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Extraction, Separation and Characterization of Antibiotic Compound produced by *Streptomyces rimosus* (ACW9) Active against MDRs Uropathogens

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Abstract: ***Soil is a well-studied ecological niche for the discovery of beneficial biologically active chemicals like therapeutically relevant antibiotics. Actinobacteria have been recognised as one of the largest microbial groups in soil that are capable of producing a wide range of beneficial secondary metabolites and compounds with varying characteristics. Therefore, the current study aimed to assess antimicrobial and antioxidant activities of Streptomyces rimosus ACW9 isolated from wheat farm soil. Molecular identification (16S rRNA ribotyping) was used to identify strain ACW9. Ethyl acetate crude extract displayed promising antibacterial activity against clinically isolated uropathogens, Enterobacter* **sp.** *(20 mm) and Bacillus subtilis (14 mm). In addition, the extract showed 62.3% DPPH scavenging with*** IC_{50} ***= 1.829 mg/ml. The characterization of the purified metabolites from* **strain** *ACW9 was done by GCMS, FTIR, and HPLC techniques. The profound metabolites of ethyl acetate extract were identified by GCMS were cyclohexane, (-2-Nitro-2-Propyl), hexadecane, and pentanoic acid. It may be concluded that actinobacteria isolated from wheat farm soil is a promising source for medicinally important bioactive compounds.***
Keywords: Actinobacteria; Uropathogens; Antibacterial activity; GCMS; FTIR; HPLC

I. INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections that can occur in any part of the urinary system, including the kidneys, ureters, bladder, and urethra [1], [2]. The bladder and urethra are the most infected parts of the lower urinary tract [3]. Each year, around 150 million people globally have UTI, resulting in huge socioeconomic costs. UTIs are more frequent in women than men due to many risk factors, including coitus, female anatomy, vaginal infection, diabetes, and an individual history of UTI. Nearly 50-60 % of women experienced this infection at least once in their lives [4], [5]. Gram-negative bacteria such as *Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae,* and *Citrobacter* spp. Among Gram-positive bacteria, *Staphylococcus saprophyticus, Enterococcus faecalis,* and *Streptococcus agalactiae* are the most common and predictable types of bacteria that can cause UTI infections. Antibiotics are routinely used to treat UTIs, which results in long-term changes in the susceptibility and resistance patterns of different bacterial strains implicated in the pathogenesis [6], [7]. The increased frequency of multi-drug resistance (MDR) pathogens as well as cross resistances with existing antibiotics pose a serious threat to public health [8].

The search for a novel bioactive compound is desperately needed to combat this drug resistance issue. Therefore, the classic strategy of isolating and cultivating novel microbes from natural environments such as wheat field soil is still beneficial and has resulted in the identification, production, and commercialization of antibiotics. Soil microorganisms are important for the development of novel pharmaceuticals, nutriment materials, cosmetics, enzyme inhibitors, immune modifiers, and vitamins [9], [10]. Among the soil microorganisms, the actinomycetes constitute a major group with 80 species [11], practically all of which are from terrestrial soils, where they live mostly as saprophytes and endophytes with significant chemical and morphological variation but originating from a distinct evolutionary route [12]. Actinobacteria are gram-positive bacteria with high G+C content in their DNA and a characteristic filamentous appearance [13]. They are recognised as the most ubiquitous source of secondary metabolites, particularly antibiotics as well as anticancer, antiparasitic, antiviral, and antifungal compounds [14], [15]. More than 70–80% of all known antibiotics have been isolated from actinomycetes and are used in medicine and agriculture [16]. The genus *Streptomyces* is the biggest producer of antibiotics. Several microbial secondary metabolites are reported to be rich sources of therapeutic drugs [17].

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The present study aimed to evaluate some actinomycetes from soil samples collected from wheat farm fields for harnessing their antimicrobial activity against certain pathogens that are known to cause urinary tract infections.

II. MATERIALS AND METHODS

A. *Isolation of Actinobacteria from soil sample*

For the isolation of strain ACW9, soil sample was collected in a Nasco sampling bag (Himedia) from wheat farm field in Haridwar, India. Actinobacterial strains were isolated on actinomycetes isolation agar (AIA) using the 10-fold serial dilution technique. All the plates were incubated at 28°C for 6-7 days. The isolated colonies were maintained on glycerol yeast agar medium plates at 4°C until further analysis.

B. *Test Human Uropathogens*

In our study, the following uropathogens, *Enterobacter tabaci* (MW785203), *Pseudomonas aeruginosa* (MW970251), *Bacillus subtilis* (MW785179), *Staphylococcus aureus* (MW952818) were used to assess the antimicrobial activity. All these uropathogens were clinical isolates. All test strains were maintained in nutrient agar slant at 4°C.

C. *Primary Screening By Cross Streak Method*

The antagonistic activity of isolated strain ACW9 was determined using the cross-streak method. Strain ACW9 was streaked in the centre of the plate and incubated at 28°C for 5-6 days. After that, freshly grown test microbial strains were streaked perpendicular to the edge of the initial streak and incubated at temperature 35°C for another 24-48 hours. The distance of inhibition between strain ACW9 and the test uropathogens was used to calculate inhibitory activities [18].

D. *Characterization Of Active Actinobacteria ACW9*

Morphological characteristics were studied by culturing ACW9 on AIA and broth medium, as described in Bergey's Mannual of Determinative Bacteriology.

Scanning electron microscopy was used to examine the morphological spore pattern [19]. For physiological characterization, different carbon sources were accompanied in basal medium at a concentration of 1% and incubated at different temperatures (20°C, 25°C, 30°C, 35°C, 40°C), pH (5-9), and NaCl (1-5 % w/v) ranges. Biochemical attributes like production of some specific enzymes were studied using catalase, oxidase, urease, and gelatin hydrolysis. All the results were demonstrated as either positive or negative.

E. *Molecular identification -16S rRNA ribotyping of ACW9*

16S rRNA gene sequencing method was employed for molecular characterization. The genomic DNA of ACW9 was isolated using method described by [20].

Actino specific forward primer 16S (5'-GGATGAGCCCGGCCTA-3') and 16S reverse primer (5'-CGGTGTGTACAAGGCCCGG-3') were used to amplify the 16S ribosomal sequence from genomic DNA in a thermal cycler. The Blast tool was used to compare the similarity of the obtained sequence.

The phylogenetic tree was built using neighbour joining method using MEGA X. The 16S rRNA sequence was submitted to NCBI GenBank using Banklt protocol.

F. *16S rRNA Bioinformatics*

Mfold webserver (http://unfold.rna.albany.edu/) was used to predict the secondary structure stability of 16S rRNA in terms of Gibbs free energy. The sequence of *Streptomyces rimosus* was submitted to the Mfold server and run on the default setting. Furthermore, restriction sites were also assessed using the NEB cutter (nc2.neb.com/nebcutter2/).

G. *Bioactive production and fermentation of ACW9*

Seed culture of ACW9 was inoculated in 500 mL of ISP-2 medium and incubated at 180 rpm, 30°C for 7 days. After incubation period, culture broth was centrifuged at 5000 rpm for 20 min and supernatant was collected separately.

Bacterial metabolites from supernatant were extracted in a separating funnel thrice with an equal volume of ethyl acetate (1:1). Finally, organic ethyl acetate layer containing metabolite was collected and concentrated using the vacuum rotatory evaporator. The crude extract thus obtained was recuperated in Dimethyl sulfoxide (DMSO) and bio-assayed against test organisms.

