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ORIGINAL ARTICLE

Isolation and Structure Elucidation of Helalomycin-1, a New Antitumor Antibiotic Produced by a New Marine Bacterium *Streptomyces* sp. Strain HuGu-11

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ABSTRACT

In our continuing search for new compounds and biologically active substances from marine microorganisms, new potent antitumor antibiotic designated helalomycin-1(chlorohydronaphthol) was isolated from the fermentation broth of a new marine bacterium *Streptomyces* sp. HuGu-11. The producer strain was found to have morphological and chemotaxonomic characteristics typical of streptomycetes. Cultural, physiological characteristics and phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene of this isolate indicated that this strain is identified as *Streptomyces* sp. strain HuGu-11. The nucleotide sequence of 16S rRNA of this isolate was deposited in the NCBI GenBank with an accession number: JX013911. In its culture supernatant, this organism could produce one major bioactive compound separated by silica gel column chromatography and then purified on sephadex LH-20 column. The isolated compound exhibited strong antibacterial activities mainly against Gram-positive bacteria; *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* MRSA with MIC values of 0.5, 1.5 and 1.3 µg/mL respectively, also it showed a potent cytotoxic activities against cell lines viz. HCT116 (colon carcinoma), MCF7 (breast carcinoma), HEPG2 (hepatic carcinoma) and HELA (cervix carcinoma) with IC_{50} values of 2.23, 7.04, 8.24 and 25.24 µg/mL respectively. The intact structure of the isolated compound was deduced by spectroscopic (UV, FTIR, Mass, ^{1}H and ^{13}C NMR spectra) and X-ray crystallographic analyses. Via comparison to the reference data in the relevant literatures and in the database search, this compound was differing from the available known antibiotics therefore; it was identified as a new antitumor antibiotic and designated helalomycin-1.

Key words: *Streptomyces* sp. HuGu-11, Biological activity, Phenotypic and phylogentic identification, Spectroscopic and cyrstallogarphic analyses, Chlorohydronaphthol.

Introduction

The search for novel drugs is still a priority goal for cancer therapy. This is mainly fueled by the rapid resistance development of carcinogenic cells towards multiple chemotherapeutic drugs. Furthermore, the high toxicity usually associated with cancer chemotherapy drugs and their undesirable side effects increase the demand for novel antitumor drugs that cause fewer side effects and/or with greater therapeutic efficiency against untreatable tumors (Demain and Sánchez, 2009). Moreover, some patients are more sensitive to certain chemotherapeutic drugs than the others and there are even certain infectious diseases that cannot be controlled using commercially available drugs. Thus, to combat the multi drug-resistant organisms, introduction of new antimicrobial compounds or antibiotics from a new source is undeniably an urgent matter (Saha *et al*., 2010).

Naturally fermented products have long been studied as attractive targets for this purpose due to their amazingly diverse and complex chemical structures as well as biological activities. As such, a number of revolutionary drugs developed from naturally fermented products have contributed to global human health (Nagao *et al*., 2010). Natural products remain either the source or inspiration for a significant proportion of the new small-molecule chemical entities introduced as drugs (Newman *et al*., 2003).

Actinomycetes are the producers of the most natural products with different biological activities, including antitumor properties. These antitumor compounds belong to several structural classes such as anthracyclines, enediynes, indolocarbazoles, isoprenoides, macrolides, non-ribosomal peptides, and others (Carlos *et al*., 2009). Among the actinomycetes, around 7,600 compounds are produced by the *Streptomyces* species. Many of these secondary metabolites are potent antibiotics, making Streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Berdy, 2005).

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Streptomycetes produce clinically useful antitumor drugs such as anthracyclines (aclarubicin, daunomycinand doxorubicin), peptides (bleomycinand actinomycinD), aureolicacids (mithramycin), enediynes (neocarzinostatin), antimetabolites (pentostatin), carzinophilin, mitomycins and isoprenoids, monoterpenes, sesquiterpenes, and diterpenes (Dairi, 2005).

Microbial natural products are an important source of both existing and new drugs and the secondary metabolites produced by actinomycetes possess a wide range of biological activities other than antitumor, such as antibacterial, antifungal, antioxidant, and antiviral (Wu *et al*., 2007). All actinomycetes including streptomycetes use the MEP pathway for the formation of isopentenyl diphosphate, but there are also some actinomycetes strains that use the mevalonate pathway for production of terpenoids as secondary metabolites. Such metabolites possess antibacterial, antitumor or antioxidative characteristics as well (Kuzuyama and Seto, 2003).

During our screening program for a novel bioactive principle from marine actinomycetes, we discovered that one of the most active strains is *Streptomyces* sp. HuGu-11. The culture filtrate of this strain was characterized by its antimicrobial and cytotoxic activities. The structure of the active compound was elucidated as chlorohydronaphthol based on spectroscopic and X-ray crystallographic analyses. It was then defined as a new derivative of bicyclic sesquiterpenoids.

Materials and Methods

Sample collection and isolation of strain:

An actinomycete strain, HuGu-11, found to be a common isolate of sandy sediments were collected from different coastal sites within the El-Gouna region, Hurghada, Egypt. From each location, 15 g of sediment sample was collected from 50 to 100 cm below the ground surface. These samples were put into small pre-labeled plastic bags and tightly sealed. The samples were then pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37°C for 4 days. After that, the samples were subjected to serial dilution (up to 10^{-6} dilution) using the soil dilution plate technique (Williams and Cross, 1971) in a glycerol asparagine agar medium [International *Streptomyces* Project (ISP)-5] (Shirling and Gottlieb, 1966) that contained 50% natural seawater and was maintained at 28°C for 7-10 days.

Biological activities of the isolated compound:

Antimicrobial spectrum (MIC):

Minimum inhibitory concentrations (MICs) of the isolated compound were determined by agar plate diffusion assay against opportunistic and pathogenic bacteria as well as fungi according to the method of Cappuccino and Sherman (2004). Nutrient agar medium was used for culturing test bacteria that include *Bacillus subtilis* (ATCC 6633); *Staphylococcus aureus* (ATCC 6538); *Staphylococcus aureus* (MRSA); *Escherichia coli* (ATCC 7839); *Pseudomonas aeruginosa* (ATCC 9027) and *Pseudomonas fluorescens* (ATCC 13525). Czapek-Dox agar was employed in culturing the test fungi, namely *Candida albicans* (ATCC 10231); *Aspergillus niger* (ATCC 16404) and *Aspergillus flavus* (ATCC 16883).

Cups of about 6 mm were cut into the agar with the help of a sterilized corck borer. Aliquots of the purified compound were dissolved in DMSO at concentrations ranging from 0 to 1000 µg/mL. The dissolved compound was subsequently added into the cups and the inoculated plates were incubated for 24h at 37°C for bacteria and 48-72h at 28°C for fungi. The lowest concentration of the bioactive compound exhibiting antimicrobial activity against the test microbes was considered as the MIC of this compound.

