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BIOACTIVITY AND *IN-SILICO* ANALYSIS OF R-ALPINE BORANE PRODUCED BY *STREPTOMYCES TOXYTRICINI* JAR4

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ABSTRACT

The present work deals with isolation, identification of bioactive metabolite produced by *Streptomyces toxytricini* JAR4 for various biological activities. The bioactive metabolite (1s,5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane produced by the strain JAR4 was obtained from the optimized culture medium through solvent extraction and molecular weight of the metabolite was characterized through Gas-chromatography mass spectrometry. The active constituents obtained after extraction and partial purification from the isolate JAR4 was tested against clinical pathogens and it was found to be efficiently inhibiting *Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus* and *Klebsiella pnuemoniae* among various gram positive and gram negative pathogens. The *in silico* studies of the isolated bioactive compound reveal the binding with glutamine 6-phosphate synthase at active binding site when compared with standard antibiotic anticapsin through molecular docking performed by Autodock vina. Actinomycetes are the prolific source of antimicrobial metabolites therefore the present investigation reveals in vitro as well as *in silico* antimicrobial potential of terrestrial actinomycetes JAR4 against medically important clinical pathogens.

KEYWORDS: **Antibiotics; Actinomycetes; Pathogens; *Streptomyces toxytricini* JAR4; GC-MS**

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INTRODUCTION

The worldwide increasing antibiotic resistant and the lack of new effective antibiotics make it critical for the search of novel and new drugs to combat multi-drug resistant microorganisms.[1] The development of new bioactive compounds is crucial to fight against emerging antibiotic resistance in pathogenic microorganisms.[2] Over the past 50 years, there is remarkable number of effective antibiotics and bioactive metabolites which have been produced by microbes.[3] Microbial sources are the leading factories of natural products and therapeutic agents. Actinomycetes are gram positive, free living and saprophytic bacteria with tremendous capacity to produce secondary metabolites with diverse chemical structures and biological activities.[4] They are the largest producers of bioactive metabolites with novel pharmaceutical background among bacteria and fungi. The *Streptomyces genus* represents 50% of the total population of soil actinomycetes and 80% of the commercially available antibiotics have been derived from this genus.[5] The *Streptomyces* sp. continues to be the prolific sources of secondary metabolites with efficient biological activity as antimicrobials, anticancer agents or other pharmaceutical compound.[6] They are the most efficient source of bioactive metabolites that have important applications in medicine and in agriculture.[7] The majority of *Streptomyces* sp. produces different antibiotics that can be classified into different ways based on the bacterial spectrum, chemical structure and the type of biological activity. There are several classes of antibiotics which can be distinguished, as: alkaloid derivatives possessing anticancer and antimicrobial properties[8,9] phthalate derivatives which are biologically active compounds[10] nonadecene compounds known for their antioxidant[11] and antifungal activities[12] diketopiperazine (DKP) derivatives having useful biological properties[13], etc. It has been proposed that the rate of discovery of new compounds from terrestrial actinomycetes has decreased whereas the reisolation of known compounds has increased.[14] There is an urgent need to isolate novel *Streptomyces* from less explored areas which produces potent bioactive compounds. The molecular systematic data act as indispensible tool for them to reduce the synonyms of previously described species.[15,16]

In the present investigation, we describe the identification of *Streptomyces toxytricini* JAR4 isolated from the manure sample by conventional and molecular methods. The bioactive metabolites were extracted and characterized through extraction of fermentation medium and spectroscopic techniques. The biological activity of bioactive metabolite has been reported against clinical pathogens. The molecular docking studies have been performed in accordance to antibacterial activity where bacterial proteins are preferable targets.[17] Glucosamine-6-phosphate synthase (EC 2.6.1.16) can be considered as target for antibacterial activity.[18] It is the major enzyme which catalyzes synthesis of D-glucosamine-6-phosphate (GlcN-6-P) which is the precursor of UDP-N-acetylglucosamine (UDP-GlcNAc).[19,20] This UDP-N-acetylglucosamine is the principal intermediate in the biosynthesis of all amino sugar containing macromolecules. Thus, the inhibition of GlcN-6-P by (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane has the impact on the microbial growth.

