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Production, purification, and identification of Glycine, N-(m-anisoyl)-methyl ester from *Pseudomonas aeruginosa* with antimicrobial and anticancer activities.

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Abstract

The present research is a trial to extract antimicrobial and anticancer substance(s) from *Pseudomonas aeruginosa*. The bacterial isolate S1B20 exhibited the highest antimicrobial activity that was identified based on 16S rRNA gene sequence as *Pseudomonas aeruginosa* MG429777. Extraction of various antimicrobial substance(s) (both proteinous and non-proteinous substances) were carried out. Chloroform was established to be the strongest solvent for extraction of the antimicrobial substance, while the 60% ammonium sulfate saturation stage was the brightest to obtain active bacteriocin fraction. The molecular weight of the refined bacteriocins estimated as 124 kDa. Qualitative and quantitative results of GC-mass spectral analysis and IR spectroscopy of non-proteinous and proteinous antimicrobial agents were Oxime methoxyphenyl and Glycine, N-(m-anisoyl)-methyl ester. The research concerned the proteinous compound and proved to have a distinct antibacterial and antitumor force against three carcinoma cell lines.

Keywords: Anticancer; GC-mass; Glycine; N-(m-anisoyl)-methyl ester; *Pseudomonas aeruginosa*.

1. Introduction

One of the interesting research spots develops from multi-resistant pathogenic microorganisms as negative effects of wastewater and sewage allocation in varied environmental conditions.The microbial products of secondary metabolism provide a unique plan for the biosynthesis of many medications. Microbial bioactive products are as mycotoxins or bacteriocins determined from specific microorganisms (Woappi *et al*., 2013). *Pseudomonas aeruginosa* is a ubiquitous gram-negative γ-proteobacterium that has been isolated from diverse origins, covering from environmental samples (water, rhizosphere) to humans (Pirnay *et al*., 2009). To support its diversity in the community, it gives various toxic proteins, identified as bacteriocins. A particular *P. aeruginosa* strain also encounters other Pseudomonads within the same antheory. To compete with these intimately similar organisms, *P. aeruginosa* excretes bacteriocins to the surrounding environment. These toxic chemicals are furnished under stressful conditions, like nutrient deficiency or overpopulation (Dingmans *et al*., 2016).

As in a previous study (Enayat *et al*., 2017), we obtained the optimized medium in which the antimicrobial agent (s) produced by *Pseudomonas aeruginosa* S1B20 gave the highest inhibitory effect, the most antibiotic-resistant bacteria. The obtained optimal medium condition was achieved at pH 7, temp. of 37 ºC, inoculum size 2ml and

incubation period of six days under shaking condition (150 rpm) in minimal salt medium supplemented with mannitol as the best C source (4%) and yeast extract as the best nitrogen source (2%) in the conical flask of 100 and 250 ml capacities. Moreover, aspartic acid is the best amino acid with additives such as vitamin B and $MgCl_2$ (50ppm). Following the preceding study, we realize that to complete the picture of purity and characterize the produced antimicrobial agent (s), reduce the cost of production, and study its functional activity against multi-drug resistant microbes and antitumor action to three carcinoma cell lines.

2. Materials and methods

All the chemicals used were of high grade purchased from Sigma Aldrich. And fine chemicals from Fermentas.

2.1. Production of antimicrobial agent with optimized medium and preparation of cell-free filtrate (CFF)

The most potent bacterial isolate *Pseudomonas aeruginosa* S1B20 allowed growing on the production medium under all the optimal conditions previously achieved and exhibited the highest antimicrobial production as earlier work (Primary identification of the potent strain followed the API biochemical testes (Enayat *et al*., 2017), which conformities by molecular tool analysis in this study.

2.2. Molecular Identification of the selected bacterial isolate by 16S ribosomal RNA.

Isolation of bacterial DNA made by using Genomic DNA Purification (Qiagen, USA).

