Star Anise Essential Oil As An Enhancer And Restorer Of The Antibacterial Action For Certain Antibiotics Against Gram Positive Or Negative Multi-Drug Resistance Isolates.

Abdel Khalek H [*1], Mattar Z [1], Ibrahim M [2], Azam Y [1]

[1]Department of Radiation Microbiology, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

[2]Department of Microbiology, Faculty of Science, Ain Shams University, Al Qalyubia Governorate, Egypt

Abstract

To our knowledge, this is the first research on the use of Star Anise Essential Oil (SAEO) to activate and recover antibiotics that are now ineffective in medical therapy toward MDR bacteria. In the current study, the SAEO was investigated for its antibacterial properties and to evaluate the ability of Cephradine, Amoxicillin, Chloramphenicol and Tetracycline activity against both Gram-negative bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, and Klebsiella pneumoniae) or Gram-positive (Streptococcus pneumoniae and Staphylococcus aureus) MDR isolates. GC of the essential oil showed as major compounds the trans- anethole (90.07%). SAEO showed broad effectiveness against studied isolates. The antibacterial activity of SAEO was not changed by gamma irradiation at 10 and 30 kGy. Significant synergistic effects were noted with SAEO when it's associated with tested antibiotics. In combination with SAEO, a significant decrease in MICs values of all tested antibiotics was observed. Synergy was detected when SAEO/antibiotics combinations tested against MDR Gram-positive and Gram-negative bacteria with FICI ranging from 0.13 to 0.52 and from 0.26 to 0.56, respectively. The synergy in this study, using combinations of Star anise essential oil with antibiotics can enhance or restore the antibacterial activity of many antibiotics currently useless. Thus, SAEO could be an important and effective tool to be used individually or in combination with some therapeutic drugs (antibiotics) that are now ineffective to control bacterial MDR. Also, star anise contains shikimic acid (Tamiflu), which is a key ingredient in the pharmaceutical synthesis of the antiviral and anti-influenza drugs have a possible effect against thus; it may COVED-19.

Keywords: Star anise essential oil, antibacterial action, MDR isolates, enhancement and restore, antibiotics

Accepted on 23 December, 2022

Introduction

Infectious illnesses cause significant level morbidity and death of a human and are still one of the world's major health problems especially in developing countries. According to the world health organization, bacterial diseases are the world's second cause of death and the leading cause of loss of productive life. Bacterial diseases are answerable for about 7 0 % microorganism relate deaths[1,2]. In ancient times, the use of antibiotics and hygiene laws helped combat bacterial infections. Antibiotics are now "endangered species" facing extinction due to the rise of antibiotic resistance (ABR) worldwide. Resistance to antibiotics is already a severe and widespread problem, causing high death rate every year in both developing and developed countries [3,4]. In the European Union, estimated 25,000 deaths occur yearly from MDR-bacterial infections [5]. Although antibiotic resistance may not be new, the rising number of bacteria that are resistant to a specific drug, geographical sites impacted by these resistant pathogens [6]. Cancer and Immune-compromised patients especially children are the most affected by bacterial antibiotic resistance, which require the discovery of new drugs to solve this problem. But the problem remains, the appearance of resistant microbial strains to this new drug within a few years after its first clinical use is also a big challenge to public health [7]. The world trend is now, looking for alternative drugs and/or novel methods of solving the problem of drug-resistant bacteria [8]. To overcome the challenges, the inhibitory action of natural products on the multidrug-resistant efflux pump discovered in most resistant strains has been explored and the studies indicate they can be an excellent enhancer of a new antibacterial drug [9,10]. The World Health Organization (WHO) to have confirmed that plant extracts are among the main sources for new drugs [11]. Factors like the cost of certain conventional drugs, the resistance of bacteria to existing drugs, social cultures, in addition to the searching for treatments for some incurable diseases, led to the trend towards in the use of plant extracts with their progressive incorporation into the primary and secondary health systems of some developing countries [12,13].

Drug combinations between plant extracts and antibiotics can help decrease the lots of side effects of artificial drug combinations. Toxicity assessment shows antibiotics to be toxic to humans (especially synthetic ones). While the toxic effects of some antibiotics are within the human range permitted, the toxicity level may surpass the permitted limit if two or more of

these antibiotics are used [14]. Exploring the combination therapy between artificial antibiotics and active compounds from plant extracts may overcome the multiple toxic effects [15]. Also, this could lead to an inexpensive drug combination therapy that could address the issue of drug regimens especially of patients who suffer from chronic diseases in developing countries that needing cheaper treatment regimen [16] The combination between antibiotics and plant extracts that are synergistic may provide a novel approach to treating Malti-drag resistance bacteria since they are intended to (re)enable the use of antibiotics which are no longer (or less) therapeutically impact. Effective use of plant extracts combinations was observed in antibacterial therapy, HIV, inflammation, cancer treatment, stress-induced insomnia, hypertension, osteoarthritis and antiviral [17].

Synergistic activity by essential oils in the treatment of bacterial infections has been shown to minimize the required minimum dose of antibiotics, which decreases the antibiotic negative impacts [18]. The use of essential oils to combat resistant bacteria is considered to be more effective since essential oils are multi-component compared to several synthetic antibiotics that only have a single target site. Combining antibiotics with essential oils targeting resistant bacteria may have different mechanisms of action and may lead to new ways of overcoming the microbial resistance attack [19]. Star Anise (Illicium verum) derived from the star-shaped pericarp of Illicium verum, widely used as a spice [20] . Star anise is a source of shikimic acid, which is the primary precursor to the synthesis of anti-influenza drugs hence; it may have a potential effect against the emerging COVED 19. [21]. Aniseeds contain 1.5–5 % essential oil and are used as a gastrointestinal spasm flavoring, digestive, carminative, and relaxation. Antimicrobial, antiviral and antioxidant properties and significant potential for anticancer have been confirmed [22,23]. The purpose of this research is to develop a new generation of aromatherapy (Star Anise Essential Oil) that can be used alone or in combination with synthetic antibiotics. This new generation of phytomedicine may offer new credibility to phytotherapy. Hence they can be used as an alternative for antibiotics with no or limited effect. Production of a new formula of star anise oil and some synthetic antibiotics to overcome antibiotic-resistant bacteria. The potential use of this new combination as antiviral drugs, especially the Coronavirus.

Materials and Methods

Plant material

Star aniseed seeds were collected from a local farmers market in Cairo, Egypt, and then divided into three groups. The first was left without irradiation and was considered as control, whereas the second and third groups were subjected to γ-irradiation at 10 kGy and 30 kGy doses respectively; The irradiator source dose rate was 2,519 kGy / hour, and 2, 4911 kGy / hour, respectively. Gamma irradiation was performed using a source of cobalt-60 irradiator (Gamma Chamber 4000 India), located at the National Radiation Research and Technology Center (NCRRT), Nasr City, Cairo, Egypt.