MATERIALS AND METHODS

(i) Organisms

The strain JAR4 was isolated from dumping area soil of VIT University, Vellore, Tamil Nadu on starch casein nitrate agar medium (soluble starch $10g^{-1}$, casein $0.3g^{-1}$, Nacl $2g^{-1}$, KNO_3 $2g^{-1}$, K_2HPO_4 $2g^{-1}$, $MgSO_4.7H_2O$ 0.5^{-1}, $CaCO_3$ $0.02g^{-1}$, $FeSO_4.7H_2O$ $0.01g^{-1}$) at pH 7.0 , incubated at 28°C for 2 weeks. The isolated strain JAR4 was further maintained on yeast extract-malt extract-dextrose (YMD) agar medium at 4°C.[21]

(ii) Phenotypic Characterization

The cultural and color of mature sporulating aerial and substrate mycelium of *Streptomyces toxytricini* JAR4 were monitored after 14d of

incubation on International *Streptomyces* project (ISP) and non-ISP medium by following[22] protocol. The utilization of sole carbon sources by *Streptomyces toxytricini* JAR4 was investigated using method described by.[22] The production of melanin was observed on peptone-yeast extract-iron (ISP medium 6) agar and tyrosine (ISP medium 7) agar. The antibiotic sensitivity of *Streptomyces toxytricini* JAR4 was determined against various antibiotic discs using Kirby-Bauer method.[23] The spore morphology of *Streptomyces toxytricini* JAR4 was depicted using scanning electron microscopy (HITACH, Model S-3400N) coated with gold to avoid charging.

(iii) Genotypic characterization

The genomic DNA was extracted from *Streptomyces toxytricini* JAR4 following the protocol of.[24] PCR amplification of 16S-rRNA gene of strain JAR4 was carried out by using forward primer of 400ng 5'-AGAGTRTGATCMTYGCTWAC-3' and reverse primer of 400ng 5'-CGYTAMCTTWTTACGRCT-3', 2.5mM each of dNTPs, 10X Taq polymerase assay buffer and Taq DNA polymerase enzyme keeping the reaction volume upto 100µl. The amplification reaction was further followed by initial denaturation at 94°C for 5 mins; to improve the denaturation of the DNA 5% (v/v) DMSO was added to the reaction mixture. After denaturation annealing at 55°C for 30 s was carried out leading to final extension at 72°C using $MgCl_2$ with 1.5mM final concentration. The amplified product was sequenced with the primer using ABI 3730xl genetic analyzer (Amnion Biosciences Pvt. Ltd.). The homology search was performed using BLAST search algorithm. The nucleotide sequence of the whole gene 16S rRNA gene has been submitted in the Gen Bank (EMBL) under accession number KC509577. The multiple sequence alignment was performed by CLUSTAL W at European Bioinformatics Institute website.[25] The phylogenetic tree of the *Streptomyces toxytricini* JAR4 was constructed using Neighbor joining algorithm.[26]

(iv) Optimization of culture medium

The optimization of antibiotic production medium by strain JAR4 was carried out in the presence of various carbon and nitrogen sources using inorganic salt medium composed of $(NH_4)_2SO_4$ 2.64g^{-1}; KH_2PO_4 2.38g^{-1}; $MgSO_4.7H_2O$ 1.00g^{-1}; $CuSO_4.5H_2O$ 0.0064g^{-1}; $FeSO_4.7H_2O$ 0.0011g^{-1}; $MnCl_2.4H_2O$ 0.0079g^{-1}; $ZnSO_4.7H_2O$ 0.0015g^{-1} at pH 8. The different carbon sources including glucose, lactose, starch, sucrose, maltose and nitrogen sources including peptone, sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl), yeast extract, casein and soybean meal were added to the basal medium at 1% of concentration. 5% of spore suspension was added to 50ml basal medium supplemented with various carbon and nitrogen sources and were incubated on rotary shaker at 28°C for 15d. The inorganic salt medium without carbon and nitrogen sources served as control. The biomass consisting the bioactive metabolite was recorded at 600nm (optical density) and antimicrobial activity was determined against clinical pathogens after 3d, 6d, 9d, 12d and 15d.

(v) Test organisms

The gram negative bacteria including *Escherichia coli, Shigella* sp., *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella* sp. and gram positive bacteria *Staphylococcus aureus, Enterococcus* sp. were procured from Microbial Biotechnology Lab, SBST, VIT University, Vellore, India. Bacterial and fungal pathogens were maintained on Nutrient agar. The antibiosis studies of clinical and plant pathogenic isolates were determined against standard antibiotics vancomycin (30mcg/disc), tigecycline (15mcg/disc), erythromycin (15mcg/disc), ciprofloxacin (30mcg/disc), penicillin (10mcg/disc), ofloxacin (5mcg/disc) were screened by disc-diffusion method.