2.3. PCR amplification of 16S rRNA gene

The Reaction mixture for amplification of the 16S rRNA gene from isolated genomic DNA containing; a 10-ng aliquot of DNA; 10 WM of each universal primer to the 16S rRNA of bacteria, 8F(5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R(5'GGTTACCTTGTTACGACTT-3'); 10U buffer; 1 mm each dNTPas; 2.0 mm $MgCl_2$; and 1 U Promega Taq (Fermentas, Germany) up to 50 µl with distilled water. PCR amplification was performed using a multigene gradient thermocycler (Labnet, USA).

Reactions were first incubated at 94ºC for for 3 min; 35 cycles as follows: 94ºC for 45 s, 55ºC for 1 min. And 72ºC for 1 min 30 *s*. Reactions were then incubated at 72ºC for another 10 min. The obtained PCR products were visualized on Ethidium bromide-stained 1% agarose gel under UV UV transilluminator (Biorad, Germany) to confirm the presence of a full length of 16S rRNA gene about 1, 5 kb band.

2.4. 16S rRNA sequencing

The amplified products of approximately 1485bp band were purified using the PCR Purification Kit (Qiagen, Germany). Purified PCR product was directly bi-directional sequencing using primers 8F and 1492R by Sanger sequences protocol using a Big Dye terminator cycle sequencing kit (Applied Biosystems, CA).

2.4.1. Phylogeny analysis

Obtained sequencing products were aligned with other similar sequences downloaded from Gene Bank using Cluster X (Thompson *et al*., 1997) and determined its identical or unique sequence. Moreover, the Sequences were compared to the compilation of 16S rRNA gene sequences available in databases NCIB/BLAST (Altschul*et al*., 1997) to determine the highest similarity to Gene Bank and EMBL database sequences.

2.5. Solvent extraction of non-proteinous antimicrobial agent

Different organic solvents used for the extraction for the extraction of non-proteinous

(non-bacteriocin) antimicrobial agent from *Pseudomonas aeruginosa* S1B20 to detect the best one for the extraction procedur*e.* Equal volumes of cell-free filtrate and each organic solvent except chloroform added in duple volume, separately mixed thoroughly by shaking in 250 ml separating funnel and allowed to stand for 30 min. Two layers separated the aqueous and organic layers, which contained the solvent and the antimicrobial agent. The organic layer collected and stored at 4ºC till tested. The aqueous layer re-extracted, and the organic layer was added to the other organic layer. The evaporation continued until the viscous syrup was obtained. The residual syrup dissolved in the least amount of the solvent and filtered through Whatman No. 1 filter paper. Antibacterial activity test for each syrup carried out against the most resistant bacteria isolates *S. aureus* I37 and *E. Coli* I48 using well diffusion assay as described by (Balouiri *et al*., 2016).

2.5.1. Ammonium Sulfate extraction of proteinous (bacteriocins) antimicrobial agent

The cell-free filtrate (CFF) is referred to as the 'crude bacteriocins preparation.' Ammonium sulfate precipitation partly purified this CFF (Sayed & Safia, 2015), and the antimicrobial activity at each concentration was analyzed by agar well diffusion assay.

2.5.2. Dialysis

Removal of the traces of ammonium sulfate (by cellulose ester membrane) is called the dialysis proces*s.* It is re-suspending in sodium phosphate buffer 50 mM (pH 7.0) and inserted into a special pre-treated plastic dialytic bag (bags previously boiled to soften in distilling boiling water) overnight. Agar well diffusion assay was used to document antibacterial behavior of the desalted protein

2.5.3. Fractional purification by applying on column chromatography packed with Sephadex G-75

The dialyzed components were partially purified and applied to a column (2.5 cm x 30 cm) using Sephadex G-75, as described by (Naz *et al*.,2015). Four ml of the bacteriocins specimen prepared were added to the top gel layer. By running the column, it allowed the gel to pass through. It gathered 25 fractions (5 ml each). For each separate fraction, the antimicrobial assay was performed.

2.5.4. Polyacrylamide gel electrophoresis (SDS-PAGE) technique

The active fraction obtained had been subjected to PAGE (13 % polyacrylamide gel) using a vertical plate gel (Bio-Rad, USA) for detecting its molecular weight by comparing with protein marker (covering from 26 to 315 kDa, Sigma). Running buffer without any air bubbles flowed into the electrophoresis container. The current is disclosed when the Bromophenol blue dye reaches 1cm above the bottom of the gel. The gel was washed twice then it was submerged and thrown into the developing solution till the bands were created. A molecular weight measurement of the antimicrobial material relative to a marker was estimated.