Essential oil extraction

Using a domestic model grinder, 50 grams of irradiated and non-irradiated star anise seeds (SAS) were powdered and hydro-distilled for 4 hours using a Clevenger-type device to produce essential oils [24]. The oils were then dried with anhydrous sodium sulphate to eliminate traces of moisture and preserved at low temperature (4°C) in sealed vials before use.

Gass-Chromatography (GC) analysis of essential oils: GC analysis of Star Anise Essential Oil (SAEO) performed with Hewlett- Packard GC- 5890 series II with a flame ionization detector (FID). Analysis performed using capillary column (30 m x 0.25 mm I'd; 0.10 μm HP-1 film thicknesses).

The amount of samples was 0.2 μl. The operational method is as follows: temperatures for the injector and the detector were 250 and 280 ° C, respectively. Helium (He) was used as carrier gas with a flow rate of 1 ml / min; oven temperature programmed at a rate of 4 ° C / min, 60-240 ° C.

Drugs (antibiotics)

Antibiotics were chosen in this study in such a way that various antibiotics have different bacterial targets (cell wall synthesis, nucleic acid, protein synthesis,). The antibiotics selected for this work were Cephradine (CE), Amoxicillin (AM), Chloramphenicol (C) and Tetracycline (TE) (Sigma Co., St. Louis, MO, USA). These antibiotics are commonly used in clinics to treat the selected isolates infection and selected bacteria often show resistance to these antibiotics. All antibiotics were dissolved in sterile water and all solutions were formulated according to the National Committee for Clinical Laboratory Standards NCCLS guidelines [25].

Bacterial isolates

The bacterial isolates used in this study were: Staphylococcus aureus, Streptococcus pneumoniae (Gram-positive), Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli and Acinetobacter baumannii and (Gram-negative). They were collected from different clinical specimens (secretion, wound, urine, cerebrospinal fluid, diabetic foot, sputum, prostatic end of the endotracheal tube, blood and tip of a urinary catheter,) over 6 months.

Theses isolate collected from the Clinical Microbiology Laboratory of the Arab Contractors Medical Center, Cairo, Egypt, then on nutrient agar slants its maintained at 4 oC. The cells had been cultured for 24 h in Heart Infusion Agar (Difco Laboratory, Ltd., Detroit, MI, USA) and medium brain heart infusion (BHI, Difco Laboratories Inc.) prior to the assay. Using Kirby-Baurer disc diffusion technique [26], all clinical isolates have been tested for antibiotic resistance. Use 18 various antibiotics that impact the cell wall, DNA and protein synthesis.

Antibiogram screening test of star anise essential oil (SAEO)

Before the combination assays of SAEO-antibiotics, the antibiogram of unirradiated and irradiated (10 and 30 kGy) SAEO were determined by agar disc diffusion assay [27,28]. Standardized inoculums of collected clinical isolates have been placed on the surface of sterile Muller-Hinton agar (MHA) plates, and a sterile cotton swab has been used for inoculum distribution. 6 mm philtre paper discs were placed on the inoculated plate surface and impregnated with 40 µL (100 mg / ml) of known oil concentration. Sterile paper discs carrying Dimethyl sulfoxide alone was considered as control. The plates were placed for 2 h at 4oC, and then incubated for 24 h at 37oC. The growth inhibition rings were assessed after incubation, by measuring the diameter of the inhibition zone in mm. There were three replicates held for each test.

Modulatory effect of SAEO on antibacterial drugs

Synergistic studies of SAEO/antibiotics were conducted with three tests: antibiogram screening with disc diffusion method, Minimum Inhibitory Concentration (MIC) method and the Fractional Inhibitory Concentration Index (FICI).

Antibiotics modulation assay by disc diffusion technique

Susceptibility tests were screened using the disc diffusion method on agar solid Mueller-Hinton (Oxoid) per the protocols of the Institute for Clinical and Laboratory Standards [29].. Petri dishes were prepared before inoculation, containing 20 mL Mueller-Hinton agar. The multidrug-resistant bacterial isolates were picked from overnight cultures in Brain Heart agar (Merck Company, Darmstadt, Germany), and suspensions were ready in a normal saline by adjusting the turbidity to 0.5 McFarland standards. In the first group of experiments, sterile filter paper discs (6 mm in diameter) (Oxoid, England) impregnated with 15 µl of irradiated and non-irradiated SAEO resuspended in 10 per cent dimethyl sulfoxide (DMSO) (Sigma, Taufkirchen, Germany) to achieve a final concentration of 2.0 mg. ml-1 was placed on the agar plate seeded with the respective bacteria. The second group, the activities of the selected antibiotics cephradine (CN), amoxicillin (AM), chloramphenicol (CH) and tetracycline (TE) on the test isolates were then evaluated using laboratory-prepared antibiotic discs by Kirby-Baurer's diffusion techniques. In the third group, 15 µl of SAEO was impregnated with selected antibiotic discs. DMSO has been used as a control. Inoculated Petri dishes were incubated at 37 ° C overnight. All substances in triplicate, the antibiogram were represented as the mean of zones of inhibition (mm) ± SEM (standard mean error).

Antibiotic modulation assay by MIC and MBC determination

SAEO's MICs and MBCs were calculated using the agar plate dilution technique as recommended by the Clinical and Laboratory Standard Institute [29]. Two-fold serial dilutions of SAEO dissolved in DMSO and prepared ranging from 1 to 1024 µl/ml. Suitable dilutions were then mixed thoroughly with molten agar that have been allowed to equilibrate in water bath (45 to 50°C). Plates were poured and allowed to solidify at room temperature. An aliquot of standardized inoculums (turbidity of the 0.5 McFarland standards) from overnight culture of selected multidrug resistant isolates were applied to the agar surface. Control plates (without oils) were inoculated before and after starting with the concentrations to ensure there was no contamination or significant antibacterial carryover during the inoculation. Inoculated plates were allowed to stand at room temperature. Inverted plates were incubated for 18 to 24 hours at 37°C. The MBCs were determined by selecting plates that showed no growth during MIC determination; a loopful from each plate was subcultured onto free agar plates, incubated for further 24 hours at 37 °C. The least concentration, at which no growth was observed, was noted as the MBC [30]. To determine the possible modulatory impact of the essential oil on microbial resistance; the SAEO was tested at the sub-inhibitory concentration (i.e, MIC/2) in combination with antibiotics (cephradine, amoxicillin, chloramphenicol and tetracycline. One hundred microliters (100 µ/L) of solution containing culture medium, the inoculum (10%) and the essential oil was distributed in alphabetical order, then 100 µ/L of antibacterial or antibiotic standard drugs was added and mixed. The final concentrations of antibacterial standard drugs used in this study ranged from 1 to 1024 µl/ml. The plates were incubated at 37 oC for 24 h [31].

The Fractional inhibitory concentration index (FICI)

The MICs of all combinations were calculated as described previously. For each isolate, the fractional inhibitory concentrations (FIC) values of antibiotics and oil were detremined using equation 1and 2:

FIC A (oil) = MIC of the oil in combination / MIC of the oil alone

……. Equation (1)

FIC B (antibiotic) = MIC of the antibiotic in combination / MIC of antibiotic alone

…….. Equation (2)

The sum of FIC A and FIC B gives the FIC Index (Equation 3) from where the interaction can be detected:

FIC Index = FIC A + FIC B Equation (3).