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H. Antagonistic action against test uropathogens

Disc diffusion method was used to assess the antimicrobial activity of crude extract against test uropathogens. Test organisms were subcultured into broth medium and incubated at 34°C for 24 hours. Using sterile nutrient broth, the growth density was adjusted until the desired turbidity at the 0.5 McFarland standard was obtained and swabbed on Muller Hinton agar plates. Paper discs were loaded with 100 µl of crude extract, and DMSO and levofloxacin were used as negative and positive controls, respectively. The loaded discs were placed on test uropathogen cultured plates and incubated for 24-48 hours at 34°C. Following incubation, the zone of inhibition around the discs was measured. Each experiment was carried out in triplicate.

I. DPPH radical scavenging activity of active isolate ACW9

DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay of crude extract was measured using the method described by [21]. In brief, different concentrations of methanolic extract of ACW9 ranging from 0.5-2.5 mg/mL were added with DPPH solution (0.005% in methanol). After 30 min of incubation at room temperature, the absorbance (OD_{517} nm) of the sample was recorded using methanol as a base line correction. The average absorbance value was calculated after recording all three readings in triplicate. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and calculated by using the formula:

% DPPH radical scavenging activity = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

The (IC_{50}) value was also calculated. The IC_{50} (The half -inhibitory concentration) value is the dose required to cause 50% inhibition.

J. Partial identification of the extracted secondary metabolites

1) FT-IR Analysis

The IR spectra of ACW9 ethyl acetate extract were recorded in FTIR, Perkin Elmer, USA Spectrum RX1. For analysis, KBr pellets were prepared by adding 1mg of dried extract to 300 mg of KBr (IR grade), dried under vacuum at 100°C and compressed to disc. The pellet thus obtained was scanned in a frequency range of 400-4000cm^{-1} with a resolution of 4cm^{-1}. The spectrum plot of intensity vs wavenumber thus obtained was interpreted.

2) Gas Chromatography-Mass Spectrometry (GCMS)

The crude extract of *Streptomyces rimosus* strain ACW9 was analysed using Gas Chromatography-Mass Spectrometry (GC-MS) on a Perkin Elmer-SQ8T-680. The apparatus includes a DB 35-MS capillary standard nonpolar column with dimensions of (30mm × 0.25mm × 0.25um) film. Helium gas (1ml/min) was used as the carrier gas to inject 0.2um-filtered, with temperature ranging from 350°C to 60°C for 15 minutes to 30 minutes. The compounds were identified using the NIST and Willey library attached to GCMS instrument.

3) HP-LC analysis

HPLC is a chromatographic technique for separating a mixture of compounds in order to identify, measure, and purify the individual components of the mixture. In this study, we used the Shimadzu LC-2010AHT to analyse an unknown compound. Column (C18) was used for sample analysis, and different mobile phases were prepared based on the samples. Before injection, the sample was mixed 50:50 with the solvent and filtered through a Millipore filter. Approximately 10 µL of the sample filtrate was injected into column C18 (250mm × 4.6mm × 5mm).

The sample was run for 30 min and the retention time was noted. The UV-VIS detector was set at 245nm and the flow rate was 0.50ml/min. The ACW9 chromatogram's peaks were identified by comparing the elution time to the standard, and the compound was determined.

III. RESULTS AND DISCUSSION

A. Primary screening of strain ACW9

As a part of our research for more potent antibiotic, one promising isolate, ACW9 was found to produce a broad-spectrum activity with strong antibacterial activity against several pathogenic multi drug resistant uropathogens.

The primary screening clearly shows that ACW9 possessed good antimicrobial potential against all the tested uropathogens. This strain was, therefore selected for further isolation of bioactive metabolites, purification, characterization.

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B. Identification of ACW9

The bacterial strain ACW9 was found to be Gram-positive, chain forming bacterium with rough surface (Fig.1). The colony showed diffusible brown pigment along the entire colony margin. The morphological, physiological, and biochemical traits of strain ACW9 are mention in detail in (Table.1).

The 16S rRNA gene coding sequence of the strain was analyzed for sequence homology by BLAST tool in NCBI platform. The sequence with highest similarity to closely related strains was retrieved from the database in FASTA format and aligned by CLUSTALW program. Neighbor joining method was used to study the phylogenic relationships among different strains (Fig.2). The strain got maximum homology with strain *Streptomyces rimosus* NBRC12907 accession number NR_112332.1 (99.49%). Therefore, on the basis of 16S rRNA gene sequence, the isolate ACW9 was designated as *Streptomyces rimosus* ACW9. The 16S rRNA sequence of ACW9 was deposited in GenBank, NCBI with accession number (MW 767045).

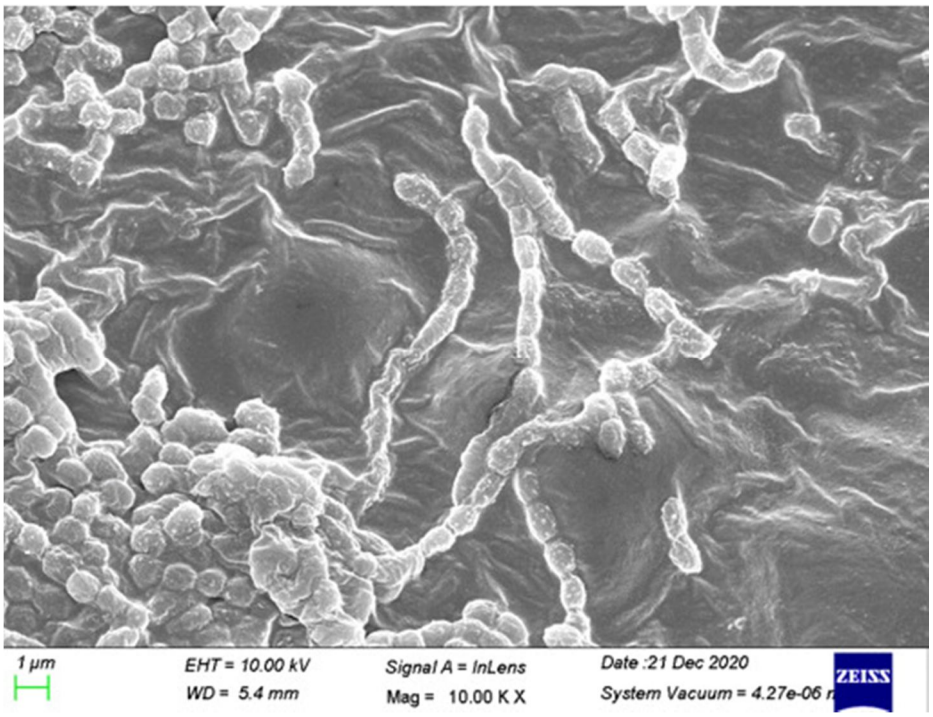


Fig. 1 Scanning electron microscope (SEM) photomicrograph of *Streptomyces rimosus* ACW9

Table 1. Cultural, morphological, physiological and biochemical characterization of *Streptomyces rimosus* ACW9

Properties	Result
Cultural characteristics	
Colony margin	Filamentous
Colony colour	Yellow
Substrate mycelium	Brown
Morphological characteristics	
Gram-reaction	+
Shape	Spore forming colony
Physiological characterisation	
pH range for growth	6-8
NaCl tolerance	3%
Biochemical characterisation	
Catalase	+
Oxidase	_
Citrate	_
Nitrate	+

MR test	+
VP test	_
Gelatine hydrolysis	_
Starch hydrolysis	+
Urea	+
Carbohydrate utilization	
Glucose	+
Lactose	_
Sucrose	+
Maltose	_
Fructose	+

Abbreviations Used: (+) = Positive, (-) = Negative.

