GROWTH CHARACTERISTICS AND PRODUCTION OF SECONDARY METABOLITES FROM SELECTED NOVEL *STREPTOMYCES* SPECIES ISOLATED FROM SELECTED KENYAN NATIONAL PARKS

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Abstract
The aim of study was to characterize growth of novel *Streptomyces* isolates as well as the secondary metabolites they were producing. Four Streptomyces isolates from Chyulu National Park (Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3) and one from Ruma National Park (Ruj 7-1) were studied. The isolates grew well at pH 6, 7, 9 and temperatures of 27.5 ^{o}C, 30 ^{o}C, and 32.5 ^{o}C. They preferentially utilized glucose and xylose and also required sodium chloride (0 g/l – 17.5 g/l) for growth. Antimicrobial products were extracted using ethyl acetate and the crude secondary metabolite extracts analyzed using Gas Chromatography- Mass Spectrophotometer (GC-MS). 0.54 g/l, 0.62 g/l, 0.41 g/l, 0.3 g/l and 0.14 g/l yields of crude secondary metabolites were extracted from the isolates. The crude secondary metabolites had different levels of activity against Gram positive and Gram negative test bacteria. Characterization of the crude secondary metabolites indicated presence of chemical compounds ranging from amides, amines, acids, pyrrolizidines, butenolides, alcohols and hydrocarbons.

Key words: *Streptomyces,* antimicrobial activity, gas chromatography, mass Spectrophotometer, secondary metabolites

1.0 Introduction

Streptomyces are mostly filamentous bacteria that are widely distributed in a variety of natural and man-made environments. They constitute a significant component of the microbial population in most soils (Watve *et al*., 2001). They are about 1μm in diameter. Their colonies appear like a mass of unicellular mycelium, with branching filament extensions of the original cell or cells, in addition to spores and degradation products (Waksman, 1950). They reproduce by fission or by spores and this is why they were originally classified as fungi. The optimal pH range in which they grow is between 7 and 8. Most of them grow at temperatures between 15 and 30°C (Waksman, 1950, 1962 and 1967). They are also characterized by their gram-positive nature and high G-C content of their genomes and have an unparalleled ability to produce diverse secondary metabolites (Berdy, 2005).

Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered (Berdy, 2005). Among Actinomycetes, around 7,600 compounds are produced by *Streptomyces* species (Berdy, 2005).

Streptomyces produce chemically diverse secondary metabolites that are structurally related, as well as structurally unrelated types of secondary metabolites from the same culture. Production of these secondary metabolites is usually during the stationary or slower stages of growth (Alexandra, 1997). Their production however is increased by inducing a biosynthetic enzyme (synthase) or increasing the limiting factor. Much of the published data indicates that the most important environmental signal triggering secondary metabolism is nutrient starvation, particularly that of phosphate (Sola-Landa *et al.,* 2003). The signaling networks behind the regulation of secondary metabolism in Streptomyces have recently been reviewed (Bibb, 2005).

Many of these secondary metabolites are potent antibiotics, which has made Streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Berdy, 2005). Members of this group are producers, in addition, of clinically useful antitumor drugs such as anthracyclines (aclarubicin, daunomycin and doxorubicin), peptides (bleomycin and actinomycin D), aureolic acids (mithramycin), enediynes (neocarzinostatin), antimetabolites (pentostatin), carzinophilin, mitomycins (Newman and Cragg, 2007; Olano *et al.,* 2009).

However, besides antibiotics which present the largest group of bioactive secondary metabolites, the Streptomycetes compounds show several other biological activities. These are: firstly, antagonistic agents that include; antibacterials, antifungals, antiprotozoans as well as antivirals: secondly, pharmacological agents that shows the following activities; antitumor, immunomodulators, neurological agents and enzyme inhibitors: thirdly, agrobiologicals comprising of; insecticides, pesticides and herbicides: fourthly, compounds with regulatory activities such as; growth factors, siderophores or morphogenic agents (Sanglier *et al.,* 1996; Berdy 1995, 2005) (Table 1).

Table 1: Examples of antibiotics produced by Streptomyces sp. (Kieser et al., 2000)

Antibiotic	**Producer**	**Chemical Class**	**Target**	**Application**
Actinomycin D	*Streptomyces* sp.	Peptide	Transcription	Antitumor
Actinomycin A	*Streptomyces* sp.	Macrolide	Cytochrome system	Telocidal
Avermectin	*S. avermetilis*	Macrolide (PK)	Chloride ion channels	Antiparasitic
Daptomycin	*S. roseosporus*	Lipopeptide	Lipoteichoic acid	Antibacterial

Nystatin	*S. noursei*	Polyene	Membrane (pore former)	Antifungal
Nikkomycin	*S. tendae*	Nucleoside	Chitin biosynthesis	Antifungal; insecticidal
Neomycin	*S. fradiae*	Aminoglycoside	Protein synthesis	Antibacterial
Phleomycin	*S. verticillus*	Glycopeptide	DNA strand breakage	Antitumor
Polyoxins	*S. cacaoi var asoensis*	Nucleoside- peptide	Chitin biosynthesis	Antifungal

Due to the ability in synthesizing numerous compounds that exhibit extreme chemical diversity, *Streptomyces* strains have become a major part of industrial strain collection used in screening for new bioactive molecules (Demain and Davies, 1999). This study was therefore geared towards unveiling and assessing novel secondary metabolites that could be of commercial importance hence improving industrial and pharmaceutical applications as well as in other sectors where they may be of use.

2.0 Materials and Methods

2.1 Growth of *Streptomyces* Isolates

The isolates were fermented in a differential broth media in a shaker incubator (Gallen Kamp, Germany) (200 rpm, 28ºC) for 96 h. The original stocks of the isolates from which the working stocks were prepared were kept in a freezer (Sanyo MDF-594 AT, Japan) at - 80°C.

2.2 Physiochemical Characterization of the Actinobacteria Isolates

In order to carryout screening and extraction of antimicrobial compounds from the isolates, optimization of growth conditions and media composition was done to achieve good results.

2.2.1 Sugar Fermentation

Sugar fermentation test was carried to determine the ability of the isolates to degrade and ferment various carbohydrates. Bromothymol blue (Sigma Aldrich, Germany) dye was added to basal broth media containing the various sugars, inoculated with the isolates and incubated in a shaker incubator (Gallen Kamp, Germany) (30°C for 96 h at 100rpm). Utilization of the various sugars was ascertained by growth of the isolates. This was determined by measuring the optical densities of the broth cultures using a UV spectral photometer (Shimadzu UV 240, Japan) at 600nm (Williams *et al*., 1989).

2.2.2 Effect of pH on Growth of the Actinobacteria Isolates

An optimum pH requirement for the isolates was determined. International Streptomyces Project (ISP_2) broth media adjusted to varying pH ranges of 3, 6, 7 and 9, using 1N sodium hydroxide and 1N hydrochloric acid was used. The cultures were incubated in a shaker incubator (Gallen Kamp, Germany) (30ºC for 48 h at 100rpm) and optical density readings were read at 600nm using a UV spectral photometer (Shimadzu UV 240, Japan).

2.2.3 Effect of Temperature on Growth of the Actinobacteria Isolates

Growth of the five isolates was monitored by spectrophotometric measurement of the optical density at 600 nm. Experiments were performed at 15, 20, 25, 27.5, 30, 32.5, 35, 36, 37, and 38°C. Prior to the experiments, bacteria were acclimatized to the temperature conditions in the growth experiments. All cultures were grown in liquid International Streptomyces Project (ISP_2) media on a rotary shaker incubator (Gallen Kamp, Germany) (15, 20, 25, 27.5, 30, 32.5, 35, 36, 37, and 38°C for 12 h at 100 rpm) in the dark. Precultures of acclimatized strains that were used for setting up growth experiments were grown overnight. Experiments were performed in 100-ml Erlenmeyer

flasks in triplicate for each isolate. The medium used for the experiments was preincubated in a flat bed incubator for 6 h under the same temperature conditions as the temperature conditions in the experiment. Measurement of the optical density was started 72 h after inoculation. The optimal growth temperature was determined graphically.

2.2.4 NaCl tolerance Test

For this test, NaCl broth was used. 50 ml of the medium was autoclaved at 121°C for 15 min in clean 100 ml conical flasks. 100µl of test strain inoculums were inoculated into the medium contained in conical flasks and incubated on rotary shaker (Gallen Kamp, Germany) (30°C for 96 h at 100 rpm). Growth of the five isolates was monitored spectrophotometrically by measuring the optical density at 600 nm.

2.3 Extraction of Secondary Metabolites

Differential broth cultures of the isolates were prepared in 500 ml conical flasks and incubated in a rotary shaker (Gallen Kamp, Germany) (at isolate's optimum temperature for 240 h at 100 rpm). Filtration of the broth cultures by use of Whatman filter paper no.1 was done after fermentation to remove bacterial cells. The cell free culture filtrates were extracted three times with ethyl acetate at volume ratio of 1:1 by use of a separating funnel.

The extract was passed through a pad of anhydrous sodium sulphate to remove excess water and thereafter evaporated to dryness using a rotary vacuum evaporator (RE 100B, Bibby Sterilin, United Kingdom). Yields of the extracts were determined and recorded. The crude extracts were used for biological activity tests as well as Gas Chromatography- Mass Spectrophotometry (GC-MS) analysis of the compounds contained in them.