The combinations were classified as synergistic, additive, indifference and antagonistic, if the FIC Index $< 1, = 1, > 1 \leq 2$ and > 2 respectively [32].

Scanning electron microscopy (SEM)

The bacterial cells of Pseudomonas aeruginosa exposed to tetracycline, SAEO alone and in combination and treated according to the procedure described by [33, 34] with modifications. Bacterial cells were fixed in a solution of distilled water containing 2.5glutaraldehyde (%, v/v). After fixation, the cells were washed repeatedly with distilled sterile water to remove salt crystals. The grains were dehydrated in a graded ethanol series (10, 20, 30, 40, 50, 60, 70, 80, 90 and 96%, each change 5 min) and subsequently in hydroxyl mexamethyldisilazane (HMDS). After drying, the cells were attached to SEM stubs using double-sided conductive tape and

sputter-coated with gold. The samples were examined using a JOEL JMS 5600 scanning electron microscopy located in central laboratories at National Center for Radiation Research and Technology.

Statistical Analysis

Data significance was assessed using ANOVA Two-way Variance Analysis. All analysis was carried out with version 9.1 of the SAS software package.3.

Results and Discussion

Essential oils contain many chemical compounds that affect its bioactivites. Thus, the assessing the chemical structure of the oils is very critical before to evaluating their bioactivities. Changes in the volatile oil components of SAEO pre and post-γ-irradiation at doses of 10 and 30 kGy (necessary for microbial decontamination) were investigated by GC-analysis; chromatograms showed the presence of 30, 35 and 35 peaks in each of the non-irradiated and irradiated (10 and 30 kGy) SAEO, respectively (Fig. 1A, 1B &1C).

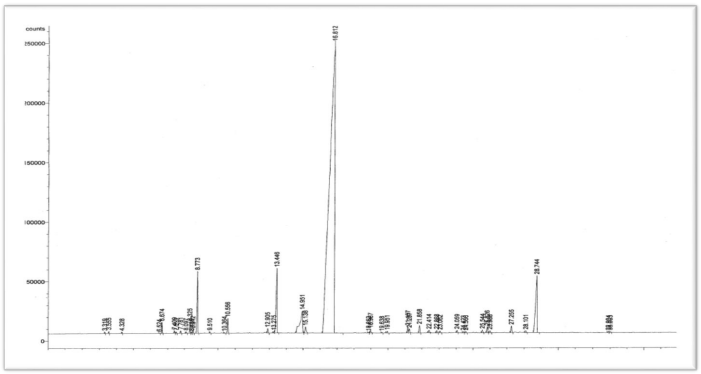

Figure 1A. *Chromatogram of non-irradiated Chinese anise essential oil (CEO)*

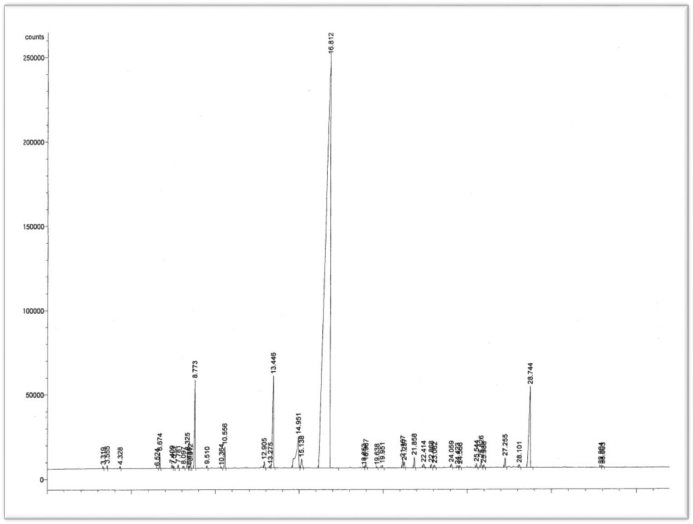

Figure 1B. *Chromatogram of 10 kGy irradiated Chinese anise essential oil (CEO)*

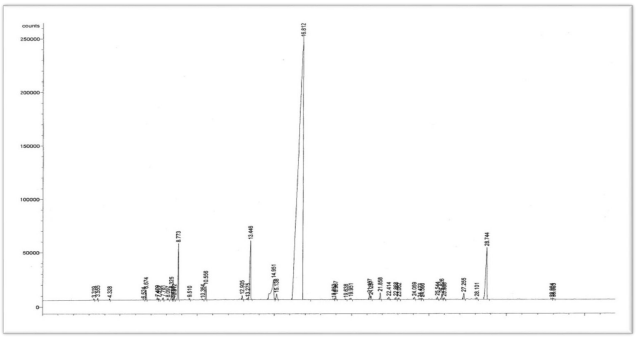

Figure 1B. *chromatogram of 30 kGy irradiated Chinese anise essential oil (CEO).*

Chromatograms obtained from SAEO revealed the presence of one major constituent, which is trans-anethole (90.07%). High percentage area indicated trans-Anethole was the most abundant compound in all the star anise essential oils (Fig 1A). Similar findings were reported by [35] who stated that the main component of star anise essential oil is trans-anethole, which accounts for 80-90 %. While [36] finding the concentration of trans-anethole in star anise essential oil was 75.62%. Compared to other studies; the observed differences in Anatole content could be attributed to plant type, growth conditions, harvest season, geographic origin, and extraction procedure [37]. Results showed that γ-irradiation at doses of 10 and 30 kGy resulted in a loss of 1.45 % and 2.74 % of the total concentration of the main component (trans-Anatole). In contrast to our results, [38] detected little increase in t-Anatole in irradiated anise seeds essential oil (0.42, 0.65, 0.67 and 1.04 %) when exposed to gamma irradiation doses of 4, 8, 16 and 32 KGy, respectively. The observed changes in anethole concentration in irradiated and non-irradiated oils can be attributed to extraction efficiency, its chemical and structural stabilities when the seeds exposed to γ-radiation and the effect of γ-radiation on the isomerization and the transformation between the different constituents. Few studies are available on the antibacterial activity of Star Anise Essential Oil (SAEO). The antibiogram potential SAEO was qualitatively evaluated against collected MDR clinical isolates (S. pneumoniae, S. aureus, K. pneumoniae, E. coli, A. baumannii and P. aeruginosa) (Tables 1). SAEO showed significant antibacterial activity against all MDR clinical isolates as determined by the diameter of the inhibition zone of the oil. The high values recorded for SAEO may be due to the use of the oil in the crude form, include of active compounds (anethole and other compounds). Many studies have confirmed that biological activity of crude oils from plant extracts within a very range of inhibition zone diameter [39].