2.5.5. Determination of minimum inhibitory concentration (MIC) of the most potent active fraction against the most resistant bacteria isolates

The active fraction with the highest antimicrobial activity to find its minimum inhibitory concentration (MIC) against the most resistant bacteria isolates, *S. aureus* I37 and *E. Coli* I48 using a suitable diffusion assay. Different concentrations of the antimicrobial active fraction were prepared between 0.5- 16 mg/ml with a double fold

serial dilution. Each well filled with 100 microns of a specific concentration of the active compound on agar plates seeded with the test organisms. We kept the plates in the refrigerator for 2hr and then incubated at 37ºC for 24 hr. The response was observed as a clear zone around the well. MIC determined as the lowest concentration causing inhibition(Vamanu, 2012).

2.6. Identification of antimicrobial compounds produced by *Pseudomonas aeruginosa* S1B20 Identification of both proteinous and nonproteinous bioactive compounds produced by *Pseudomonas aeruginosa* S1B20 carried out by Gas Chromatography/Mass Spectroscopy (GC/MS) and Infra-Red spectrum (IR) techniques.

2.6.1. Analysis (GC/MS) of the most potent fractions

The GC-MS study was performed in the Shimadzu GC-MS-QP 2010 Plus with the capillary column RTX-5 (60 m 9 0.25 mm 9 0.25 lm). It was set at a standard temperature of 70°C and held for 2 minutes at this temperature. At this stage, an addition of 5°C / min of the oven temperature grew up to 280°C and stayed for 9 min. The injection port temperature was as high as 260°C, and the Helium flow rate was as low as 1 ml/min. The voltage of ionization was 70 ev. In the split procedure, the samples are injected as 10:1. Spectral scale for mass scanning adjusted to 45–450. The discovery of bioactive compounds found in extracts identified with reports from NIST05 (National Institute of Standards and Technology, US), WILEY 8, and FFNSC1.3 (Flavour and Fragrance Natural and Synthetic Compounds) libraries.

2.6.2. Fourier transform infrared spectroscopy (FTIR) of the most potent fractions

FTIR of solid samples recorded over a spectral region from 4000 to 650 cm^{-1} using SHIMADZU FT-IR (Germany) spectrophotometer model. Spectra for specimens in solution form recorded over a spectral region from 10,000 to 300 cm^{-1} using Perkin Elmer Spectrum GX FT-IR provided with spectrum v 5.3.1 software. A quartz cuvette was used to investigate the liquid specimens.

2.6.3. Antifungal activity assay of the effective compound Glycine, N-(m-anisoyl)-methyl ester

The antifungal activity brought out using the agar diffusion technique, according to (Balouiri *et al*.,2016), against four pathogenic fungal strains, i.e., *Aspergillus fumigatus* (RCMB 002008), *Candida albicans* RCMB 005003(1) ATCC 10231, *Syncephalastrumracemosum* RCMB 016001 (1), and *Penicillium expansum* RCMB 001001 (1) IMI 28169.

2.6.4. Antitumor activity Assay of the active compound Glycine, N-(m-anisoyl)-methyl ester

2.6.5. Antitumor activity assessment using viability assay

The purified compound checked against three lines of mammalian cells: the hepg-2 cells (human Hepatocellular carcinoma), cells MCF-7 (human cell line of breast cancer), and HCT-116 (cell line of colon carcinoma). The approach was defined in this antitumor viability experiment (Wilson, 2000). Data analysis of the cell viability percentage measured using the ExcelMicrosoft. Cell viability rate determined according to the following figure:The proportion of cell viability = [1–(ODt/odc)] x 100%, Where ODt is the mean optical density of wells treated with the investigated compounds and ODt is the mean optical density of untreated cells. It also correlated the standard compounds using IC50 value, i.e., the concentration of an individual compound contributing to 50% cell death determined from the graphical figure of recovering cells vs. compound concentrations (Gomha *et al*., 2015).