Antitumor bioassay:

The isolated compound was tested in vitro for antitumor activity by MTT assay against various human solid tumor cell lines such as human brain carcinoma cells (U251); human breast carcinoma cells (MCF7); human cervix carcinoma cells (HELA); human liver carcinoma cells (HEPG2); human colon carcinoma cells (HCT 116) and human lung carcinoma cells (H460). This testing was done at the National Cancer Institute (NCI), Cairo, Egypt according to the method described by Mosmann (1983) and Alley *et al*. (1988). In brief, the tumor cells taken from the exponential phase of cultures were seeded into 96-well plates in 180 µL RPMI 1640-medium at between $2x10^4$ and $3x10^4$ cells/mL and then incubated at 37°C with 5% CO_2 for 24h.

Different concentrations of the compound under scrutiny were diluted in the RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo, and U.S.A). About 20 µL of the sample along with 20 µL of RPMI 1640 medium acting as the negative control were then added to the plates in triplicate. This gave the final dilution fold of 0, 2.5, 5, 10, 20 and 40 for the diluted compound. The cells were incubated for an initial period of 44h. This was

followed by the addition of 20 µl of MTT (5 mg/mL) and the cells were incubated again for another 4h. After pouring out the tested dilutions, 150 µl of DMSO (Sigma Chemical Co., St. Louis, Mo, and U.S.A) was added to dissolve the formazan product. Absorbance at 490 nm was measured using an ELIZA microplate reader (Meter tech. ∑ 960, U.S.A.).

The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line. The results were expressed as the drug concentration that inhibited cell growth by 50% as compared to the controls (IC_{50}). The IC_{50} values were calculated using the software, Origin 6.1.

Taxonomic characterization of the isolated actinomycete, HuGu-11:

Conventional taxonomy:

The characterization of the HuGu-11 strain followed the guidelines adopted by the International *Streptomyces* Project (ISP). The melanin pigment production, nitrate reduction, utilization of C and N sources, and the culture characteristics were studied in accordance with the guidelines established by the ISP (Shrilling and Gottlieb, 1966). Colors were assessed on the scale adopted by Kornerup and Wanscher (1978). Diaminopimelic acid isomers in the cell-wall were analyzed by using the methods of Becker *et al.* (1965); Lechevalier and Lechevalier (1980).

The electron microscope study was carried out using a scanning electron microscope (JEOL JSM 5300, JEOL Technics Ltd., Japan) in accordance to the method described by Bozzola and Russell (1999). The physiological and biochemical characteristics such as the activities of lipase (Elwan, 1977), protease (Chapman, 1952), α-amylase (Cowan, 1974) and catalase (Jones, 1949) were tested. Additionally, the hydrogen sulfide production was analyzed; oxidase tests were also performed (Cowan, 1974).

Molecular and phylogenetic identification:

The actinomycete isolate, HuGu-11, was used to inoculate 50 ml of ISP-2 broth and the culture was incubated at 200 rpm and 28°C for 24h. The total genomic DNA was extracted according to the method of Sambrook *et al.* (1989).

The 16S rRNA of the strain was amplified by PCR using a GeneAMP PCR System 9700 from PE Applied Biosystems (Perkin Elmer, Ohio, USA). The following primers were used in the Biolegio BV software: F27; 5'-AGAGTTTGATCMTGGCTCAG-3' and R1492; 5'-TACGGYTACCTTGTTACGACTT-3' (Biolegio, Nijmegen, the Netherlands) according to Edwards *et al.* (1989). The PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were detected using a gel documentation system (Alpha-Imager 2200, CA, USA). The PCR products were sequenced using the Gene Analysis Unit in the genetics laboratories of an Egyptian Company for Production of Vaccines, Sera and Drugs (VACSERA) at El-Dokki, Cairo, Egypt.

The DNA sequences were determined using an ABI PRISM 377 DNA sequencer and ABI PRISM BigDye Terminator Cycle Sequencing Kit (Perkin Elmer, Ohio, US) at a sequencing facility of Cornell University in the U.S. BLAST (www.ncbi.nlm.gov). A multiple sequence alignment and molecular phylogenetic analysises were performed using the BioEdit software (Hall, 1999). The phylogenetic tree was constructed using the TreeView program (Page, 1996). The nucleotide sequence of the 16S rRNA gene of strain was deposited in the NCBI Genbank with accession number: JX013911. The strain was maintained on yeast–malt extract agar medium (ISP-2) at 4°C for further study.

Fermentation and isolation of the bioactive compound:

HuGu-11 strain grown on ISP-2 agar slants (14-day old) was used to inoculate 25 Erlenmeyer flasks (1000 mL) with each containing 400 mL of sterile starch-nitrate medium. The medium consisted of (g/L) Starch: 20.0; $NaNO_3$: 2.0; K_2HPO_4: 1.0; $MgSO_4 \cdot 7H_2O$: 0.5; KCl: 0.5; trace salt solution: 1.0 ml [(g/L): $CuSO_4 \cdot 5H_2O$: 0.64; $FeSO_4 \cdot 7H_2O$: 0.11; $MnCl_2 4H_2O$: 0.79 and $ZnSO_4 \cdot 7H_2O$: 0.15]; natural sea water: 500 mL and distilled water: 500 mL; the medium pH was adjusted to 7.0 before sterilization. The inoculated flasks were incubated on a rotary shaker at 30°C and 220 rpm for 7 days.

The culture broth (35 L) obtained after filtration was extracted twice with ethyl acetate and subsequently concentrated under reduced pressure using a rotary evaporator (Büchi, R-114, Switzerland).The temperature was maintained to be less than 50°C to give 4.8 g of crude extract which was then dissolved in 5 ml methanol and applied to silica gel column (22 X 5 cm, Silica gel 60; Merck) as stationary phase. The column was eluted using a gradient polarity of solvent system; ethyl acetate:methanol (10:1 to 1:10). 50 mL fractions were collected and the fractionation process was monitored through TLC analysis. The fractions that exhibited similar TLC profiles

were combined to give a final total of 13 fractions (H_1-H_{13}) collected at 20, 95, 130, 35, 65, 128, 59, 158, 71, 96, 119, 212, and 420 mg respectively.

Bioactivity of the obtained fractions was evaluated for antimicrobial activity against *B. subtilus* (ATCC 6633) as the most sensitive test organism to the original crude extract through the agar plate diffusion assay (Cappuccino and Sherman, 2004). The bioactive fractions (fractions having highest antibacterial activity) were tested again for cytotoxic activity against human colon cancer cell line (HCT116) as the most sensitive cancer cell line to the original crude extract by MTT assay Mosmann (1983) and Alley *et al.* (1988). Fractions that exhibited cytotoxic activity were applied on sephadex LH-20 column (2 X 15 cm) using 100% methanol as the eluent solvent (500 mL) and lastly, 5 mL of the fractions were collected. The sub-fraction 89 (250 mg) was separated as a single band to yield purified active compound.