In our study, the SAEO show high effectiveness against S. pneumonia and S. aureus (Gram-positive) by producing inhibition zone diameters 24.6 and 23.0 mm, respectively. Also SAEO showed pronounced activity against K. pneumoniae, E. coli, A. baumannii and P. aeruginosa isolates (Gram-negative); the inhibition zones were found to be 25.0, 17.0, and 21.6 and 22.3 mm, respectively, the largest inhibition zones were obtained with K. pneumoniae, in comparison to E. coli, was found to be the least sensitive one against the tested oil. The

findings obtained were very similar to other studies documented by [40, 41] they reported that star anise oil was found to exhibit strong antifungal and antibacterial activity.

The high antibacterial activity of SAEO is probably due to the high concentration of anethole in the oil or as a result of the synergism of its components.

Anethole was known to possess antimicrobial and antioxidant properties [37,42]. Also, star anise has shown anti-viral effects, especially influenza viruses, since it contains shikimic acid (Tamiflu), which is a key ingredient in the pharmaceutical synthesis of the antiviral and anti-influenza drugs thus, it may have a possible effect against the Corona-virus [43,21].

SAEO includes several compounds with proven antimicrobial activity in addition to the anethole in their chemical composition as limonene, a-pinene, acetoanisole, b- pinene, safrole, linalool, estragole camphene, cis-anethole and anisaldehyde.

Many other components are identified from its acetone [35,44], those components can affect synergically by increasing the antimicrobial activities. The essential oils have no fixed target in the bacterial cell, due to the different active compounds. The lipophilic structure enables the passage of essential oil via the membranes and destabilizes ion and solute transport.

This process leads to ion loss and reduced membrane possibility, with the consequent collapse of the proton blast, ATP depletion causes apoptosis and necrosis which leads to cell death [45-47].
Gamma-irradiation is used as a safe technique for the decontamination of herbal drugs that are used as alternative medicines. Our data (Table 1) indicated that γ-irradiation at 10 and 30 kGy did not effect on the antibacterial activities of SAEO.

It is understood that ionizing radiation induces quantitative and qualitative differences in plant matter, increasing, reduction, or inducing secondary materials. [48] Found radiation exposure induced effect on the physical and chemical components of phenolic extracts of cashew leaves, and improved tannin amounts.

So the antibacterial activities of leaves of cashew extract increased against Staphylococcus aureus. [49] Reported that the antibacterial activity of anise oil was increased against Bacillus cereus and Escherichia coli with irradiation at doses of 5 and 10 kGy and maximum stimulation was achieved at a dosage of 20 kGy. [50] reported that the antibacterial activity of volatile oil extracted from irradiated fennel seeds was increased against Bacillus cereus and Staphylococcus aureus, while decreased activity was observed against Escherichia coli.

No data found in a review of literature about the use of star anise essential oil as a modulator of antibacterial action for a standard antibiotic so this study was done. Figure. 2

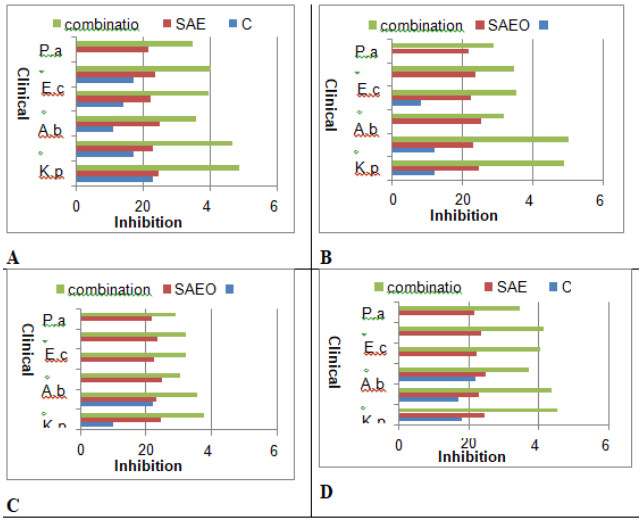

Figure 2. *Combination between SAEO and tested antibiotics. A: with Cephradine, B: with Amoxicillin, C: with Tetracycline, D: with Chloramphenicol, CEO: S.p: Streptococcus pneumoniae, S.a: Staphylococcus aureus, K.p: Klebsiella pneumoniae, A.b: Acinetobacter baumannii, E.c: E.scherichia coli, P.a: Pseudomonas aeruginosa.*

Table 1. *Antibiogram activity of unirradiated and irradiated Star anise essential oil.*

Clinical isolate	Inhibition zone (mm)		
	SAEO		
	NI	10 kGy	30 kGy
Streptococcus	24.6ac± 0.6	24.0ac± 0.5	23.3ac± 0.6
pneumoniae			
Staphylococcus aureus	23.0af± 0.5	24.6af± 0.5	24.0af± 0.6
Klebsiella pneumoniae	25.0ac± 0.5	24.0ac± 0.0	23.6ac± 0.3
Escherichia coli	17.0ad± 0.3	26.0ad± 0.3	25.0ad± 0.5
Pseudomonas aeruginosa	21.6ae ± 0.3	21.0ae ± 0.3	20.3ae ± 0.5
Acinetobacter baumannii	22.3ag ± 0.3	22.06ag ± 0.3	21.06ag ± 0.3

Shows the Inhibition Zone (IZ) of combinations between SAEO and tested antibiotics, significant synergistic effects were noted with SAEO when it's associated with several antibiotics. The extension of the inhibition zones reveals a positive interaction (synergism). Data revealed that SAEO was more effective against the MDR isolates when compared to standard antibiotics and the combinatory impact exceeded their individual efficiency and produce increasing the antibacterial activity of tested antibiotics. Complementary interaction between two agents indicates their combined effects are higher than the total of each agent effects. In general, the inhibition zones in antibiotic / SAEO plates are approximately 13-40 mm larger than the zones of inhibition in the control plates depending on the bacterial isolates. The extension of the