2.7. Statistical analysis

The results were displayed as mean using SPSS statistical software. With Duncan's test at $p \leq 0.05$, the differences between mean values were assessed.

3. Results and Discussion

3.1 Identification of the most potent bacterial isolate using molecular methods

Sequence analysis of 16S rRNA gene of the nuclear-encoded rRNA(1485 bp) of the promising *Pseudomonas aeruginosas* S1B20 examined through BLAST and FASTA searches and direct analogies with potentially related taxa. The sequence analysis indicated significant alignments 97–100% and satisfactory correlation with selected *Pseudomonas aeruginosa* strain corresponding to the sequences from the NCBI Gene Bank database using cluster X. They strongly classified the isolated strain of bacteria according to the alignment analysis and given Submission No. SUB2959477 and Accession number MG429777 from Gene Bank.

3.2 Molecular phylogeny

According to the preliminary analysis of the 16S rRNAgene sequence of *Pseudomonas aeruginosa* MG429777 and sequences of 6 related strains downloaded from Gene Bank database for construct phylogenetic analysis tree using MEGA 6.0 software, see (Figure 1). Kim *et al*.,2017, reported that the 16S rRNA gene sequence is a valuable tool for phylogenetic and characteristic distinction (differentiating unknown isolates to species or strain level), which can be genetically modified in a successive phonetic study and various ancestral studies according to ordinary taxonomic techniques. The behavior of huge clonal groups of these microorganisms in the ecosystem (Streeter &Katouli, 2017) has resulted in enormous diversification.

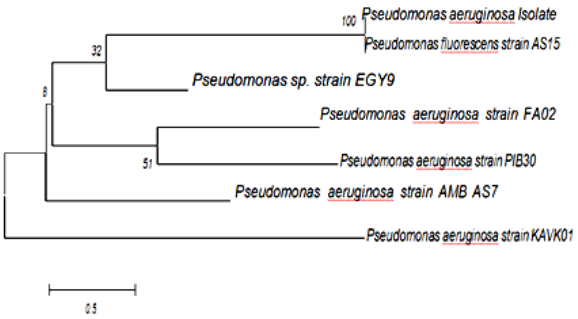


Fig. 1. A dendrogram showing the sequence relationships between *Pseudomonas aeruginosa* MG429777 and other *Pseudomonas* strains

3.3. Extraction, separation, and purification of antimicrobial agent produced by *Pseudomonas aeruginosa* MG 429777

In all the tested solvent systems, chloroform was the best solvent for the extraction of antimicrobial substances (Figure 2).Other workers recorded various solvents for extracting the antimicrobial agent biosynthesized by different kinds of microorganisms, viz. Chloroform (Agrawal & Chauhan, 2016; Özyürek*et al*.,2016).

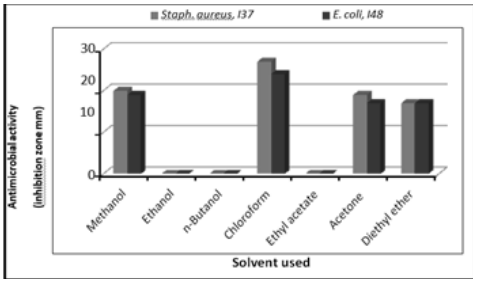


Fig. 3. Ammonium Sulphate extraction of proteinous(bacteriocin) compound against *S. aureus*, I37and *E. Coli*, I48.

Data represented in (Figure 3) signed the most active bacteriocin fraction at 60% ammonium sulfate saturation level. We carried out the extraction of the proteinous antimicrobial agent (bacteriocins) produced by *Pseudomonas aeruginosa* MG 429777 fractional precipitation by ammonium sulfate since it is highly soluble in water, cheap, and has no deleterious effect on the structure of the protein(Saeed *et al*., 2005). Results showed that rising ammonium sulfate concentration up to 60% saturation indices the antimicrobial activity, with 32 to 27.8 mm inhibition areas

