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Genetic Identification of Bacteria Producing Antibacterial Agent Isolation from Soil and Study of their Effectiveness as Antioxidants

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ABSTRACT

Twenty-five soil samples were collected from five different sites. Three isolates were identified for their antibacterial activity against pathogenic bacteria through primary screening protocol and on their inhibitory activity against the pathogenic bacteria. These were tested for their antibacterial activity through secondary screening protocol. These isolates were identified and diagnosed using many physiological, biochemical tests and amplification of 16S rRNA gene sequence. The isolates were identified as *Bacillus subtilis* (B1) and two *Bacillus polymyxa* (B4) and (B7). These had high inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* with inhibition zone reaching to 34 mm and less activity against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. B1 isolate was characterized by greater inhibitory activity against pathogenic bacteria. The antibacterial agents that produced by these isolates were characterized by three different methods. First : The ultraviolet spectroscopy (UV) for measuring the λmax, the results showing the range of this assay from 234 to 350 nm. Second : Infrared spectroscopy (FT-IR) in the range from 400 to 4000/cm. Third : Liquid chromatography by mass spectrometer analysis. The effect of temperature on the growth of the two isolates showed that they were developing well in temperature 40 and 45°. Both isolates were unable to grow at a temperature of 5°C and the effect of salinity on the growth of the isolates showed that they were developing well in 3% NaCl concentration. Only B1 was able to grow till 6% NaCl concentration, while both isolates were unable to grow at 10% concentration of sodium chloride. Activity of antioxidant was determined by using DPPH (2,2-diphenyl-2-picrylhydrazyl). The extract bacterial had the highest antioxidant activity with median effective dose (IC50) as 5.24±8.0 µg/ml.

Key words : 16S rRNA, antioxidant activity, *Bacillus*, DPPH, IC50

INTRODUCTION

In ancient history, humans relied on several methods of treatment, including very primitive ones, using plant or animal materials. Some of these substances have effect on pathogenic organisms. The Greeks were the first to use (plant resins) to treat many infections and diseases. The Chinese also resorted to using soybeans to treat many skin infections (Abbas *et al.*, 2020). Microorganisms that produce antibiotics are widely found in nature, where they are found in soil, water and the remains of flora and fauna. Soil is the main source of isolation. Many researchers have turned to soil for microorganism that had ability to produce new antibiotics (Egorov, 2015; Kumari *et al.,* 2019).

Bacillus produces different secondary metabolites and has multiple benefits in several areas, the most important of which is its production of anti-pathogenic factors (Al-Thubiani *et al.*, 2018; Mannaa and Kim, 2018). Moreover, *Bacillus* produces some important vitamins such as vitamin B12 through some of its metabolic pathways. Scientific evidence has emerged on the efficiency of microorganisms in the production of antibiotics and it included many types of organisms and the *Bacillus* is the most important species (Tyor and Kumari, 2016).

The term antibiotics was first used in 1942 by the scientist Waksman, who defined as : Antibiotics are important substances produced as secondary metabolites by some microorganisms to affect the growth of other organisms that are harmful and pathogenic to humans (Havare *et al*, 2019). Antibiotics have been known as one of the secondary metabolites produced by microorganisms after their growth reaches stationary phase (also known as production phase or idio phase). Some

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sources have suggested that these could be called Idiolites (Mannaa and Kim, 2018).

This study was used to isolate local bacteria from the soil of the city of Hilla and to detect efficient isolation in the production of ant microorganisms from the experimental isolates and test their tolerance to the conditions of different temperature and concentrations of saline.

MATERIALS AND METHODS

Twenty-five samples were collected from different soils and taken from different depths ranging from 5-10 cm. Fifty grams of soil was taken from each site and the soil was placed in sterile containers, full information was recorded on it and taken to the laboratory. Decimal dilutions were made for soil samples and then cultured on a nutrient agar medium and placed in an incubator for 48 h at a temperature ranging from 15 to 45°C.

Bacterial isolates obtained were : *S. aureus, P. eruginosa, E. coli* and *K. pneumonia)* from Marjan hospital in Hilla city of Babil province. These isolates were diagnosed using physiological and biochemical tests and based on the global scientific sources for diagnosis of bacteria. The diagnosis included the following tests : microscopic examination, tumor traits, motion examination catalase test, oxidase test (Mannaa and Kim, 2018), red blood test, VP test, urea test, nitrate reduction test, blackboard composition test, coagulation enzyme test, blood analysis capacity, gelatin melting test and sugary fermentation test.