inhibition zone by over than 5 mm was considered significant. Such results may be due to the effect of the active compounds or a potential inhibition by other oil compounds of one or more modes of MDR bacteria mechanisms. Combining effects of cephradine, amoxicillin, tetracycline and chloramphenicol with SAEO showed the inhibition zones 25.6, 36.3, 27.6, 27.3 mm and 29.6, 38.0, 13.6, 26.3 mm wider than controls to S. pneumoniae and S. aurous (gram-positive), respectively. The star anise essential oil can enhance the sander antibiotic against positive MDR isolates by indirect contact during the disk diffusion test, indicates that the SAEO has the ability to be used as adjuvant therapy in antibiotic drugs. The increase in antibiotic activity observed may be explained by the presence of bioactive compounds in SAEO. Our study is in agreement with earlier studies [51] reported that the greatest synergism was observed in the combination of amoxicillin with lemongrass essential oil, followed by amoxacillin with cardamom oil towards certain clinical isolates of Staphylococcus aurous (methicillin-resistant). A synergistic effect was achieved by a combination of Zingier cassumunar volatile oil and some of the antibiotics (fluoroquinolones, aminoglycosides and tetracycline's) and folate mechanism inhibitors that can form the basis for the production of a new drug against MDR bacteria [15]. The results of that study (Fig 2) showed that antibiotics alone did not demonstrate antibacterial activity towards most negative MDR isolates (no inhibition zones), however, the association SAEO with antibiotics resulted from synergistic action. The antibiotic/ SAEO combination that is synergistic will proffer a new method of treating negative MDR isolates and (re)enabling the use of antibiotics that are no therapeutically successful. This is a very significant outcome for solving the problem of increasing drug resistance progress in human diseases. Similarly, [52] reported that Geraniol has shown strong efficacy in the modulation of antibiotic resistance against P. aeruginosa, Enterobacter aerogenes and E. coli (Gram-negative) by targeting efflux pumps and may recover drug susceptibility in over-expressing efflux pump strains. The modulation in antibiotic resistance by essential oils is more apparent for drugs like fluoroquinolones, β-lactams and chloramphenicol [14]. Our study found this association could enhance the antibacterial activity of the antibiotics tested against S. pneumoniae and S. aureus (Gram-positive) and may contribute to re-sensitize K. pneumoniae, E. coli, P. aeruginosa and A. baumannii (Gram negative) for the action of cephradine chloramphenicol, tetracycline and amoxicillin. Volatile oils from several plants have shown that they often possess the capability to interact with the conventional antibiotic and show a powerful trend in potentiating them, in addition to their antimicrobial activities. In this study, star anise essential oil inhibits the bacteria's growth and their combined activity with antibiotics leads to an improved antibacterial action and continued useful antibiotic life. The combined action mechanism for plant extracts and antibiotics is still unknown. Some researchers state that phytochemicals disrupt the cell wall or increase the cytoplasmic membrane's permeability, thus facilitating the inflow of antibiotics, creating efflux pump inhibitors, or inhibiting proteins that bind penicillin. Fig 3 revealed that MICs and MBCs of SAEO and its combination with tested antibiotics. The antibiotic MIC values, and also the SAEO values, were first identified as a point of reference for the identification of interactions. SAEO was potentially active against both positive and negative bacteria with MICs values ranging from 4 to 16 µl/ml, except for P. aeroginosa which was less sensitive to oil with MIC value of 64 µl/ml (Fig 3). The obtained results agree well for those obtained with the technique the disc diffusion According to [53,54], when the MICs are below 100 µg / mL, the antibacterial activity of a plant extract is considered significant, moderate when 100 ≤ MIC ≤ 625 µg / mL and low when MIC higher than 625 µg / mL. So in this study, the antibacterial activities reported of SAEO can mostly be regarded as significant. It is also important to mention that the MBC values was equivalent to or slightly increased from the MIC values, indicating a bactericidal action of oil (Fig. 3). The MICs values of SAEO against test clinical MDR isolates were lower than the MICs of test antibiotics; in another meaning, the different bacterial isolates exhibited higher sensitivity to the oil than antibiotics (Fig. 3). This may be due to the variation in the mechanism of action of various compounds found in the SAEO, to which the bacterial isolates were never previously exposed and therefore never had any chance of developing resistance. A combination of conventional antibacterial drugs and essential oils is a new concept; the combinations of star anise essential oil with antibiotics were tested against clinical isolates (Fig. 3). A significant reduction in the MICs of all antibiotics was observed when the SAEO was associated with the antibiotics. The MICs of cephradine, amoxicillin, tetracycline and chloramphenicol were lowered 4, 8, 128 and 64 fold, respectively when combined with SAEO against St. pneumoniae, while 8, 4, 128 and 16 fold reduction, respectively has been observed in the presence of SAEO against S. aureus.

These results corroborate reports of [55,56]. Also Fig. 3 shows the antibacterial activity of 4 widely used antibiotics against G- negative bacteria isolates in the presence of SAEO. SAEO has considerably increased the efficacy of the antibiotics tested against most of the bacteria studied. SAEO showed significant synergistic activity towards K. penumoniae ($P < 0.001$), reduction the MIC by 1024 fold for cephradine, by 2048 fold for amoxicillin, by 64 fold for tetracycline and chloramphenicol. Positive interaction of SAEO with cephradine, amoxicillin, tetracycline and chloramphenicol were evidenced by lowering the antibiotics' MICs by 2048, 512, 128 and 32 fold against A. baumannii and by 256, 2048, 128 and 16 against E. coli, respectively. Synergy also was apparent when SAEO was combined with chloramphenicol, amoxicillin, tetracycline and cephradine; in all cases, 8192, 4096, 16, and 128 fold reduction of MICs was observed toward P. aeruginosa, respectively.

In the checkerboard assays, the fractional inhibitory concentration index (FICI) ranged from 0.1 to 0.6, suggesting that all combinations tested had a synergistic impact (FICI < 1) in all clinical isolates, irrespective of the mode of action of the antibiotic studied.(Table 2).

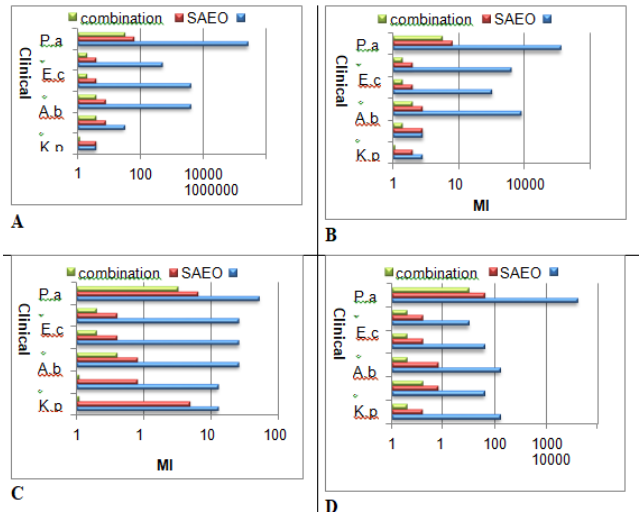

Figure 3. *MIC of the Combination between SAEO and tested antibiotics, A: with Cephradine, B: with Amoxicillin, C: with Tetracycline, D: with Chloramphenicol: S..: Streptococcus pneumoniae, S.a: Staphylococcus aureus, K.p: Klebsiella pneumoniae, A.b: Acinetobacter baumannii, E.c: E.scherichia coli, P.a: Pseudomonas aeruginosa.*

Studies on S. aureus and S. pneumonia showed a synergistic pattern with FIC indices ranging from 0.13 to 0.52 Our results revealed that the synergistic effect was verified from the combination of different antibiotics with SAEO. Although Gram -negative bacterial isolates showed high resistance to tested antibiotics, synergistic activity was shown by this oil with all tested antibiotics. Synergy was detected when SAEO/ antibiotics combinations toward E. coli, K. pneumoniae, A. baumannii and P. aeruginosa with FICI ranging from 0.26 to 0.56. Some essential oils modify the resistance to antibiotics by attacking efflux pathways in many Gram-negative bacteria species [57].

Among all tested combinations in this study antagonistic and additive interactions were not observed (Table 2).