Structural elucidation of the bioactive pure compound:

Structural elucidation of the obtained pure compound was achieved via spectroscopic analyses: UV spectra in methanol using a 20 UV/VIS spectrophotometer (Perkin Elmer, Ohio, U.S.), Fourier Transform Infrared (FTIR) using KBr method of (JASCO FT/IR–6100) spectrophotometer. Nuclear magnetic resonance (NMR) spectra using a Varian Mercury VX-300 NMR spectrometer operated at 300 MHz for ^{1}H and 75 MHz for ^{13}C in $CDCl_3$. Electron Ionization Mass Spectroscopy (EIMS) spectra were obtained with a Direct Inlet part DI-50 connected to the mass analyzer m Shimadzu GC/MS-QP5050, at the Micro-Analytical Center, Faculty of Science, Cairo University, Egypt.

Molecular modeling:

The structural model was built using the BUILDER module in the Spartan 08 program (Spartan, 2008). The optimization conformational analyses of the built molecules were performed in a two-step procedure. Firstly, this compound was submitted to the energy minimization tool. Secondly, the geometry of the compound was optimized using the AB initio/STO-3G with Restricted Hartree-Fock (RHF) and an RMS gradient of 0.05 Kcal/moL.

Results and Discussion

Biological Activity:

Antimicrobial spectrum of isolated compound:

The isolated compound was evaluated for its antimicrobial potency against bacteria and fungi and this was expressed as MIC. The compound proved active against a number of Gram-positive and Gram-negative bacteria, but Gram-positive bacteria were more sensitive to the compound than Gram-negative representatives. On the other hand, the compound has weaker effect against *C. albicans* whereas *A. niger* and *A. flavus* were even less susceptible to the compound (Table 1). Many related terpenoidcompounds isolated from marine *Streptomyces* were found to have antibacterial activity against *Staphylococcus aureus* 209P JC-1 (MIC recorded at 2.0 and 3.7 µg/mL), *Bacillus subtilis* ATCC6633 (MIC recorded at 1.0 and 3.7 µg/mL), *Enterococcus faecalis* ATCC19433 (MIC recorded at 31.6 and 14.8 µg/mL), *Enterococcus faecium* ATCC19434 (MIC recorded at 15.8 and 14.8 µg/mL) and *Streptococcus pyogenes* ATCC12344 (MIC recorded at 7.8 µg /mL) (Keiichiro *et al*., 2008b).

Table 1: Antimicrobial activities (MIC µg/mL) of helalomycin-1 produced by *Streptomyces* sp. HuGu-11.

Test organism	MIC (µg/mL)
Bacteria	
Bacillus subtilis ATCC 6633	0.5
Staphylococcus aureus ATCC 6538	1.5
Staphylococcus aureus MRSA	1.3
Escherichia coli ATCC 7839	2.1
Pseudomonas aeruginosa ATCC 9027	3,8
Pseudomonas fluorescens ATCC 13525	2.8
Yeasts	
Candida albicans ATCC 10231	5.2
Fungi	
Aspergillus niger ATCC 16404	>50
Aspergillus flavus ATCC16883	>50

Cytotoxic activities of isolated compound:

In the cytotoxicity tests (Fig. 1), the isolated compound exhibited strong cytotoxicity against human colon cancer carcinoma cells with an IC_{50} of 2.24 µg/mL. Its cytotoxicity is moderate against human liver carcinoma cells with an $IC_{50}$of 8.24 µg/mL and human breast carcinoma cells with an IC_{50} of 6.58 µg/mL. On the other hand, it has a weaker effect against human breast carcinoma cells (MCF7) with an IC_{50} of 25.24 µg/mL, and remained inactive against human lung carcinoma cells (H460) and human brain carcinoma cells (U251). In fact, there are several chlorinated dihydroquinones with a mixed terpenoid/polyketide origin produced in saline culture of the actinomycete strain CNQ-525 and isolated from ocean sediments near La Jolla, California that have been found to be cytotoxic toward human colon carcinoma HCT-116 cells with IC_{50} of 2.4, µg/mL (Soria-Mercado *et al*., 2005).

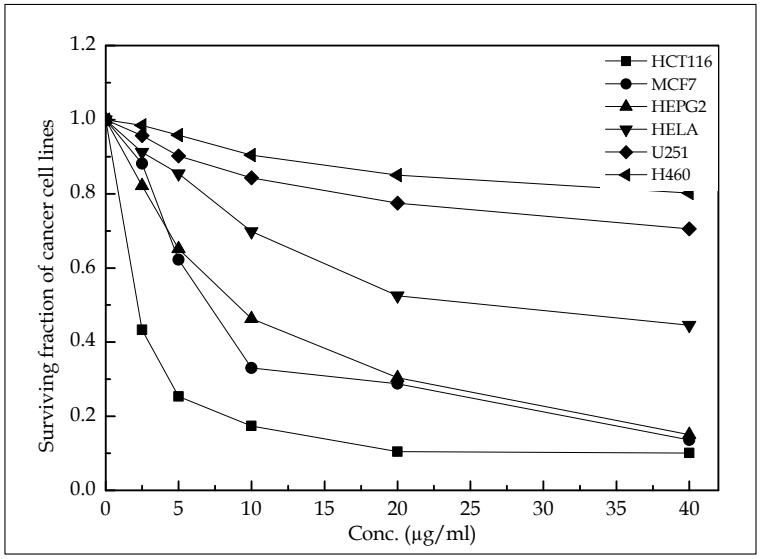


Fig. 1: Cytotoxicity profile of helalomycin-1 against different human cell lines.

Fermentation, purification and isolation of the bioactive compound:

In the present work, 35L of *Streptomyces* sp. strain HuGu-11 were cultured. The exhaustive extraction of the bacterial spent culture broth yielded 4.8 g of crude extract. Bioassay-guided fractionation of the crude extract against the tested bacterial strain exhibited that fractions denoted as H-3, H-4, H–5, H-12 and H-13 are the active ones (Fig. 2). Among these fractions, only two fractions (H-3 and H-13) proved cytotoxic activity against the tested cancer cell line (Fig. 3). The fraction H-13 (420 mg) was the more promising one so it was thus subjected to further purification to give pure bioactive compound with a net yield of 250 mg.

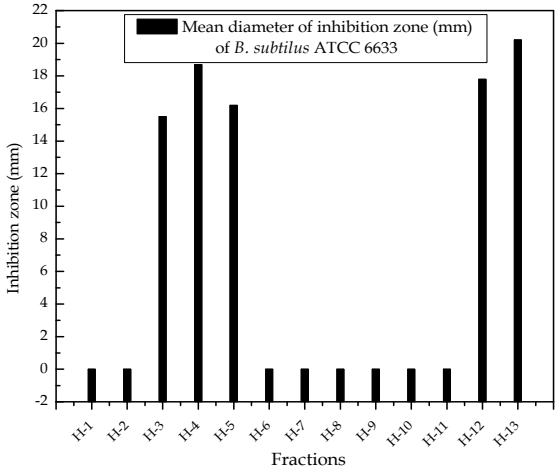


Fig. 2: Antibacterial activities of *Streptomyces* sp. HuGu-11 crude extract fractions.