against Staph *aureus*137 and *E. Coli* I48 respectively, we notice a decrease in antimicrobial activity above this value. Antimicrobial activity was noticed to be lowered over this value. Based on the current results, Sayed&Safia,2015, demonstrated that *Pseudomonas aeruginosanrrl* B-227 peptide antibiotic was treated to 60 % saturation with ammonium sulfate. Muhammad *et al*.,2015, stated that *Pseudomonas luteola* showed an inhibitory zone higher than initial in optimized growth conditions and following partial purification with 60% ammonium sulfate precipitation. The bacteriocins that *Pseudomonas aeruginosa* SA 188 produces with 70% ammonium sulfate precipitation are partially purified by Naz &Rasool, 2013.On the contrary, Shanmugaraju *et al*., 2012, also revealed that a *Pseudomonas aeruginosa* peptide antibiotic was treated with 30% saturation of ammonium sulfate.
The variation in antimicrobial activities of different extraction methods against *S. aureus* I37 isshown (see Plate 1).

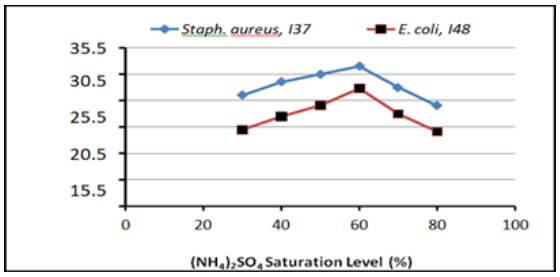


Fig. 3. Ammonium Sulphate extraction of proteinous(bacteriocin) compound against *S. aureus*, I37and *E. Coli*, I48.

Since the antimicrobial activity of the proteinous antimicrobial agent (bacteriocin) was higher than that of a non-proteinous antimicrobial substance, further studies were carried out. The most active proteinous antimicrobial agent was dialyzed against 50 mM sodium phosphate buffer (pH 7), then it was applied to the Sephadex G-75 column chromatography. Twenty-five fractions were obtained (each of 5 ml). All fractions were organisms using the agar diffusion technique (see plate 1). The antimicrobial activity was recorded in fractions 7 up to 17. tested for their activities against the target

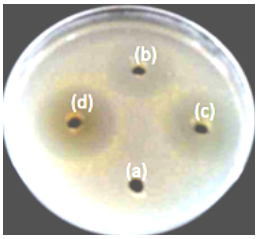


Plate 1. Differences in the antimicrobial activities of different extraction products against *S. aureus*, I37.
A = Chloroform only, B= Extract of chloroform.
C = 60% Ammonium sulfate precipitate after dialysis.
D = Fraction No. 17 obtained by column chromatography.

The highest activity was represented in fraction 17, as it showed inhibition zones of 32 &27.8 mm against *S. aureus* I37 and *E. coli* I48, respectively (see Figure 4). Naz *et al*.,2015, found that bacteriocins obtained by *Pseudomonas aeruginosa* SA189 were purified by applying Sephadex G-75 column chromatography. On the other hand, gel filtration through Sephadex G-150 was reported by Shanmugaraju *et al*.,2012.

Table (1): Determination of minimum inhibitory concentration(MIC) of the most potent fraction against *S. Aureus*, I37 and *E. Coli*, I48*Aureus*, I37 and *E.Coli*, I48.

Conc. (mg/ml)	**Antimicrobial activity (inhibition zone mm)**	
	S. Aureus, I_{37}	***E. Coli,*** I_{48}
16	24±0.56	21±**0.45**
8	15±**0.34**	13.5±0.50
4	**12.9±0.34**	**11.8±0.23**
2	0	0
1	0	0
0.5	0	0

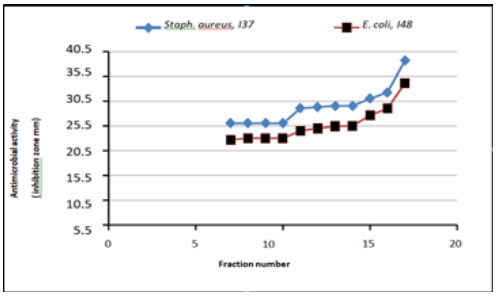


Fig. 4.. Fractionation pattern of bacteriocin produced by *pseudomonas aeruginosa*.