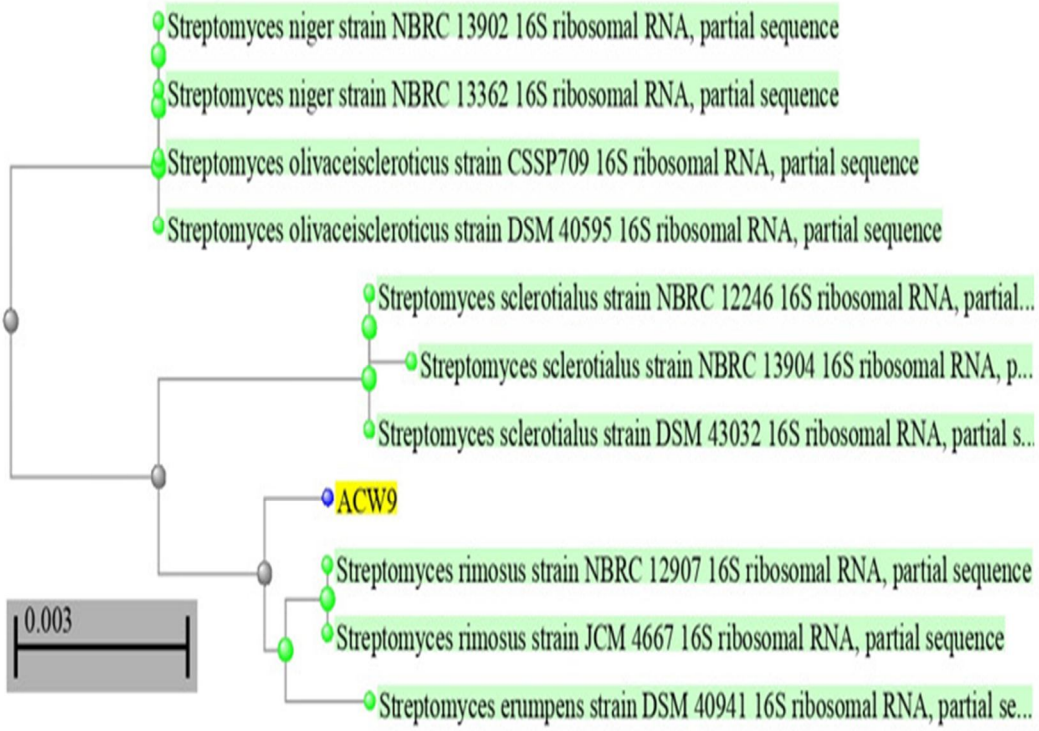
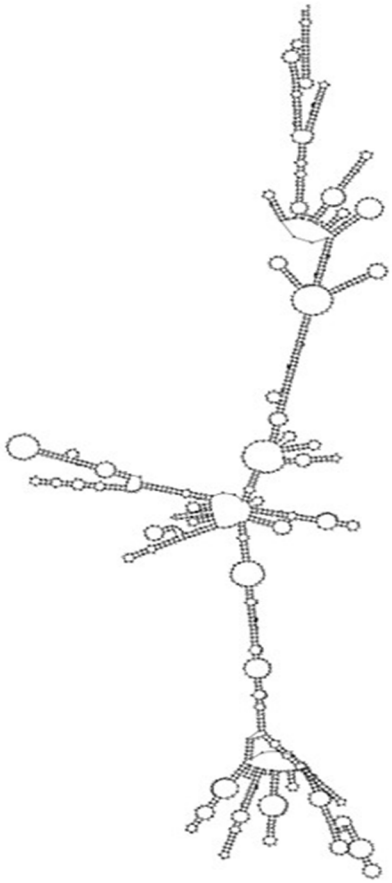


Fig. 2 Phylogenetic tree based on 16S rRNA coding sequences demonstrating relationships between strain ACW9 and *Streptomyces* members. Numbers at nodes represent levels of bootstrap value of 1000 resembled datasets based on neighbour-joining analysis; only values greater than 50% are reported. Bar represents 0.003 substitutions per nucleotide position.

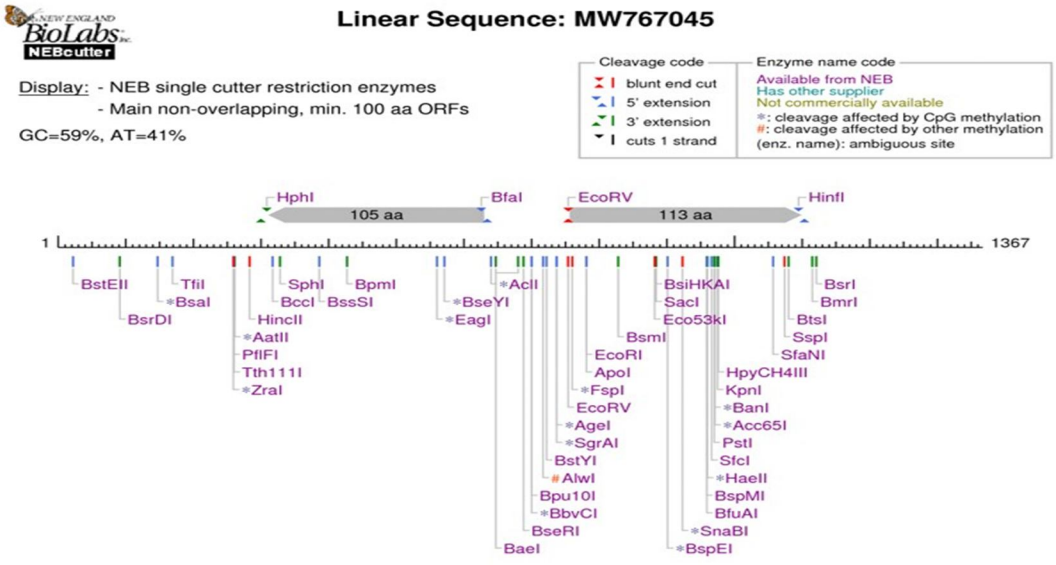
C. Prediction of the secondary structure of 16S rRNA sequence and analysis of restriction sites

The folding of 16S rRNA was predicted to fully understand the thermodynamic stability of the gene sequence (Fig.3a). The Gibbs free energy of 16S rRNA sequence in folded state was found to be -480.10Kcal/mol. This study suggests that minimum free energy (MFE) of the 16S rRNA sequence, indicating high nucleotide stability in strain ACW9 with optimal configuration. GC-AT content from 16S rRNA coding sequence comes out to be 59% and 41% respectively (Fig.3b). The concept of studying free energy associated with the folding of 16S rRNA gene sequence might provide preliminary information to make a concurrent prediction on the stability of the genes. In our study, *Streptomyces rimosus* ACW9 showed −480.10 kcal/mol free energy of secondary structure, which is more or less in accordance with the previous studies [22], [23].



dG = -431.20 [Initially -497.60] MW767045

(a)



(b)

Fig. 3: Base pair graph representation of 16S rRNA secondary structure analysis of *Streptomyces rimosus* ACW9 obtained with RNA fold: (a) Predicted minimum free energy structure. (b) Restriction site analysis through NEB cutter tool.

D. Antimicrobial activity of ACW9 crude extract against MDRS of UTI infection

The strain ACW9 was grown in ISP-2 medium for production of biometabolites. ISP-2 medium was extracted using ethyl acetate solvent. Ethyl acetate was extensively used as a specific solvent for biometabolite extraction. The antimicrobial activity of ethyl acetate crude extracts was assessed by disc diffusion method. The ethyl acetate extract of strain ACW9 was found to be effective against all the tested human uropathogens. The strain ACW9 showed a broad range of antimicrobial activity against both Gram-negative and Gram-positive pathogens. The results of antimicrobial activity of strain ACW9 are given in (Table. 2). The researcher [24] observed a marked difference in the crude extracts in comparison to pure drug that was already in clinical use. In another study, [25] reported extracellular ethyl acetate extract of *Methylobacterium* sp. with zone of inhibition of 9 mm against *B. subtilis*. In several reports, ethyl acetate was mostly used as an extraction solvent to isolate the crude extracts from actinomycetes [26], [27]. Earlier, several studies reported that most of the antimicrobial secondary metabolites were from extracellular actinomycetes [28], [29], [30].