Table 2. *Fractional inhibitory concentrations (FIC) values for the combination between some antibiotics and SAEO.*

Clinical Isolate	Antibiotic FIC	SAEO FIC	FIC Index	Interaction
Cephradine				
Streptococcus pneumoniae	0.25	0.25	0.5	Synergistic
Staphylococcus aureus	0.125	0.25	0.375	Synergistic
Klebsiella pneumoniae	0.0009	0.25	0.2509	Synergistic
Acinetobacter baumannii	0.0009	0.5	0.5009	Synergistic
Escherichia coli	0.0039	0.5	0.5039	Synergistic
Pseudomonas aeruginosa	0.0001	0.5	0.5001	Synergistic
Amoxicillin				
Streptococcus pneumoniae	0.125	0.25	0.375	Synergistic
Staphylococcus aureus	0.125	0.0625	0.1875	Synergistic
Klebsiella pneumoniae	0.0009	0.5	0.5009	Synergistic
Acinetobacter baumannii	0.0009	0.125	0.1259	Synergistic
Escherichia coli	0.0004	0.5	0.5004	Synergistic
Pseudomonas aeruginosa	0.0002	0.5	0.5002	Synergistic
Tetracycline				
Streptococcus pneumoniae	0.0078	0.25	0.2578	Synergistic
Staphylococcus aureus	0.0078	0.0625	0.0703	Synergistic
Klebsiella pneumoniae	0.0156	0.25	0.2656	Synergistic
Acinetobacter baumannii	0.0156	0.5	0.5156	Synergistic
Escherichia coli	0.0078	0.5	0.5078	Synergistic
Pseudomonas aeruginosa	0.0625	0.5	0.5625	Synergistic
Chloramphenicol				
Streptococcus pneumoniae	0.0156	0.5	0.5156	Synergistic
Staphylococcus aureus	0.125	0.5	0.625	Synergistic
Klebsiella pneumoniae	0.0312	0.25	0.2812	Synergistic
Acinetobacter baumannii	0.0625	0.5	0.5625	Synergistic
Escherichia coli	0.0625	0.5	0.5625	Synergistic
Pseudomonas aeruginosa	0.0078	0.5	0.5078	Synergistic

Results of FICI in our study confirm some other studies [58, 59, 9], synergistic activity was shown by the essential oils with the tested antibiotics. In the following four mechanisms, synergy can be discussed based on recent research findings in basic pharmacological, genomic-biological, and clinical data. Synergistic impacts may occur if the components of the plant extract impact various targets or associate with each other to enhance the efficacy and thus improve the bioactivity of one and several substances of a plant extract [60].

The morphological alterations observed in bacteria are the most direct evidence to reveal the antibacterial activity of SAEO alone and in combination with tested antibiotics. Scanning electron microscopy (SEM) was established to study the effect of SAEO and tetracycline alone and their combination against P. aeruginosa. The continued emergence

of multidrug-resistant P. aeruginosa strains has been increasingly reported worldwide and it's the most resistant bacteria to antibiotics [61]; therefore P. aeruginosa was selected for SEM testing. Overuse of tetracycline leads to an increase in the bacteria that are resistant to it and a loss of its effectiveness against the bacteria [62]. In this study, untreated cells of P. aeruginosa in SEM study showed no changes in the structure of the cells, the surface of the bacterial cells were morphologically intact, regular, smooth and showed typical characters of rod shape (Fig.4 A), cells treated with teteracycline, led to cell surface malformation, changed size and shape and cell membrane injury (Fig 4 B).

While, transitions include total destruction of the cell, damage to the cell wall, swelling in cells, shrunk and reduced cell size and cell deformation, treated with SAEO were observed. Degradation of the

P. aeruginosa 's exterior and interior membrane may be attributed to the SAEO's penetration with the discovery of several spaces and openings in the weakened cells (Fig. 4 C). Several reports have shown that the active constituents present in EOs can bind to the cell surface, and then penetrate the cell membrane's phospholipid bilayer. The membrane's structural integrity is disrupted by its accumulation, which can negatively influence the metabolic processes that cause cell death [63-65].

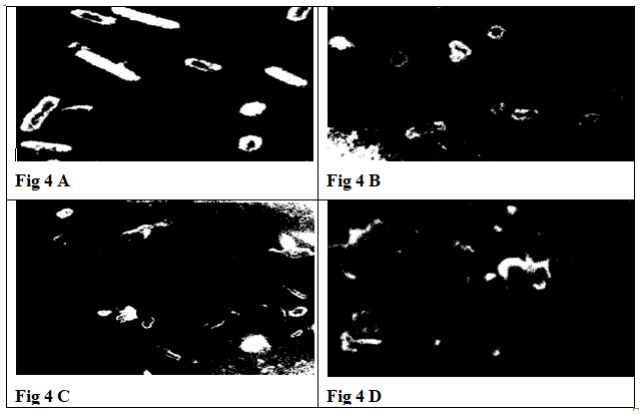

Figure 4. *(A, B, C &D): Scanning electron micrograph of Pseudomonas aeruginosa (P2), A: untreated cells, B: after exposure to tetracycline: C: after exposure to SAEO, D: after exposure to SAEO + tetracycline in combination.*

In our study, the cells treated with SAEO and teteracycline in combination resulted in all previously mentioned effects occurred more frequently, plus the observation of many pitted, shrivelled, gaps and openings in damaged cells, furrowed cells and disintegrated cell materials, leakage of cellular content, aggregated with loss of initial rod structure and loss of membrane integrity and increased permeability, which led to killing them eventually (Fig.4 D).

Observed changes in cells treated with SAEO support the results of MIC of combinations and FICI study indicating that SAEO could destroy the bacterial cell membrane, resulting in cell lysis followed by the losses of intracellular dense materials and death. From misshapen, ruptured and damaged cells observed in micrographs, we can hypothesize that the bactericidal effect of star anise essential oil could be due to affecting the structure and permeability of cell membrane followed by losing of intracellular materials and death. The use of volatile oils to prevent resistant bacteria is supposed to be more successful since volatile oils are multi-component compared to other antibiotics drugs that only have one target site. It was also presumed that the role of the major components is controlled by other minor components which assist to improve the synergic activity [66].

Conclusion

The modifying assay (IZ, MIC, MBC, FICI and SEM) of Star anise essential oil/antibiotics showed that this combination could enhance the antibacterial efficiency of Cephradine, Amoxicillin, Chloramphenicol and Tetracycline especially against Gram-positive MDR isolates and may contribute to re-sensitize Gram-negative MDR isolates (P. aeruginosa, A. baumannii, K. pneumoniae, and E. coli,) for the action of those antibiotics.

The synergy in this study, using combinations of Star anise essential oil with antibiotics can enhance or restore the antibacterial activity of many antibiotics currently useless. Also, star anises contain shikimic acid (Tamiflu) makes it potential to have an effect on coronavirus. The combination of antibiotics with Star anise essential oil may be an effective basis for developing a new resistance-modifying agent strategy, as the use of essential oil shows a low risk of increasing bacterial resistance to their action. The essential oil contains mixes of different active compounds, which make microbial ability to adapt very difficultly comparing to single-constituent antibiotics.