2.4 Bio Assay of the Crude Extracts Against Gram –ve and +ve Bacteria

Agar diffusion method was used to determine antibacterial activity of the crude extracts. The bacteria used comprised, *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* (NCTC 10788). Paper discs were prepared and impregnated with 10 µl of the sample crude extract prepared by dissolving the dry crude extracts in 1 ml ethyl acetate. The impregnated paper discs were allowed to dry in a fume chamber and then placed on agar seeded with the test organisms. Incubation was done in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (37 ºC for 24 h) and diameters of zones of inhibitions measured using a ruler and recorded.

2.5 GC-MS Analysis of Secondary Metabolites from the Actinobacteria Isolates

Chemical screening of the active compounds present in the crude extracts was done by use of a GC-MS (Figure 2.10) to detect the active compounds as well as their quantity and quality ratios. Each samples was reconstituted using 1ml DCM (Dichloromethane (≥99.8%; Aldrich chemical co. ltd., USA.) and passed through a glass wool to remove solid materials. 40 µl of the collection in triplicate was transferred into auto sampler glass vials having Teflon caps and analyzed using GC-MS whose conditions are given below (Table 2.10 a and b).Agilent Technologies 7890A system was used. Oven conditions set during the analysis were: 1 minute for equilibration time; 35 °C for 5 minutes, 10 °C/minute to 280 °C for 10.5 minutes and 50 °C/min to 285 °C for 9.9 minutes as the oven program while the running time was 50 minutes. Injection was done in splitless mode and the conditions used were as follows: 250 °C for the heater, 8.8271 Psi as the pressure, a total flow of 10.2 ml/ min, septum purge flow of 3 ml/ min, gas saver at 20 ml/ min after 2 minutes and Purge flow to split vent at 6mL/ min at 0.8minutes. The column used was HP-5MS, (5% methyl silox), (30 m × 250 µm × 0.25 µm) .The compounds identified were generated from a computer program that involved calculation by the data system of a similarity index, match factor or purity between the unknown spectrum and library (reference) spectra. For this analysis, NIST/EPA/NIH MASS SPECTRAL LIBRARY (NIST 05) and NIST MASS SPECTRAL SEARCH PROGRAM Version 2.0d were used.

2.6 Data Analysis

ANOVA tests were used to analyze the data. SAS 9.1 version was the software used to perform the analysis and separation of means was done by use of Tukeys' test. Means were used to draw graphs and tables.

3.0 Results

3.1 Physiochemical Characterization

3.1.1 Utilization of Sugars by the Isolates

Glucose resulted in highest growth of isolate Chy 4-10 (OD_{600}= 0.601nm). However, sucrose (OD_{600}= 0.473nm) and xylose (OD_{600}= 0.383nm) also led to a considerable growth of the isolate. (Figure 1a)

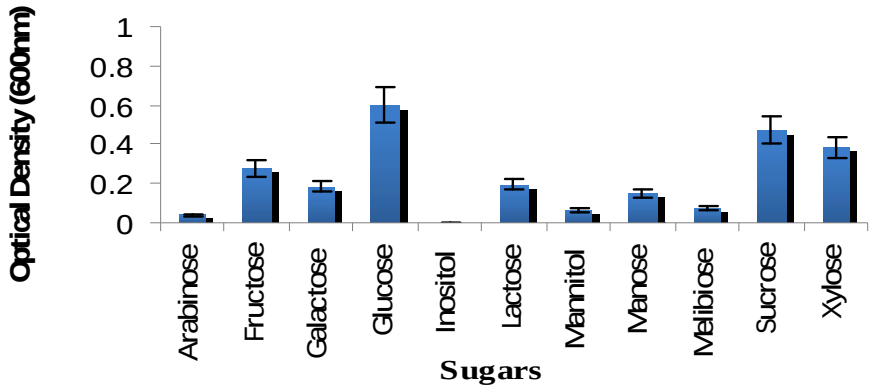

Figure 1a: Utilization of sugars by isolate CHY 4-10. (P < 0.05)

For isolate Chy 15-10, xylose led to highest growth (OD_{600}= 0.899nm). It provided optimum growth whereas mannitol (OD_{600}= 0.010nm), inositol (OD_{600}= 0.017nm), lactose (OD_{600}= 0.019nm) and arabinose (OD_{600}= 0.037nm) did not support high growth of the isolate. Glucose (OD_{600}= 0.509nm) and mannose (OD_{600}= 0.499nm) were also utilized although growth of the isolate was at a lower level from xylose (Figure 1b).

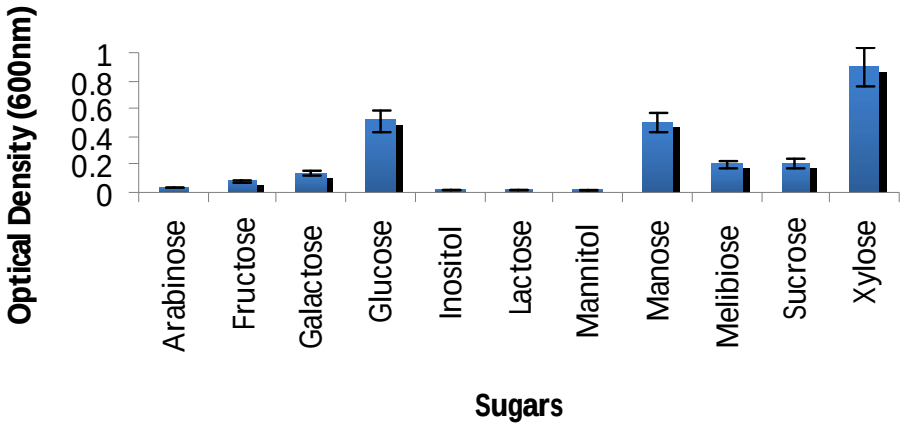

Figure 1b: Utilization of sugars by isolate CHY 15-10. (P < 0.05)

For isolate Chy 15-5, glucose had the highest growth (OD_{600}= 0.719nm) compare to the other sugars. Considering the difference in growth between the various sugars, optimum growth for the isolate would only be realized with glucose as the carbon source (Figure 1c).

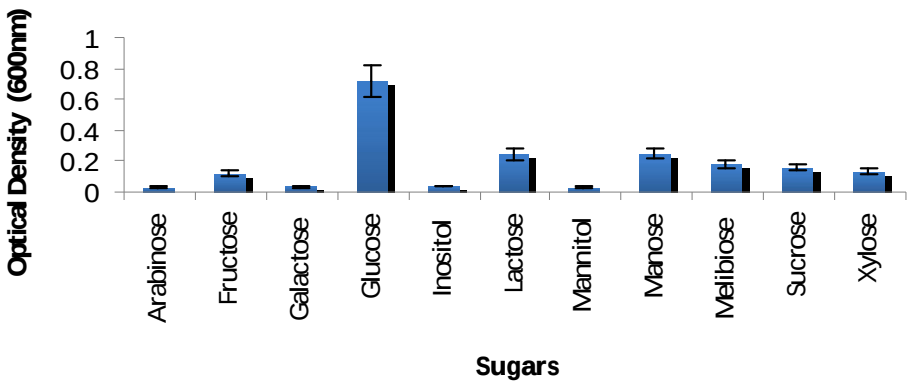

Figure 1c: Utilization of sugars by isolate CHY 15-5. (P < 0.05)

For isolate Chy 2-3, glucose (OD_{600}= 0.475nm) similarly led to high growth whereas mannitol (OD_{600}= 0.021nm) led to lowest growth of the isolate (Figure 1d).

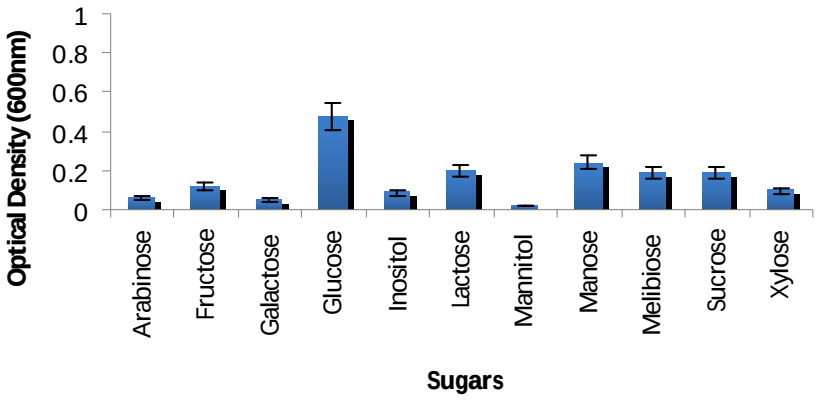

Figure 1d: Utilization of sugars by isolate CHY 2-3. (P<0.05)

And finally for isolate Ruj 7-1, xylose supported high growth (OD_{600}= 0.900nm) followed by glucose though at a much lower optical density (OD_{600}= 0.325nm) (Figure 1e).