(vi) Isolation and purification of biological active compounds

Streptomyces toxytricini JAR4 was cultivated in Yeast-extract Malt-extract dextrose medium in rotary shaker at 220 rpm at 28°C for 48h. 10% of the seed medium was inoculated into the optimized fermentation medium for the

production of bioactive metabolites consisting of soluble starch 10g^{-1}, K_2HPO_4 1g^{-1}, $MgSO_4.7H_2O$ 1.0^{-1}, NaCl 2g^{-1}, $(NH_4)_2SO_4$ 2.0g^{-1}, $CaCO_3$ 2.0g^{-1} at pH 7.0. The fermentation was carried out for 144h at 28°C in rotary shaker with continuous agitation at 220 rpm. Three liters of production medium was centrifuged to remove the biomass. The cell free filtrate was extracted twice with ethyl acetate using separating funnel and organic layer was concentrated in vacuum to dryness. The metabolic product was recovered successfully after extraction. The dry extract was spotted and developed in hexane:formic acid:methanol (2:1:1). The bands were visualized under UV 254nm and at 365 nm. The fractions were eluted by various solvent systems and were characterized using Gas-chromatography mass spectrometry (GC-MS).

(vii) Biological assays
Antimicrobial screening

The antimicrobial potential of the collected fraction was investigated by Kirby-Bauer method on Muller-Hinton Agar. The Muller-Hinton agar plates were seeded with 100µl of test organism, 6mm diameter of four cups was punctured onto agar plates. The active elute with different concentration of 25µl, 50µl, 75µl and 100µl was added into four wells. The agar plates were further incubated at 37ºC for 24h and zone of inhibition was measured.

Minimum Inhibitory concentration

The minimum inhibitory concentration (MIC) against bacteria clinical pathogens was determined by using Boruwa et al.[27] protocol. The inoculums size of 3×10^5 colony forming units of test microorganisms was inoculated in 10ml of nutrient broth. The active elute fraction with different concentration of 50µg, 100µg, 150µg, 200µg, 250µg was added in nutrient broth supplemented with test organisms. The serial dilution of the test organism with active elute was performed and 100µl of the suspension was plated onto nutrient agar plates at different intervals. MIC of the test organisms was determined after 24h of incubation. The cell survivability rate of inhibited test organism with time dependent studies of the metabolite was determined using Dubey et al.[28] protocol.

(viii) Molecular docking studies

All the docking studies were carried out by Autodock Vina which is recently introduced by Scripps Research Institute.[29] The crystal structure of GlcN-6-P synthase (PDB ID: 1GDO) was selected and downloaded from PDB (www.rcsb.org/pdb). Crystallographic water molecules and ligand were removed from GlcN-6-P and A chain was preferred for docking. GlcN-6-P was firstly modified by adding polar hydrogen atoms and kollman charges using AutoDock Tools- ADT.[30] The torsional bonds of ligand were set free by Ligand module in AutoDock Tools- ADT. Grid points of 36 × 38 × 48 with 0.375 Å spacing were calculated around the docking area for all the ligand atom types using Autodock Vina default optimization parameters. Docking results from each calculation were clustered on the basis of root mean square deviation (RMSD) between the cartesian coordinates of ligands and were ranked according to binding energy. The conformer of each ligand with lowest binding free energy was chosen for docking.

RESULTS AND DISCUSSION

(i) Morphology and cultural characteristics

The phenotypic characteristics of *Streptomyces toxytricini* JAR4 was observed after 14d on ISP1-7 medium. The isolated strain JAR4 developed good aerial and vegetative mycelium on 6d and 7d. The color of aerial mycelium appeared to be peach after 6d of incubation and remain white although 14d whereas substrate mycelium changed color from pale yellow to dark yellow. The cultural characteristics of the JAR4 strain are depicted in Table 1. The strain JAR4 produced diffusible pigment on IPS-2, ISP-7 and Maltose-tryptone agar medium. Melanin production was observed on ISP-2, ISP-7 and maltose-tryptone agar. Figure 1 depicts the spore morphology of the *Streptomyces toxytricini* JAR4.