In this study, PCR method was used for identification. DNA was extracted using DNA extraction protocol, forward, RW01 (5-AACTGGAGGAAGGTGGGGAT-3) and reverse, DG74 (5-AGGAGGTGATCCAACCGCA-3). The PCR product was sequenced. The obtained sequences were compared with the sequences reported in the gene bank (Mellmann *et al.,* 2018).

Two methods were used for detecting the capacity of isolated microorganisms on the production of antibiotics : the method of agar well diffusion assay was used for the purpose of detecting the ability of isolates to produce antibiotics (Nanjwade *et al.*, 2020). Using the selected isolates bacteria to test their ability to produce antibiotics on Muller Hinton agar containing pathogenic bacteria. It was incubated at a temperature of 37 ^{O}C for 24 h and the detection of its ability to produce antibiotics through appeared inhibition zoon round the well.

The second method was the method of cross-streaks, where the abandoned organisms to be detected in the production of antibiotics in a straight line on the tip of the dish containing the center of Muller Hinton agar and incubated the occlusion at a temperature of 37 ^{O}C for 48 h. The planted isolates test bacteria was in the form of lines (which was to be tested for its activity in the production of antibiotics), so that the transplant from the remote end of the microorganism and ends at the line of growth incubated the dishes at a temperature of 37°C for 24 h. The bacteria were considered a product of antibiotic through the presence of inhibition for the growth of one or more of the bacteria test in the near-line living microorganism areas (Yupparach *et al.*, 2019).

The positive results obtained from the primary screening protocol were used to extract the extracellular agent to determine the antimicrobial activities of the isolates. All isolates were incubated in flasks ranging from 500-1000 ml in nutrient broth and pH was adjusted at 7.2 for 48 h. After growth occurs at the incubation period, the nutrient broth was filtered through filter paper. After collecting the filtrate, centrifugation was carried out at 6000 rpm for 15 min. Then the filtrate was taken and separated using volume/volume ethyl acetate by shaking vigorously for an hour to obtain two layers, an aqueous layer and an organic layer. The active substance was present in the organic layer. Using a rotary evaporator, the alcohol residue was removed and the material was dried using an oven to obtain the final extract (Hoh *et al.,* 2019; Rajesh *et al.*, 2019).

All extraction from isolates was measured by λmax in the range from 200 to 800 nm wave lengths as described by Immanual *et al.* (2016). Infrared (FT-IR) spectroscopy was measured in the Department of Chemistry at the University of Babylon**.** Liquid chromatography (LC) analysis was carried out using UFLC Prominence series (Shimadzu, Japan). The separation was accomplished using an X Bridge C18 column (2.5 µm, 2.1 × 50 mm). The protocol was used as described by Butler (2015).

The sensitivity of the isolates against seven types of commercial antibiotics was tested by

making three replicates and using seven tablets of these antibiotics on Muller Hinton label and incubated for 24 h at 37°C.

For testing the effect of temperature on growth of bacteria, the bacteria were incubated at varying degrees of temperatures : 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C on nutrient agar media.

Different concentrations of sodium chloride were added to compute the effect of salt concentration on bacteria : 1, 2, 3, 4, 5 and 7 % to the nutritious medium and incubated with bacteria for 2 to 4 days. The results were positive when turbidity appeared (Lee *et al.*, 2015).

To calculate antioxidant activity, the standard and test solution were prepared as shown in Tables 1 and 2.

RESULTS AND DISCUSSION

Samples of different soils were collected from different areas of the city of Hilla (Table 3).

The isolating results showed 10 bacterial isolates producing the antibiotics from the total of 25 soil samples used in the isolation process. These isolates had ability to produce different antibiotic agents. The diameter of the inhibition against the bacteria used in test differed between isolates and depending on it; the powerful isolates were from agricultural vegetables and river sediment. Three isolates were given the codes B1, B4 and B7 for their high ability to produce antibodies from the rest of the isolates and were from different locations of the city as shown in Table 3. These isolates were selected for *vitro* and *chemo* tests for the purpose of final diagnosis and identification of genus of bacteria.

