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Bioactivity of Brevibacillus laterosporus againt fungal and bacterial growth

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ABSTRACT

The acquisition and spread of antibiotic resistance among pathogenic bacteria pose a major threat to public health. Thus, there is a need for new antimicrobials that can be used as bacteriostatic alternatives to conventional antibiotics. Antibacterial compounds produced by genus Brevibacillus have been studied in recent years. They are an important tool for biological control. Different species of Brevibacillus show broad-spectrum antimicrobial activity, including activity against bacteria and fungi. A variety of molecules, including proteins and antibiotics, have been associated with their observed pathogenicity and mode of action. The antifungal and antibacterial properties of some Brevibacillus species found medical interest, linked to the production of antibiotics with therapeutic effects. The aim of this study is to isolate and diagnose Brevibacillus spp. from soil and show its toxic activity against bacteria and fungi by studying the protein extract of the bacteria. Soil samples were collected, Brevibacillus laterosporus was isolated, the protein mass was extracted from these bacteria, and its bioactivity was tested on the growth of the selected bacterial strains and yeast strains. We found that the protein extracted has in vitro bioactivity that inhibits the growth of bacteria and fungi under the current study. Minimum Inhibitory and Bactericidal Concentration (MIC&MBC) of extracted protein was identified against Staphylococcus aureus, Serratia spp., Candida albicans, and Candida tropicalis. We found that we can prepare a protein extract with MIC and MBC values from Brevibacillus laterosporus as an antibiotic, further study is required to confirm these results.

Keywords: *MIC, MBC, Bioactivity, Brevibacillus laterosporus, antibiotic*

1.INTRODUCTION

Brevibacillus is a genus of rod-shaped, oval, Gram-positive or Gram-variable bacteria that live in a variety of environments [1], [2]. The many strains of the genus Brevibacillus are noted for their capacity for facultative anaerobic and aerobic fermentation [3]. On common medium, the majority of species produce smooth, flat, yellowish-gray colonies. Due to the extensive variety within the genus Brevibacillus and the poor responsiveness to traditional biochemical identification techniques, it is particularly challenging to identify between species [4]. Brevibacillus is a highly heterogeneous phylogenetic genus [5]. There are currently 23 species of Brevibacillus with names that have been published [6]. Brevibacillus laterosporus is a rod-shaped, gram-positive bacteria that produces resistant spores. It differs from other bacilli by having the capacity to create a canoe-shaped parasporal body (CSPB) on one side of the spore. Additionally, B. laterosporus creates secondary metabolites with a variety of biological functions, including antibiotic compounds. As

biological control agents, these factors make the species a valuable resource for the biological control of widespread pests and illnesses [7]. There are multiple virulence factors produced by B. laterosporus that are involved in a variety of ecological processes and give the bacterium competitive advantages [8]. Strong antimicrobial properties were shown by Brevibacillus laterosporus against fungi, Gram-positive and Gram-negative bacteria [9].

The present study investigated the antibacterial activity of the protein extract of Brevibacillus laterosporus in vitro to find out its efficacy against bacteria and fungi.

2.MATERIAL AND METHODS

2.1. Isolate Brevibacillus spp and choose the isolation environment.

Forty-eight samples were collected, each time 16 samples were collected from the north, south, east and west of Al-Diwaniyah governorate from eight sites of the sanitary landfill areas for waste from each site two depths of 5 cm and 10 cm, and that was in each of the months August and September/2022, while the last collection of samples was on October 18/2022. And according to the fundamental methods, where they were placed in plastic containers and then labeled and kept in the incubator at 37 ° C, which is the appropriate temperature for the growth of bacteria until they are used in the study.

