الآية

وَعِندَهُ مَفَاتِحُ الْغَيْبِ لَا يَعْلَمُهَا إِلَّا هُوَ ۚ وَيَعْلَمُ مَا فِي الْبَرِّ وَالْبَحْرِ ۚ وَمَا تَسْقُطُ مِن وَرَقَةٍ إِلَّا يَعْلَمُهَا وَلَا حَبَّةٍ فِي ظُلُمَاتِ الْأَرْضِ وَلَا رَطْبٍ وَلَا يَابِسٍ إِلَّا فِي كِتَابٍ مُّبِينٍ.

سورة الأنعام: الآية (59)

DEDICATION

To the soul of my dear mother

To my dear father

To my family members

To my friends

To my teachers

ACKNOWLEDGEMENT

First of all thank to ALMIGHTY ALLAH.

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ABSTRACT

Bacterial resistance to antibiotics is growing up day by day in both community and hospital setting, increasing morbidity and mortality, the continuous development of bacterial resistance leading to an urgent requirement for new antibacterial compounds.

This study was conducted during the period 2012-2016 to isolate and characterize antibacterial compounds from *Streptomyces* strains in Red Sea, Port Sudan.

A total of fifty soil samples were collected from different depths in Red Sea, Port Sudan. Different culture media were used for the isolation of promising *Streptomyces* with antibacterial activities. The isolates were characterized by microbiological methods and confirmed by molecular techniques using both conventional and multiplex PCR. Antibacterial activity of the isolates were done by agar wells diffusion method and perpendicular streak method using *Staphylococcus aureus* and *Escherichia coli* as indicator organisms. The cultural conditions were optimized for the maximum growth and antibacterial activity with different physical and chemical conditions including; temperature, pH, incubation period, effect of nitrogen source, trace elements, light & dark. The extraction of antibacterial compound was done by ethyl acetate solvent then evaluated for antibacterial activities against test microorganisms. Thin layer chromatography (TLC) technique and gas chromatography-mass spectrometer (GC-MS) analysis were used to characterize the active compounds.

The results revealed that 21 (43%) isolates were recovered as promising organisms. Identification of these isolates showed that 9/21 (48%) were *Streptomyces* spp. Sequencing of the PCR products were compared with Gen Bank database and showed that all identified isolates were related to the genus

Streptomyces species with similarity ranged from (92% to 99%). Of the broad spectrum *Streptomyces*, two (PS1 and PS28) were chosen for production of antibacterial compounds. Study on optimization of cultural conditions for PS1 and PS28 revealed that maximum growth and antibacterial activities were obtained when grown in a medium having the following properties (gm./L); Soybean, 2.5; $MgSO_4.7H_2O$, 1.0; K_2HPO_4, 2.5; dissolved in Sea water, when adjust pH to 7.5, and incubated at 30°C for seven days. GC-MS characterization of the ethyl acetate crude extract showed 54 and 96 compounds from PS1 and PS28 respectively. Of them eight (n=8) and eleven (n=11) respectively were antibacterial compounds.

This study concluded that *Streptomyces* isolates from the Port Sudan coast are promising sources for antibacterial compounds. Further investigations for antifungal, antiviral and antitumor compounds are required.

المستخلص

مقاومة البكتيريا للمضادات الحيوية ينمو يوما بعد يوم، في كل من المجتمع والمستشفيات، وزيادة معدلات الامراض و الوفيات نتيجة ذلك. مع التطور المستمر وانتشار مقاومة البكتيريا مما يؤدي إلى مطلب ملح لمركبات جديدة مضادة للجراثيم.

هذه الدراسة امتدت للفترة من 2012 الى 2016 الكشف وعزل وتوصيف مركبات المضادات الحيوية من سلالات بكتريا الستربتومايسيس المعزولة من البحر الأحمرفي بورتسودان.

لقد تم جمع خمسين عينه من التربة، من اعماق مختلفة في البحر الأحمرفي بورتسودان، واستخدمت مجموعة متنوعة من الاوساط الزرعية لعزل بكتريا الستربتومايسيس الواعدة بفعاليتها المضادة للجراثيم، وقد تمت هذه الخطوة بالطرق المايكروبايولوجية، وتم تشخيصها بالتقنيات الجزيئية بأستخدام تقنية تفاعل البوليميرز المتسلسل التقليدية و المتعددة. النشاط المضاد للبكتريا تم عمله للعزلات بواسطة تقنية الانتشار بواسطة الحفر و بطريقة التخطيط العمودي للبكتريا بأستخدام بكتريا المكورات العنقودية الذهبية وبكتريا الايشيريشيا القولونية كبكتريا معيارية.

ظروف الوسط الزرعي تم تعديلها للوضع الامثل بواسطة تغيير عدد من الظروف الكيمياوية و الفيزياوية، و قد تضمن درجة الحرارة، الاس الهيدروجيني،المحتوي النيتروجيني ، وقت التحضين، ظروف الضوء و الظلام و تراكيز العناصر الكيمياوية النادرة. أستخلاص مضادات البكتريا تم بأستعمال خلات الاثيل كمذيب، و تم فحص الفعالية المضادة للبكتريا لها بأستخدام البكتريا المعيارية المستخدمة في هذا البحث. وصف المركبات المضادة للجراثيم تمت بأستخدام تقنيتي الطبقة الرقيقة اللونية، و تحليل المطياف اللوني للكتلة الغازي.

أظهرت النتائج ان 21(43%) من العزلات هي ذات فعالية واعدة مضادة للجراثيم. التشخيص المخبري أظهر 21/9 (48%) كبتريا الستربتومايسيس. التحليل التسلسلي لنتاج فحص تفاعل البوليميرز المتسلسل بالمقارنة ببنك الجينات أن جميع العينات هي عائدة لبكتريا الستربتومايسيس بنسبة تطابق بين (92% الى 99%). من حلال فحص الطيف لمضادات الجراثيم تم اختيار العزلتين PS1 و PS28 لأنتاج مضادات الجراثيم. ظروف الوسط الزرعي المثلى للعزلتين PS1 و PS28 للحصول على اعلى نمو وانتاج لمضادات الجراثيم حيث ضبطت الظروف الزرعية على: (غم/لتر) الشوفان 2.5, كبريتات المغنيسيوم المائة عند

1.0, فوسفات الهيدروجين ثنائي البوتاسيوم عند 2.5 أذيبو في ماء البحر, وتثبيت الاس الهيدروجيني عند 7.5 على درجة حرارة 30 درجة مئوية لمدة سبعة ايام.

تحليل المطياف اللوني للكتلة الغازي من المستخلص الخام بواسطة خلات الاثيل أظهرت 54 و 96 مركبا من العزلتين PS1 و PS28 على التوالي. من هذه المركبات ثمان و احد عشرمركبا من العزلتين PS1 و PS28 على التوالي أظهرت فعالية مضادة للجراثيم.

وخلصت هذه الدراسة إلى أن بكتريا الستربتومايسيس المعزولة من ساحل بورت سودان هي من المصادر الواعدة للمركبات المضادة للجراثيم, وتشجع على مزيد من الدراسات لهذه المنطقة لاستخلاص المركبات المضادة للفطريات، المضاد للفيروسات ومضادة للأورام.

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