Table 1
Morphological and cultural characterististics of Streptomyces toxytricini JAR4

S. No.	Culture Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusable pigment	Melanoid Pigment
1	Tryptone-yeast agar medium (ISP-1)	Poor	Creamish white	-	-	-
2	Yeast extract malt extract agar (ISP-2)	Very Good	Peach	Pale yellow	Yellow	-
3	Oatmeal agar (ISP-3)	Good	Peach	-	-	-
4	Inorganic salt-starch agar (ISP-4)	Very Good	White	Pale yellow	-	-
5	Glycerol asp.aragine agar (ISP-5)	Good	White	-	-	-
6	Peptone yeast iron agar (ISP-6)	Very Poor	Transleucent	-	-	-
7	Tyrosine agar (ISP-7)	Good	White	-	-	-
8	Starch-casein nitrate agar	Good	White	Light red	-	-
9	Sabourad agar	Poor	White	-	-	-
10	Maltose-tryptone agar	Good	White	-	-	+

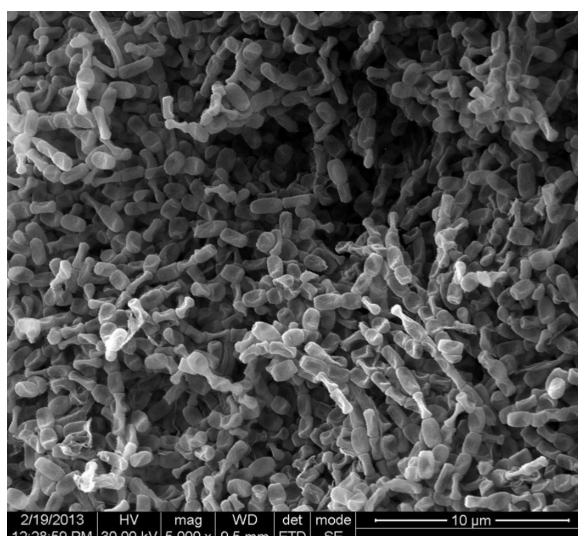

Figure 1
Scanning electronic micrograph showing the spore morphology and arrangement of strain JAR4.

(ii)Physiological Characteristics
The various carbon sources have been utilized by the JAR4 strain indicating its wide pattern of carbon assimilation. The strain JAR4 did not exhibit melanoid pigment on Tyrosine-agar (ISP-7 medium). The strain exhibited salt tolerance upto 5%. The strain was found to be negative for biochemical test including citrate utilization, H_2S, urease and indole production as shown in Table 2. The strain JAR4 showed resistance to methicillin and was found to be sensitive to tetracycline, penicillin, chloramphenicol, streptomycin, clindamycin, vancomycin, gentamicin, kanamycin, ciprofloxacin and erythromycin.

Table 2
Phenotypical characteristics of Streptomyces toxytricini JAR4

Sr no.	Carbon sources	JAR4	Antibiotic	Zone of inhibition in mm
1	D-glucose	P	Tigecycline (15mcg/disc)	S (44)
2	D-sucrose	W	Penicillin (10mcg/disc)	S (17)
3	D-mannitol	W	Streptomycin (10mcg/disc)	S (30)
4	D-lactose	P	Chloramphenicol (30mcg/disc)	S (28)
5	D-Fructose	P	Vancomycin (30mcg/disc)	S (15)
6	Arabinose	W	Gentamicin (10mcg/disc)	S (15)
7	D-xylose	P	Ampicillin (10mcg/disc)	S (20)
8	Maltose	P	Kanamycin (30mcg/disc)	S (20)
9	Inositol	P	Ciprofloxacin (30mcg/disc)	S (30)
10	Rhamanose	W	Erythromycin (15mcg/disc)	S (20)
11	H_2S production	N	Methicillin (10mcg/disc)	R
12	Citrate utilization	N	Tetracycline (30mcg/disc)	S (22)
13	Gelatin	N	Fluconazole (25mcg/disc)	S (20)
14	Urease	N	Voriconazole (5mcg/disc)	S (21)

(iii) Phylogenetic analysis

The amplified 16S rRNA region and the sequence of the strain were examined by nucleotide BLAST analysis. The 16S rRNA genome sequence of the strain showed 99% similarity with *Streptomyces toxytricini*. The 16S rRNA gene sequence of strain JAR4 (1,303) has been deposited in NCBI GenBank database with accession number KC509577. Figure 2 represents the phylogenetic position of 16S rRNA gene nucleotide sequences between the *Streptomyces toxytricini* JAR4 and reference sequences retrieved from NCBI Gen Bank constructed through the neighbor joining method.