A quantity of soil was taken and placed in a sterile plastic test tube Microfuge, after that it was transferred to the laboratory to be cultivated, where 1 gram of soil was taken and mixed with 9 ml of distilled water in a plastic test tube using the shaker device (Shaiker) for 1 15 minutes. minutes in order for the soil to be completely dissolved, where the dilution here was (10^1), then 1 ml of the previous mixture was taken and added to 9 ml of distilled water and mixed using the shaking device for 15 minutes 2 in order to mix well, and here the dilution became (10^2), After that, 1 ml was taken from the second mixture and added to 9 ml of distilled water, and it was mixed using the shaking device for 15 minutes in order to mix completely 3, as the dilution here became (10^3), and finally 1 ml was taken from the third mixture and added to 9 ml of distilled water and mixed well using the shaker for 15 minutes. Here, the dilution became (10^4), the purpose of these four dilutions is to reduce the number of microorganisms present in the soil, and the dilution was taken until the sixth dilution (10^6) is obtained. Then, each tube was cultured on a nutrient agar plate, Then the samples were activated with BHI broth, then we were planted on plates of muller hinton agar medium, where growth appeared for most of the isolates, bacillus subtilis and bacillus cereus. As for samples No. 2 and No. 4, which were taken from a depth of 5 cm, they were closest to Brevibacillus in terms of phenotypic diagnosis.

2.2. Biochemical identification of Brevibacillus spp.

The biochemical identification was made as described in some references [10], [11], [12], and [13]. All biochemical assays and culture media are shown in table (1).

2.3. The assay for antibacterial

2.3.1. Bacterial strains

The bacterial strains Staphylococcus aureus, & Serratia spp. and yeast strains Candida albicans & Candida tropicalis were used in this work supplied by unite of zoonotic disease/ college of vet. Medicine / University of Al-Qadisiyah.

2.3.2. Determination of MIC and MBC

The MIC and MBC value was calculated for prepared extracted protein via using broth microdilution method. Two fold serial dilutions of tested material range from 0.25 µg/ml to 500 µg/ml were prepared directly in a microtiter plate containing Mueller Hinton broth or Sabouraud dextrose broth to obtain various concentrations. The bacterial or yeast inoculum was added to give a final concentrationof 5×10^5 CFU/mL in each well. The negative control was used containing DMSO. The plate was covered with a sterile sealer and incubated for 24 h at 37°C. Resazurin was added in each well of the microtiter plate and was incubated at 37°C for 30 min. The wells containing the bacterial growth or yeast growth turned into pink color whereas the well without bacterial or candida growth remained blue. The MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth. MBC is the lowest concentration that can prevent the growth of bacteria or yeast as confirmed by subculture. The experiment was performed in

triplicate for each bacterium and negative control was also included [14].

2.4. Small scale protein expression

Bacteria cells for protein expression were utilized in small volume protein production to really look at the capacity of the cell to deliver the protein and to decide the ideal condition, which yielded an elevated degree of solvent protein. Cells were plated first on LB agar and incubated for 18 hrs and then a single colony was utilized to inoculate 5 ml LB media. A 1 ml volume of short-term culture was utilized to inoculate 50 ml LB media in 250 volume container, which were utilized for better air circulation condition.

The total volume sample was incubated at 37 ˚C in a shaking incubator until log development stage assessed from an OD 600nm of 0.6-0.8. Then the temperatures changed to35 ˚C. Pellet of all out volume culture were put away at - 20 ˚C after centrifugation at 5X rpm/sec for 20 min to take a look later for protein concentration by Nanodrop device [15].

2.5. Large volume overexpression

The ideal production conditions detailed from small volume overexpression measures were performed to increase to huge volume of target proteines. The objective cells were plated for the time being to get new single colony, and afterward to contaminate 50 ml media. From the short-term culture (50 ml) an amount of 5 ml was utilized to inoculate a 500 ml volume of reasonable culture media (LB) in a 2000 ml flask, and afterward the enhanced circumstances were utilized to deliver the proteins. At last, the cells were reaped by pouring the culture into 50 ml centerfuge tubes, turning down in an Avanti rotator (Beckman), involving a F500 rotor at 8500 rpm for 30 minutes at 4 ˚C. The supernatant was disposed off, and the pellet was re-suspended into 50 ml falcon tubes and centrifuged again at 5000 rpm for 15 minutes at 4 ˚C. Cell pellets were put away in - 20 until required [15].

2.5. Protein collection

Putting away pellets from 2000 ml volume of culture were re-suspended in 1g/3ml of lysis buffer and afterward lysed on ice by sonication utilizing beats of approx. 15 kHz for 3×20 seconds in a Soniprep 150 at 3 micron sufficiency. The suspension was centrifuged for 10 minutes at 45000 x g, the supernatant was moved into 1.5 ml falcon tubes and equal amount of water was added to re-suspend the debris [16].