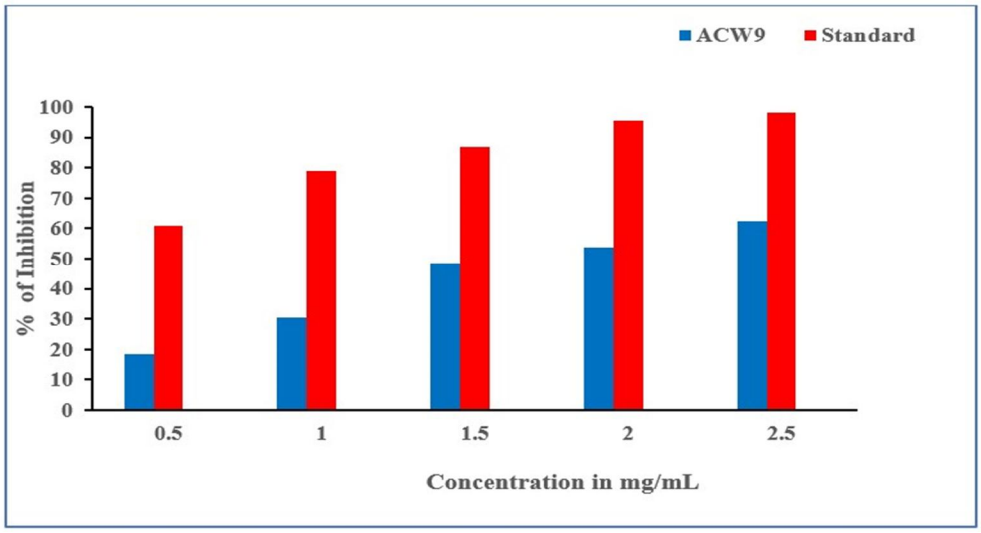
Table 2. Antibacterial activity of ethyl acetate extract of *S. rimosus* (ACW9) against selected Uropathogens

Test human uropathogens	Ethyl acetate extract of ACW9 ZOI	Levofloxacin (5µg/ disc) ZOI	DMSO (Negative Control) ZOI
Pseudomonas aeruginosa P1	6.3 ±0.33	19.3±0.33	ND
Enterobacter tabaci P2	19.3±0.33	20.6±0.33	ND
Bacillus subtilis P5	13.3±0.33	15.3±0.66	ND
Staphylococcus aureus P6	7.3±0.66	20.6±0.66	ND

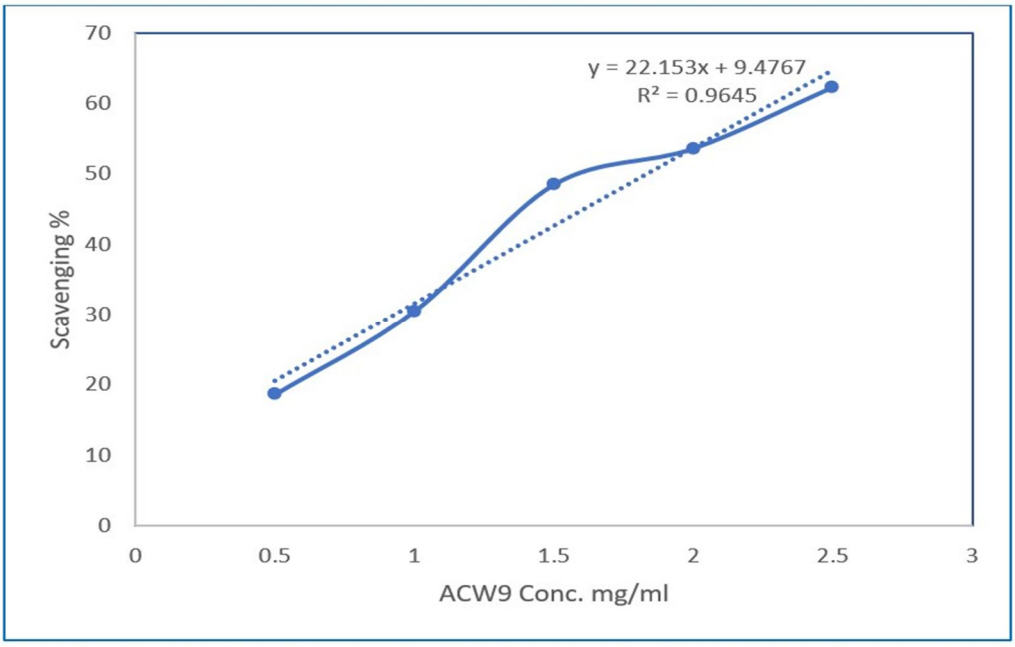
± (Stander error), ND (non detected)

E. Antioxidant property of crude extract

Several diseases are implicated in high levels of reactive oxygen species (ROS) in the body, including diabetes [31], cardiovascular disease, inflammation and arthritis, cancer, and neurodegeneration [32], [33], [34]. Furthermore, there seems to be growing interest in the use of naturally derived antioxidants rather than synthetic antioxidants by the food industry [35]. DPPH is a stable free radical with maximum absorbance at 517 nm. The decolorization of violet colour of DPPH into yellow colour indicated the reduced form of DPPH demonstrated the extract scavenging ability. The extract of ACW9 showed substantial scavenging activity in range between 18.5±0.033 to 62.3±0.033 with significant dose dependent inhibition of DPPH activity with an IC_{50} value of 1.829 mg/ml. The results of inhibition are clearly presented in (Fig. 4a-b).



(a)



(b)

Fig .4 (a) DPPH scavenging activity of crude extract S. *rimosus* (ACW9) (b) Evaluation of antioxidant Ic50 of crude extract ACW9

F. FTIR analysis

The bioactive compound produced by *Streptomyces rimosus* ACW9 was identified using FT-IR spectroscopy. The region of IR spectra displayed between 400-4000cm-1 is used to identify functional groups present in biometabolites (Fig.5). The crude extract revealed peaks of different functional groups at 3786.27 cm^{-1}(O-H stretching), 3427.92cm^{-1} (O-H and N-H stretching), 1583.95 cm^{-1}(C=C stretch and N-H bending), 1403.79 cm^{-1}(OH bending), 699.78 cm^{-1}(C=C bending). The OH group detected is able to form hydrogen bonding, that probably related with the higher inhibitory activities of alcohols and phenolics against microorganisms [36], [37], [38], [39] also indicated the presence of –OH group, and the appearance of bands between 3000-2900 cm^{-1} is an indication of C aromatic compounds [40]. Due to the presence of aromatic compound, vibrations of carbon stretching in the aromatic ring show absorptions in the regions 1618-1660 cm^{-1}. It is suggested that, less hydroxyl groups in the molecular structure of compound may reduce the antimicrobial activity [41]. On the other hand, phenolic compounds containing hydroxyl groups increase their affinity for microbial lipid membranes, resulting in higher antibacterial activity [42].