The results of the culturing and biochemical tests of the bacterial isolates produced (based on Somma *et al.*, 2020) of *Bacillus* species

Table 3. Different soil collecting sites and types of soil

S. No.	Isolates symbol	Soil type
1.	B1	Agriculture
2.	B2	Sediment
3.	B3	Agriculture
4.	B4	Sediment
5.	B5	Agriculture
6.	B6	Agriculture
7.	B7	Sediment
8.	B8	Sediment
9.	B9	Sediment
10.	B10	Sediment

Table 1. Preparation of standard solution

Materials	How to prepare	Conditions of preparation	Analyzed
50 mg of ascorbic acid 100% of ethanol 2, 2- diphenyl 1-picralhydrazyl (DPPH) solution	1. Dissolving ascorbic acid in 50 ml ethanol 2. Ascorbic acid was used by concentrations from 1, -, - 0.032 mg/ml 3. DPPH solution in concentration 0.002 mg/100 ml **(DPPH = 2, 2- diphenyl 1-picralhydrazyl)	1. Kept in a dark container for 30 min. 2. Absorbance was read by use of spectrophotometer at 517 nm 3. Drawing standard curve to calculate DPPH scavenging activity of radical 4. Calculating IC50	All tested concentrations were analyzed in duplicates (Budi, 2017)

Table 2. Methods for preparing test solutions

Materials	How to prepare	Conditions of preparation	Analyzed
2, 2- diphenyl 1-picralhydrazyl (DPPH) 100 ml ethanol extract of B*acillus subtillus*	1. 0.002 g DPPH dissolved in 100 ml ethanol 2. *Bacillus subtilis* extract was mixed with ethanol to make dilution 3. Using the hand or the mixer, the mixture was shaken for 2 min to obtain a homogeneous mixture	1. Kept for 30 min in dark place 2. Using a visible spectrophotometer at 517 nm, DPPH de-colorization was determined 3. The antioxidanti activity was expressed and calculated as IC50 (mg/ml)	All samples were analyzed in duplicates (Budi, 2017)

DPPH radical scavenging activity % = [(A0-A1)/A0] × 100

Where, A0 = The control absorbance; A1 = The sample absorbance.

(Table 4) was *B. subtilis*. The local code B1 was isolated from agricultural soil. The results of the tests for this isolation depended on biochemical and microscopic test that they were chains or pairs of spores and were positive *bacilli*. There were obligated aerobic colonies with a yellow-coloured circular colour. The edges of the colony were positive to oxidase and indole. They were positive for catalase and the motion and red-methyl were negative for the urea test. Nitrate was reduced to acid-producing nitrite and H2S Gelatin synthesis, sugary fermentation and blood hemolysis (Vestby *et al.,* 2020).

Table 4. Biochemical test to identify the active isolates

	B1	B4	B7
Gram stain	++	++	++
Motility	++	++	++
Heamolysis	++	--	--
Citrate	++	--	--
Nitrate test	++	++	++
Oxidase	--	+-	++
Manitol	++	--	--
Urea	--	--	--
Catalase test	++	++	++
MR test	++	++	++
Indol test	--	--	--
H2Sitest	++	++	++
iVP	--	++	++
Spore stain	++	++	++
Gelatinase	++	++	++

Two isolates, B4 and B7 were recovered from the river sediments and found to be returning to *Bacillus polymyx.* The microscopic examination showed that the cells were single or double *bacilli* gram positive, which were composed of spores which were elliptical and fell to the tip of the sub-terminous, leading to the bulging of the cell. The ability of these bacterial isolates was in the production of oxidase and catalase enzyme and the analysis of gelatin and starch. An important advantage of this isolation was its ability to produce acid and gas from most sugars and they grew in circles consciousness button and liquid had been turbid which proved that this isolation was *Bacillus* (Somma *et al.*, 2020). The bacterial isolation was able to produce acid and gas from the sugar of glycose and on this basis was identified as *polymyxa.* Vestby *et al. (*2020) isolated three strains of this type. The diagnostic properties coincided with our isolation.

The obtained PCR product had a 370 bp specific band on the gel (Fig. 1). The inhibitory activity of all local isolates against test bacteria was identified (Table 5). Effectively, the isolates B1, B4 and B7 had higher effect against pathogenic bacteria and were able to produce antibacterial agents against pathogenic bacteria. It was observed that the isolates had the highest activity against *S. aureus* and *E. coli. B. subtilis* had greater activity than *B. polymyxa* in inhibition of *E. coli. B. subtilis* (30-34 mm), while *B. polymyxa* had the highest diameter inhibition (20-22 mm).

The ability of the extracts to inhibit the test bacteria was a very important factor, especially that *S. aureus* and *P. aeruginosa* were among the most virulent and resistant to antibiotics (Moor *et al.,* 2017; Adel *et al.,* 2018).