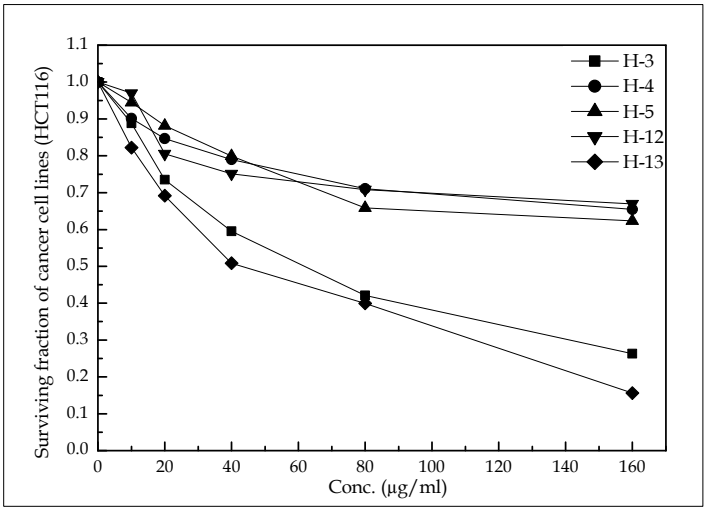


Fig. 3: Cytotoxicity profile of the bioactive fractions of *Streptomyces* sp. HuGu-11.

Taxonomic characterization of the actinomycete isolate, HuGu-11:

Conventional methods:

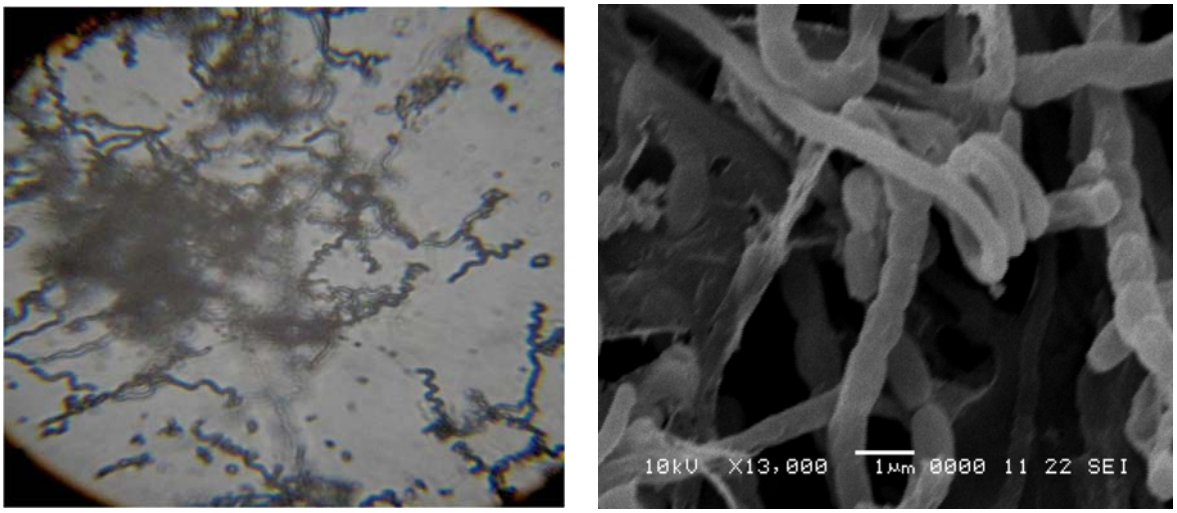
The cultural characteristics of actinomycete isolate, HuGu-11, grown on different ISP and non-ISP media (Table 2) showed that the aerial hypha of the strain was gray. Therefore, it was assigned to the gray series with slight dark yellow substrate mycelium. In the culture media tested, the strain was found to produce light orangey yellow diffused pigments.

Table 2: Cultural characteristics of *Streptomyces* sp. HuGu-11, on different culture media.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
Tryptone yeast extract broth (ISP-1)	Weak	White (ISCC-NBS 263)	Slightly yellow (ISCC-NBS 84)	None
Yeast -malt extract agar (ISP-2)	Good	Moderate gray (ISCC-NBS 265)	Dark yellow (ISCC-NBS 88)	Light orange yellow (ISCC-NBS 70)
Oat meal agar (ISP-3)	Good	Light Gray (ISCC-NBS 264)	Pale yellow (ISCC-NBS 89)	Light orange yellow (ISCC-NBS 70)
Inorganic-trace salt- starch agar (ISP-4)	Good	Moderate gray (ISCC-NBS 265)	Pale yellow (ISCC-NBS 89)	Dark yellow (ISCC-NBS 88)
Glycerol asparagine agar (ISP-5)	Good	Light Gray (ISCC-NBS 264)	Pale greenish yellow (ISCC-NBS 104)	Dark yellow (ISCC-NBS 88)
Peptone yeast extract iron agar (ISP-6)	No growth	-	-	-
Tyrosine agar (ISP-7)	Moderate	Deep gray (ISCC-NBS 266)	Dark yellow ISCC-NBS 88)	Deep olive brown (ISCC-NBS 96)
Starch nitrate agar	Good	Moderate gray (ISCC-NBS 265)	Pale greenish yellow (ISCC-NBS 104)	Light orange yellow (ISCC-NBS 70)
Nutrient agar	Weak	White	Colourless	None
Czapek-Dox agar	No growth	-	-	-

Micro-morphological studies of HuGu-11 strain through light and SEM microscope revealed that the spore chains of the strain were spirals with smooth spore surface (Fig. 4a, b). The entire hydrolysate cell of this strain contained LL-diaminopimelic acid (LL-DAP), indicating that the strain has a chemo-type I cell wall. The presence of LL-DAP in the cell wall also signifies that, this strain is *Streptomyces* as identified by Lechevalier and Lechevalier (1970) they reported that cell-wall composition analysis is one of the main methods that can be employed to identify the chemotaxonomic characteristics of *Streptomyces*.

The physiological and biochemical properties; C- and N-sources utilization; tolerance to NaCl; growth pH; growth temperature; growth inhibitors and sensitivity to antibiotics were presented in table 3. The cultural and physiological properties of the isolated strain were compared to those reported for actinomycetes as described in Bergey's Manual of Determinative Bacteriology; it is then established that this strain belongs to the genus *Streptomyces* (Lechevalier *et al*., 1989).



(a) **(b)**

Fig. 4: (a) Light microscopy image of the aerial mycelium showing a spiral spore chain (G x 400); **(b)** Scanning electron micrograph (X13,000) showing smooth spore surface cells of *Streptomyces* sp. HuGu-11 grown on yeast-malt extract agar (ISP-2) for 14 days at 28 °C.

Table 3: Morphological, physiological and biochemical characteristics of *Streptomyces* sp. HuGu-11.