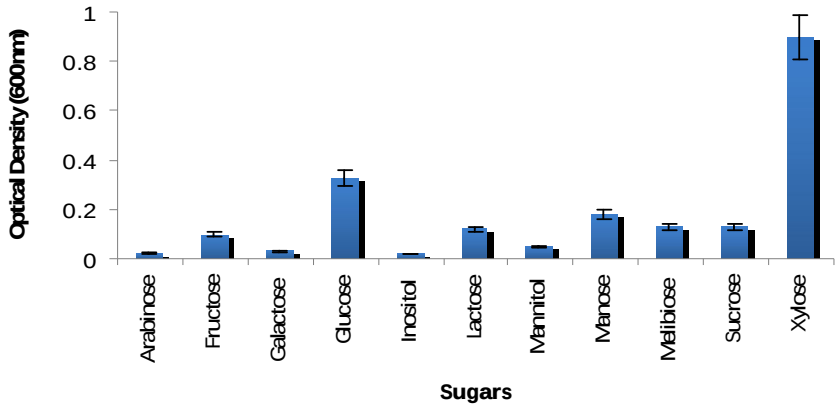

Figure 1e: Utilization of sugars by isolate RUJ 7-1. (P < 0.05)

3.1.2 PH tolerance by the Isolates

All the isolates were able to grow at acidic, neutral and alkaline pH conditions. However, the different pH conditions yielded different growth levels for the various isolates. PH 7 led to highest growth of isolate Chy 4-10 with an optical density (OD_{600}= 0.604nm). Lowest growth of the isolate was yielded at pH 3 (OD_{600}= 0.173nm) (Figure 2a). Increased growth of isolate Chy 15-10 was yielded at pH 9 (OD_{600}= 0.610nm) whereas minimal growth was at pH 3 (OD_{600}= 0.178nm) (Figure 2b). For isolate Chy 15-5, pH 9 (OD_{600}= 0.591nm) led to the highest growth of the isolate followed by pH 7 (OD_{600}= 0.549nm). Minimal growth of the isolate was yielded at pH 3 (OD_{600}= 0.205nm) (Figure 2c). For isolate Chy 2-3, increased growth of the isolate was yielded at pH 9 (OD_{600}= 0.489nm) whereas pH 3 had the lowest growth with an optical density (OD_{600}= 0.193nm) (Figure 2d). Lastly, highest growth of isolate Ruj 7-1was yielded at pH 6 (OD_{600}= 0.619nm) whereas minimal growth was at pH 3 (OD_{600}= 0.215nm) (Figure 2e).

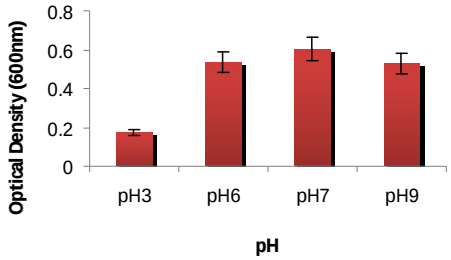

Figure 2a: Effect of pH on growth of the isolate CHY 4-10. (P<0.05)

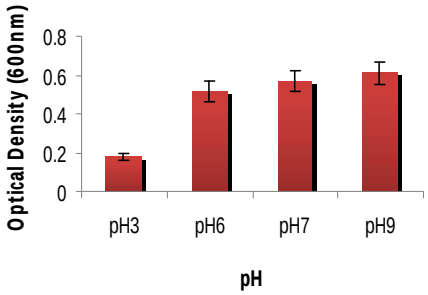

Figure 2b: Effect of pH on growth of the isolate CHY 15-10. (P<0.05)

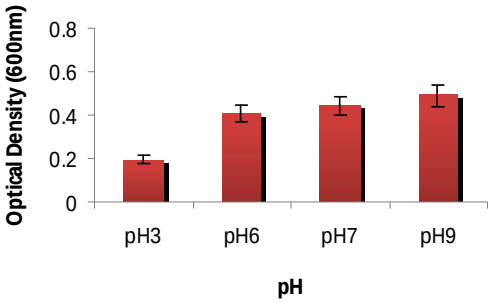

Figure 2c: Effect of pH on growth of the isolate CHY 15-5. (P<0.05)

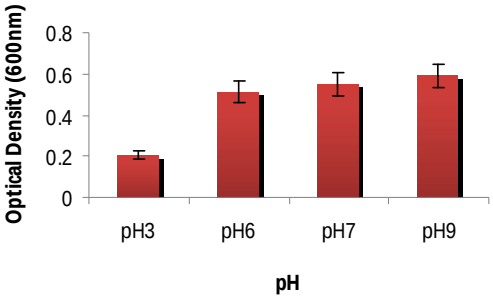

Figure 2d: Effect of pH on growth of the isolate CHY 2-3. (P<0.05)

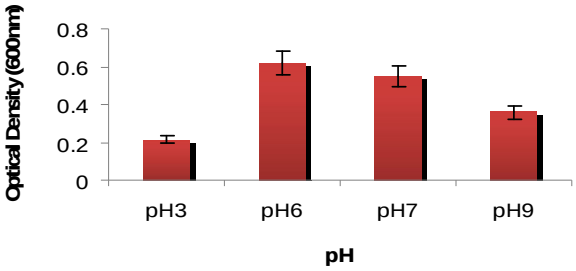

Figure 2e: Effect of pH on growth of the isolate RUJ 7-1. (P<0.05)

3.1.3 Temperature Tolerance by the Isolates

In all the isolates investigated, there was a linear increase in the temperature-dependent growth curves at the temperature range 15 °C to 32.5 °C. With further increases in temperature, the growth curves showed either a plateau or a linear decrease. The optimal growth temperature had the highest OD at 600nm. The optimum growth temperature for isolate Chy 4-10 was 30 °C (OD_{600}=0.610nm). Beyond 30 °C, there was a decline in growth of the isolate (Figure 3a). For isolate CHY 15-10, 32.5 °C (OD_{600}=0.602nm) was the optimum temperature for growth of the isolate (Figure 3b) whereas 30 °C (OD_{600}=0.562nm) was the optimum temperature for growth of isolate Chy 15-5 (Figure 3c). For isolate Chy 2-3, the optimum temperature for growth was 32.5 °C (OD_{600}=0.518nm) (Figure 3d). Lastly, 27.5 °C was the optimum temperature for growth of isolate Ruj 7-1 as it gave the highest growth (OD_{600}=0.653nm) when compared with other temperature regimes (Figure 3e).

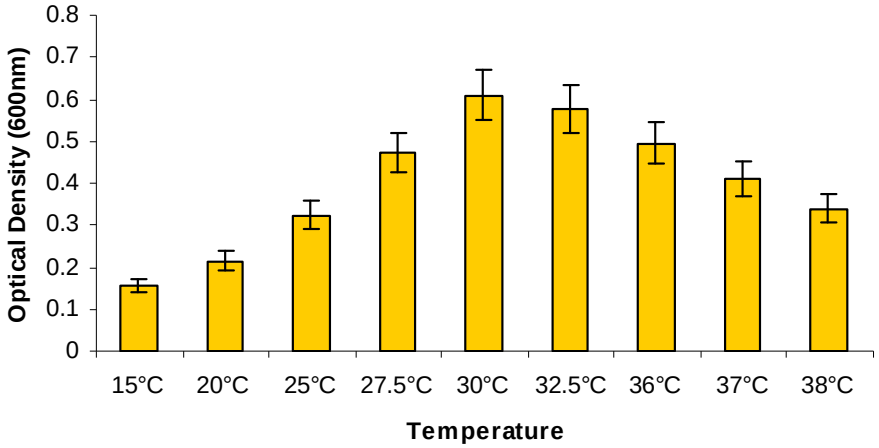

Figure 3a: Growth of isolates Chy 4-10 under different temperature ranges. (P<0.05)

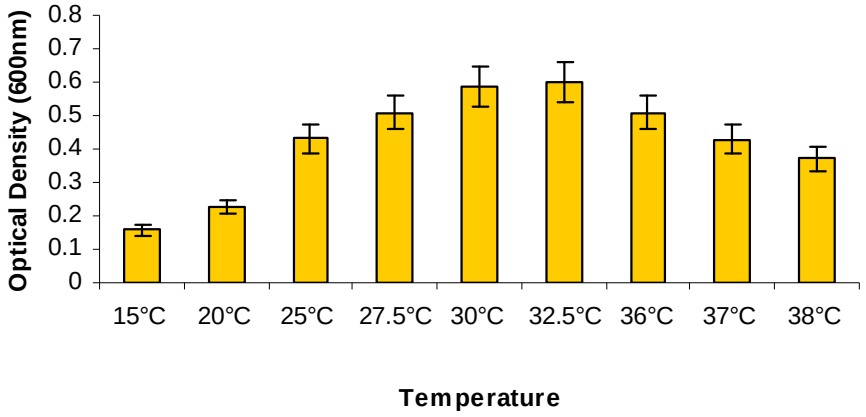

Figure 3b: Growth of isolates Chy 15-10 under different temperature ranges. (P<0.05)

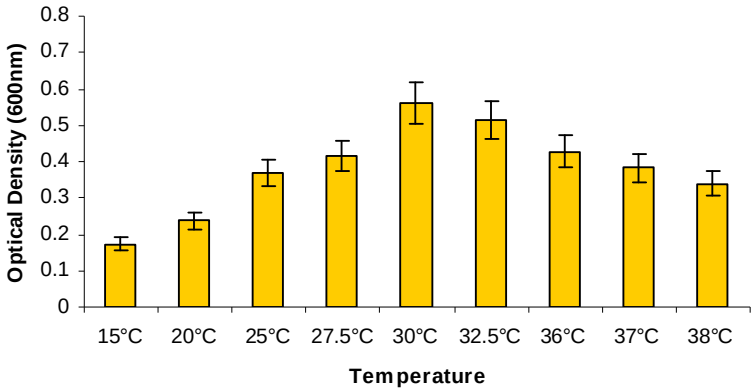

Figure 3c: Growth of isolates Chy 15-5 under different temperature ranges. (P<0.05)