The findings of this study have entered the possible utilization of star anise oil as a potential replacement therapy represents 'a new phytopharmaceutical, the promising results presented will open the door for more research into the related field in developing countries, particularly in Egypt; discovery will be faster than bacteria evolution.

References

1. Yu Z, Tang J, Khare T, Kumar V. The alarming antimicrobial resistance in ESKAPEE pathogens: Can essential oils come to the rescue? Fitoterpia, 2020:104433.
2. Fankam A, Kuiate J, Kuete VP. Antibacterial and antibiotic resistance modulatory activities of leaves and bark extracts of Recinodindron heudelotii (Euphorbiaceae) against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine 2017; 17:168.
3. Founou L, Founou R, Essack S. Antibiotic Resistance in the Food Chain: A Developing Country Perspective. 2016; 7:1881.
4. Khalil N, Fikry S, Salama O. Bactericidal activity of Myrrh extracts and two dosage forms against standard bacterial strains and multidrug resistant clinical isolates with GC/MS profling. 2020 AMB Expr 10:21.

5. ECDC/EMEA. The bacterial challenge: time to react, Joint Technical Report from ECDC and EMA," Stockholm, Sweden: European Centre for Disease Prevention and Control 2009.
6. Gharaibeh MH, Khalifeh MS, Zattout EM, Abu-Qatouseh LF. Potential antimicrobial effect of plant essential oils and virulence genes expression in methicillin-resistant Staphylococcus aureus isolates. EISSN. 2020; 2231-0916
7. Sibanda T, Okoh A. In vitro evaluation of the interactions between acetone extracts of Garcinia kola seeds and some antibiotics. Afr J Biotechnol 2008; 7(1): 1672-1678.
8. Baylor College of Medicine Emerging Infectious Diseases
9. Gadisa E, Weldearegay G, Desta K, Tsegaye G, Hailu S, Jote K, Takele A. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. BMC Complementary and Alternative Medicine. 2019; 19(1):24.
10. Man A, Santacroce L, Jacob R, Mare A, Lidia Man L. Antimicrobial Activity of Six Essential Oils Against a Group of Human Pathogens: A Comparative Study. Pathogens. 2019; 8-15.
11. Nascimento G, Juliana L, Paulo C, Giuliana L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 2000; 31: 247-256.
12. El-Mahmood A, Ameh J. In vitro antibacterial activity of Parkia biglobosa (Jacq.) root bark extract against some microorganisms associated with urinary tract infections. Afr J Biotechnol 2007; 6(1): 1272-1275.
13. World Health Organisation (WHO) Traditional Medicine Strategy: 2014-2023.
14. Knezevic P, Aleksic V, Simin N, Svircev E, Petrovic A, Mimica-Dukic N. Antimicrobial activity of Eucalyptus camaldulensis essential oils and theirinteractions with conventional antimicrobial agents against multi-drug resistant Acinetobacter baumannii. J Ethnopharmacol 2016; 178:125-36.
15. Boonyanugomola W, Kraisriwattana K, Rukseree K, Boonsam K, Narachai P. In vitro synergistic antibacterial activity of the essential oil from Zingiber cassumunar Roxb against extensively drug-resistant Acinetobacter baumannii strains. J Infect Public Health 2017; 10:586-592.
16. Gharaibeh MH, Khalifeh MS, Zattout EM, Abu-Qatouseh LF. (2020) Potential antimicrobial effect of plant essential oils and virulence genes expression in methicillin-resistant Staphylococcus aureus isolates. EISSN 2020; 2231-0916
17. Hemaiswarya S, Kruthiventi A, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine 2008; 15: 639-652.
18. Veras H, Rodrigues F, Botelho M, Menezes I, Coutinho H, Costa J. Enhancement of aminoglycosides and b-lactams antibiotic activity by essential oil of Lippia sidoides Cham. and the Thymol. Arabian J Chemistry 2017; 10: S2790-S2795.
19. Luís A, Sousa S, Wackerlig J, Dobusch D, Duarte A, Pereira L, Domingues F. Star anise (Illicium verum Hook. f.) essential oil: Antioxidant properties and antibacterial activity against Acinetobacter baumannii. Flavour and Fragnance 2019; 34:260-270.
20. Sun W, Shahrajabian M, Cheng Q. Anise (Pimpinella anisum L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes. Cogent Biology 2019; 5: 1673688.
21. Nadjib B M. Effective Antiviral Activity of Essential Oils and their Characteristic Terpenes against Coronaviruses: An Update. J Pharmacol & Clinical Toxicology. 2020 8: 1138
22. Yang JF, Yang CH, Chang HW, Yang CS, Wang SM, Hsieh MC, Chuang LY. Chemical composition and antibacterial activities of Illicium verum against antibiotic-resistant pathogens. J. medicinal food 2010; 13(5): 1254-1262.
23. Shabaan H, Ramadan A, Edris A. Ranking the Antimicrobial Activity of Four Principle Essential Oil Components Against Some Food Pathogenic Bacteria. Int J Microbiolo Research 2020; 11:01-03.
24. Senthilkumar A, Kannathasan K, Venkatesalu V. Antibacterial activity of the leaf essential oil of Blumea mollis (D. Don). Merr World J Microbial Biotechnol 2009; 25: 1297-1300.
25. National Committee for Clinical Laboratory Standards. (NCCLS): Performance standards for antimicrobial disk susceptibility tests. Approved standard. NCCLS document M2-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa 2009.
26. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, United Kingdom. 2000; 151-220.
27. Gupta N, Mittal M, Parashar P, Mehra V, Khatri M. Antibacterial Potential of Elletaria cardamomum, Syzygium aromaticum and Piper nigrum, their synergistic effects and phytochemical determination. J Pharmacy Research 2014; 8:1-7.
28. Casella S, Leonardi M, Melai B, Fratini F, Pistelli L. The role of diallyl sulfides and dipropyl sulfides in the in vitro antimicrobial activity of the essential oil of garlic, Allium sativum L., and leek, Allium porrum L. Phytotherapy Research 2013; 27:380-383.
29. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition M07-A9 2012.
30. Mathur R. Phytochemical and antimicrobial evaluation of plant extracts of Enicostemma hyssopifolium. J Pharmacogn Phytochem 2013; 2: 30-36.
31. Coutinho H, Costa J, Siqueira-Júnior J, Lima E. In vitro anti-staphylococcal activity of Hyptis martiusii Benth against methicillin-resistant Staphylococcus aureus: MRSA strains. Rev Bras Farmacogn 2008; 18:670–675.