Character	Results	Character	Results
Morphological characteristics:		N-sources utilization	
Spore chain	Spiral	L-Asparagine	+
Spore mass	Light gray	L-Glutamic	++
Spore surface	Smooth	L-lysine	+
Motility	Non motile	L-Ornithine	Wg[f]
Color of substrate mycelium	Grayish	L-Tyrosine	+
Diffusible pigment	Light orange yellow	L-Valine	++
Cell wall hydrolysis:		L-Histidine	+
Diaminopimelic acid (DAP)	LL-DAP	Glycine	+++
Sugar pattern	ND[a]	L-leucine	+
Physiological characteristics:		L-Phenyl alanine	-
Melanin pigment:		L-Serine	+++
Peptone-yeast extract iron agar	-[b]	L-Methionine	-
Tyrosine agar	+[c]	L-Cysteine	++
Tryptone-yeast extract broth	-	Tolerance to NaCl (%)	
Hydrolysis of Protein	+	3:6	+++
Hydrolysis of Starch	+	7:10	++
Hydrolysis of Lipid	-	11	+
Catalase production	+	12	Wg[f]
H_2S production	-	13	-
Nitrate reduction	+	Growth inhibitors	
Tyrosine degradation	+	Crystal violet (0.0001%)	+
Urea test	-	Crystal violet (0.0002%)	-
C-sources utilization		Sodium azide (0.02)	+
D-Glucose	+++[d]	Phenol (0.1%)	+
D-Mannose	+	Growth temperature °C	
D-Mannitol	+	10	-
D (+) trehalose	++[e]	25:37	+
L-Rhamnose	-	Growth pH	
D-Fructose	+	5:7	+++
Meso-Inositol	+	8	+
D-Galactose	-	9	-
Sucrose	+	Sensitivity to antibiotics	
Maltose	-	Rifampicin (50 µg mL^{-1})	+
Starch	+++	Erythromycin (15 µg mL^{-1})	-
Cellulose	+	Penicillin (10 µg mL^{-1})	+
Salicine	-		
L(+)Arabinose	++		
Raffinose	-		

[a]ND= not detected, [b](-) = negative, [c](+) = moderate , [d](+++) = abundant, [e](++) = good growth, [f](wg)= weak growth.

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Molecular and phylogenetic identification:

To further confirm the identity of the isolated strain, the 16S rRNA sequence of the local isolate was compared to sequences of 10 *Streptomyces* sp. through multiple sequence alignment. The primer pair, F27/R1492, was used to amplify the fragments of the genomic DNA's expected size (1500 bp); this DNA was isolated from the positive control strain, *S. griseus*. This primer pair was especially used to amplify the 27-bp and 1492-bp fragments. The results obtained are in agreement with those of Edwards *et al.* (1989) who found that these primers are specific for bacteria. Hongyu *et al.* (2011) isolated and identified a marine *Streptomyces* sp. W007 on Gause's synthetic agar containing 50% natural seawater from the marine sediments of Kiaochow Bay, Qingdao. A phylogenetic tree was derived from the distance matrices using a neighbor-joining method (Fig. 5). A good congruence was found between the 16S rRNA sequences of the *Streptomyces* sp. MS-266 and of strain HuGu-11.

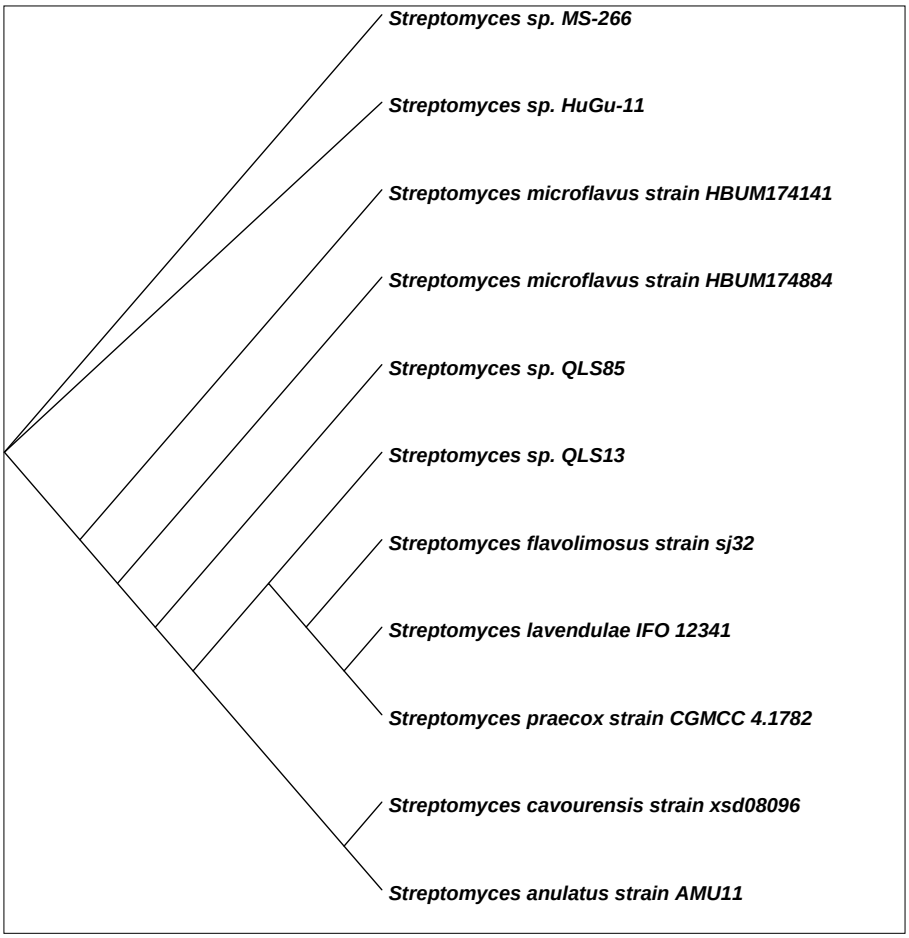

Fig. 5: The phylogenetic position of *Streptomyces* sp. HuGu-11 among neighboring method showing 16S rDNA tree of the phylogenic similarity comparing with the sequences of other known *Streptomyces* species.

At the turn of the century, a shift driven by new technologies started in search of exploitable biology. This shift is exemplified by the extent of biodiversity now revealed and recognized by biologically informative and data-rich methods functioning at the molecular scale. Such methods are often employed for characterizing organisms and defining taxon-property relationships through high-throughput screening and the PCR and DNA sequencing (Alan *et al.,* 2000). In this study, the phylogenetic analyses coupled with a conventional methods related to HuGu-11 indicated that the most closely-related strain is *Streptomyces* sp. MS-266 therefore, *Streptomyces* sp. HuGu-11 is proposed as its name. The use of genotypic and phenotypic techniques gives a better resolution in species-level identification (Mizui *et al*., 2004).

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Spectroscopic studies on pure isolated active compound:

The compound was isolated as a colorless amorphous solid [UV (MeOH): λmax (log €) nm, 237 (3.6) nm (Fig. 6); IR (KBr): *v*max 3417 cm^{-1} (OH), 1620 cm^{-1} and 1384 cm^{-1} (C=C) (Fig. 7)]. The molecular formula was established as $C_{18}H_{29}ClO$ based on EIMS (Fig. 8) that showed the presence of molecular ion (M^+) at m/z 296 and other important ions at m/z 123, 79 and 71 (base peak). The ^{1}HNMR spectrum of helalomycin-1 (Fig. 9) exhibited signals at δ_H: 0.97-2.02 (m, 19H, [4(3H,CH_3)+2H, CH_2CHOH, 2H,CH,$CHCH_3$+1H,CH,CHCH(CH_3)2 + 2H,2H,CH-alicyclic ring]); 2.33 (broads,1H,OH); 3.5 (m, 2H, CH_2-CH_2Cl); 4.110-4.114 (m, 2H, [2H,CH_2-CH C in ring+ 1H,OH, CHOH]; and 5.34-5.384 (m, 2H, 2CH- CH CH_2). Carbon signals in the ^{13}CNMR spectrum (Fig. 10) displayed the presence of 20 carbons, 1 quaternary, 4 methines, 10 methylenes, and 5 methyl groups.