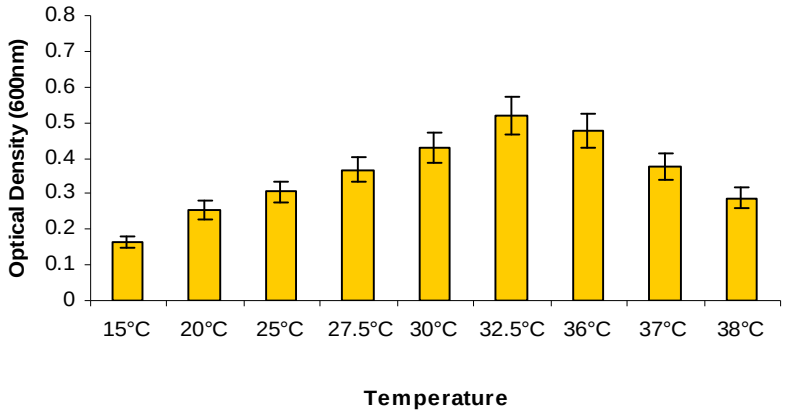

Figure 3d: Growth of isolates Chy 2-3 under different temperature ranges. (P < 0.05)

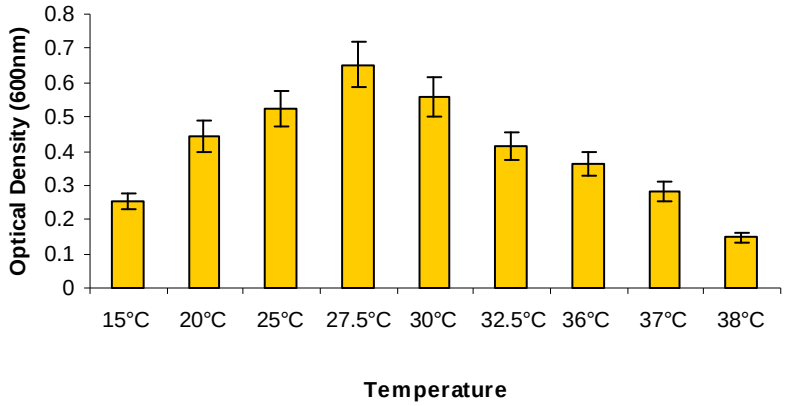

Figure 3e: Growth of isolates Ruj 7-1 under different temperature ranges. (P < 0.05)

3.1.4 NaCl Tolerance by the Isolates

Isolates were subjected to different sodium chloride concentrations to determine the concentration for optimum growth for each isolate. All the isolates were observed to utilize sodium chloride for growth. Optimum growth for isolate Chy 4-10 was achieved in 5 g/l (OD_{600}=0.411nm) whereas the lowest growth was recorded in 32.5 g/l (OD_{600}=0.026nm) concentration (Figure 4a). 5 g/l (OD_{600}=0.423nm) gave the highest growth of isolate Chy 15-10. Lowest growth on the other hand was recorded in 32.5 g/l (OD_{600}=0.010nm) concentration (Figure 4b). Sodium chloride concentration that recorded highest growth of isolate Chy 15-5 was 10 g/l (OD_{600}=0.169nm) whereas 32.5 g/l (OD_{600}=0.023nm) was the concentration with the lowest growth of the isolate (Figure 4c). For isolate Chy 2-3, optimum growth was recorded in 5 g/l (OD_{600}=0.388nm) whereas lowest growth was at 32.5 g/l (OD_{600}=0.009nm) concentration (Figure 4d). And lastly, optimum growth of isolate Ruj 7-1 was recorded in 17.5 g/l (OD_{600}=0.584nm) sodium chloride concentration (Figure 4e).

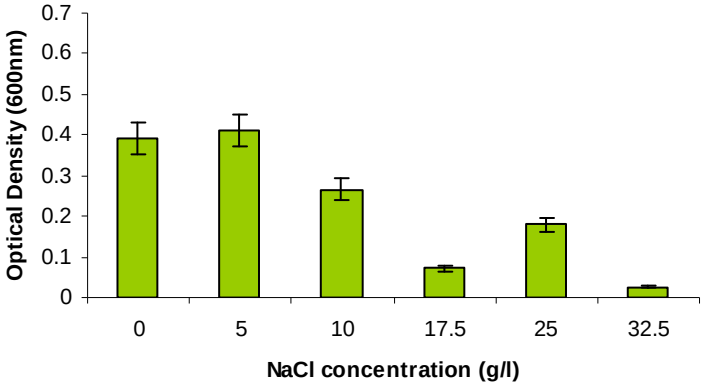

Figure 4a: Growth of isolate Chy 4-10 under different sodium chloride concentrations (P<0.05).

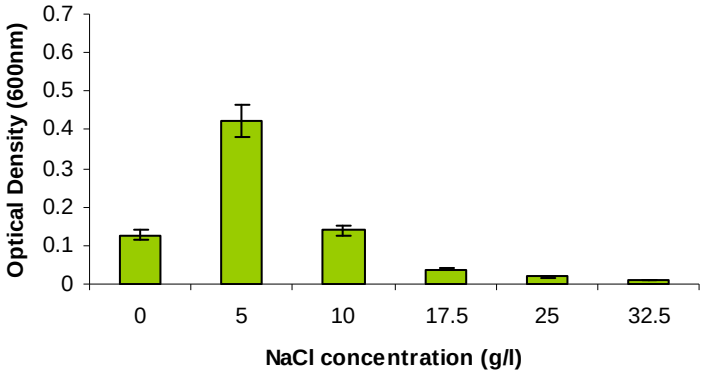

Figure 4b: Growth of isolate Chy 15-10 under different sodium chloride concentrations (P<0.05)

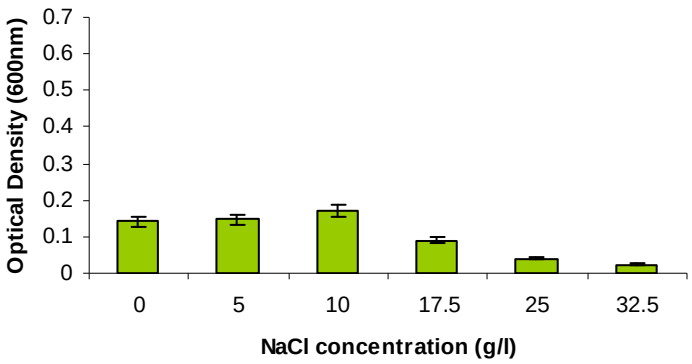

Figure 4c: Growth of isolate Chy 15-5 under different sodium chloride concentrations (P<0.05)

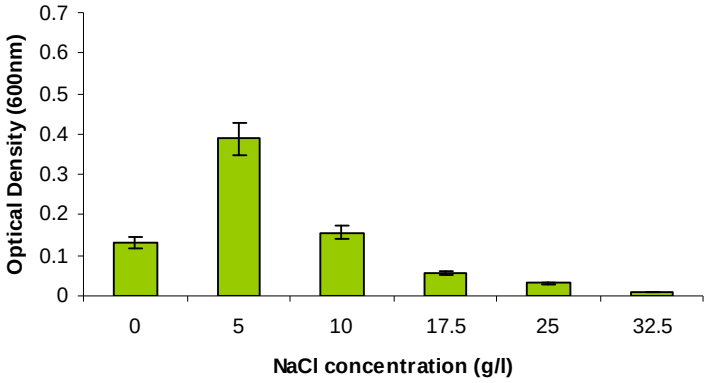

Figure 4d: Growth of isolate Chy 2-3 under different sodium chloride concentrations (P<0.05).

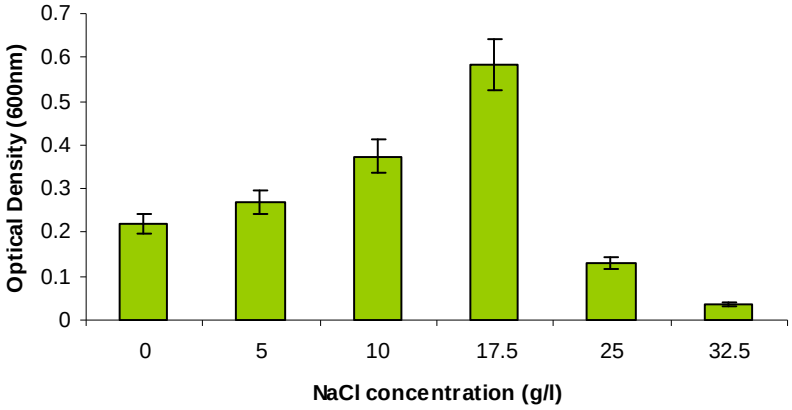

Figure 4e: Growth of isolate Ruj 7-1 under different sodium chloride concentrations (P<0.05).

3.2 Extraction and Activity of Secondary Metabolites

Production of secondary metabolites by the isolates was observed in differential broth cultures by a change in colour of the media from colorless to; red (Chy 4-10), dark brown (Chy 15-10), light brown (Chy 15-5), Orange (Chy 2-3) and black (Ruj 7-1) (Figure 5a – 5e).