Ultraviolet (UV) spectroscopy was used for measuring the λ max for antibacterial and this ranged from 234 to 350 nm. This gave idea about the types of these compounds (Fig. 2).

After separating the antibacterial agents from the genera of *Bacillus,* they were diagnosed based on measurement using ultraviolet rays, and it was found that they ranged from 234 to 350 nm, which could be linked to the types of

Table 5. Inhibitory effect of B1, B4 and B7 against pathogenic bacteria

Isolates No.	Diameter inhibition (mm)			
	S. aureus	*K. pneumonia*	*P. aeruginosa*	*E. coli*
B1	34±0.1	30±0.4	31±0.6	30±0.2
B2	7.3±0.5	18±0.4	6.9±0.1	18±0.4
B3	12.8±0.6	12±0.3	8.5±0.1	9.5±0.1
B4	21±0.3	20.5±0.2	20±0.4	22±0.1
B5	18.4±0.3	17.4±0.4	9±0.1	7.7±0.2
B6	13±0.4	15.5±0.2	9.8±0.2	10.9±0.1
B7	21.9±0.2	20.1±0.1	21±0.3	22.7±0.7
B8	18±0.4	12±0.4	17.8±0.3	16.3±0.4
B9	16.3±0.3	13±0.4	14±0.1	15.5±0.2
B10	11.9±0.1	7.3±0.5	17.5±0.6	9.9±0.1

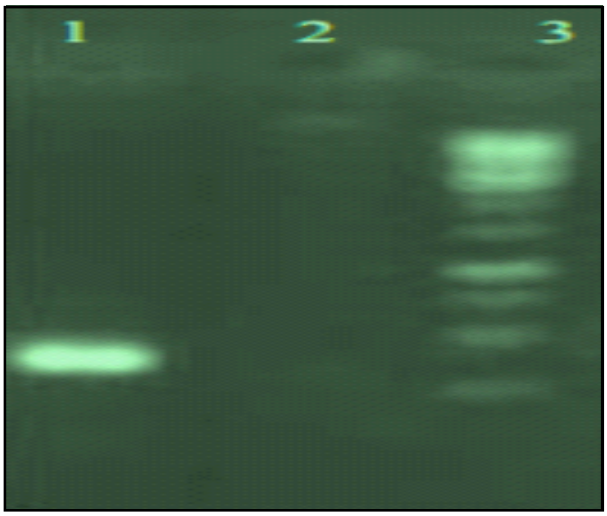
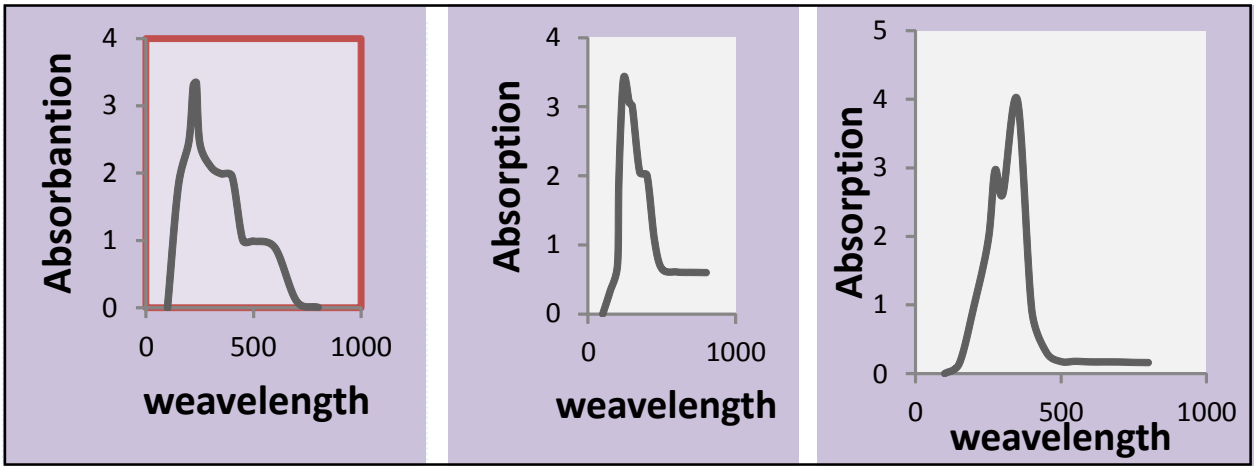


Fig. 1. Electrophoresis of the PCR product of isolated 16S rRNA gene from the bacteria isolates from soil on 1% agarose gel.

bonds in a particular molecule, and were of value in determining the functional groups within the molecule (Taware *et al.*, 2014). These results are in accordance with those of Abu-Khumrah (2014). Also, most antibiotics had a maximum absorption at range 210-230 and 270-280 nm (Motta and Brandelli, 2015; Sudha and Masilamani, 2019). In addition at 223-225 and 332 nm, Ababutain *et al.* (2016) found that the maximum absorption peak in UV was at 269 nm.