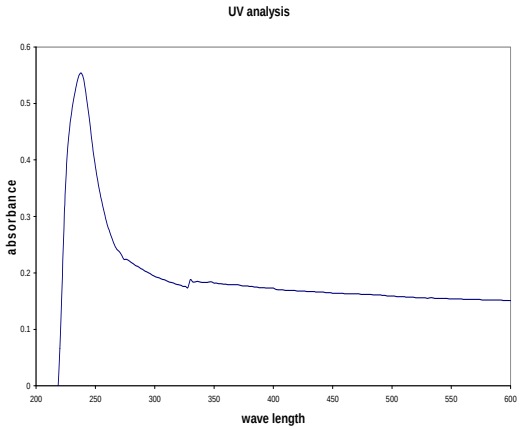

Fig. 6: UV-Spectrum of helalomycin-1 in methanol.

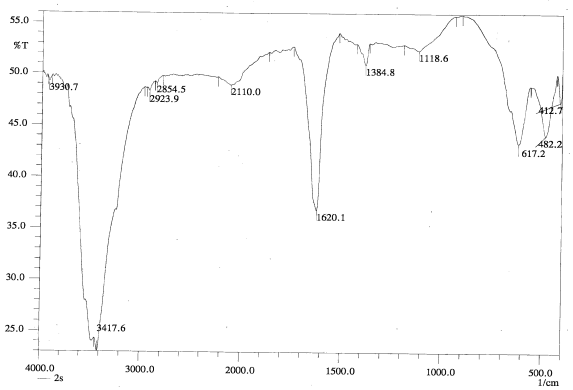

Fig. 7: IR-Spectrum of helalomycin-1 in KBr.

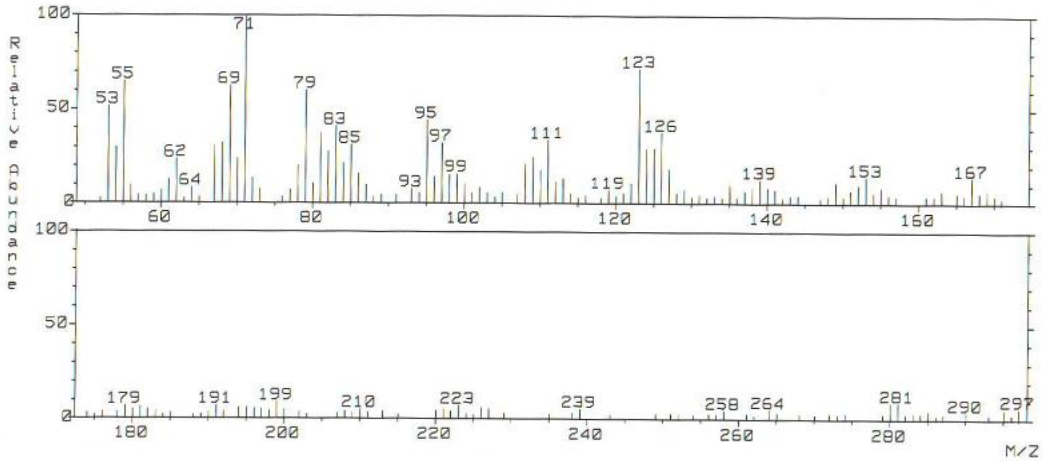

Fig. 8: MS spectrum of helalomycin-1**.**

A signal was detected at δ: 16.101-19.858, 22.58 and 22.670 (4**C**H_3); 24.421 (**C**H(CH_3)$_2$); 24.42-25.08 (2C, **C**HCH_3 $_{ringA}$); 27.92 (**C**H_2Cl); 28.65 (**C**H-$_{ringB}$); 32.84 (**C**H_{2ringA}); 36.6 (**C**H(CH_3)$_2$); 37.83, 37.31 (2C, **C**H_2**C**H_2Cl); 39.31, 39.86 (2C,**C**HCH_3 $_{ringA}$); 59.25 (**C**HOH);123.024 (C C) and 140.066 (**C** CH_2). The computed bond length and bond angels since helalomycin-1 were calculated as well (Table 4). From the aforementioned spectroscopic data, the molecular structure of helalomycin-1 was identified as chlorohydronaphthol(6-(2-chloroethyl)-5-isopropyl-3,4-dimethyl-7-methylene-1,2,3,4,4a,5,6,7 octahydronaphthalen-1-ol (Fig. 11a). Ball and stick rendering of the chlorohydronaphthol compound was as calculated by PM3 semi-empirical molecular orbital calculations (Fig. 11b).