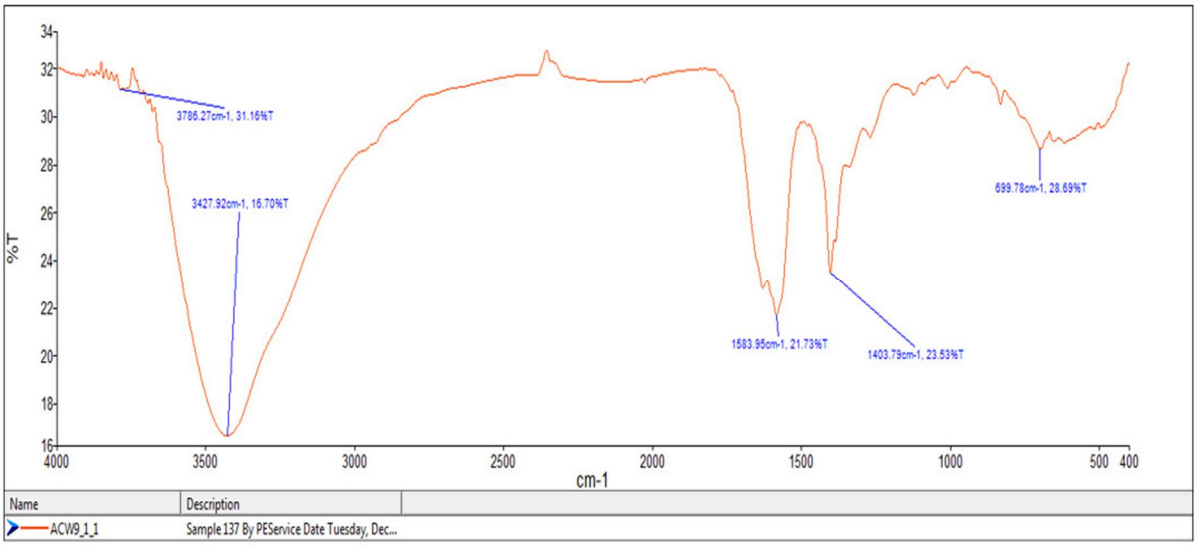


Fig. 5 FTIR analysis of ethyl acetate crude extract of ACW9.

G. GC-MS analysis

The ethyl acetate extract was subjected to GC-MS analysis, and the fractional profiles revealed the presence of various chemical compounds, each with their specific retention time (Fig.6) which depicted that the ethyl acetate extract generated a total of 13 peaks after 30 minutes of run time. The NIST database was used to locate the probable compound as well as its molecular weight for all the compounds present at respective peaks (Table. 3). The major identified compounds were (1). Pentanoic acid, (2). Dodecane, 1-fluoro, (3). Hexadecane, (4). Cyclohexane, (2-Nitro-2- Propyl), (5). 1-Docosene, (6). 5-Ecosane-E, (7). 1-Heptacosanol, (8). N-Tetracosanol-1, (9). Octadecanoic acid, (10). Heptadecane, (11). Pentadecanoic acid, (12). Tetradecanoic acid (13). 9-Octadecanoic 1,2,3 Propanetriyl ester [EEE] is present in the sample ACW9. However, when compared to other chemical components in the extract, Cyclohexane, (-2-Nitro-2-Propyl), and Hexadecane had the highest peak area 15.2% and 16.8%, respectively, and are thus recognised as main contributors. The present results showed the presence of different kinds of fatty acids which are biosurfactants similar results were obtained for glycolipid biosurfactant properties displayed by *S. puniceus* RHPR9 can be understood [43]. GC-MS analysis revealed the following compounds, cyclohexane, (2-Nitro 2-Phenyl) and hexadecane, which have both been previously reported as antimicrobial [44], [45], [46]. Hexadecane has been reported as an antimicrobial and antifungal compound [47]. Hexadecane plays a major role in many biological activities as a preservative in food as well as in drugs [48]. The bioactive compounds reported in the present study, dodecane, hexadecanoic acid, 1-Heptacosanol, N-tetracosanol, were previously reported as antifungal and antibacterial [49]. Hexadecanoic acid is the most effective antibacterial fatty acid in mice, and it is also the most effective antibacterial fatty acid in humans [50], [51], [52]. Other studies have found octadecanoic acid and tetradecanoic acid to be antioxidants, antimicrobials, and immunomodulators [53]. Despite the fact that it is known to have antibacterial and antifungal properties, there have been few reports on tetradecanoic acid [54], [55]. Octadecanoic acid has antifungal, antitumor, and antibacterial properties [56]. Even though it is known to have antibacterial and antifungal properties, there have been few reports on tetradecanoic acid [57], [58].

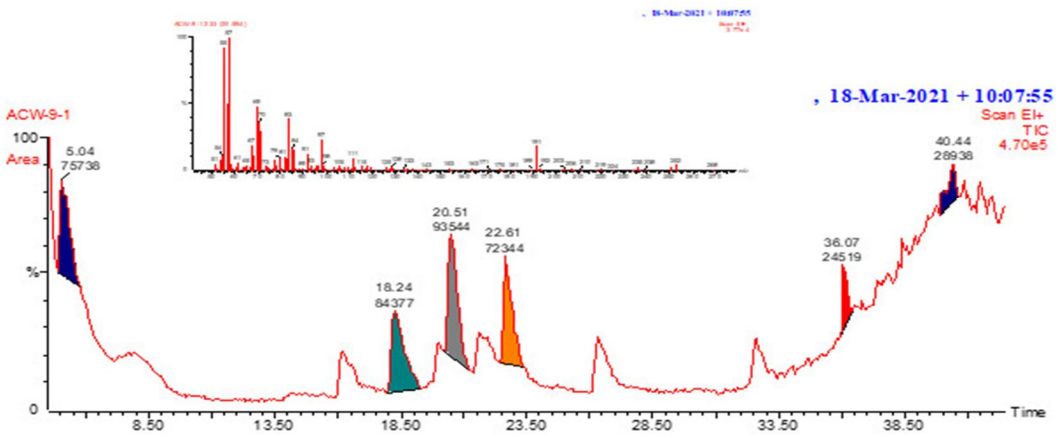


Fig.6 GCMS analysis of crude extract of S. *rimosus* (ACW9)

Table 3. Major compounds identified from ethyl acetate extract of *Streptomyces rimosus* ACW9

S.NO	Retention time	Compound	Molecular Formula	Molecular weight (MW)	Area %	Reported activity	References
1	5.04	Pentanoic acid	C5H10O2	102	13.6	Antimicrobial, Antioxidant and Anticancer activities	[59]
2	16.22	Dodecane,1-Fluoro-	C12H25F	188	5.8	Antimicrobial and Antioxidant	[50]
3	18.24	Hexadecane	C16H34	226	15.2	Antibacterial activity	[47]
4	20.51	Cyclohexane, (2-Nitro-2-Propenyl)	C9H15O2N	169	16.8	Antimicrobial activity	[46]

5	21.60	1-Docosene	C22H44	308	5.7	Antimicrobial activity	[60]
6	22.61	5-Eicosene, (E)-	C20H40	280	13	Antimicrobial activity	[44, 45]
7	26.3	N-Tetracosanol-1	C24H50O	354	5.7	Antibacterial, Antiproliferative	[54]
8	32.62	1-Heptacosanol	C27H56O	396	5	Antimicrobial, Antioxidant	[61]
9	36.07	Octadecanoic acid	C18H36O	284	4.4	Antifungal, Antitumor, Antibacterial	[62]
10	38.93	Heptadecane	C17H36	240	2.9	Antifungal, Antimicrobial, Antibacterial, Antioxidant	[62,63]
11	39.77	Pentadecane	C15H36	212	3.1	Antifungal, Antimicrobial, Anticancer	[48]
12	40.44	Tetradecanoic acid	$C_{14}H_{28}O$	228	5.2	Used in cosmetics as antioxidant	[64]
13	41.53	9-Octadecanoic1,2,3-Propenetriyl ester, [EEE]	C57H104O6	885	3.4	Immunomodulatory	[62]

H. HPLC analysis

The antibacterial compounds produced by ACW9 were detected using ethyl acetate extract via HPLC analysis. The sample was prepared as mentioned in USP. The obtained chromatogram of ACW9 was compared with the standard antibiotic based on retention time. HPLC chromatogram of ACW9 revealed a major peak with a retention time of 3.457 minutes, which resembles that of standard oxytetracycline. The study suggests, that the strain ACW9 produces oxytetracycline along with other compounds. Corn-cob was used as a substrate in the production of oxytetracycline by *Streptomyces rimosus* TM-55 in a solid-state fermentation [65]. In the HPLC report of ACW9, major compound may be similar to oxytetracycline, like in the previous report [66]. At retention time 3.4, the extract of isolate ACW9 showed a major peak (Fig.7). When the peak was compared to standards, it was observed that it resembled the oxytetracycline standard peak, intimating that oxytetracycline is the second of the broad-spectrum tetracycline group of antibiotics. Tetracycline is an antibiotic that is used to treat a variety of Gram-positive and Gram-negative pathogens, but it is most effective against *Enterobacter* sp. and *Pseudomonas* sp. in the present research. Tetracycline is an antibiotic that is effective against multidrug resistant pathogens. *Streptomyces* strain ACW9 is a potent antimicrobial producer that can be further studied for other bioactive against different resistant uropathogens due to the presence of all of the important antimicrobial metabolite producers. The current study's findings suggested that the potential activity of the extract against uropathogens could be attributed to cyclohexane and hexadecane, which were also identified by [58, 46].