The isolates were tested for inhibition of growth on test organisms *S. aureus* (NCTC 10788) and *E. coli* (NCTC 10418) (Table 3). Isolates Chy 4-10, Chy 15-5 and Ruj 7-1 were found to suppress the growth of the two test bacteria while isolates Chy 15-10 and Chy 2-3 only suppressed growth of *S. aureus* (NCTC 10788) (Table 3). Isolate Chy 4-10 (24mm) and Chy 2-3 (20mm) showed stronger inhibition on *S. aureus* (NCTC 10788) while isolates Chy 15-5 (18mm) and Ruj 7-1 (15mm) showed stronger inhibition on *E. coli* (NCTC 10418) (Table 3). Isolates Chy 4-10, Chy 15-5 and Ruj 7-1 showed strong inhibition on both test organisms hence broad-spectrum activity (Table 3). This showed the potential of the isolates to produce antibiotics. The positive control consisted of commercial Kanamycin antibiotic (1mg/ml) while negative control consisted of un-innoculated plate.

Yields of the secondary metabolites produced were determined as shown in (Table 3). Isolate CHY15-10 produced the highest yield (0.62g/l) whereas RUJ7-1 produced the least (0.14g/l).

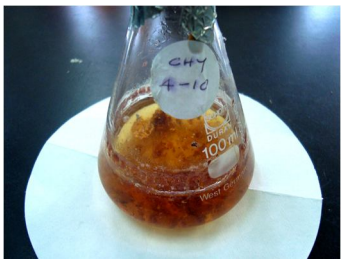

Figure 5a: Broth culture showing production of secondary metabolites by isolate CHY4-10.

Figure 5b: Broth culture showing production of secondary metabolites by isolate CHY15-10.

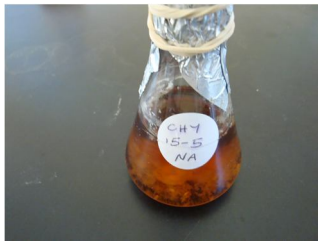

Figure 5c: Broth culture showing production of secondary metabolites by isolate CHY15-5.

Figure 5d: Broth culture showing production of secondary metabolites by isolate CHY2-3.

Figure 5e: Broth culture showing production of secondary metabolites by isolate RUJ7-1

Table 3: Bio-assays of secondary metabolites crude extracts from the Streptomyces isolates on S. aureus (NCTC 10788) and E. coli (NCTC 10418)

Isolate	Activity		Disc assay (diameter in mm)		Yield (g/l)
	S. aureus (NCTC 10788)	*E. coli* (NCTC 10418)	*S. aureus* (NCTC 10788)	*E. coli* (NCTC 10418)	
Chy 4-10	+	+	24	9	0.54
Chy 15-10	+	-	13	-	0.62
Chy 15-5	+	+	15	18	0.41
Chy 2-3	+	-	20	-	0.3
Ruj 7-1	+	+	19	15	0.14
+ve control	+	+	15	19	
-ve control	-	-	0	0	

* Zones of inhibition measured in mm.
+ = Inhibition
- = No inhibition.

3.3 GC-MS Analysis Of Crude Extracts From The Actinobacteria Isolates

Mass spectra and retention time analysis were used to determine the chemical compounds present in the respective isolate metabolites. Among the compounds identified comprised of amides, amines, acids, pyrrolizidines, quinones, alcohols and hydrocarbons. Amides identified from isolate Chy 4-10 were: Propanamide, N, N-dimethyl- (17.667 min), Acetamide,2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)- (23.715min) and Formamide, N-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)- (29.000min). Amines comprised of: Pyrimidine, 2-methoxy-5-methyl- (18.698 min), Pyridine, 2-methoxy-5-nitro- (22.057 min), Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)- (29.023 min) and l-Phenylalanine, N-(2,6-difluorobenzoyl)-, methyl ester (29.291 min). Acids: Benzenepropanoic acid (16.391min), Sulfurous acid, 2-ethylhexyl hexyl ester (17.063 min), 2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)- (21.408 min), 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (23.468 min), n-Hexadecanoic acid (23.468 min), Phosphonic acid, bis(1-methylethyl) ester (24.028 min), Octadecanoic acid (25.327 min) and 1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodeca hydro-1,4a,7-trimethyl-, methyl ester, [1R-(1.alpha.,4a. (27.813 min). Pyrrolizidines: Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- (21.587 min) and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- (26.940 min). Quinone: p-Benzoquinone, 2-hydroxy-5-(methylthio)- (25.932 min). Alcohols: Propenylguaethol (17.175 min), Phenol, 3-methoxy- (17.555 min) and Phenol, 3, 5-dimethoxy- (22.416 min). Hydrocarbons: Tridecane, 1-iodo- (20.669 min), Tridecane, 5-propyl- (20.736 min), 7-Acetyl-1,7-diazabicyclo[2.2.0]heptane (21.206 min), Heneicosane (21.744 min), Nonadecane (22.774 min), 1,4-Dioxaspiro[4.5]decane, 6-methylene- (23.222 min), Heptadecane (24.678 min), Hexacosane (28.821 min), Triacontane (29.560 min), Nonacosane (31.240 min), and Tetracosane (33.592 min). Other chemical compounds identified were: Caprolactam (15.248 min) and Squalene (30.658 min) (Table 4).

Table 4: Compounds identified from isolate CHY 4-10

Retention Time (min)	Compound Name	% of total	Quality
6.648	2-Pentanone, 4-hydroxy-	0.275	83
10.187	Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	0.717	72
12.001	2-Pentene, 4,4-dimethyl-, (Z)-	1.618	79

14.151	2-Piperidinone	1.003	72
15.248	Caprolactam	1.610	74
15.808	5-Methoxy-2,4-dimethyl-furan-3-one	12.237	70
16.391	Benzenepropanoic acid	0.763	90
17.063	Sulfurous acid, 2-ethylhexyl hexyl ester	0.334	72
17.175	Propenylguaethol	0.230	75
17.555	Phenol, 3-methoxy-	0.690	70
17.667	Propanamide, N,N-dimethyl-	0.362	73
18.698	Pyrimidine, 2-methoxy-5-methyl-	0.855	70
19.213	4-Methylformanilide	0.325	77
19.526	Borane, diethyl(decyloxy)-	0.230	72
19.952	Benzene, 3,5-dimethyl-1-(phenylmethyl)-	0.658	91
20.512	2,6-Diisopropylnaphthalene	0.290	91
20.669	Tridecane, 1-iodo-	0.188	70
20.736	Tridecane, 5-propyl-	0.309	77
20.937	(Z)-2-Methylimino-4,5-tetramethylenetetrahydro-1,3-oxazine	0.320	75
21.206	7-Acetyl-1,7-diazabicyclo[2.2.0]heptane	0.560	72
21.408	2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)-	0.589	75
21.587	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	1.761	86
21.744	Heneicosane	1.230	86
22.057	Pyridine, 2-methoxy-5-nitro-	0.236	72
22.416	Phenol, 3,5-dimethoxy-	0.560	83
22.528	Phthalic acid, isobutyl nonyl ester	0.616	83
22.774	Nonadecane	0.400	90
23.065	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.890	99

23.222	1,4-Dioxaspiro[4.5]decane, 6-methylene-	1.002	72
23.468	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	1.560	78
23.513	n-Hexadecanoic acid	0.560	94
23.715	Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-	2.260	83
24.028	Phosphonic acid, bis(1-methylethyl) ester	0.966	83
24.364	1,4-Anthracenedione, 5,6,7,8-tetrahydro-2-methoxy-5,5-dimethyl-	0.484	89
24.678	Heptadecane	0.656	83
25.327	Octadecanoic acid	3.307	99
25.932	p-Benzoquinone, 2-hydroxy-5-(methylthio)-	9.493	78
26.940	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	1.992	90
27.477	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodeca hydro-1,4a,7-trimethyl-, methyl ester, [1R-(1.alpha.,4a.	0.651	73
27.813	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7 -(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	0.923	94
28.485	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester		83
28.821	Hexacosane	0.368	90
29.000	Formamide, N-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)-	1.960	72
29.023	Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)-	1.230	82
29.291	l-Phenylalanine, N-(2,6-difluorobenzoyl)-, methyl ester	1.600	78
29.560	Triacontane	0.676	91

30.344	Heptadecane	0.604	91
30.658	Squalene	0.369	93
31.240	Nonacosane	0.599	96
32.315	Triacontane	0.314	94
33.592	Tetracosane	0.246	83
36.817	Heptamethyl-3-phenyl-1,4-cyclohexadiene	0.796	77

Chemical compounds identified from isolate Ruj 7-1 included: Amide: Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)- (23.715 min). Alcohols: 1, 4-Dioxane-2, 6-dimethanol (15.584 min), 1-Tridecanol (22.707 min) and Phenol, 4, 4'-(1-methylethylidene) bis [2, 6-dichloro- (29.359 min). Acids: Dodecanoic acid (19.101 min), Tetradecanoic acid (21.340 min), Diethyldithiophosphinic acid (22.236 min), Octadecanoic acid (25.260 min), 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (28.485 min) and Oxalic acid, monoamide, monohydrazide, N-(2,5-dimethylphenyl)-N2-(4-methylbenzylideno)- (29.919 min). Hydrocarbons: Hexadecane (19.526 min), Heptadecane (27.253 min), Octadecane (28.060 min), Hexacosane (28.821 min), Heptacosane (29.560 min), Octadecane, 1-iodo- (30.031 min), Octacosane (30.882 min), Tetracosane (31.240 min), Nonacosane (31.890 min) and Triacontane (32.315 min) (Table 5).

