Effect of Irradiated and Non- irradiated Egyptian and Chinese anise on the Susceptibility of Multidrug Resistant Bacterial Isolates to Different Antibiotics

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بسم الله الرحمن الرحيم

نَرفَعُ دَرَجَاتٍ مَن نَّشَآءُ

وَفَوقَ كُلِّ ذِى عِلمٍ

عَلِيمٌ

DEDICATION

To my parents ***Mr. Mostafa Mahmoud Azzam*** *and* ***Mrs. Fatma Amin Abu Elso'oud,*** *thank you for your love support and your tremendous help. Your constant motivation and faith has always given me hope. Now, this was your dream through me.*

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اتقدم بخالص الشكر و التقدير و الامتنان الي أساتذتي الأفاضل الذين كانوا عونا لي في البحث و العمل.

كل التقدير و الاحترام الي **أ.د./ محمد خالد ابراهيم**: أستاذ البكتريولوجي و رئيس قسم الميكروبيولوجي, كلية علوم, جامعة عين شمس و ذلك لإشرافه و مساعدته و تشجيعه لي.

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أ.م.د./ حنان حسن عبد الخالق: استاذ مساعد الميكروبيولوجي, قسم الميكروبيولوجيا الاشعاعيه بالمركز القومي لبحوث و تكنولوجيا الاشعاع, هيئه الطاقه الذريه, لها مني كل الاحترام و التقدير لإشرافها و مساعدتها في كل خطوات البحث, فقد كانت بمثابه الأخت الكبيره و المعلمه الجليله.

Abstract

Antibiotic resistance in bacteria is becoming a serious problem, especially after the emergence of multidrug-resistant strains. To overcome this problem, new and effective antibacterials or resistance modulators are highly needed and plant kingdom represents a valuable source of these compounds. In this study we investigated the antibacterial activity of Egyptian anise Essential Oil (EEO), Egyptian anise Waste Residue Extract (EAWRE), Chinese anise Essential Oil (CEO) and Chinese anise Waste Residue Extract (CAWRE) against 100 isolates belonging to two Gram positive (*Streptococcus* spp. and *Staphylococcus* spp.) and four Gram negative bacteria *(Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp. and *Pseudomonas* spp.), also we investigated the resistance modification activity of both oils and post-distillation extracts against six clinical isolates (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* , *Acinetobacter baumannii* and *Pseudomonas aeruginosa*). Phenolic compounds of anise wastes (EAWRE and CAWRE) were determined by HPLC and the components of EEO and CEO were detected by Gas Chromatography. The antibacterial activity of anise waste extracts and anise essential oils assays were performed by using inhibition zone diameters, MIC and MBC. Synergistic evaluation of anise waste extracts and anise essential oils combined with certain known antibiotics like cephradine, chloramphenicol**,** tetracycline and amoxicillin was carried out using disc diffusion method, MIC, MBC and the fractional inhibitory concentration (FIC) indices. Assessment of bacterial morphology of *Pseudomonas aeruginosa* treated with tested oils and extracts alone and in combination with tetracycline was carried out using scanning electron microscopy (SEM). The results showed that HPLC method has been developed for the determination of 25 phenolic compounds from anise waste extracts; while GC analysis detected the presence of trans-anethole as the major component of both oils. EAWRE, CAWRE, EEO and CEO have significant antibacterial activity against all of the test bacteria. CAWRE was found to have higher amounts of phenolic compounds contents that might be responsible for its comparatively higher antibacterial activity than EAWRE. Also CEO was found to possess higher anethole content that could be responsible for its higher effectiveness compared to EEO. Neither the antibacterial activity of both post-distillation extracts nor the activity of both essential oils was significantly affected by irradiation at 10 and 30 kGy. The combination of anise waste extracts and the tested antibiotics mostly showed synergistic effect. Synergistic interaction was most expressed against *Streptococcus pneumoniae* and *Staphylococcus aureus* by tetracycline and chloramphenicol; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Escherichia*

coli by cephradine, amoxicillin and tetracycline. In contrast, all tested combinations of EEO and CEO with tested antibiotics resulted in synergistic interaction against all tested isolates. SEM observations confirmed the antibacterial activity of EEO, CEO, EAWRE and CAWRE; as in all cases the bacteria lost its original shape and irregularity was observed. Synergism was also confirmed through the detected severe morphological changes in cells of *Pseudomonas aeruginosa*. Synergistic interaction resulted from the combination of EAWRE, CAWRE, EEO and CEO with commercial antibiotics could be useful in fighting emerging drug-resistant bacteria. These results suggest that both aniseeds and star anise waste residue methanolic extract (post-distillation) and essential oils could be good economic sources of multidrug resistance inhibitors. The results also indicate that indiscriminate co-administration of antibiotics with some aromatic and medicinal plant wastes such as those from aniseeds and star anise wastes could be therapeutically wasteful. Their use in combination with conventional antibiotics should be further studied for *in vivo* which may lead to the development of much needed drug enhancing preparations.

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