Infrared (FT-IR) spectroscopy was used for knowing the important chemical functional groups present in produced antibiotic (Fig. 3). It showed that bands referred to absorption bands : each one referred to specific functional chemical group.

Active agent was identified using LC/MS analysis (Fig. 4). It showed the positive and

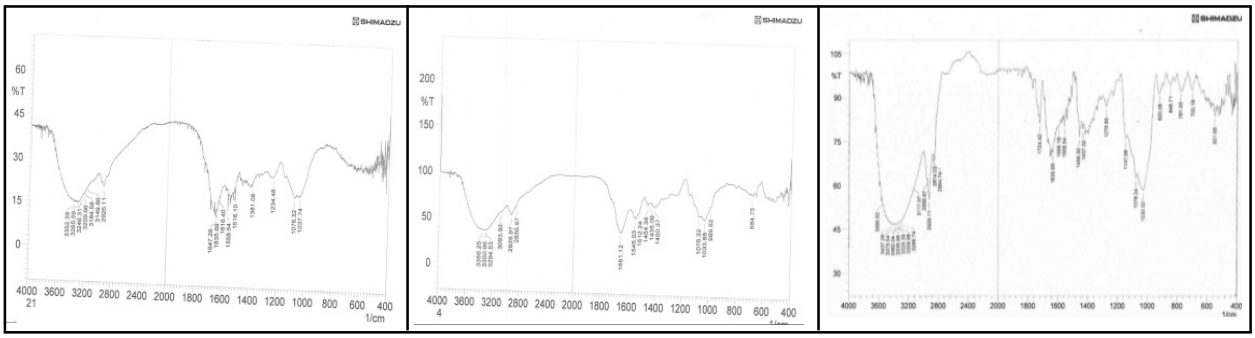


A : This figure shows the λ max was 234.00 nm for B1 isolate.

B : This figure shows the λ max was 240.00 nm for B4 isolate.

C : This figure shows the λ max was 350.00 nm for B7 isolate.

Fig. 2. Ultraviolet spectrum for extracellular antibacterial extracts.



A : Infrared spectrum for B1 isolate extracellular antibacterial extract.

B : Infrared spectrum for B4 isolate extracellular antibacterial extract.

C : Infrared spectrum for B7 isolate extracellular antibacterial extract.

Fig. 3. Infrared spectrum.

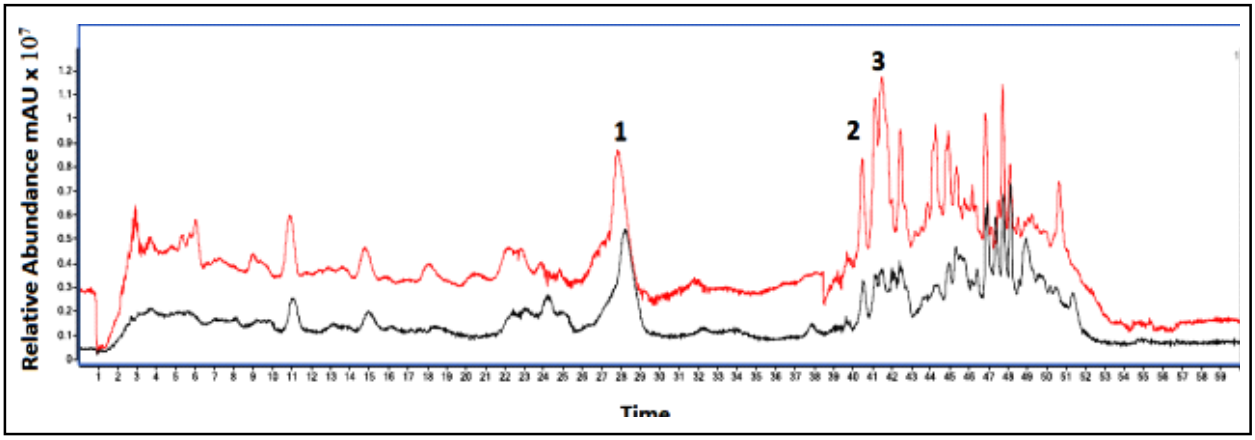


Fig. 4. LC chromatogram of antibacterial extract monitored at 190 nm.

negative UV chromatogram of antimicrobial agents at 190 nm. By comparing data received from LC/MS all peaks were analyzed and identified (Chumroenphat *et al.*, 2021).