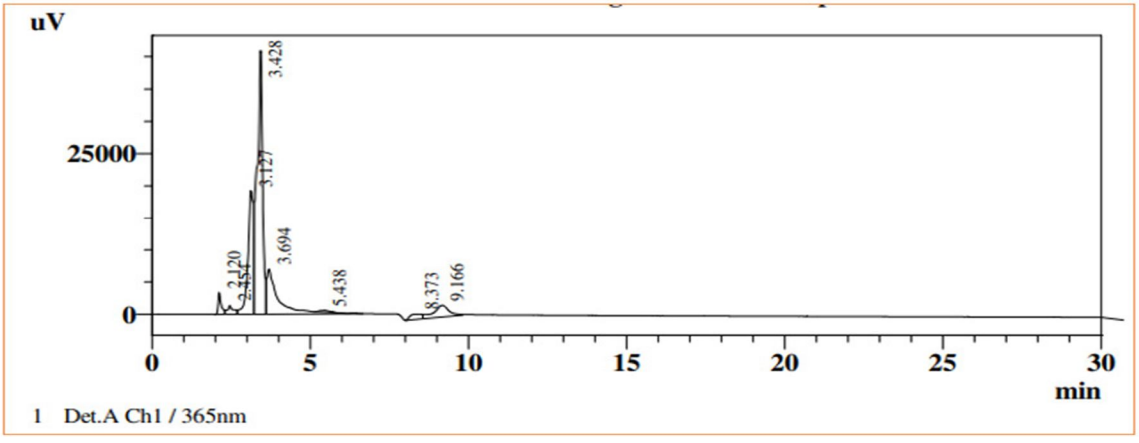


Fig.7 HPLC profile of *S. rimosus* (ACW9)

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IV. CONCLUSION

In conclusion, the extracellular crude extract of *Streptomyces rimosus* ACW9 showed good activity against all the tested uropathogenic bacteria. The present results suggest that the isolated actinomycete *S. rimosus* (ACW9) could be used as an antioxidant and antibacterial agent against the tested microbial pathogens.

V. AUTHORS' CONTRIBUTIONS

Archana Singh conceptualized the study, developed the methodology, wrote the original draft, performed the experiment, and contributed to the validation process. Padma Singh provided supervision throughout the study. Both authors have reviewed and approved the manuscript for submission to your esteemed journal.

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REFERENCES

[1] Li M, Yang F, Lu Y, Huang, W. Identification of Enterococcus faecalis in a patient with urinary-tract infection based on metagenomic next-generation sequencing: a case report. BMC Infect Dis 2020; 20:1-7. https://doi.org/10.1186/s12879-020-05179-0
[2] Klein RD, Hultgren SJ. Urinary tract infections: microbial pathogenesis, host–pathogen interactions and new treatment strategies. Nat Rev Microbiol 2020; 18:211-226. https://doi.org/10.1038/s41579-020-0324-0
[3] Shaheen G, Akram M, Jabeen F, Ali Shah SM, Munir N, Daniyal M, Riaz M, Tahir IM, Ghauri AO, Sultana S, Zainab R. Therapeutic potential of medicinal plants for the management of urinary tract infection: A systematic review. Clin Exp Pharmacol Physiol 2019; 46:613-24. https://doi.org/10.1111/1440-1681.13092
[4] Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol 2019; 11:1756287219832172 https://doi.org/10.1177/1756287219832172
[5] Nickel JC, Saz-Leal P, Doiron RC. Could sublingual vaccination be a viable option for the prevention of recurrent urinary tract infection in Canada? A systematic review of the current literature and plans for the future. Can Urol Assoc J 2020; 14:281 https://doi.org/10.5489/cuaj.6690
[6] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015; 13:269-84. https://doi.org/10.1038/nrmicro3432
[7] Mareș C, Petca RC, Petca A, Popescu RI, Jinga V. Does the COVID Pandemic Modify the Antibiotic Resistance of Uropathogens in Female Patients? A New Storm. Antibiotics 2022;11:376 https://doi.org/10.3390/antibiotics11030376
[8] Nadar RG, Chackaravarthy G, Ramachandran G, Manoharan N, Zubair SM, Alharbi NS, Alobaidi AS, Li WJ. Isolation and molecular identification of biofilm producing P. aeruginosa and K. pneumoniae from urinary tract infections patient urine sample. J Infect Public Health 2021;14 :1875-80 https://doi.org/10.1016/j.jiph.2021.11.004
[9] Bhatti AA, Haq S, Bhat RA. Actinomycetes benefaction role in soil and plant health. Microb Pathog 2017; 111:458-67 https://doi.org/10.1016/j.micpath.2017.09.036
[10] Sarika K, Sampath G, Govindarajan RK, Ameen F, Alwakeel S, Al Gwaiz HI, Komuraiah TR, Ravi G. Antimicrobial and antifungal activity of soil actinomycetes isolated from coal mine sites. Saudi J Biol Sci 2021. 28:3553-8 https://doi.org/10.1016/j.sjbs.2021.03.029
[11] Singh A, Singh P. Production of bioactive compounds by Streptomyces sp. and their antimicrobial potential against selected MDR uropathogens. J. Appl. Biol. Biotechnol 2021;9 :7-9. https://doi.org/10.7324/JABB.2021.9609
[12] Vander Meij A, Worsley SF, Hutchings MI, van Wezel GP. Chemical ecology of antibiotic production by actinomycetes. Microbiol FEMS Rev 2017; 41:392-416 https://doi.org/10.1093/femsre/fux005
[13] Puttaswamygowda GH, Olakkaran S, Antony A, Purayil AK. Present status and future perspectives of mari ne actinobacterial metabolites. InRecent Developments in Applied Microbiology and Biochemistry. Academic Press 2019;307-319
[14] Wang C, Lu Y, Cao S. Antimicrobial compounds from marine actinomycetes. Arch Pharm Res 2020; 43:677-704. https://doi.org/10.1007/s12272-020-01251-0
[15] Al-Shaibani MM, Radin Mohamed RM, Sidik NM, Enshasy HA, Al-Gheethi A, Noman E, Al-Mekhlafi NA, Zin NM. Biodiversity of secondary metabolites compounds isolated from phylum actinobacteria and its therapeutic applications. Molecules 2021; 26:4504. https://doi.org/10.3390/molecules26154504
[16] Sanglier JJ, Haag H, Huck TA, Fehr T. Section review; anti-infectives: review of actinomycetes compounds 1990–1995. Expert Opin Investig Drugs 1996;5: 207-223. https://doi.org/10.1517/13543784.5.2.207
[17] Selim, MSM., Abdelhamid S.A. Mohamed S. Secondary metabolites and biodiversity of actinomycetes. J Genet Eng Biotechnol 2021;19: 72 https://doi.org/10.1186/s43141-021-00156-9
[18] Rajivgandhi G, Vijayan R, Kannan M, Santhanakrishnan M, Manoharan N. Molecular characterization and antibacterial effect of endophytic actinomycetes Nocardiopsis sp. GRG1 (KT235640) from brown algae against MDR strains of uropathogens. Bioact. Mater 2016; 1:140-150. https://doi.org/10.1016/j.bioactmat.2016.11.002
[19] Abirami M, Gopal JV, Kannabiran K. Extraction and identification of antibacterial compound from marine Streptomyces sp. VITAK1 isolated from the coast of Andaman and Nicobar Islands, India. Appl. Biochem. Microbiol 2015; 51: 406-10. https://doi.org/10.1134/S000368381504002X
[20] Ganesan P, Reegan AD, David RH, Gandhi MR, Paulraj MG, Al-Dhabi NA, Ignacimuthu S. Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India. Alex J Med 2017; 53:101-110 https://doi.org/10.1016/j.ajme.2016.03.004