2.6. Antimicrobial Assay of purified protein

Antibacterial assay of different concentration of protein extract were performed by agar well diffusion method in Mueller Hinton Agar (MHA) for bacteria or Sabouraud dextrose agar for candida. The test organisms were inoculated in Nutrient broth or Sabouraud dextrose broth and incubated overnight at 37◦ C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. Medium (15-20 ml) was poured into sterile Petri dishes and allowed to solidify for 30 min.

protein extract of 20, 40, 80 mg/ml concentrations were prepared in Dimethyl Sulfoxide (DMSO). Thereafter, 0.1 ml of inoculum (bacterial or fungal strain in saline) was spread on an agar plate, and the excess was removed via draining. four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with 50 μl of protein, negative control (negative/solvent) control (DMSO). It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compound. The zone of inhibition was measured in millimeters. The assay was repeated thrice for confirmation [17].

2.7. Statistical analysis

Data analysis was performed using the SPSS program. In order to compare the data on the diameter of the inhibitory zone at various extracted protein concentrations on two microorganism, one-way ANOVA and the post test of the LSD test were utilized. A significant level was defined as $P< 0.05$.

3. RESULTS AND DISCUSSION

The protein extract was made to be tested against S. aureus bacteria, and the MIC and MBC values were 32 µg/ml and 250 µg/ml, respectively. The protein extract prepared for testing against Serratia spp. had a 64 µg/ml MIC value and a 250 µg/ml MBC value as show in Table (2).

Three concentrations of the extracted protein, 20, 40, and 80 mg/mL, were examined to see if they could stop the development of Serratia spp. and Staphylococcus aureus. For all concentrations, albeit in various degrees, the outcomes were favorable. Where the zone of inhibition emerged around each protein extract concentration of Brevibacillus laterosporus in the culture medium compared to DMSO. As a result, there was three inhibition zone formed around the bacterial growth of S. aureus when we measured these clear zone (Mean ± SD) mm, there was (11.48±0.82, 12.96±0.45, and 14.68±0.24) mm for three concentrations of the extracted protein (20, 40, and 80) mg/ml respectively, and also, there was three inhibition zone formed around the bacterial growth of Serratia spp. (11.02±0.98, 11.48±0.72, and 12.27±0.8) mm for three concentrations of the extracted protein (20, 40, and 80) mg/ml respectively, and there were no significant differences P = 0.42 (P>0.05) between S. aureus and Serratia spp. in susceptibility to extracted protein. The result shows there was a significant emergence of inhibition zone around bacterial growth for S. aureus and Serratia spp. compared to the activity of negative control (negative/solvent) control (DMSO) (0±0) mm against bacterial growth. as shown in table (3), Figure (1) & (2).

These results clearly indicate that the protein extract of Brevibacillus laterosporus possesses biological activity against the bacterial growth, Therefore, these results are consistent with the results of other studies conducted for the same purpose [18], [19], and [20]. where Zayed and his group confirmed in (2022) the biological effectiveness of Brevibacillus laterosporus against pathogenic bacteria under study Staphylococcus aureus, Escherichia coli, , Salmonella typhi, and Klebsiella pneumoniae [19]. The studies also confirmed that the with high molecular weight protein (bacteriocin) of Gram-positive bacteria, including Brevibacillus laterosporus, has antibacterial activity, as the disc diffusion assay was used to determine the antagonistic activities (Babar et al., 2022). Without more funding for drug discovery, it will be impossible to treat the bacteria that are resistant to certain medications. The use of bacteria that produce bacteriocin including Brevibacillus laterosporus for the treatment of infectious diseases brought on by drug-resistant pathogens is appropriate. Although the MIC of bacteriocin is greater for anti-drug-resistant pathogens, they have the ability to change resistance. Consequently, medications made from bacteriocin can aid in the fight against drug resistance [21].

The results of this study show the extracted protein prepared for testing against Candida albicans had a MIC value of 64 µg/ml and an MBC value of 500 µg/ml. The protein extract was produced for testing against Candida tropicalis and had MIC values of 32 µg/ml and MBC values of 250 µg/ml. table (4).