The antibacterial extracts were analyzed using LC-MS, and the results showed that some compounds were in a negative mode (negative ion), while only one compound was within negative ionization. The first peak was of pentacyclic triterpenoids lantadene C (Fig. 4). The peak was set at 28.4 min with protonated and deprotonated molecule [M+H]+of 551 m/z, while the second peak was at 40.4 min with [M+H]- at m/z 567. This peak was identified as icterogenin (Fig. 4). Third peak was eluted at 42.3 min, which showed a molecular ion [M+H]- at m/z 551. Two compounds were recognized at this elution, which were lantadene A and lantadene B (Chumroenphat *et al.*, 2021). Sharma *et al.* (2020) explained that two peaks could be separated in successive times, especially the two adjacent ones, one for club B, with a retention time of 17 min and the other for club A with a retention time of 18 min.

The ions detected were pentacyclic triterpenoids extracted from *B. subtilis* using LC/MS or NMR at m/z 567.3 (Litaudon *et al.*, 2019), 531.3 and 568. These findings are in concordance with the results obtained before. The results of the growth of the three isolates at different temperatures showed that the best temperature of growth through measure optical density (OD) was 40 to 45°C and the lowest growth (not able to grow) at the level of 5°C (Fig. 5). The difference in temperature had an impact on the characteristics of bacteria both on the physiological and phenotypic. Regenerative regeneration will affect growth

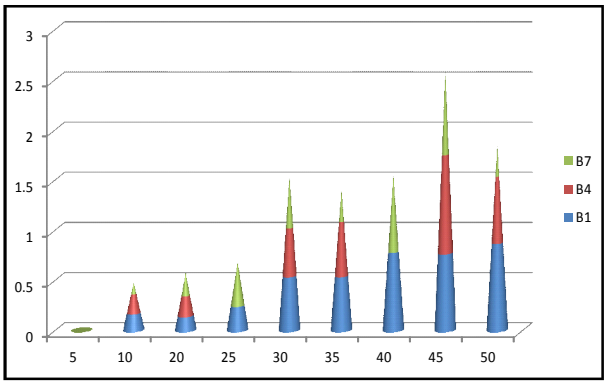


Fig. 5. Effect of temperature on concentration of growth of isolates (bacteria).

and reduce its production of antibacterial agents as metabolic compounds (secondary metabolic) by influencing enzyme activity.

The effect of NaCl on the growth of the isolates showed that they developed well in NaCl concentration till 3% and only B1 was able to grow till 6% NaCl concentration, while all isolates were unable to grow at 10% concentration of sodium chloride (Fig. 6).

After extracting the antibacterial agents by

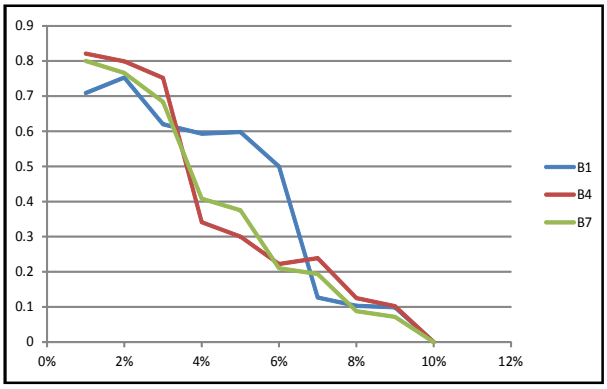


Fig. 6. Effect of NaCl concentration of growth of bacteria.

ethyl acetate solution (v/v) antioxidant activity was estimated**.** Gradually scavenging free radical increased till reaching to 91% (the formed DPPH free radical). This increase was caused when the extracellular crude extract concentration increased until reaching to 20 mg/ml (Mbwambo *et al.*, 2017). The IC50 of the obtained extract (extracellular crude) was 5.24 mg/ml meaning it inhibited 50% of free radical formed by DPPH, while the IC50 of standard (the ascorbic acid) was 0.492 mg/ml (Fig. 7). Ascorbic acid was used as a standard to measure scavenging activity of the isolates by making a standard curve (Fig. 8) and calculating the IC50 (Braca *et al.,* 2021).