Table 5: Compounds identified from isolate RUJ7-1

Retention Time (min)	**Compound Name**	**% of total**	**Quality**
9.156	2-Butenal, 3-methyl-	0.360	73
15.584	1,4-Dioxane-2,6-dimethanol	18.055	78
18.071	1,1'-Bicyclohexyl, 2-methyl-, trans-	0.630	71
19.101	Dodecanoic acid	1.077	95
19.302	Naphthalene, 1,6-dimethyl-	0.890	58
19.526	Hexadecane	0.456	97
21.340	Tetradecanoic acid	2.988	95
22.236	Diethyldithiophosphinic acid	0.690	78
22.505	Phthalic acid, isobutyl octyl ester	1.181	90
22.707	1-Tridecanol	0.919	91
23.065	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	6.090	70
23.446	Dibutyl phthalate	27.546	94

23.715	Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-	2.175	98
25.260	Octadecanoic acid	3.804	99
26.940	4,8,12,16-Tetramethylheptadecan-4-olide	0.804	95
27.253	Heptadecane	1.389	97
28.060	Octadecane	1.580	97
28.485	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	2.297	91
28.821	Hexacosane	1.615	98
29.359	Phenol, 4,4'-(1-methylethylidene)bis[2,6-dichloro-	0.990	93
29.560	Heptacosane	1.954	98
29.919	Oxalic acid, monoamide, monohydrazide, N-(2,5-dimethylphenyl)-N2-(4-methylbe nzylideno)-	0.980	93
30.031	Octadecane, 1-iodo-	1.009	93
30.658	Squalene	2.283	97
30.882	Octacosane	1.008	90
31.240	Tetracosane	1.023	97
31.890	Nonacosane		96
32.315	Triacontane	1.003	99

Chemical compounds identified from isolate Chy 2-3 included: Amides: Propanamide, 2-methyl- (9.694 min), N-Methoxymethyl-N-methylformamide (11.015 min), L-Prolinamide (21.072 min), Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)- (23.737 min) and Formamide, N-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)- 29.157 min). Amines: 2-Ethylpiperidine (12.830 min) and Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)- (29.381 min). Acids: Acetic acid, 2-methylpropyl ester (5.416 min), 2-Propenoic acid, 2-methyl- (8.709 min), Hexanoic acid, 2-methyl- (9.044 min), Pentanoic acid (9.515 min), Benzeneacetic acid (15.450 min), Benzenepropanoic acid (16.480 min), 5-Oxohexanethioic acid, S-t-butyl ester (17.802 min), Dodecanoic acid (19.146 min), n-Hexadecanoic acid (23.535 min), Octadecanoic acid (25.327 min), p- Fluorophenoxyacetic acid (25.574 min), 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (28.508 min) and Octadecanoic acid, ethenyl ester (41.700 min). Alcohols: 1,2-Ethanediol, monoacetate (7.678 min), p-Dioxane-2,5-dimethanol (16.032 min), Cyclohexanol, 4-methoxy- (18.272 min) and Phenol, 3,5-dimethoxy- (22.528 min). Ketones: 2-Pyrrolidinone (12.449 min), 2, 5-Piperazinedione, 3, 6-bis (2-methylpropyl) - (25.910 min) and 16-Hentriacontanone (36.795 min).

Hydrocarbons: Cyclohexane (13.681 min), Tetradecane (17.063), Hexadecane (19.526 min), Hexacosane (28.821 min) and Tetracosane (30.344 min). Pyrrolizidines: Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- (21.766 min) and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl (26.985 min). Caprolactam was also detected at (15.808 min) retention time (Table 6).

Table 6: Compounds identified from isolate CHY2-3

Retention Time (min)	**Compound Name**	**% of total**	**% Quality**
5.416	Acetic acid, 2-methylpropyl ester	0.073	83
7.678	1,2-Ethanediol, monoacetate	0.397	78
8.709	2-Propenoic acid, 2-methyl-	1.742	70
9.044	Hexanoic acid, 2-methyl-	0.960	72
9.515	Pentanoic acid	0.662	83
9.694	Propanamide, 2-methyl-	0.630	80
11.015	N-Methoxymethyl-N-methylformamide	0.313	78
12.449	2-Pyrrolidinone	0.520	89
12.516	Furan, tetrahydro-2,5-dipropyl-	0.072	75
12.830	2-Ethylpiperidine	0.230	86
13.681	Cyclohexane	0.500	72
14.778	Thiophene, 2,3-dihydro-	2.319	86
15.450	Benzeneacetic acid	1.319	76
15.808	Caprolactam	1.419	83
16.032	p-Dioxane-2,5-dimethanol	0.566	76
16.480	Benzenepropanoic acid	0.0103	97
17.063	Tetradecane	0.332	98
17.802	5-Oxohexanethioic acid, S-t-butyl ester	0.822	75
18.272	Cyclohexanol, 4-methoxy-	0.890	75
18.630	4-Ethyl hydrogen itaconate	0.385	72
19.146	Dodecanoic acid	0.900	99
19.526	Hexadecane	0.860	96

20.579	2,6-Diisopropylnaphthalene	0.369	93
21.072	L-Prolinamide	0.290	77
21.363	3-Pyrrolidin-2-yl-propionic acid	2.133	86
21.766	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	2.710	83
22.528	Phenol, 3,5-dimethoxy-	2.890	83
22.953	Bicyclo[3.1.0]hex-2-ene, 5,6-diphenyl-	1.900	72
23.088	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1.256	99
23.311	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1',2'-d]pyrazine	1.230	79
23.535	n-Hexadecanoic acid	1.036	98
23.737	Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-	1.089	78
25.327	Octadecanoic acid	2.364	99
25.574	p-Fluorophenoxyacetic acid	1.357	82
25.910	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	13.999	72
26.581	Benzene, 1-ethyl-4-(1-methylethyl)-	1.230	75
26.985	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	1.520	93
28.508	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	0.980	91
28.821	Hexacosane	0.900	95
29.157	Formamide, N-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)-	2.530	78
29.381	Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)-	1.710	77
30.344	Tetracosane	0.254	97
36.795	16-Hentriacontanone	0.301	78
41.700	Octadecanoic acid, ethenyl ester	0.532	88

Secondary metabolites from Chy 15-5 analyzed had the following chemical compounds, Amide: Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)- (23.715 min). Acids: Hexanoic acid (10.814 min), Heptanoic acid (12.426 min), Cyclohexanecarboxylic acid (13.165 min), Benzenecarboxylic acid (14.263 min), Nonanoic acid (15.428 min), n-Decanoic acid (16.659 min), Dodecanoic acid (19.123 min), Tetradecanoic acid

(21.363 min), Pentadecanoic acid (22.393 min), n-Hexadecanoic acid (23.558 min), 9-Octadecenoic acid, (E)- (25.081 min), Octadecanoic acid (25.372 min), 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1R (1.alpha.,4a.beta.,10a.alpha.)]-(27.791min),Hexadecane acid, 4-nitrophenyl ester (36.817 min) and Octadecanoic acid, ethenyl ester (41.744 min). Ketone: 2-Pentadecanone, 6, 10, 14-trimethyl- (22.236 min). Hydrocarbons: Tetradecane (17.085 min), Pentadecane (18.339 min), Hexadecane (19.526 min), Pentadecane, 2,6,10,14-tetramethyl- (20.736 min), Hexadecane, 2,6,10,14-tetramethyl- (21.856 min), Nonadecane (22.774 min), Heneicosane (24.700 min), Docosane (25.596 min), Octadecane (26.447 min), Eicosane (28.821 min), Tricosane (29.583 min), Squalene (30.658 min), Nonacosane (31.262 min), Tetracosane (32.315 min) and Octacosane (33.592 min) (Table 7).

Table 7: Compounds identified from isolate CHY15-5

Retention Time (min)	**Compound Name**	**% of total**	**% Quality**
10.814	Hexanoic acid	0.590	83
12.426	Heptanoic acid	0.003	79
13.165	Cyclohexanecarboxylic acid	0.360	97
14.263	Benzenecarboxylic acid	0.895	93
15.248	Caprolactam	2.010	91
15.428	Nonanoic acid	0.532	93
16.010	Phthalic anhydride	0.870	81
16.659	n-Decanoic acid	0.191	98
17.085	Tetradecane	0.348	97
17.152	Vanillin	0.850	97
18.048	alpha-Cedrene oxide	0.197	76
18.339	Pentadecane	0.600	97
18.586	Tributyl phosphate	0.185	73
19.123	Dodecanoic acid	0.680	99
19.526	Hexadecane	0.603	98
19.862	Dodecanoic acid, 1-methylethyl ester	0.166	90
20.310	1,1'-Biphenyl, 4-(1-methylethyl)-	0.585	96
20.512	2,6-Diisopropylnaphthalene	1.370	95
20.736	Pentadecane, 2,6,10,14-tetramethyl-	0.330	94