Three concentrations of the extracted protein, 20, 40, and 80 mg/mL, were examined to see if they could stop the development of Candida tropicalis and Candida albicans. For all concentrations, albeit in various degrees, the outcomes were favorable. Where the zone of inhibition emerged around each protein extract concentration of Brevibacillus laterosporus in the culture medium compared to DMSO. As a result, there was three inhibition zone formed around the bacterial growth of Candida albicans when we measured these clear zone (Mean ± SD) mm, there was (11.35±0.61, 12.04±0.92, and 12.84±0.38) mm for three concentrations of the extracted protein (20, 40, and 80) mg/ml respectively, and also, there was three inhibition zone formed around the bacterial growth of Candida tropicalis (11.52±0, 12.28±0.36, and 13.02±0.23) mm for three concentrations of the extracted protein (20, 40, and 80) mg/ml respectively, and there were no significant differences P = 0.461 (P>0.05) between Candida albicans and Candida tropicalis in susceptibility to extracted protein. The result shows there was a significant emergence of inhibition zone around bacterial growth for Candida albicans and Candida tropicalis compared to the activity of negative control (negative/solvent) control (DMSO) (0±0) mm against bacterial growth. as shown in table (5), Figure (3) & (4).

The results of this study indicate that there is a biological activity of the protein extract from Brevibacillus laterosporus against the yeasts under study. These results agree with the results of previous and recent studies conducted to detect the biological activity of Brevibacillus laterosporus against fungi [22], [23], and [24].

There is a wide spectrum of antimicrobial action in the Brevibacillus species, including activity against fungus and bacteria. Just two of the numerous substances whose apparent

pathogenicity and modes of action have been connected are proteins and antibiotics. Before, it was believed that Brevibacillus species served as a biological control agent for disease, and because of their relationship to the development of medicines with therapeutic benefits, some Brevibacillus species' antifungal and antibacterial properties prompted interest in medicine [25].

4. CONCLUSIONS

We conclude from this study that we can prepare the protein extract with MIC and MBC values from Brevibacillus laterosporus as an antibiotic against some microbes and test its effectiveness by perforation method.We conclude from this study that Brevibacillus laterosporus has a biological activity that inhibits the growth of both The bacterial strains (Staphylococcus aureus) (Serratia spp.) and yeast strains. (Candida albicans) (Candida tropicalis). further future studies are required to confirm the bioactivity of the protein extract of Brevibacillus laterosporus against bacteria and fungi.

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CONFLICTS OF INTEREST

No Conflicts of interest

Source of funding

Entirely self-funded

Ethical clearance

In vitro study

REFERENCE

1. Inan, K., Ozer, A., Guler, H.I., Belduz, A.O., Canakci, S., 2016. Brevibacillus gelatini sp. nov., isolated from a hot spring. International Journal of Systematic and Evolutionary Microbiology 66 (2), 712e718.
2. Johnson, E.T., Dunlap, C.A., 2019. Phylogenomic analysis of the Brevibacillusbrevisclade: a proposal for three new Brevibacillusspecies, Brevibacillusfortissp. nov., Brevibacillusporterisp. nov. and Brevibacillusschislerisp. nov.Antonie Van Leeuwenhoek 112 (7), 991e999.
3. Panda, A. K., Bisht, S. S., DeMondal, S., Senthil Kumar, N., Gurusubramanian, G., & Panigrahi, A. K. (2014). Brevibacillus as a biological tool: a short review. Antonie Van Leeuwenhoek, 105, 623-639.
4. Goto, K., Fujita, R., Kato, Y., Asahara, M., Yokota, A., 2004. Reclassification of Brevibacillusbrevisstrains NCIMB and DSM 6472 (¼NRRL NRS-887) as Aneurinibacillusdanicussp. nov. and Brevibacilluslimnophilussp. nov. Int. J. Syst. Evol. Microbiol. 54 (2), 419e427.
5. Ammanagi, A., CT, S., Badiger, A., &Ramaraj, V. (2021, September). Functional and structural characterization of melanin from Brevibacillusinvocatus strain IBA. In Doklady Biological Sciences (Vol. 500, No. 1, pp. 159-169). Pleiades Publishing.
6. Ray, S., Patel, N., & Amin, D. (2020). Brevibacillus. In Beneficial Microbes in Agro-Ecology (pp. 149-167). Academic Press.
7. Marche, M. G., Mura, M. E., & Ruiu, L. (2019). Rapid polymerase chain reaction assays for Brevibacillus laterosporus detection. Journal of basic microbiology, 59(8), 853-857.
8. Hamze, R., & Ruiu, L. (2022). Brevibacillus laterosporus as a Natural Biological Control Agent of Soil-Dwelling Nematodes. Agronomy, 12(11), 2686.
9. Wu, Y., Zhou, L., Lu, F., Bie, X., Zhao, H., Zhang, C., ... & Lu, Y. (2019). Discovery of a novel antimicrobial lipopeptide, brevibacillin V, from Brevibacillus laterosporus fmb70 and its application on the preservation of skim milk. Journal of agricultural and food chemistry, 67(45), 12452-12460.
10. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2002). Baily and Scotts. Diagnostic Microbiology. 11th ed. Mosby, company Baltimore. USA .
11. Reddy, M. S., Naresh, B., Leela, T., Prashanthi, M., Madhusudhan, N. C., Dhanasri, G., & Devi, P. (2010). Biodegradation of phenanthrene with biosurfactant production by a new strain of Brevibacillus sp. Bioresource technology, 101(20), 7980-7983.
12. Zhen, S., Kaiqi, L., Changxu, L., Jian, Y., Ruicheng, J., & Xunli, L. (2011). Isolation and characterization of a potential biocontrol Brevibacillus laterosporus. African Journal of Microbiology Research, 5(18), 2675-2681.
13. Katrodiya, N., Patel, N., Patel, S., Animasaun, D. A., Oyedeji, S., Dhanji, P., ... & Krishnamurthy, R. (2022). Isolation and Identification of Brevibacillus and Escherichia spp. from Urinary