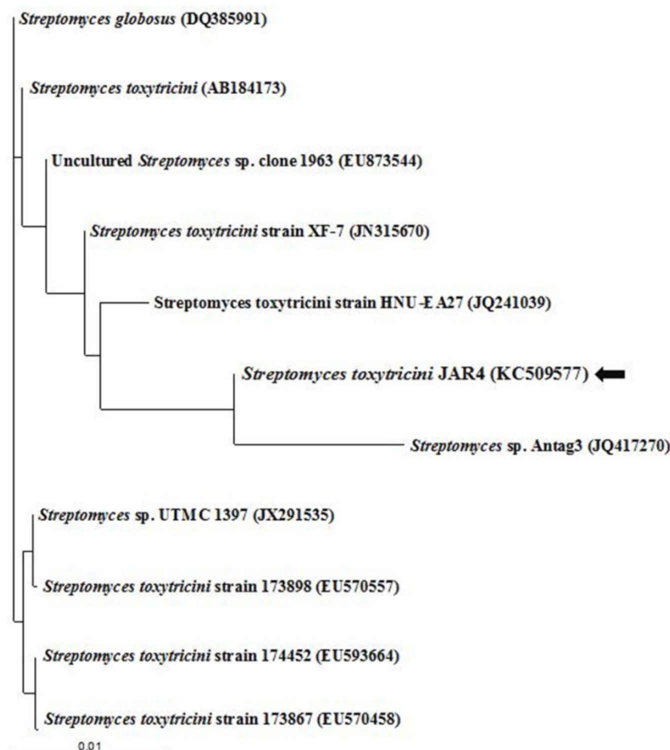

Figure 2
Phylogenetic relationship based on 16S rRNA gene nucleotide sequences between the Streptomyces toxytricini JAR4 and reference sequences retrieved from NCBI Gen Bank constructed through the neighbor joining method

(iv) Optimization of culture medium
The optimization of bioactive metabolite production was carried out in submerged fermentation employing various carbon and nitrogen sources and their effect on the antimicrobial activity was also studied. The strain JAR4 was able to grow in all carbon as well at nitrogen sources. The maximum biomass was yielded by starch and soybean meal on 9d of the fermentation as shown in Figure 3a and Figure 3b respectively. The other carbon sources like glucose, starch, sucrose also favored the antibiotic production but it was less when compared to starch. Soybean meal was found to be effectively utilized by strain JAR4 for biomass production.

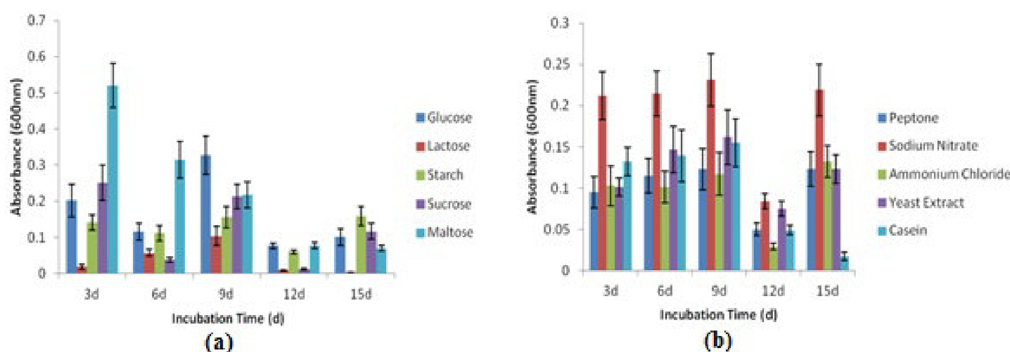

Figure 3
(a) Optimization of carbon sources for fermentation medium of Streptomyces toxytricini JAR4.
(b) Optimization of nitrogen sources for the fermentation medium of Streptomyces toxytricini JAR4.

This article can be downloaded from www.ijpbs.net

(v)Extraction, charcterization and identification of bioactive metabolites

The fermented broth obtained after culturing the strain JAR4 in optimized culture medium was extracted twice with ethyl acetate and concentrated to vaccum to yeild dark reddish color compound. It was further purified by preparative TLC using $CHCl_3$:MeOH (96:4 v/v) as mobile phase. The biological active fraction was analyzed GC-MS. The GC-MS analysis showed (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane with molecular formula $C_{18}H_{31}B$ and molecular weight of 258.25 as shown in Figure 4. In the previous reports related to *Streptomyces toxytricini* it has been known to produce lipstatin a potent irreversible inhibitor of pancreatic lipase. To our knowledge this is the first time that organoborane compound which is chemical synthesized organic compound has been produced by *Streptomyces toxytricini* strain JAR4.