20.893	2-(p-Tolylmethyl)-p-xylene	0.590	78
21.363	Tetradecanoic acid	1.867	99
21.856	Hexadecane, 2,6,10,14-tetramethyl-	0.629	95
22.236	2-Pentadecanone, 6,10,14-trimethyl-	0.780	89
22.393	Pentadecanoic acid	0.623	99
22.528	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.530	73
22.774	Nonadecane	1.411	80
22.931	Benzene, (1-methyldodecyl)-	1.980	76
23.087	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	1.862	86
23.558	n-Hexadecanoic acid	6.182	98
23.715	Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-	3.010	73
24.364	Heptadecanoic acid	2.270	91
24.700	Heneicosane	1.312	98
25.081	9-Octadecenoic acid, (E)-	3.361	99
25.372	Octadecanoic acid	11.471	99
25.596	Docosane	3.260	99
26.447	Octadecane	1.911	97
27.477	Androst-5-en-17-ol, 4,4-dimethyl-	1.903	75
27.791	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7 -(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	2.650	99
28.485	Phthalic acid, decyl 2-ethylhexyl ester	2.224	72
28.821	Eicosane	1.570	98
29.583	Tricosane	1.346	98
30.658	Squalene	1.416	98
31.262	Nonacosane	1.723	99
32.315	Tetracosane	0.887	97

33.592	Octacosane	0.804	98
36.817	Hexadecanoic acid, 4-nitrophenyl ester	0.367	78
37.959	Stigmastanol	0.467	78
41.744	Octadecanoic acid, ethenyl ester	4.108	83

Isolate CHY15-10 produced secondary metabolites with the following chemical compounds, Amides: L-Prolinamide (20.982 min) and N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide (23.289 min). Amines: Pyrimidine-2(1H)-thione, 4, 4, 6-trimethyl-1-(1-phenylethyl) - (29.336 min). Acids: Butanoic acid, 3-methyl- (8.395 min), Butanoic acid, 2-methyl- (8.619 min), Benzenecarboxylic acid (14.420 min), Benzeneacetic acid (15.338 min), Benzenepropanoic acid (16.413 min), Dodecanoic acid (19.146 min), n-Hexadecanoic acid (23.535 min), Heptadecanoic acid 924.364 min), 9-Octadecenoic acid, (E)- (25.081 min), Octadecanoic acid (25.350 min), 1,2- Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (28.463 min) and Hexadecanedioic acid (29.717 min). Hydrocarbons: Tetradecane (17.085), Hexadecane (19.526 min), Nonadecane (22.774 min), Tridecane (23.759 min), Heneicosane (26.447 min), Tetracosane (27.276 min), Pentacosane (28.060 min), Octacosane (29.538 min), Triacontane (30.344 min), Squalene (30.658 min), Nonacosane (31.240 min), Heptadecane, 9-octyl- (32.024 min) and Heptadecane (32.315 min). Alcohols: 1,4-Dioxane-2,6-dimethanol (15.719 min) and Phenol, 3,5-dimethoxy- (22.460 min). Pyrrolizidines: Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- (21.744 min) (Table 8).

Table 8: Compounds identified from isolate CHY15-10

Retention Time (min)	**Compound Name**	**% of total**	**% Quality**
7.633	1,2-Ethanediol, monoacetate	0.230	74
8.395	Butanoic acid, 3-methyl-	0.560	83
8.619	Butanoic acid, 2-methyl-	0.123	74
9.224	Dimethyl sulfone	0.269	85
14.196	2-Piperidinone	1.481	86
14.420	Benzenecarboxylic acid	0.560	95
14.845	1H-Pyrrole-2-carboxylic acid	0.234	91
15.338	Benzeneacetic acid	3.036	64
15.540	Caprolactam	0.589	76
15.719	1,4-Dioxane-2,6-dimethanol	1.002	89
16.413	Benzenepropanoic acid	1.117	97
17.085	Tetradecane	1.230	97
17.17	Vanillin	0.840	93

19.146	Dodecanoic acid	0.980	99
19.526	Hexadecane	0.589	97
20.579	2,6-Diisopropylnaphthalene	0.258	95
20.982	L-Prolinamide	0.789	77
21.273	3,7-Dimethyloctyl acetate	3.088	78
21.744	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	12.539	96
22.460	Phenol, 3,5-dimethoxy-	3.409	77
22.528	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.968	94
22.774	Nonadecane	0.879	98
23.087	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	1.125	79
23.289	N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide	0.456	91
23.535	n-Hexadecanoic acid	11.074	98
23.759	Tridecane	2.067	94
24.364	Heptadecanoic acid	1.456	95
25.081	9-Octadecenoic acid, (E)-	1.790	97
25.350	Octadecanoic acid	5.746	99
26.044	cis-2-Thioxo-5,6-trimethylene-2,3,5,6-tetrahydropyrimidin-4(1H)-one	26.891	77
26.447	Heneicosane	2.369	98
26.962	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7 -(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta.,	1.386	75
27.276	Tetracosane	0.646	99
27.477	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodeca hydro-1,4a,7-trimethyl-, methyl ester, [1R-(1.alpha.,4a.	0.890	86
27.791	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7 -(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	0.789	99

28.060	Pentacosane	0.855	98
28.463	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	3.480	87
29.336	Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)-	1.470	78
29.538	Octacosane	0.929	96
29.717	Hexadecanedioic acid	0.456	74
30.344	Triacontane	0.976	98
30.658	Squalene	1.009	91
31.240	Nonacosane	1.467	98
32.024	Heptadecane, 9-octyl-	0.870	93
32.315	Heptadecane	0.892	96

4.0 Discussion

The isolates had been isolated from two Kenyan National Parks namely Chyulu and Ruma National parks. Differential media was used to revive the isolates. The medium had fermentable carbohydrate, nitrogen, organic and inorganic compounds. Morphological studies were carried out and characteristics of the isolates compared with the standard characteristics described in Bergy's manual. They formed colored, tough and leathery colonies that were hard to pick from the culture media. Microscopic studies also showed that the isolates cells formed long branched network of mycelia a characteristic of *Streptomyces sp* as previously described by Kieser *et al.,* (2000).

Physiochemical characterization of the isolates Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3 on pH showed optimal growth of the isolates at pH range of 6 - 9. These results were in accordance to Gava (1998) who reported that majority of Actinomycetes isolated from rhizosphere and non-rhizosphere soil grows at a pH range varying from 6.5 to 8.0. Isolate Ruj 7-1 yielded good growth, characterized by abundant mycelium, in culture media with pH 6.0 suggesting its tolerance to acidic condition. The optimum pH level allows for optimal metabolic reactions characterized by enzymes hence the increase in growth of microorganisms (Moreira & Siqueira, 2002). Isolates CHY4-10, CHY15-10, CHY15-5, CHY2-3 and RUJ7-1 showed optimum growth at 30 °C, 32.5 °C, 30 °C, 32.5 °C and 27.5 °C respectively. Minimal growth was recorded below 27.5 °C and above 32.5 °C. These results also confirmed that isolates Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3 originated from a relatively warmer ecosystem than isolate Ruj 7-1 (27.5 °C). According to Goodfellow *et al.* (1990), bacterial growth rates increase with temperature up to the optimum temperature, at which the growth rate is maximal. Enzymatic processes are thought to limit further increases in growth rates at temperatures above the optimum temperature (Goodfellow *et al.,* 1990).

Growth of the isolates in culture medium with varying NaCl levels (0 g/l to 32.5 g/l) confirmed tolerance to saline conditions. All the isolates recorded growth in absence of sodium chloride but isolate Chy 4-10, Chy 15-10 and Chy 2-3 indicated an increase in growth at 5 g/l sodium chloride concentration which was similar to that of *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete (Chun *et al.,* 2000). Isolate Chy 15-5 and Ruj 7-1 grew optimally at higher NaCl concentrations (10 g/l and 17.5 g/l) respectively meaning they were more tolerant and also required higher NaCl concentrations for them to grow.

Most microorganisms obtain their energy through a series of orderly and integrated enzymatic reactions leading to the biooxidation of a substrate that is frequently a carbohydrate. The organisms use the carbohydrate differently depending on their enzyme complement. Some organisms ferment sugars such as glucose anaerobically, while others use the aerobic pathway (Williams *et al*., 1989). Glucose was the best carbon source for growth of isolates

Chy 4-10 (0.601nm), Chy 15-5 (0.719nm) and Chy 2-3 (0.475nm). This meant that glucose would give optimum growth during fermentation processes than the other sugars. Sucrose also indicated a considerable growth of the isolate Chy 4-10 (0.473nm) meaning it could act as an alternative source of carbon for this isolate. Mannose would also be an alternative source of carbon to isolate Chy 15-5 (0.250nm) besides glucose. Xylose was the preferred carbon source for isolates Chy 15-10 (0.899nm) and Ruj 7-1 (0.900nm) hence it would be the ideal carbon source during fermentation processes of isolates.

Actinomycetes are a prolific source of secondary metabolites. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered (Berdy, 2005). Among Actinomycetes, around 7,600 compounds are produced by *Streptomyces* species (Berdy, 2005). Streptomyces species are distributed widely in aquatic and terrestrial habitats Pathom-aree *et al.* (2006) and are of commercial interest due to their unique capacity to produce novel bioactive compounds. The main source for the bioactive secondary metabolites is soil streptomycetes, but a wide variety of structurally unique and biologically active secondary metabolites have recently been isolated from marine Actinomycetes, including those from the genus *Streptomyces* (Cho *et al.,* 2001; Sanchez-Lopez *et al.,* 2003; Lee *et al.,* 2005; Jensen *et al.,* 2005).