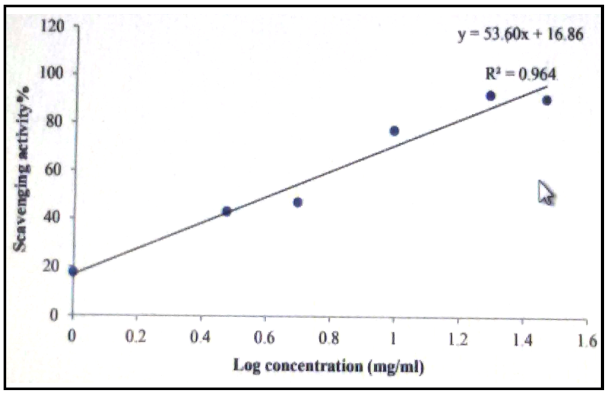


Fig. 7. Antioxidant activity of extract (extracellular crude) and DPPH scavenging activity.

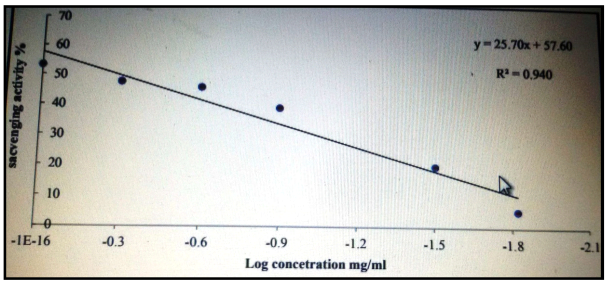


Fig. 8. Antioxidant activity of ascorbic acid in different concentrations and DPPH scavenging activity.

REFERENCES

Ababutain, I. M., Abdul Aziz, Z. K. and AL-Meshhen, N. A. (2016). Lincomycin antibiotic biosynthesis produced by *Streptomyces* sp. isolated from Saudi Arabia soil. II. Extraction, separation and ourification of lincomycin. *Cana. J. Pure Appl. Sci.* **6** : 1905-1911.

Abbas, F., Hafeez-ur-Rehman, M., Ashraf, M., Iqbal, K. J,, Andleeb, S. and Khan, B. A. (2020). Mucus properties of Chinese carp and Indian carp : Physical barrier to pathogens. *Iran J. Fish Sci.* **19** : 1224-1236.

Abu-Khumrah, N. M. H. (2014). Characterization of antimicrobial agents produced by some *Streptomyces* species isolated from agricultural soils in Babylon. M. Sc. thesis, Department of Biology, Babylon University.

Adel, M., Safari, R., Soltanian, S., Zorriehzahra, M. J. and Esteban, M. A. (2018). Antimicrobial activity and enzymes on skin mucus from male and female *Caspian kutum* (Rutilusfrisiikutum Kamensky, 1901) specimens. *Slov. Vet. Res.* **55** : 235-243.

Al-Thubiani, A., Maher, Y. A., Fathi, A., Abourehab, M., Alarjah, M. and Khan, M. (2018). Identification and characterization of a novel antimicrobial peptide compound produced by *Bacillus megaterium* strain isolated from oral microflora. *Saudi. Pharm. J.* **26** : 1089-1097.

Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M. and Morelli, I. (2021). Antioxidant principles from *Bauhinia terapotensis. J. Nat. Prod.* **64** : 892-895.

Budi, M. (2017). Performance of the selected main tree of liberoid coffee in the peatland of Kepulauan. Meranti, Riau. *J. Wetlands Environ. Manag.* **5** : 32-36.

Butler, M. S. (2015). The role of natural product chemistry in drug discovery. *J. Nat. Products* **12** : 2141-2153.

Chumroenphat, T., Saensouk, S. and Saensouk, P. (2021). Chemical composition and antioxidant activity of three species of *Cornukaempferia* in Thailand. *BIODIVERSITAS* **22** : 4036-4044.

Egorov, N. S. (2015). Antibiotics–A scientific approach. Mir Publishers, Moscow.

Havare, D., Basavaraj, K. and Murthy, P. S. (2019). Coffee starter microbiome and in-silico approach to improve Arabica coffee. *LWT* **114** : 108382.

Hoh, D. Z., Lin, Y. F., Liu, W. A., Sidique, S. N. M. and Tsai, I. J. (2019). Nest microbiota and pathogen abundance impact hatching success in sea turtle conservation. *BioRxiv. DOI : 10.1101/776773.*

Immanual, G., Dhanusa, R., Prema, P. and Palavesam, A. (2016) Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *Int. J. Environ. Sci. Technol.* **3** : 25-34.