3.3.1. Polyacrylamide gel electrophoresis (PAGE) system

We dealt with the purified proteinous antimicrobial compound (bacteriocins) formed by *Pseudomonas aeruginosa* MG 429777 to SDS-PAGE analysis. We assessed the molecular value of the pure bacteriocins giving 124 kDa. Plate2. showed that the purified bacteriocins which contributed to *Pseudomonas aeruginosa* MG 429777 contained the individual protein band. Naz *et al*.,2015, documented that, electropherogram of purified bacteriocins SA189 revealed an individual band, which corresponded to the molecular size of almost 66 kDa. Ling *et al*.,2010,showed a single band comparable to molecular weight 62.4 kDa on the SDS-PAG*E*. On the other hand, (Muhammad *et al*.,2015) evaluated the molecular size of the antimicrobial peptide made by *Pseudomonas luteola* to be fewer than 20 kDa. In another review, (Fontoura *et al*.,2017) illustrated a sizable band of about 30 kDa on SDS–PAG*E*. Each value represents the mean of three replicate*s*. P< 0.05.

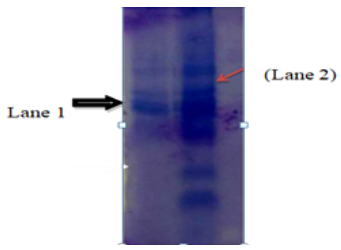


Plate 2. SDS-PAG*E*. Of purified bacteriocins, Lane (1): Purified bacteriocins and Lane (2): Crude bacteriocin*s*.

3.3.2. Determination of minimal inhibitory concentration (MIC) of the proteinous antimicrobial compound made by *Pseudomonas aeruginosa* MG 429777

We exposed the most potent fraction of various dilutions to check the MIC, as displayed in Table 1. The MIC of the effective compound was 4mg/ml.

3.3.3. Recognition of antimicrobial compounds produced by *Pseudomonas aeruginosa* MG 429777

Firstly, we deal with identifying nonproteinous antimicrobial compounds where GC-mass spectral evaluation of non-pretentious antimicrobial agents extracted by chloroform from *Pseudomonas aeruginosa* MG429777 (see Figure 5).

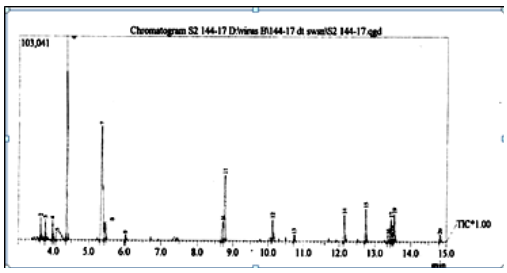


Fig. 5. GC-mass spectral analysis of non-proteinous antimicrobial agent extracted by chloroform from *Pseudomonas aeruginosa* MG429777.

GC-MS Chromatograph of *Pseudomonas aeruginosa* MG429777 chloroform extract had proved an acute peak at retention time 4.381 minutes that gave an intense molecular peak at 133 m/z.

3.4. Infra-red (IR) spectrum of the non-proteinous antimicrobial compound produced by *Pseudomonas aeruginosa* MG429777

As demonstrated in (see Figure 6), The bands detected at 3437.26 cm^{-1} and 1639.55 cm^{-1} were referred to as the stretching pulses of primary and secondary amines (Vigneshwaran *et al*., 2006). Similarly, (Vithiya & Sen,2010) have declared

functional groups as –C– O–C–, –C–O– and –C=C– were obtained from proteins (heterocyclic compounds).

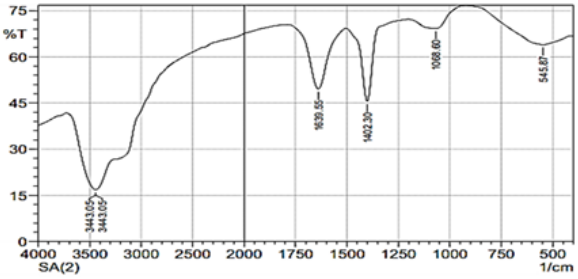
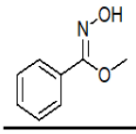


Fig. 6. The IR spectrum of bioactive compound obtained by chloroform extraction by *Pseudomonas aeruginosa* MG429777.