32. Okore V. Principles of Pharmaceutical Microbiology. 2ndedn. Nigeria, Ephrata Publishers 2009.
33. Weise W, Rheinheimer G. Scanning electron microscopy and epifluorescence investigation of bacterial colonization of marine sand sediments. Microbial Ecol 1978; 4: 175-188.
34. Novitsky J, MacSween M. Microbiology of high-energy beach sediment: evidence for an active and growing community. Mar Biol Prog Seer 1989; 52: 71- 75.
35. Padmashree A, Roopa N, Semwal A, Sharma GK, Agathian G, Bawa A. Star- anise (Illicium verum) and black caraway (Carum nigrum) as natural antioxidants. Food Chem 2007; 104:59-66.
36. Ariamuthu S, Balakrishnan V, Srinivasan M. Chemical composition and antibacterial activity of essential oil from fruits of Illicium verum Hook. F. Int J Res Phytochem Pharmacol 2013; 3: 85-89. Wong Y, Lee P, Nurdiyana W. Extraction and Antioxidative Activity of Essential Oil from Star Anise (Illicium verum). Orient J Chem 2014; 30: 1159-1171.
37. Mohamed G, Botros H, Ahmed B, Abo-El-Seoud M. Gamma Radiation Impact on the Survival Microflora and Biochemical Constituents of Stored Anise Seeds (Pimpenella anisum L.). J Rad Res Appl Sci 2010; 3: 261-276.
38. Karmegam N, Karuppusamy S, Prakash M, Jayakumar M, Rajasekar K. Antibacterial potency and synergistic effect of certain plant extracts against food borne diarrheagenic bacteria. Int J biomedical and pharmaceutical Scie 2008; 2:88-93.
39. Tian L, Li P. Study on the soxhlet's extraction of star anise oil and preliminary investigation of its antibacterial activity. In: Advances in Applied Biotechnology 2015. (Ed.) Zhang, T. and Nakajima, M. Springer Berlin Heidelberg. Volume 333 of the series Lecture Notes in Electrical Engineering, p: 509-518
40. Mohamed H, Abdelgadir W, Almagboul A. In vitro antimicrobial activity of Anise seed (Pimpinella anisum L.). Int J Advanced Research, 2015; 3: 359-367.
41. Amer AM, Aly UI. Antioxidant and antibacterial properties of anise (Pimpinella anisum L.). Egyptian Pharmaceutical J IP 2019; 45:108.77.
42. Estévez A, Estévez R. A short overview on the medicinal chemistry of shikimic acid. Mini Rev Med Chem 2012; 12: 1443–1454.
43. Zhou J, Lu G, Zhong X, Wen H. Quantitative determination of anethole in the fruit of Illicium verum from various places of Guangxi province. Zhong Yao Cai 2005; 28: 106–107.
44. Sikkema J, De Bont J, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 1994; 269: 8022-8028.
45. Yoon H, Moon S, Kim N, Park B, Jeong M, Yoo Y. Genistein induces apoptosis of RPE-J cells by opening mitochondrial PTP. Biochem Biophys Res Commun 2000; 276:151-156.
46. Turina A, Nolan M, Zygadlo J, Perillo M. Natural terpenes: self-assembly and membrane partitioning. Biophys Chem 2006; 122: 101-113.
47. Santos G, Silva E, Silva B, Sena K, Lima C. Influence of gamma radiation on the antimicrobial activity of crude extracts of Anacardium occidentale rich in tannins. Brazilian J Pharmacognosy 2010; 21: 444-449.
48. Mohamed G. Antimicrobial activity of essential oils extracted from gamma irradiated and stored anise (Pimenella anisum) and black cumin (Negilla sativa) seeds. Isotope & Rad Res 2011; 43: 271-284.
49. 50. Ali S, Mohamed A, Sameeh M, Darwesh O, Abd El-Razik T. Gamma-Irradiation Affects Volatile Oil Constituents, Fatty Acid Composition and Antimicrobial Activity of Fennel (Foeniculum vulgare) Seeds Extract. RJPBCS 2016; 7: 524- 532.
50. Abdel-Khalek H, Mohamed E. Synergistic Effect of Certain Medicinal Plants and Amoxicillin against Some Clinical Isolates of Methicillin – Resistant Staphylococcus aureus (MRSA). IJPA 2012; 3: 387-398.
51. Lorenzi V, Muselli A, Bernardini A, Berti L, Pagès J, Amaral L, Bolla J. Geraniol Restores Antibiotic Activities against Multidrug-Resistant Isolates from Gram- Negative Species. Antimicrob Agents Chemother 2009; 53: 2209-2211.
52. Rios J, Recio M. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 100:80–4.
53. Kuete V. Medicinal Plant Research in Africa. In: Pharmacology and Chemistry, Kuete V (Ed.) editor. Published by Elsevier Science Publishing Co Inc, United States 2013.
54. El-Bashiti T, Abou Elkhair E, Abu Draz W. The antibacterial and synergistic potential of some Palestinian plant extracts against multidrug resistant Staphylococcus aureus. J. Medicinal Plants Studies 2017; 5: 54-65.
55. Abd EL-Tawab A, Ammar A, Hamouda A, El-Hofy F, Elgamal A. Synergistic antimicrobial combination with cumin extract and some antibiotics on Staphylococcus aureus from chickens. Benha Veterinary Medical J 2017; 32: 7-12.
56. Devi K, Nisha S, Sakthivel R, Pandian S. Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J Ethnopharmacol 2010; 130: 107-115.
57. Haroun M, Al-Kayali R. Synergistic effect of Thymbra spicata L. extracts with antibiotics against multidrug-resistant Staphylococcus aureus and Klebsiella pneumoniae strains. Iran J Basic Med Sci 2016; 19:1193-1200.
58. El Atki Y, Aouam I, El Kamari F, Taroq A, Nayme K, Timinouni M, Lyoussi B, Abdellaoui A. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J Advanced Pharmaceutical Technology & Research IP 2020; 197: 46.87.5.
59. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. J Natural Remedies 2009; 9: 121-141.

60. Sharma S, Srivastava P) Resistance of Antimicrobial in Pseudomonas aeruginosa.
61. Int J Curr Microbiol App Sci 2016; 5(3): 121-128.
62. Jenner L, Starosta A, Terry D, Mikolajka A, Filonava L, Yusupov M, Blanchard S, Wilson D, Yusupova G. Structural basis for potent inhibitory activity of the antibiotic tigecycline during protein synthesis. Proc Nat Acad Sci 2013; 110:3812-3816.
63. Allam N, Eldrieny E, Mohamed A. Effect of combination therapy between thyme oil and ciprofloxacin on ulcer-forming Shigella flexneri. J Infect Dev Ctries 2015; 9: 486-495.
64. Cui H, Zhou H, Lin L, Zhao C, Zhang X, Xiao Z, Li C. Antibacterial activity and mechanism of cinnamon essential oil and its application in milk. J Animl Plant Sci 2016; 26: 532-541.
65. Mahmoud A, Abd El-Baky R, Ahmed A, Gad. Antibacterial Activity of Essential Oils and in Combination with Some Standard Antimicrobials against Different Pathogens Isolated from Some Clinical Specimens. Is J Microbiol Res 2016 4: 16-25.
66. Bassolé IHN, Juliani HR. Essential Oils in Combination and Their Antimicrobial Properties. Molecules 2012; 17: 3989-4006.

*Correspondence to:

Abdel Khalek H

Department of Radiation Microbiology,

National Center for Radiation Research and Technology

Cairo

China

E-mail: hanan.hassan210@gmail.com