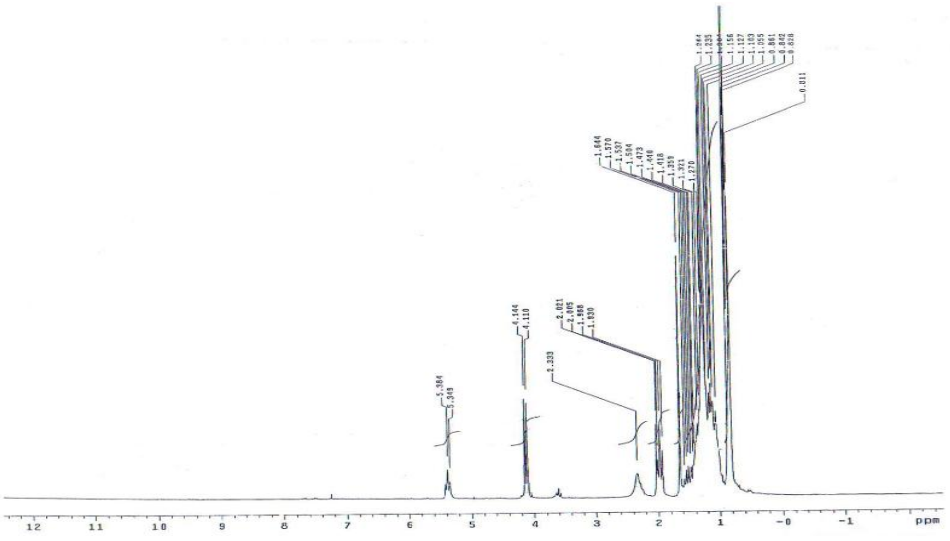

Fig. 9: ^{1}HNMR-Spectrum of helalomycin-1 in $CDCl_3$

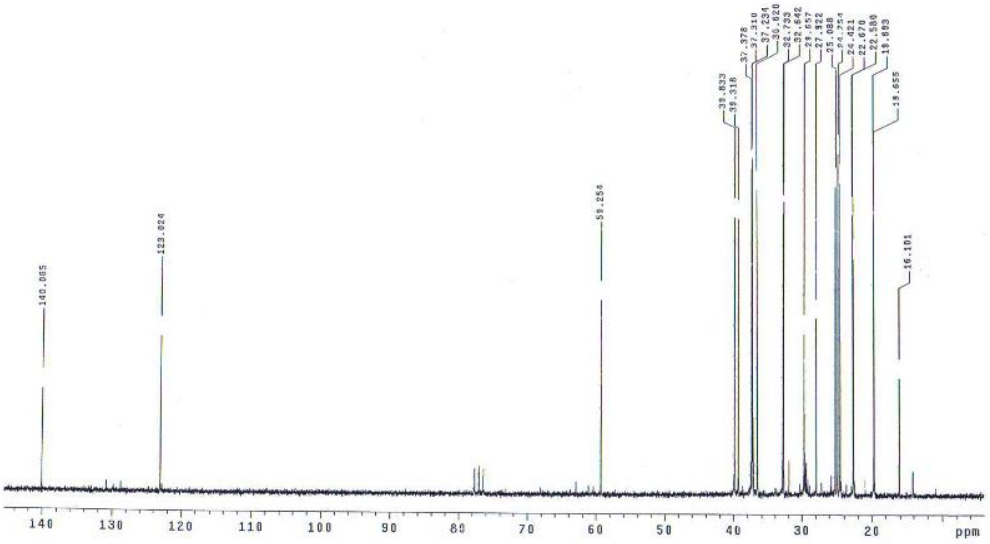

Fig. 10: ^{13}CNMR spectrum of helalomycin-1 in $CDCl_3$

Table 4: Various calculated bond lengths and bond angles of (chlorohydronaphthol) as calculated in Ab initio wit STO-3G.

Bond length A°			*Bond Angle °*		
Atom	Actual	Optimal	Atom	Actual	Optimal
C(20)-H(49)	1.0847	1.113	H(49)-C(20)-H(48)	107.7312	109.00
C(20)-H(48)	1.0861	1.113	H(49)-C(20)-H(47)	108.3830	109.00
C(20)-H(47)	1.0837	1.113	H(49)-C(20)-C(2)	111.1363	110.00
C(19)-H(46)	1.0831	1.113	H(48)-C(20)-H(47)	108.0184	109.00
C(19)-H(45)	1.0835	1.113	H(48)-C(20)-C(2)	109.3938	110.00
C(19)-H(44)	1.0860	1.113	H(47)-C(20)-C(2)	112.0352	110.00
C(18)-H(43)	1.0838	1.113	H(46)-C(19)-H(45)	108.4217	109.00
C(18)-H(42)	1.0854	1.113	H(46)-C(19)-H(44)	107.9390	109.00
C(18)-H(41)	1.0858	1.113	H(46)-C(19)-C(17)	111.9365	110.00
C(17)-H(40)	1.0832	1.113	H(45)-C(19)-H(44)	107.8205	109.00
C(16)-H(39)	1.0803	1.100	H(45)-C(19)-C(17)	111.8236	110.00
C(16)-H(38)	1.0782	1.100	H(44)-C(19)-C(17)	108.7450	110.00
C(14)-H(37)	1.0888	1.108	H(43)-C(18)-H(42)	108.3908	109.00
C(14)-H(36)	1.0902	1.108	H(43)-C(18)-H(41)	107.7465	109.00
C(13)-H(35)	1.0864	1.113	H(43)-C(18)-C(17)	111.8229	110.00

Table 4: *Cont.*

Bond length A°			*Bond Angle °*		
Atom	Actual	Optimal	Atom	Actual	Optimal
C(13)-H(34)	1.0861	1.113	H(42)-C(18)-H(41)	108.0410	109.00
C(12)-H(33)	1.0825	1.100	H(42)-C(18)-C(17)	111.0451	110.00
C(10)-H(32)	1.0919	1.113	H(41)-C(18)-C(17)	109.6628	110.00
C(9)-H(31)	1.0883	1.113	H(40)-C(17)-C(19)	106.1295	109.39
C(8)-H(30)	1.0822	1.113	H(40)-C(17)-C(18)	105.8467	109.39
C(8)-H(29)	1.0853	1.113	H(40)-C(17)-C(9)	106.2658	109.39
C(8)-H(28)	1.0859	1.113	C(19)-C(17)-C(18)	108.8142	109.51
O(7)-H(27)	0.9906	0.961	C(19)-C(17)-C(9)	117.5298	109.51
C(6)-H(26)	1.0971	1.111	C(18)-C(17)-C(9)	111.4608	109.51
C(4)-H(25)	1.0920	1.113	H(39)-C(16)-H(38)	115.3973	119.00
C(3)-H(24)	1.0896	1.113	H(39)-C(16)-C(11)	120.9254	120.50
C(2)-H(23)	1.0902	1.113	H(38)-C(16)-C(11)	123.6728	120.50
C(1)-H(22)	1.0880	1.113	H(37)-C(14)-H(36)	108.8026	109.40
C(2)-C(20)	1.5437	1.523	H(37)-C(14)-Cl(15)	106.8495	000.00
C(17)-C(19)	1.5470	1.523	H(37)-C(14)-C(13)	112.3444	109.41
C(17)-C(18)	1.5506	1.523	H(36)-C(14)-Cl(15)	106.7306	000.00
C(9)-C(17)	1.5730	1.523	H(36)-C(14)-C(13)	110.8884	109.41
C(11)-C(16)	1.3162	1.337	Cl(15)-C(14)-C(13)	110.9823	000.00
C(14)-Cl(15)	1.8181	1.777	H(35)-C(13)-H(34)	107.6805	109.40
C(13)-C(14)	1.5454	1.515	H(35)-C(13)-C(14)	107.4447	109.41
C(10)-C(13)	1.5546	1.523	H(35)-C(13)-C(10)	109.2587	109.41
C(12)-C(5)	1.3190	1.337	H(34)-C(13)-C(14)	109.4440	109.41
C(11)-C(12)	1.4931	1.503	H(34)-C(13)-C(10)	111.3980	109.41
C(10)-C(11)	1.5383	1.497	C(14)-C(13)-C(10)	111.4619	109.50
C(9)-C(10)	1.5673	1.523	H(33)-C(12)-C(5)	119.2348	120.00
C(4)-C(9)	1.5731	1.523	H(33)-C(12)-C(11)	115.8763	120.00
C(3)-C(8)	1.5474	1.523	C(5)-C(12)-C(11)	124.8868	120.00
C(6)-O(7)	1.4390	1.410	C(16)-C(11)-C(12)	120.1891	120.00
C(6)-C(1)	1.5618	1.514	C(16)-C(11)-C(10)	125.6662	121.40
C(5)-C(6)	1.5502	1.497	C(12)-C(11)-C(10)	114.1262	121.40
C(5)-C(6)	1.5502	1.497	C(12)-C(11)-C(10)	114.1262	121.40
C(4)-C(5)	1.5398	1.497	H(32)-C(10)-C(13)	105.9791	109.39
C(3)-C(4)	1.5758	1.523	H(32)-C(10)-C(11)	105.7365	109.39
C(2)-C(3)	1.5577	1.523	H(32)-C(10)-C(9)	104.3371	109.39
C(1)-C(2)	1.5457	1.523	C(13)-C(10)-C(11)	115.3101	109.51
C(1)-H(21)	1.0866	1.113	C(13)-C(10)-C(9)	113.4901	109.51
			C(11)-C(10)-C(9)	110.9187	109.51
			H(31)-C(9)-C(17)	105.5481	109.39
			H(31)-C(9)-C(10)	105.4548	109.39
			H(31)-C(9)-C(4)	105.2818	109.39
			C(17)-C(9)-C(10)	116.9876	109.51
			C(17)-C(9)-C(4)	116.7978	109.51
			C(10)-C(9)-C(4)	105.6229	109.51
			H(30)-C(8)-H(29)	108.3004	109.00
			H(30)-C(8)-H(28)	107.8168	109.00
			H(30)-C(8)-C(3)	113.2788	110.00
			H(29)-C(8)-H(28)	107.7530	109.00
			H(29)-C(8)-C(3)	110.5135	110.00
			H(28)-C(8)-C(3)	109.0012	110.00
			H(27)-O(7)-C(6)	104.3211	106.90
			H(26)-C(6)-O(7)	104.0752	106.70
			H(26)-C(6)-C(1)	107.1984	109.39
			H(26)-C(6)-C(5)	107.1797	109.39
			O(7)-C(6)-C(1)	111.5802	107.70
			O(7)-C(6)-C(5)	111.1205	000.00
			C(1)-C(6)-C(5)	114.9196	109.51
			C(12)-C(5)-C(6)	117.7846	121.40
			C(12)-C(5)-C(4)	120.8789	121.40
			C(6)-C(5)-C(4)	121.1057	117.20
			H(25)-C(4)-C(9)	102.8371	109.39
			H(25)-C(4)-C(5)	105.2028	109.39
			H(25)-C(4)-C(3)	103.5389	109.39
			C(9)-C(4)-C(5)	111.0419	109.51
			C(9)-C(4)-C(3)	118.6434	109.51
			C(5)-C(4)-C(3)	113.6824	109.51
			H(24)-C(3)-C(8)	104.5009	109.39