[21] Sánchez-Moreno C, Larrauri JA. Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food research international 1999; 32:407-412.
[22] Gupta KK, Chandra H, Sagar K, Sharma KK, Devi D. Degradation of highdensity polyethylene (HDPE) through bacterial strain from Cow faeces. Biocatalysis and Agricultural Biotechnology 2023; 48:102646.
[23] Skariyachan S, Setlur AS, Naik SY, Naik AA, Usharani M, Vasist KS. Enhanced biodegradation of low and high-density polyethylene by novel bacterial consortia formulated from plastic-contaminated cow dung under thermophilic conditions. Environmental Science and Pollution Research 2017; 24:8443-8457.
[24] Rex MA, Stuart CT, Etter RJ. Do deep-sea nematodes show a positive latitudinal gradient of species diversity? The potential role of depth. Mar Ecol Prog Ser 2021;210: 297-8.
[25] Balachandran C, Duraipandiyan V, Ignacimuthu S. Cytotoxic (A549) and antimicrobial effects of Methylobacterium sp. isolate (ERI-135) from Nilgiris forest soil, India. Asian Pac J Trop Biomed 2012; 2:712–6. https://doi.org/10.1016/S2221-1691(12)60215-9
[26] Franco CM, Coutinho LE. Detection of novel secondary metabolites. Crit Rev Biotechnol 1991; 11:193-276. https://doi.org/10.3109/07388559109069184
[27] Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y. Purification and biological evaluation of the metabolites produced by Streptomyces sp. TK-VL_333. Res Microbiol 2010; 161:335–45. https://doi.org/10.1016/j.resmic.2010.03.011
[28] Bernan VS, Montenegro DA, Korshalla JD, Maiese WM, Steinberg DA, Greenstein M. Biosxalomycins new antibiotics produced by the marine Streptomyces spp. LL-31F508: taxonomy and fermentation. J Antibiot 1994; 47:1417–24. https://doi.org/10.7164/antibiotics.47.1417
[29] Hacene H, Daoudi H, Bhatnagar T, Baratti JC, Lefebvre G. A new aminoglycosidase anti Pseudomonas antibiotic produced by a new strain of Spirillosora. Microbiols 2000; 36:102:69.
[30] Berdy J. Bioactive microbial metabolites: a personal view. J Antibiot 2005; 25:58:126. https://doi.org/10.1038/ja.2005.1
[31] Volpe CM, Villar-Delfino PH, Dos Anjos PM, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis 2018; 9:1-9. https://doi.org/10.1038/s41419-017-0135-z
[32] Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive oxygen species in metabolic and inflammatory signaling. Circ. Res 2018;122: 877-902 https://doi.org/10.1161/CIRCRESAHA.117.311401
[33] Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. Cancer Lett 2018; 387:95-105. https://doi.org/10.1016/j.canlet.2016.03.042
[34] Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. Oxid Med Cell Longev 2017; 9:1712–18. https://doi.org/10.1155/2017/2525967
[35] Caleja C, Barros L, Antonio AL, Oliveira MB, Ferreira IC. A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits. Food Chem 2017; 216:342-6. https://doi.org/10.1016/j.foodchem.2016.08.075
[36] Ashok kumar R, Ramaswamy M. Comparative study on the antimicrobial activity of leaf extracts of four selected Indian medicinal plants against Pseudomonas aeruginosa, Pseudomonas fluorescens, Penicillium chrysogenum and Penicillium restrictum J Chem Bio Phy Sci 2013; 3:1279.
[37] Radhika D, Mohaideen A. Fourier transform infrared analysis of Ulva lactuca and Gracilaria corticata and their effect on antibacterial activity. Asian J Pharm Clin Res 2015; 8:209-12.
[38] Govindasamy B, Dhayalan A, Chinnaperumal K, Paramasivam D, Dilipkumar A, Kannupaiyan J, Perumal S, Pach iappan P. Comparative extraction of Salmonella bongori derived metabolites and their toxicity on bacterial pathogens, mosquito-larvae, zebrafish-embryo and brine-shrimp: A modified approach. Ecotoxicol Environ Saf 2019; 169:192-206. https://doi.org/10.1016/j.ecoenv.2018.11.012
[39] Devnath P, Uddin M, Ahamed K. Md. Hossain F, Manchur TMA. Extraction, Purification and Characterization of pyocyanin produced by Pseudomonas aeruginosa and evaluation for its antimicrobial activity. Int Res J Biological Sci. 2017; 6:1-9.
[40] Silverstein RM, Bassler GC. Spectrometric identification of organic compounds.J Chem Educ 1962; 39:546. https://doi.org/10.1021/ed039p546
[41] Nitiema LW, Savadogo A, Simpore J, Dianou D, Traore AS. In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. Int J Microbiol Res 2012; 3:183-7. https://doi.org/10.5829/idosi.ijmr.2012.3.3.6414
[42] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12:564-82. https://doi.org/10.1128/CMR.12.4.564
[43] Ravinder P, Manasa M, Roopa D, Bukhari NA, Hatamleh AA, Khan MY, MS R, Hameeda B, El Enshasy HA, Hanapi SZ, Sayyed RZ. Biosurfactant producing multifarious Streptomyces puniceus RHPR9 of Coscinium fenestratum rhizosphere promotes plant growth in chilli. PloS one. 2022;17:e0264975. https://doi.org/10.1371/journal.pone.0273533
[44] Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express 2017; 7:1-9. https://doi.org/10.1186/s13568-017-0351-z
[45] Kumar PS, Duraipandiyan V, Ignacimuthu S. Isolation, screening and partial purification of antimicrobial antibiotics from soil Streptomyces sp. SCA 7. Kaohsiung J Med Sci 2014; 30:435-46. https://doi.org/10.1016/j.kjms.2014.05.006
[46] Nguyen TM, Kim J. Antifungal and antibacterial activities of Streptomyces polymachus sp. nov. isolated from soil. Int J Syst Evol Microbiol 2015; 65:2385–2390. https://doi.org/10.1099/ijs.0.000268
[47] Kalpana Devi, V. Shanmugasundaram R. and Mohan VR. GC–MS analysis of ethanol extract of Entada pursaetha DC seed. Biosci Discov 2012; 3:30-33.
[48] Sejalsadhu VR, Mital Aadesariya PD. Ekta Khosla. A study on phytoconstituents, bioactivity, uses: analysis of acetonitrile leaf extract of Abutilon pannosum with gas chromatography mass spectrometric technique. Cibtech j. Pharm. Sci 2016; 5:8-21.
[49] Shiyamala DS, Priya P, Sahadevan R (2014) Pyrrolo [1, 2-A] Pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- and phenol, 2,4-Bis (1,1-dimethy ethyl) novel antibacterial metabolites from a marine Kocuria sp. SRS88: optimization and its application in medical cotton gauze cloth against bacterial wound pathogens. Int J pharm Res Dev 6:44–55.
[50] Nandhini SU, Sangareshwari S, Kumari L. Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine Streptomyces. Asian J Pharm Clin Res 2015; 8:244–246.
[51] Henry GE, Momin RA, Nair MG, Dewitt DL. Antioxidant and Cyclooxygenase activities of Fatty Acids Found in Food. Journal of Agricultural and Food Chemistry 2002; 50:2231-2234.