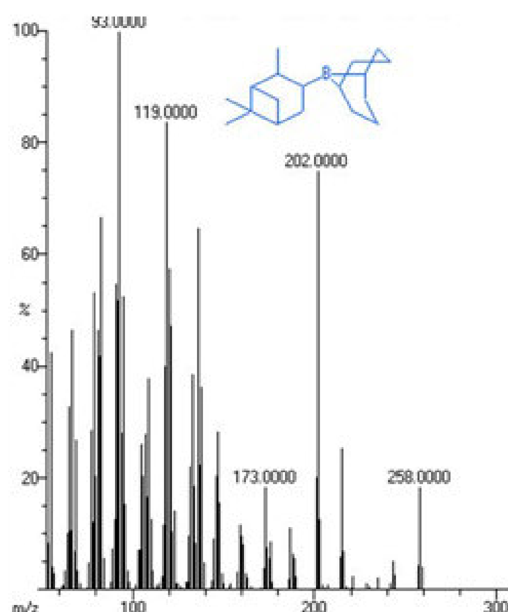

Figure 4
Mass spectrum of bioactive metabolite produced by Streptomyces toxytricini JAR4.

(vi) Biological assays

The test organisms were found to be resistant towards most of the antibiotics including vancomycin (10mcg/disc), erythromycin (15mcg/disc), tetracycline (30mcg/disc) and tigecycline (15mcg/disc). The antimicrobial activity of *Streptomyces toxytricini* JAR4 against clinical pathogens inhibited *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus*, *Klebsiella pnuemoniae*. Table 3 summarizes the antimicrobial activity of bioactive metabolite produced by strain JAR4. The isolated compound possesses effective antimicrobial effect against various pathogenic microorganisms which has been explained by mimimum inhibitory concentration test performed in our study. The minimum inhibitory concentration (MIC) determined against the pathogens *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus* and *Klebsiella pnuemoniae* was found to be 100µg/ml, 150µg/ml, 200µg/ml and 137µg/ml respectively as shown in Table 4.

Table 3
Antimicrobial activity of Streptomyces toxytricini JAR4 against gram positive and gram negative pathogenic bacteria

Sr No.	Test organisms	Diameter zone of inhibition (mm)			
		25µl	50µl	75µl	100µl
1	*Escherischia coli*	-	-	-	-
2	*Proteus mirabilis*	-	-	-	-
3	*Pseudomonas aeruginosa*	15.33±0.41	15.81±0.41	16.33±0.81	18.33±0.41
4	*Klebsiella pneumonia*	16.33±0.41	17.33±0.41	18.66±0.81	19.33±0.41
5	*Enterococcus* sp.	-	-	-	-
6	*Salmonella* sp.	13.00±0.00	13.33±0.41	14.33±0.81	16.33±0.41
7	*Shigella* sp.	-	-	-	-
8	*Staphyloccocus aureus*	15.33±0.41	16.66±0.81	21.33±0.41	22.33±0.81

Table 4
Minimum inhibitory concentration (MIC) of (1s, 5s)-9-(2,6,6 trimethylbicyclo [3.1.1]heptan- 3-yl)-9-borabicyclo[3.3.1]nonane.

Sr no.	Test Organism	MIC value (µg/ml)
1	*Pseudomonas aeruginosa*	100
2	*Klebsiella pneumoniae*	137
3	*Salmonella* sp.	150
4	*Staphylococcus aureus*	200

(vii) Molecular docking

The result of molecular docking with GlcN-6-P was evaluated on the basis of binding free energies of the molecules. The comparison of the binding energies of (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane with a standard competitive inhibitor of glutamine 6-phosphate synthase, antibiotic anticapsin in the active binding site was calculated and was be -6.6 kcal/mol whereas anticapsin involved -7.0 kcal/mol. The binding of R-alpine borane and anticapsin to active binding site has been represented in Figure 5. The *in silico* approach has been used to validate the antimicrobial studies by comparing the binding energies of (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane with standard competitive inhibitor of glutamine 6-phosphate synthase. The binding energy contributes to affinity of the molecules towards active binding site of the protein. The binding energy of R-alpine borane is equivalent to the binding energy of competitive inhibitor (anticapsin).

