus* seeds possessed a bacteriostatic, non-concentration dependent mode of action against test bacterial and fungus strains. Thus, there is a need to isolate and purify the antimicrobial principle in the aqueous extract of *C. lanatus* seed as this could serve as a potential new antibiotic.

Conflict of Interest

The authors have no conflict of interest to declare.

REFERENCES

- Adedeji, T.O. (2018). Extraction and evaluation of oil from watermelon (*Citrullus lanatus*) seed. *Journal of Nutritional Health and Food Engineering*. **8**(4): 293-295.
- Afolayan, A.J. and Meyer, J.M. (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. *Journal of Ethnopharmacology*. **57**: 177-181.
- Babaiwa, U.F., Erharuyi, O., Falodun, A. and Akerele, J.O. (2017). Phytochemical and anti-oxidant properties of *Citrullus lanatus* seeds extracts. *Nigerian Journal of Pharmaceutical Sciences*. **16**(2): 55-60.
- Braide, W., Odiong, J. and Oranusi, S. (2012). Phytochemical and antibacterial properties of the seeds of watermelon (*Citrullus lanatus*). *Prime Journal of Microbiology Research*. **2**(3): 99-104.
- Clinical and Laboratory Standards Institute (2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.
- Drusano, G.L., Neely, M., Van Guilder, M., Schumitzky, A., Brown, D., Fikes, S., Peloquin, C., Louie, A. (2014) Analysis of combination drug therapy to develop regimens with shortened duration of treatment for tuberculosis. *PLoS One*. **9**(7): e101311.
- Egbuonu, A.C.C. (2015). Comparative assessment of some mineral, amino acid and vitamin compositions of watermelon (*Citrullus lanatus*) rind and seed. *Asian Journal of Biochemistry*. **10**: 230-236.
- Englehardt, J.D. and Chiu, W.A. (2019). A general dose-response relationship for chronic chemical and other health stressors and mixtures based on an emergent illness severity model. *PLoS One*. **14**(2): e0211780.
- Erhirhie, E.O. and Ekene, N.E. (2013). Medicinal values on *Citrullus lanatus* (Watermelon): Pharmacological review. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. **4**(4):1305-1312.
- Foster, D.M., Jacob, M.E., Warren, C.D. and Papich, M.G. (2016). Pharmacokinetics of enrofloxacin and ceftiofur in plasma, interstitial fluid, and gastrointestinal tract of calves after subcutaneous injection, and bactericidal impacts on representative enteric bacteria. *Journal of Veterinary Pharmacology and Therapeutics*. **39**: 62-71.
- Hassan, L.A., Hasnah, M.S., Sakina, M.A., Waleed, S.K. and Abdelwahab S.I. (2011). *In vitro* antimicrobial activities of chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* var. Citroides (wild melon). *Journal of Medicinal Plant Research*. **5**(8): 1338-1344.
- Keepers, T.R., Gomez, M., Cleric, C., Nichols, W.W. and Krause, K.M. (2014). Bactericidal activity, absence of serum effect and time kill kinetics of ceftazidium-avibactam against beta lactam producing enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrobial Agents Chemotherapy*. **58**(9): 5297-5305.
- Konaté, K., Mavoungou, J.F., Lepengué, A.N., Aworet-Samseny, R.R., Hilou, A.,

- Souza, A., Dicko, M.H. and M'batchi, B. (2012). Antibacterial activity against β -lactam producing methicillin and ampicillin resistant *Staphylococcus aureus*; fractional inhibitory concentration index (FIC) determination. *Annals of Clinical Microbiology and Antimicrobials*. **11**: 18.
- Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., Chai, X., Wu, Y., Cai, J. and Cheng, G. (2017). Antimicrobial activity and resistance: Influencing factors. *Frontiers in Pharmacology*. **8**: 364.
- Maoto, M.M., Beswa, D. and Jideani, A.I.O. (2019). Watermelon as a potential fruit snack. *International Journal of Food Properties*. **22**(1): 355-370.
- Moore, H. (2018). How to mathematically optimize drug regimens using optimal control. *Journal of Pharmacokinetics and Pharmacodynamics*. **45**(1): 127-137.
- Murray, P.R., Baron, E.J., Tenover, F.C. and Tenover, F.C. and Tenover, R.H. (1999). *Manual of Clinical Microbiology*. 7th ed. ASM Press, Washington, DC, pp 234-239.
- Okoli, S. and Iroegbu, C.U. (2005). *In vitro* antibacterial activity of *Synclisa scabrida* whole root extract. *African Journal of Biotechnology*. **4**(9): 946-952.
- Pohl, A., Lübke-Becker, A. and Heuwieser, W. (2018). Minimum inhibitory concentrations of frequently used antibiotics against *Escherichia coli* and *Trueperella pyogenes* isolated from uteri of postpartum dairy cows. *Journal of Dairy Science*. **101**(2): 1355-1364.
- Shao, X., Mugler, A., Kim, J., Jeong, H.J., Levin, B.R. and Nemenman, I. (2017). Growth of bacteria in 3-d colonies. *PLoS Computational Biology*. **13**(7): e1005679.
- Tonoyan, L., Fleming, G.T.A., Mc Cay, P.H., Friel, R. and O'Flaherty, V. (2017). Antibacterial potential of an antimicrobial agent inspired by peroxidase-catalyzed systems. *Frontiers in Microbiology*. **8**: 680.