Tract of Infected Patients. Annals of Biology, 38(1), 134-137.
14. CLSI. (2012). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement.
15. Grabski, A. C. (2009). Advances in preparation of biological extracts for protein purification. Methods in enzymology, 463, 285-303.
16. Hames, B. D. (Ed.). (1998). Gel electrophoresis of proteins: a practical approach (Vol. 197). OUP Oxford.
17. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine, 2019, 1–5.
18. Li, Z., de Vries, R. H., Chakraborty, P., Song, C., Zhao, X., Scheffers, D. J., ... & Kuipers, O. P. (2020). Novel modifications of nonribosomal peptides from Brevibacillus laterosporus MG64 and investigation of their mode of action. Applied and environmental microbiology, 86(24), e01981-20.
19. Zayed, M., El-Garawani, I. M., El-Sabbagh, S. M., Amr, B., Alsharif, S. M., Tayel, A. A., ... & Elfeky, N. (2022). Structural Diversity, LC-MS-MS Analysis and Potential Biological Activities of Brevibacillus laterosporus Extract. Metabolites, 12(11), 1102.
20. Babar, T. K., Glare, T. R., Hampton, J. G., Hurst, M. R., Narciso, J. O., & Beattie, A. (2022). Purification of High-Molecular-Weight Antibacterial Proteins of Insect Pathogenic Brevibacillus laterosporus Isolates. Processes, 10(10), 1932.
21. Lertcanawanichakul, M., & Chawawisit, K. (2020). Bacteriocin-like protein produced Brevibacillus laterosporus that can inhibit the growth of drug resistant bacteria. Int J Pharm Sci Dev Res, 6(1), 012-015.
22. Hassi M, Guendouzi SE, Haggoud A, David S, Ibnsouda S, Houari A, et al. Antimycobacterial activity of a Brevibacillus laterosporus strain isolated from a Moroccan soil. Braz J Microbiol. 2012;43(4):1516–1522.
23. Zhao, J., Guo, L., Zeng, H., Yang, X., Yuan, J., Shi, H., ... & Qiu, D. (2012). Purification and characterization of a novel antimicrobial peptide from Brevibacillus laterosporus strain A60. Peptides, 33(2), 206-211.
24. Jiang H, Wang X, Xiao C, Wang W, Zhao X, Sui J, Sa R, Guo TL, Liu X. Antifungal activity of Brevibacillus laterosporus JX-5 and characterization of its antifungal components. World J Microbiol Biotechnol. 2015;31:1605–18.
25. Mohammed, I. H. A. H. D. (2023). Molecular Study Of Brevibacillus Laterosporus. Iraqi Journal of Humanitarian, Social and Scientific Research, 3(9).