Kumari, S., Tyor, A. K. and Bhatnagar, A. (2019). Evaluation of the antibacterial activity of skin mucus of three carp species. *Int. Aquat. Res.* **11** : 225-239.

Lee, L. W., Cheong, M. W., Curran, P., Yu, B. and Liu, S. Q. (2015). Coffee fermentation and

flavour–An intricate and delicate relationship. *Food Chem.* **185** : 182-191.

Litaudon, M., Jolly, C., Callonec, C. L., Cuong, D. D., Retailleau, P., Nosjean, O., Nguyen, V. H., Pfeiffer, B., Boutin, J. A. and Guéritte, F. (2019). Cytotoxic pentacyclic triterpenoids from *Combretum sundaicum* and *Lantana camara* as inhibitors of Bcl- xL/BakBH3 domain peptide interaction. *J. Natural Products* **72** : 1314-1320.

Mannaa, M. and Kim, K. D. (2018). Biocontrol activity of volatile-producing *Bacillus megaterium* and *Pseudomonas protegens* against *Aspergillus* and *Penicillium* spp. predominant in stored rice grains : study II. *Mycobiology* **46** : 52-63.

Mbwambo, Z. H., Moshi, M. J., Masimba, P. J., Kapingu, M. C. and Nondo, R. S. O. (2017). Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownie* roots and stem. *BMC Compl. and Alter. Med.* **7** : 9. *doi : 10.1186/1472-6882-7-9.*

Mellmann, A., Cloud, J., Maier, T., Keckevoet, U., Ramminger, I. and Iwen, P. (2018). Evaluation of matrix-assisted laser desorption ionization-time-off light, mass spectrometry in comparison to 16S rRNA gene sequencing. *J. Clin. Microbiol.* **46** : 1946-1954.

Moor, R. A., Bates, N. C. and Hancock, R. E. W. (2017) Interaction of polycationic antibiotics with *Pseudomonas aeruginosa* lipopoly saccharide and lipid a studied by using dansyl. polymyxin. *Antimicrobial Agents Chemoth.* **29** : 496-500.

Motta, A. and Brandelli, D. (2015). Characterization of an antimicrobial peptide produced by *Brevibacterium linens. J. App. Microbiol.* **52** : 357-363.

Nanjwade, B., Chandarshekhara, S., Ali, M., Goudanavar, P. and Manvi, F. (2020). Isolation and morphological characterization of antibiotics producing actinomycetes. *Tropical J. Pharma.* **9** : 21-26.

Rajesh, R. O., Mary Helen, P. A. and Jaya Sree, S. (2019). Screening of antibiotic producing actinomycetes from coconut husk retting sample. *Int. J. Res. Pharmac. Biomed.* **4** : 67-74.

Sharma, M., Sharma, P. D., Bansal, M. P. and Singh, J. (2020). Lantadene A-induced apoptosis in human leukemia HL-60 cells. *Ind. J. Pharma.* **39** : 140-144.

Somma, A. D., Moretta, A., Cane, C., Cirillo, A. and Duilio, A. (2020). Antimicrobial and antibiofilm peptides. *Biomolecules* **10** : 1-15.

Sudha, S. and Masilamani, S. M. (2019). *In vitro* cytotoxic activity of bioactive metabolite and crude extract from a new actinomycete, streptomyces avidinii strain SU4. *Int. J. Pharm. Sci.* **5** : 612-616.

Taware, R., Abnave, P., Patil, D., Rajamohananan, P. R., Raja, R., Soundararajan, G., Gopal Chandra, Kundu, G. C. and Absar Ahmad, A. (2014). Isolation, purification and characterization of Trichothecinol-A produced by endophytic fungus *Trichothecium* sp. and its antifungal, anticancer and antimetastatic activities. *Sustainable Chemical Processes* **8** : 1-9.

Tyor, A. K. and Kumari, S. (2016). Biochemical characterization and antibacterial properties of fish skin mucus of fresh water fish, *Hypophthalmichthysnobilis. Int. J. Pharm. Sci.* **8** : 132-136.

Vestby, L. K., Gronseth, T., Simm, R. and Nesse, L. L. (2020). Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics* **9** : 1-29.

Yupparach, P., Wongsuwan, P., Phokham, B., Sangnark. S. and Picheansoonthon, C. (2019). Two new species of *Cornukaempferia* (Zingiberaceae) from Thailand. *J. Jap. Bot.* **94** : 82-89.