Quantitative results of GC-mass spectral study and IR scope of non-preteinious antimicrobial agent separated by chloroform from *Pseudomonas aeruginosa* MG429777 presented in Table 2.

Table 2. Quantitative results of GC-mass spectral analysis of non-proteinous antimicrobial agent extracted by chloroform from *pseudomonas aeruginosa* MG429777.

Peak ID	Retention Time(mi)	Molecular Ion (m/z)	Molecular Weight	Name	Chemical Formula
				Oxime	$C_8H_9NO_2$
6	4.381	133	151	Methyl Phenyl	



The results proposed the chemical formula of C8H9NO$_2$, and the compound was Oxime-methoxy-phenyl with the chemical design (Table 2). Oxime- methoxy-phenyl, yielded from chloroform extraction, came out to have antibacterial action (Barghouthi *et al*.,2017) and (Abeer *et al*.,2018). Oximes with diverse structures have found multiple uses (Sørensen *et al*., 2018); in the agro-sector as plant protectants, insecticidal, fungicidal, and herbicidal activity (Wang *et al*., 2017). In the health sector is used to treat nerve agent poisoning (Singh *et al*., 2015).

3.5. Identification of proteinous antimicrobial compound

GC-mass spectral analysis of proteinous antimicrobial agent had shown a sharp peak at retention time 14.871 minutes which gave an intense molecular peak at 223 m/z. Other minor peaks were found in a negligible amo

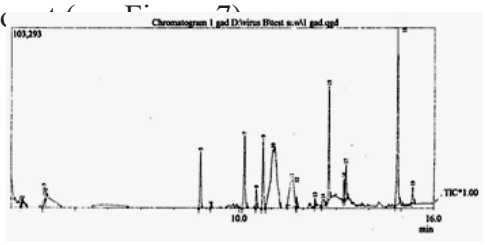


Fig.7. GC-mass spectral analysis of proteinous antimicrobial agent (bacteriocin) produced by *Pseudomonas aeruginosa* MG429777.

3.5.1. IR spectrum of the proteinous antimicrobial compound obtained by gel filtration chromatography

The absorbent bands of the compound detected by infrared (IR) were 3437,26 and1639,55 cm^{-1}, owing to the presence of amine group –NH; all bands of 3034,13 and 2941,54cm^{-1}, due to alkyl group – C – H; bands of 2403,38 and 2330,09 cm^{-1} attributed to a carboxylic acid; bands of 1535,39 cm^{-1} due to the existence of an aromatic ring of C = C, band of 1301,99 cm^{-1}, due to the presence of carboxylic acid in the group of OH group; band at 1219.05 cm^{-1} due to the presence C=O group of ketone; band at 1057.03 cm^{-1} due to the presence C–O bonding in alcohols and esters and band at 761.91 cm^{-1}due to the presence=C–H bonding in aromatic ring (see Figure 8).

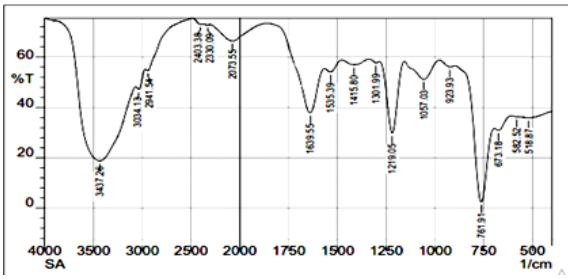


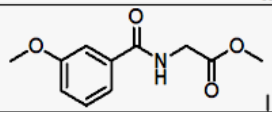
Fig. 8. The IR spectrum of bioactive compound was obtained by gel filtration chromatography by *Pseudomonas aeruginosa* MG429777.

Table 3 showed the chemical formula C11H13NO4, and the compound was Glycine, N-(m-anisoyl)-methyl ester with chemical

structure. Glycine treating depression, avoiding brain shocks, and used in Ointment for abscesses, mitigating damage. Glycine as "nutritionally unessential amino acid" NEAA is an important component of genetic balancing, cell signaling process, digestive operation, nutrient dissemination of ingredients, proteolyzes, glucose and lipid absorption, hormone conditions, mortality of men and women (Hou *et al*., 2015).