TABLE 1 : biochemical assays and culture media

Tests	**The result**
Gram stain	+
Motility	+
Catalase test	+
Methyl red test	+
Blood hemolysis	+
Oxidase test	-
Culture media	The growth
Muller Hinton agar	Growth
Blood agar	Growth
MRS agar	Growth
MacConky agar	No Growth

(+): Positive result, (-) : Negative result

TABLE 2: Values of MIC and MBC of prepared extracted protein against selected bacteria

Material	**Type of bacteria**			
	S. aureus		**Serratia spp.**	
	MIC	**MBC**	**MIC**	**MBC**
Protein extract	32 µg/ml	250 µg/ml	64 µg/ml	250 µg/ml

(S.) : Staphylococcus, (MIC): Minimum Inhibitory Concentration, (MBC): Minimum Bactericidal Concentration, (µg/ml): microgram/milliliter

TABLE 3 : Inhibition zone of prepared extracted protein against selected bacteria in culture media

Materials	**Concentration Mg/mL**	**Zone of inhibition (mm)**	
		S. aureus	**Serratia spp.**
Protein extract	20	11.48±0.82Aa	11.02±0.98Ab
	40	12.96±0.45Ba	11.48±0.72Bb
	80	14.68±0.24Ca	12.27±0.8Cb
DMSO	----	0±0Da	0±0Da
LSD(P<0.05)		0.42	

(S.) : Staphylococcus, Mg/mL: milligram/milliliter, DMSO: Dimethyl Sulfoxide, (mm) : millimeter

TABLE 4: Values of MIC and MBC of prepared extracted protein against selected

Material	Type of bacteria			
	Candida albicans		Candida tropicalis	
	MIC	MBC	MIC	MBC
Protein extract	64 µg/ml	500 µg/ml	32 µg/ml	250 µg/ml

(MIC): Minimum Inhibitory Concentration, (MBC): Minimum Bactericidal Concentration, (µg/ml): microgram/milliliter

TABLE 5: Inhibition zone of prepared extracted protein against selected bacteria in culture media

Materials	Concentration Mg/mL	Zone of inhibition (mm)	
		Candida albicans	Candida tropicalis
Protein extract	20	11.35±0.61Aa	11.52±0.22Aa
	40	12.04±0.92Ba	12.28±0.36Ba
	80	12.84±0.38Ca	13.02±0.23Ca
DMSO	----	0±0Da	0±0Da
LSD(P<0.05)		0.461	

Mg/mL: milligram/milliliter, DMSO: Dimethyl Sulfoxide (mm) : millimeter

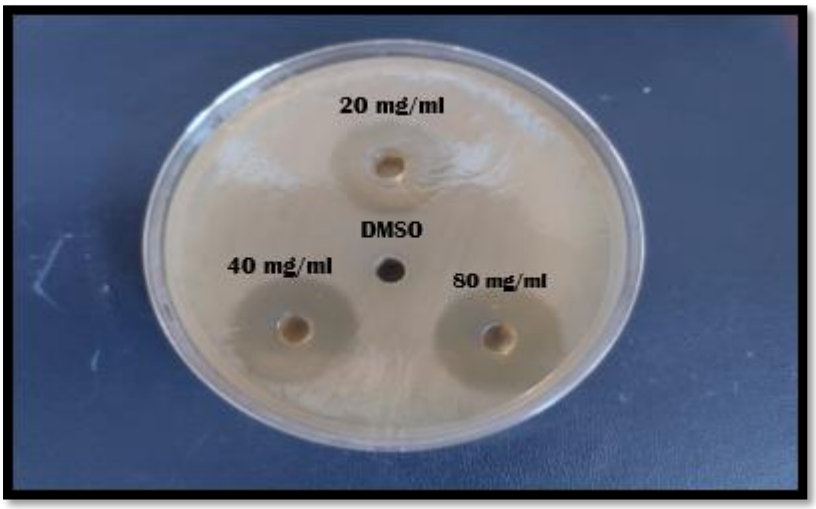

FIGURE 1 : Anti -Staphylococcus aureus activity of protein extractin culture media