[52] Praveen KP, Kumaravel S, Lalitha C. Screening of Antioxidant Activity, Total phenolics and GC-MS study of Vitex negundo. Afr J Biochem Res 2010; 4:191-195.
[53] Shah A, Singh T, Vijayvergia R. GC-MS analysis of Bioactive Phytoconstituents from Rurmex vesicarius L. Int Res J Pharm 2015;6:269-272
[54] Vergara M, Olivares A, Altamirano C. Antiproliferative evaluation of tall-oil docosanol and tetracosanol over CHO-K1 and human melanoma cells. Electron J Biotechnol 2015; 18:291-4.
[55] El-Naggar NE, El-Bindary AA, Abdel-Mogib M, Nour NS. In vitro activity, extraction, separation and structure elucidation of antibiotic produced by Streptomyces anulatus NEAE-94 active against multidrug-resistant Staphylococcus aureus. Biotechnol Biotechnol Equip 2017; 31:418-30.
[56] Hsouna AB, Trigie M, Mansour RB. Chemical composition, cytotoxicity effect and antimicrobial activity of Ceratonia silisqua essential oil with preservative effects against Listeria inoculated in minced beef meat. Int J Food Microbiol 2011;148: 66–72.
[57] Abou-Elela GM, Abd-Elnaby H, Ibrahim AHH. Marine natural products and their potential applications as anti-infective agents. World Sci J 2009; 7:872–880.
[58] Karanja EN, Boga HI, Muigai AW, Wamunyokoli F, Kinyua J, Nonoh JO. Growth characteristics and production of secondary metabolites from selected novel Streptomyces species isolated from selected Kenyan national parks. Nairobi: Jomo Kenyatta University of Agriculture and Technology 2006.
[59] Madkour HM, Ghareeb MA, Abdel-Aziz MS, Khalaf OM, Saad AM, El-Ziaty AK, Abdel-Mogib M. Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of n-hexane and methylene chloride extracts of Senna italica. Journal of Applied Pharmaceutical Science 2017;7:023-032.
[60] Hamed MM, Abdelfattah LS, Fahmy NM. Antimicrobial Activity of Marine Actinomycetes and the Optimization of Culture Conditions for the Production of Antimicrobial Agent (s). J Pure Appl Microbiol 2019;13: 2177-88.
[61] Das R, Romi W, Das R, Sharma HK, Thakur D. Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. BMC microbiology, 2018;18:1-16.
[62] Dubey P, Jayant S, Srivastava N. Preliminary phytochemical screening, FTIR and GC-MS analyses of aqueous, ethanolic and methanolic extracts of stem of Tinospora cordifolia (Willd.) Miers for search of antidiabetic compounds. Ann. Phytomed, 2020;8:2020-2024.
[63] Kim JH, Choi JY, Park DH, Park DJ, Park MG, Kim SY, Ju YJ, Kim JY, Wang M, Kim CJ, Je YH (2020) Isolation and characterization of the insect growth regulatory substances from actinomycetes. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2020; 228:108651.
[64] Abdel-Aziz, MS, Hathout AS, El-Neleety AA, Hamed AA, Sabry BA, Aly SE, Abdel-Wahhab MA. Molecular identification of actinomycetes with antimicrobial, antioxidant and anticancer properties. Comunicata Scientiae 2019;10:218-231.
[65] Yang SS, Swei WJ. Oxytetracycline production by Streptomyces rimosus in solid-state fermentation of corncob. World J Microbiol Biotechnol 1996;12: 43-46.
[66] Hussien EM. HPLC method validation for modernization of the tetracycline hydrochloride capsule USP monograph. Bull Fac Pharm Cairo Univ 2014; 52:239-44.

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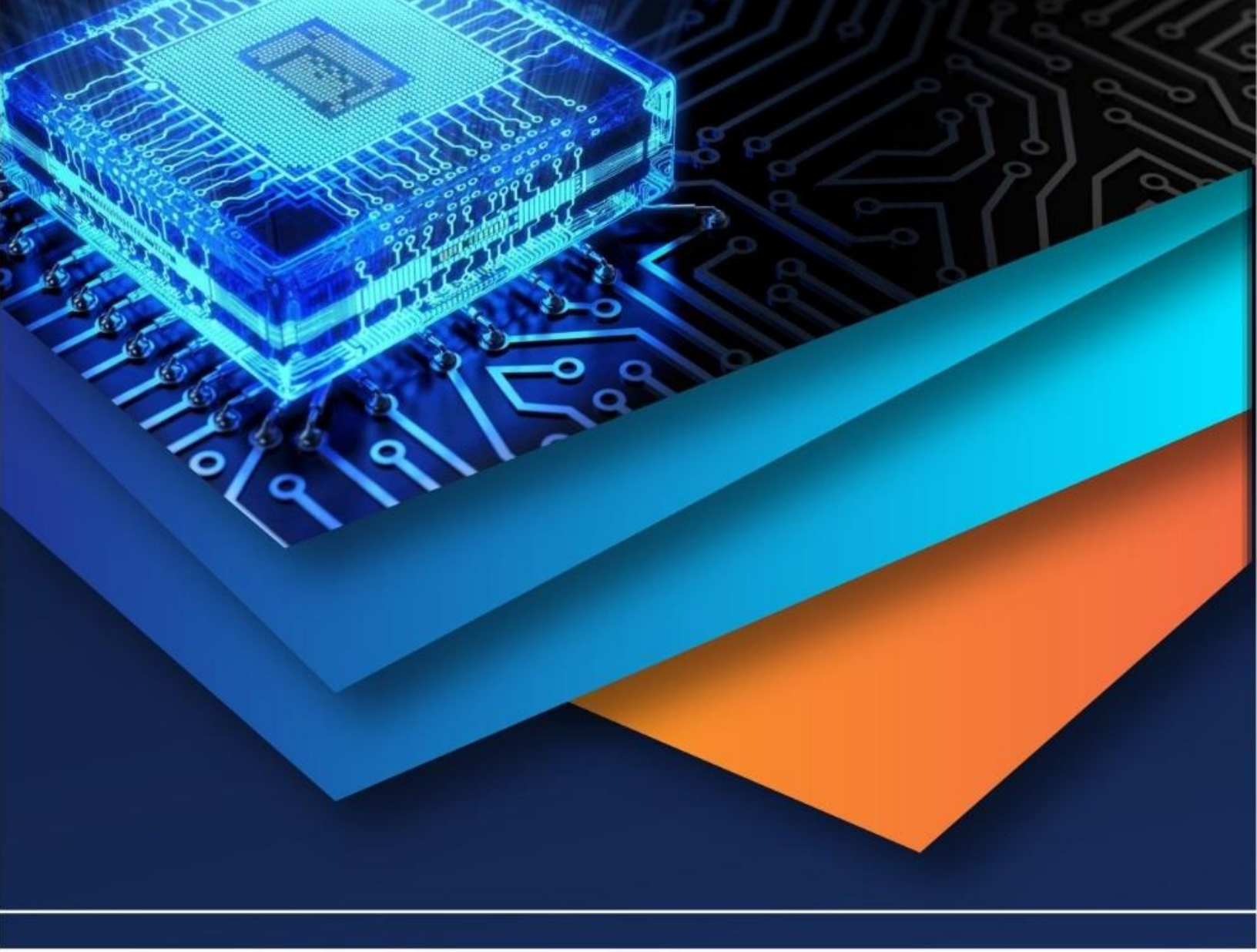

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