Table 4: *Cont.*

Bond length A°			*Bond Angle °*		
Atom	Actual	Optimal	Atom	Actual	Optimal
			H(24)-C(3)-C(4)	104.1430	109.39
			H(24)-C(3)-C(2)	103.2661	109.39
			C(8)-C(3)-C(4)	115.4446	109.51
			C(8)-C(3)-C(2)	112.7750	109.51
			C(4)-C(3)-C(2)	114.8666	109.51
			H(23)-C(2)-C(1)	106.8025	109.39
			C(20)-C(2)-C(3)	116.9499	109.51
			C(20)-C(2)-C(1)	111.8227	109.51
			C(3)-C(2)-C(1)	107.7521	109.51
			H(22)-C(1)-C(6)	107.9912	109.41
			H(22)-C(1)-C(2)	110.2910	109.41
			H(22)-C(1)-H(21)	106.9895	109.40
			C(6)-C(1)-C(2)	114.5575	109.50
			C(6)-C(1)-H(21)	108.5883	109.41
			C(2)-C(1)-H(21)	108.1541	109.41
			H(25)-C(4)-C(5)	105.2028	109.39
			H(25)-C(4)-C(3)	103.5389	109.39
			C(9)-C(4)-C(5)	111.0419	109.51
			C(9)-C(4)-C(3)	118.6434	109.51
			C(5)-C(4)-C(3)	113.6824	109.51
			H(24)-C(3)-C(8)	104.5009	109.39
			C(8)-C(3)-C(4)	115.4446	109.51
			C(8)-C(3)-C(2)	112.7750	109.51
			C(4)-C(3)-C(2)	114.8666	109.51
			H(23)-C(2)-C(20)	106.6893	109.39
			H(23)-C(2)-C(3)	106.2344	109.39
			H(23)-C(2)-C(1)	106.8025	109.39
			C(20)-C(2)-C(3)	116.9499	109.51
			C(20)-C(2)-C(1)	111.8227	109.51
			C(3)-C(2)-C(1)	107.7521	109.51
			H(22)-C(1)-C(6)	107.9912	109.41
			H(22)-C(1)-C(2)	110.2910	109.41

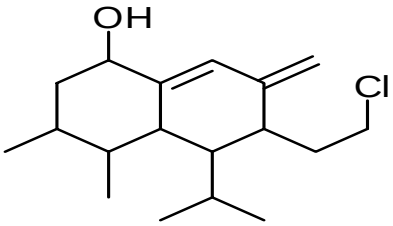

Fig. 11a: The molecular structure of helalomycin-1 (chlorohydronaphthol).

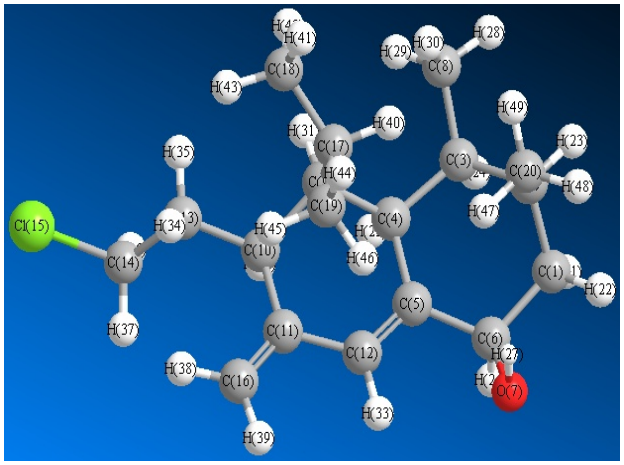

Fig. 11b: Ball and stick rendering of chlorohydronaphthol as calculated by Ab initio wit STO-3G molecular orbital calculations.

The identification of helalomycin-1 was accomplished quickly by taking advantage of the available databases of natural product structures, Antibase (Laatsch, 2010) and literatures (Pathirana, *et al.,* 1992; Keiichiro *et al.,* 2008a; Ding *et al.,* 2009) which describe the chemical structure of similar compounds from other marine *Streptomyces* species. The proposed molecular structure of the compound with its molecular weight generated by this local strain in the present investigation proved to be belongs to the bicyclic sesquiterpenoids and does not match with any terpenoid compounds produced by actinomycetes (Keiichiro *et al.,* 2008b) or antitumor compounds from marine actinomycetes (Olano *et al.,* 2009).

Conclusion:

Helalomycin-1, a new bicyclic sesquiterpenoid derivative, was isolated from the marine *Streptomyces* sp. HuGu-11. This is the first report of this compound from nature. The compound exhibited strong antibacterial activities mainly against Gram-positive bacteria and also showed cytotoxic activities against different human cancer cell lines. In conclusion, it is believed that a rich source of new drug candidates can be potentially obtained from marine organisms or their metabolites. This preliminary screening of marine actinomycetes for new antitumor antibiotic revealed their potential to yield potent bioactive compounds for drug discovery programs.

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