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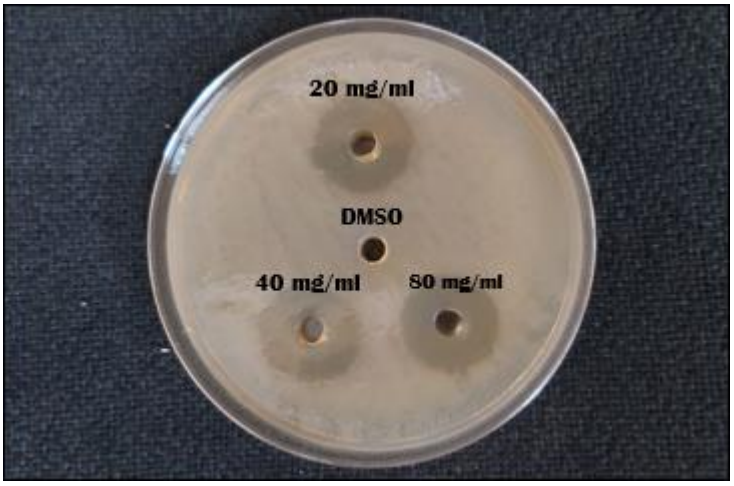

FIGURE 2 : Anti-Serratia spp. activity of protein extractin culture media

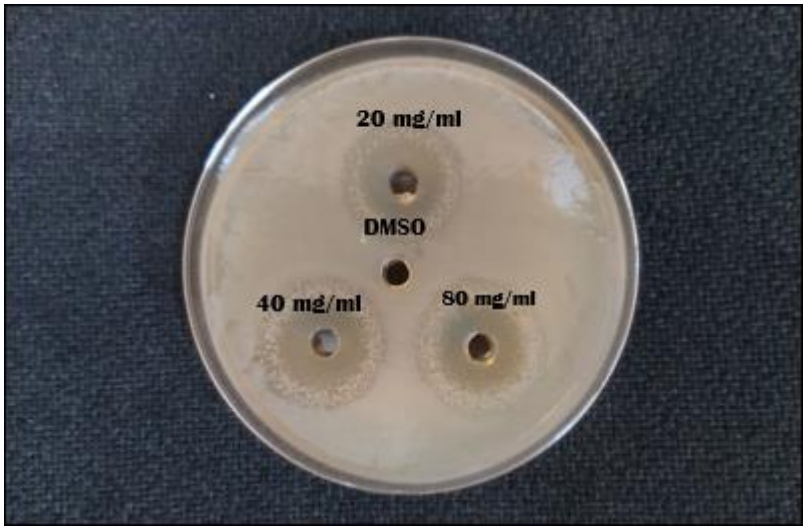

FIGURE 3 : Anti-candida albicans activity of extracted protein against in culture media

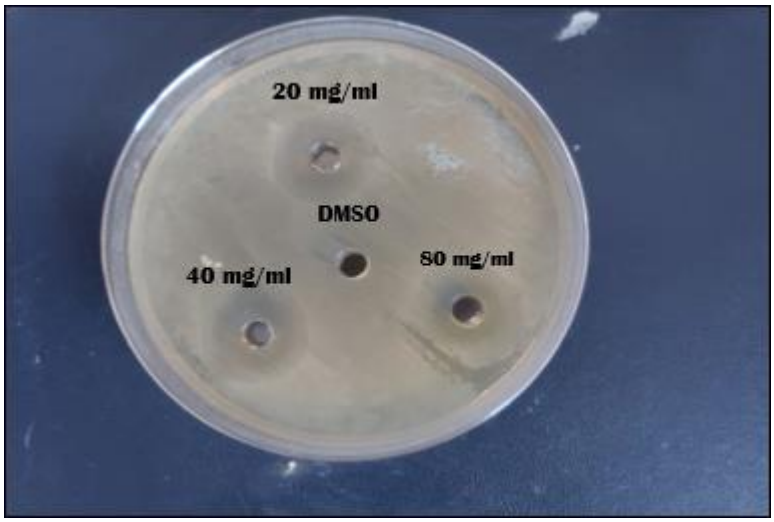

FIGURE 4 : Anti-candida tropicalis activity of extracted protein against in culture media

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