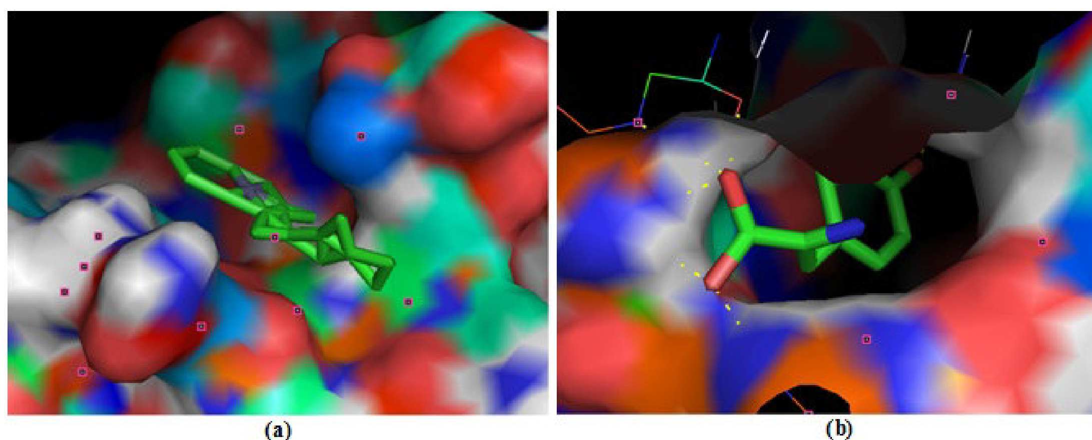

Figure 5
(a)Molecular docking of (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane with auto-dock vina. (b) Molecular docking of Anticapsin by Autodock Vina.

CONCLUSION

The present investigation deals with the *in vitro* and *in silico* antimicrobial property of (1s, 5s)-9-(2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-9-borabicyclo[3.3.1]nonane which is also known as R-alpine borane with 258.24 molecular weight. The present study concludes that R-alpine borane isolated from strain JAR4 can be used as active pharmaceutical agent categorized as microbial natural product and able to bind to active binding site of GlcN-6-P (glutamine 6-phosphate synthase) but it is not competitively inhibiting as anticapsin which inhibits active binding site more specifically. The authors report no conflicts of interest.

REFERENCES

1. Radhakrishnan M., Suganya S., Balagurunathan R., Kumar V. Preliminary screening for antibacterial and antimycobacterial activity of actinomycetes from less explored ecosystems. World J Microbiol Biotechnol, 26: 561–566, (2010).
2. Selvin J., Shanmughapriya S., Gandhimathi R., Kiran G., Ravji T., Natarajaseenivasan K., Hema T.A. Optimization and production of novel antimicrobial agents from sponge associated marine actinomycetes *Nocardiopsis dassonvillei* MAD08. Appl Microbiol Biotechnol, 83: 435–445, (2009).
3. Busti E., Monciardini P., Cavaletti L., Bamonte R., Lazzarini A., Sosio M., Donadio S. Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. Microbiol, 152: 675–683, (2006).
4. Arasu M.V., Duraipandiyan V., Agastian P., Ignacimuthu S. Antimicrobial activity of *Streptomyces* sp. ERI-26 recovered from Western Ghats of Tamil Nadu. J Mycol Méd, 18: 147-153, (2008).
5. Smaoui S., Mathieu F., Elleuch L., Coppel Y., Merlina G., Karray-Rebai I., Mellouli L. Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp. TN256 strain. World J Microbiol Biotechnol, 28: 793-804, (2012).
6. Bibb M.J. Regulation of secondary metabolism in *Streptomyces*. Curr Opion Microbiol, 8: 208-215, (2005).
7. Watve M.G., Tickoo R., Jog M.M., Bhole B.D. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol, 176: 386–390, (2001).
8. Chatterjee S., Vijayakumar E.K., Franco C.M., Maurya R., Blumbach J., Ganguli

B.N. Phencomycin, a new antibiotic from a *Streptomyces* species HIL Y-9031725. J Antibiot, 48: 1353–1354, (1995).