Production of secondary metabolites commonly precedes the development of aerial hyphae, when the growth rate of bacterial filaments has decreased and sporulation starts (Bibb, 2005). Much of the published data indicate that the most important environmental signal triggering secondary metabolism is nutrient starvation, particularly that of phosphate (Sola-Landa *et al.,* 2003). The signaling networks behind the regulation of secondary metabolism in streptomycetes have recently been reviewed (Bibb, 2005). Many of these secondary metabolites are potent antibiotics, which has made streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Berdy, 2005).

Besides antibiotics, which present the largest group of bioactive secondary metabolites, these antimicrobial compounds show several other biological activities i.e. antagonistic agents**,** including antibacterials, antifungals, antiprotozoans as well as antivirals, pharmacological agents, including antitumorals, immunomodulators, neurological agents and enzyme inhibitors, agrobiologicals, including insecticides, pesticides and herbicides, and compounds with regulatory activities, such as growth factors, siderophores or morphogenic agents. To detect simultaneous bioactivities for a given compound, pharmacological and agricultural screens are increasingly being used in combination with antimicrobial tests. This has revealed several novel therapeutic and agrobiological agents and previously unknown biological activities for antibiotics (Berdy, 1995; Sanglier *et al.,* 1996; Berdy, 2005).

Progress has been made recently on drug discovery from Actinomycetes by using high- throughput screening and fermentation, mining genomes for cryptic pathways, and combinatorial biosynthesis to generate new secondary metabolites related to existing pharmacophores (Baltz, 2008).

In this research, five Actinobacteria isolates were studied for the production of secondary metabolites. Yields of the secondary metabolites produced were determined and isolate CHY 15-10 produced the highest yield (0.62 g/l) whereas RUJ 7-1 produced the least (0.14 g/l). The five isolates were also tested for their *in vitro* activity on type culture collection of gram-positive (*S. aureus* NCTC 10788) and gram-negative bacteria (*E. coli* NCTC 10418). All the isolates had inhibitory effects on *S. aureus* but with different levels of inhibition. Isolate Chy 4-10 showed stronger inhibition against *S. aureus* NCTC 10788 with an inhibition diameter of 24mm followed by isolate Chy 2-3 (20mm) whereas isolate Chy 15-10 recorded the weakest inhibition of 13mm. The results were an indication that although all the isolates showed activity on the test microorganism, the degree of activity was varying due to the different or varying concentrations of the active ingredient. On the Gram negative *E. coli* NCTC 10418, isolates Chy 15-10 and Chy 2-3 showed no antagonistic effect on the test organism. However, isolate Chy 15-5 and Ruj 7-1 showed inhibitory effects on the test organism with zones of inhibition measuring 18mm and 15mm indicating higher chances of producing secondary metabolites that would counteract growth of Gram negative bacteria.

GC-MS analysis of the secondary metabolites was carried out and profiles of the fractions indicated the presence of different number of chemical compounds with different retention times and abundance. Among the compounds

identified comprised of amides, amines, acids, pyrrolizidines, ketones, quinones, alcohols and hydrocarbons. Some of these compounds have been detected from Actinobacteria and documented. Quinone related compounds with antitumor activity have been isolated from different marine Actinomycetes. *Streptomyces chinaensis* AUBN1/7 isolated from marine sediment samples of Bay of Bengal, India, is the producer of 1-hydroxy-1-norresistomycin and resistoflavin (Gorajana *et al.,* 2005). These compounds together with resistomycin and tetracenomycin D have also been produced by *Streptomyces* sp. B8005 isolated from sediments of the Laguna de Términos at the Gulf of México (Kock *et al.,* 2005). Resistomycin was isolated, in addition, from *Streptomyces* sp. B4842 from mud sediment of a coastal site of Mauritius, Indian Ocean (Kock *et al.,* 2005). 1-hydroxy-1-norresistomycin (Gorajana *et al.,* 2005) and resistoflavin (Gorajana *et al.,* 2007) showed cytotoxic activity against human gastric adenocarcinoma HMO2 and hepatic carcinoma HePG2 cell lines. Apart from the common or documented quinones obtained from *Streptomyces*, a unique group of quinone compound was detected from isolate Chy 4-10 in this study. p-Benzoquinone2-hydroxy-5-(methylthio)- from isolate Chy 4-10 was detected at 25.932 min retention time with an abundance of 9.493%. The potential of this compound as an antitumor is still not known.

Two new cytotoxic quinones of the angucycline class, marmycins A and B have been isolated from the culture broth of *Streptomyces* strain CNH990 isolated from a sediment sample collected at a depth of 20 m at the entrance to the Sea of Cortez, 5 km east of Cabo San Lucas, México (Martin *et al.,* 2007). In cytotoxic assays using the human cell line of colon adenocarcinoma HCT-116, marmycin A showed an IC50 of 60.5 nM, almost 18 times more potent than marmycin B, which showed an IC50 of 1.09 µM. Marmycin A was further evaluated for its *in vitro* cytotoxicity showing a mean IC50 value of 0.022 µM against 12 human tumor cell lines (breast, prostate, colon, lung, leukemia). In the same assays marmycin B was significantly less potent with a mean IC50 value of 3.5 µM (Martin *et al.,* 2007).

Streptomyces luteoverticillatum 11014 Li *et al.* (2006) isolated from underwater sediment at 20 m depth collected off the coast of Taipingjiao, Qingdao, China, is the producer of four known butenolides: (4*S*)-4,10- dihydroxy-10-methyl-undec-2-en-1,4-olide Cho *et al.* (2001), (4*S*)-4,10-dihydroxy-10-methyl-dodec-2-en- 1,4-olide (Mukku *et al.,* 2000; Cho *et al.,* 2001) and two diastereomeric (4*S*)-4,11-dihydroxy-10-methyl-dodec-2-en-1,4- olides. The four butenolides showed cytotoxic activity against human leukemia K562 with IC50 values of 8.73, 6.29, and 1.05 µmol/ml and murine lymphoma P388 cell lines with IC50 values of 0.34, 0.19, and 0.18 µmol/ml, respectively.

In this study, a butenolide was detected in isolate Ruj 7-1. 4, 8, 12, 16-Tetramethylheptadecan-4-olide was detected at 26.940 min retention time and its activity is still unknown. This butenolide could be having novel antitumoral suppression activity in its application.

A new class of 2-alkylidene-4-oxazolidinone, exhibiting an unprecedented antibiotic pharmacophore, was isolated from a marine actinomycete (NPS8920) (Macherla *et al.,* 2007; Michelle *et al.,* 2008). A series of three compounds in this class, lipoxazolidinones A to C, have been isolated, with the most potent activity against Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) and Anti-vancomycin-resistant Enterococci (VRE).

In this study, two chemical compounds relating with lipoxazolidinones were detected; 2-Piperidinone from isolate Chy 4-10 at 14.151 min retention time and 2-Pyrrolidinone from isolate Chy 2-3 at 12.449 min retention time. The activity of these two chemical compounds is unknown and further purification and testing would provide more information on their activities.

Today, the urgent need for new antimalarials requires the discovery of small and inexpensive molecules. Using an SYBR Green bioassay on the parasite's erythrocytic stages Jacques *et al.* (2008) carried out research on secondary metabolite produced by a marine actinomycete Salinispora tropica that had significant antimalarial activity. A pure amide chemical compound, salinosporamide A, was tested for its inhibitory activity against parasite development in vitro *(*P. falciparum*)* and *in vivo (*P. yoelii). The finding demonstrated that natural products remain one of the most important sources of medicines against the parasite. In this research, all the studied isolates produced amides that were detected at different retention times; N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide at 23.289 min, L-Prolinamide at 20.982 min, Acetamide,2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)- at 23.737 min, Formamide,N-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)- at 29.157 min, N-

Methoxymethyl-N-methylformamide at 11.015 min, Propanamide, 2-methyl- at 9.694 min and Propanamide, N,N-dimethyl- at 17.667 min. Activities of these amide chemical compounds need to be researched upon for they could be holding novel properties that would help in tackling the challenges of malaria at hand. Other groups of chemical compounds were also detected such as alcohols and hydrocarbons.

5.0 Conclusion and Recommendation

Physiochemical characterization of the isolates showed that optimal growth of the isolates was observed at pH values 6 and above. Optimum growth temperatures were observed between 27.5°C to 32.5°C. Growth of the isolates in culture medium at different NaCl levels (varying between 5 and 32.5g/l) indicated tolerance to salinity and an adaptability of these isolates to varying NaCl concentrations. In addition, glucose and xylose were the most utilized sugars for growth of the isolates whereas arabinose, inositol and mannitol were the least preferred sugars. The isolates showed antimicrobial activity and also produced a wide range of chemical compounds indicating they could be an important source of different antimicrobial compounds. *Streptomyces* have been well known during the last seventy years as prolific producers of new bioactive compounds, antitumor drugs included. With the increasing development of oceanographic studies leading to the isolation of new Actinomycetes from marine sources, new prolific genera in the production of useful compounds have been found, such as *Salinispora*. Moreover, protected terrestrial ecosystems may harbor a myriad of new Actinomycetes providing novel structural diversity to be discovered as evidenced by this study. Besides, the continuous effort to unravel the biosynthesis of the already known compounds and the isolation and characterization of their biosynthesis gene clusters may lead to the development of new antitumor compounds, hopefully with improved therapeutic properties, by using combinatorial biosynthesis approaches. And lastly, adoption of innovative techniques such as coculturing, cross species induction and biofilm development, may further facilitate the discovery of new and useful antimicrobial compounds.

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