Table 3. Quantitative results of GC-mass spectral analysis and IR spectroscopy of proteinous antimicrobial agent produced by *Pseudomonas aeruginosa* MG429777.

Peak ID	Retention time(min)	Molecular ion (m/z)	Molecular weight	Name	Chemical formula
18	14.871	223	223	Glycine, N-(m-aniso)-methyl ester	$C_{11}H_{13}NO_4$



3.6.. Application on the most active proteinous antimicrobial compound (Glycine, N-(m-anisoyl)-methyl ester)

Data recorded that the compound Glycine, N-(m-anisoyl)-methyl ester had no antifungal activity against the tested pathogenic fungal strain.

3.7. Evaluation of the antitumor activities of Glycine, N-(m-anisoyl)-methyl ester produced by *pseudomonas aeruginosa* MG429777

Inhibitory activity of active compound Glycine, N-(m-anisoyl)-methyl ester produced by *Pseudomonas aeruginosa* MG429777 against HCT-116 (colon carcinoma cell line), HepG-2cells (human Hepatocellular carcinoma), and MCF-7cells (human breast cancer cell line) was 94.81, 90.42 and 87.97 as percentage respectively (see Figures 9, 10, 11). Moreover, IC50 was found to be 28.8, 48.7, and 59.4 µg/ well, respectively. This finding showed a dose-dependent and cell-specific agreement with the finding of Abdi-Ali *et al*.,2004.

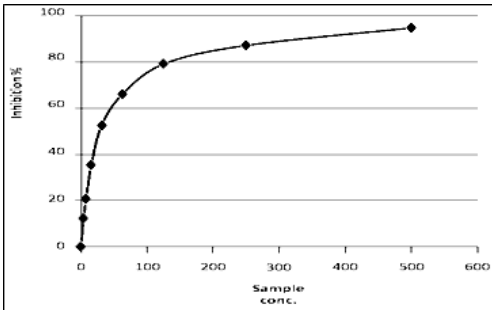


Fig. 9. In vitro inhibitory activity of Glycine, N-(m-anisoyl)-methyl ester against colon carcinoma cell line (HCT-116).

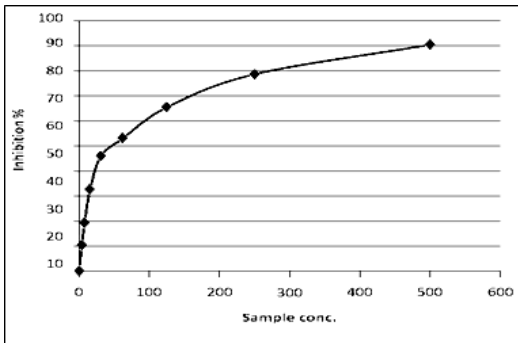


Fig. 10. In vitro inhibitory activity of Glycine, N-(m-anisoyl)-methyl ester against human Hepatocellular carcinoma cell line (HepG-2 cells).

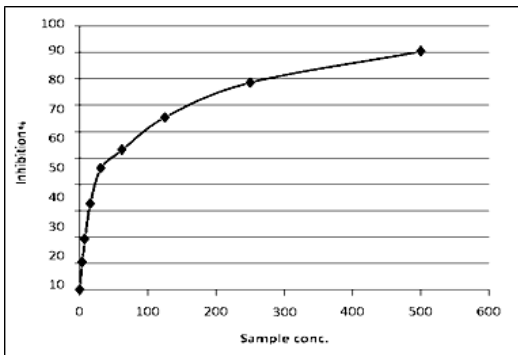


Fig. 11. In vitro inhibitory activity of Glycine, N-(m-anisoyl)-methyl ester against human breast cancer cell line (MCF-7 cells).

4. Conclusion

This study is the first report to reveal that it is possible to produce bioactive antimicrobial compounds by *Pseudomonas aeruginosa* MG429777; hence, these bacteria consider potent bioactive sources for anticancer against colon carcinoma cell line human Hepatocellular carcinoma, and human breast cancer cell line. Inhibitory activity of active compound Glycine, N-(m-anisoyl)-methyl ester encourage us to continue searching other active compounds where this strain has medical applications.

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