9. Gebhardt K., Schimana J., Krastel P., Dettner K., Rheinheimer J., Zeeck A., Fiedler H. Endophenazines A-D, New phenazine antibiotics from the arthropod associated endosymbiont *Streptomyces anulatus*. I. Taxonomy, fermentation, isolation and biological activities. J Antibiot, 55: 794–800, (2002).
10. Marchetti L., Sabbieti M.G., Menghi M., Materazzi S., Hurley M.M., Manghi G. Effects of pthalate esters on actin cytoskeleton of Py1a rat osteoblasts. Histol Histopathol, 17: 1061–1066, (2002).
11. Yassa N., Masoomi F., Rohani Rankouhi S.E., Hadjiakhoondi A. Chemical composition and antioxidant activity of the extract and essential oil of *Rosa damascena* from Iran, population of Guilan. DARU, 3: 175–180, (2009).
12. El-Sakhawy F.S., El-Tantawy M.E., Ross S.A., El-Sohly M.A. Composition and antimicrobial activity of the essential oil of Murraya exotica. Flavour Frag J, 13: 59–62, (1998).
13. Ben Ameur Mehdi R., Mellouli L., Chabchoub F., Fotso S., Bejar S. Purification and structure elucidation of two biologically active molecules from a new isolated *Streptomyces* sp. US24 strain. Chem Nat Comp, 40: 510–513, (2004).
14. Lam K.S. Discovery of novel metabolites from marine actinomycetes. Curr Opin Microbiol, 9: 245-251, (2006).
15. Lanoot B., Vancanneyt M., Dawyndt P., Crockaert M., Zhang J., Huang Y., Liu Z., Swings J. BOX-PCR fingerprinting as a powerful tool to reveal synonymous names in the genus *Streptomyces*. Emended descriptions are proposed for the species *Streptomyces cinereorectus*, *S. fradiae*, *S. tricolor*, *S. columbiensis*, *S. filamentosus*, *S. vinaceus* and *S. phaeopurpureus*. Syst Appl Microbiol, 27: 84–92, (2004).
16. Liu Z., Shi Y., Zhang Y., Zhou Z., Lu Z., Li W., Rodriguez C., Goodfellow M. Classification of Streptomyces griseus (Krainsky 1914) Waksman and Henrici 1948 and related species and the transfer of '*Microstreptospora cinerea*' to the genus *Streptomyces* as *Streptomyces yanii* sp. nov. Int J Syst Evol Microbiol, 55: 1605–1610, (2005).
17. Vidya S.M., Manjunatha V., Rajesh K.P., Bharath B.R., Manjunatha H. Antibacterial and molecular docking studies of entagenic acid, a bioactive principle from seed kernel of *Entada pursaetha* DC Med Chem Res, 21: 1016-1022, (2012).
18. Bearne S.L., Blouin C. Inhibition of Escherichia coli glucosamine-6-phosphate synthase by reactive intermediate analogues. The role of the 2-amino function in catalysis. J Biol Chem, 275: 135-140 (2000).
19. Badet-Denisot M.A., Rene L., Badet B. Mechanistic investigations on glucosamine-6-phosphate synthase. Bull Soc Chim Fr, 130: 249–255, (1993).
20. Massiere F., Badet-Denisot M.A. The mechanism of glutamine-dependent amidotransferases. Cell Mol Life Sci, 54: 205–222, (1998).
21. Williams S.T., Cross T. Isolation, purification, cultivation and preservation of actinomycetes. Methods Microbiol, 4: 295–334, (1971).
22. Shirling E.B., Gottlieb D. Methods for characterization of *Streptomyces* species. IJSEM, 3: 313-340, (1966).
23. Cappuccino J.G., Sherman N. Microbiology-laboratory Manual, Pearson Education Inc., New Delhi, India, 2004.
24. Hopwood D.A., Bibb M.J., Chater K.F., Kieser T., Bruton C.J., Kieser H.M., Lydiate D.J., Smith C.P., Ward J.M., Schremph H. Genetic manipulation of *Streptomyces*: a laboratory manual, John Innes Foundation, Norwich, 1985.
25. Thompson J.D. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res, 22: 4673-4680, (1994).
26. Saitou N., Nei M. The neighbor-joining method: a new method for reconstructing

phylogenetic trees. Mol Biol Evol, 4: 406-25, (1987).
27. Boruwa J., Kalita K., Barua N.C., Borah J.C., Mazumder S., Thakur D., Gogoi D.K., Bora T.C. Synthesis, absolute stereochemistry and molecular design of the new antifungal and antibacterial antibiotic produced by *Streptomyces* sp. 201. Bioorg Med Chem Lett, 14: 3571-3574, (2004).
28. Dubey V., Ghosh A.R., Mandal B.K. Appraisal of Conjugated Linoleic Acid Production by Probiotic Potential of *Pediococcus* sp. GS4. Appl Biochem Biotechnol, 168: 1265–1276, (2012).
29. Oleg T., Olson A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem, 30: 455-461, (2010).
30. Sanner M.F. Python: a programming language for software integration and development. J. Mol. Graph Model, 17: 57-61, (1999).