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Evaluation of antibacterial efficacy of anise wastes against some multidrug resistant bacterial isolates

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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 and resistance modulatory activity of Aniseeds waste Residue Extract (ASWRE) and Star Anise Waste Residue Extract (SAWRE) (post-distillation) against 100 isolates belonging to two Gram positive (*Streptococcus pneumoniae* and *Staphylococcus aureus*) and four Gram negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*). Phenolic compounds of anise wastes were determined by HPLC. The antibacterial activity of anise waste extracts assays were performed by using inhibition zone diameters, MIC and MBC. Evaluation of synergy interaction between anise waste extracts and certain known antibacterial drugs like Cephradine, Chloramphenicol, Tetracycline and Amoxicillin was carried out using disc diffusion method, MIC and the fractional inhibitory concentrations (FIC). The results showed that HPLC method has been developed for the determination of 25 phenolic compounds from waste extracts. Both ASWRE and SAWRE have significant antibacterial activity against all of the test bacteria. SAWRE was found to have higher amounts of phenolic compounds contents that might be responsible for their comparatively higher antibacteria activity than ASWRE. Irradiation at 10 and 30 kGy did not significantly affect the antibacterial activity of both ASWRE and SAWRE. The combination of anise waste extracts and the tested antibiotics mostly showed synergistic effect. Synergistic interaction was most expressed against *Streptococcus pneumoniae* (Sp1) and *Staphylococcus aureus* (Sa1) by Tetracycline and chloramphenicol; *Pseudomonas aeruginosa* (P2), *Klebsiella pneumoniae* (K3), *Acinetobacter baumannii* (A2) and *Escherichia coli* (E3) by cephradine, amoxicillin and Tetracycline. The combination between anise waste extracts and the test 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) could be good economic sources of multidrug resistance inhibitors, and indicate that indiscriminate co-administration of antibiotics with some aromatic and medicine 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* activities. This may lead to the development of much needed drug enhancing preparations.

1. Introduction

In the recent times the emergence and spread of multidrug resistance as a phenomenon among bacterial pathogens has been a major problem confronting the field of antibacterial chemotherapy. However, it has been found that, in addition to the production of

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intrinsic antimicrobial compounds (Stefanovic & Comic, 2012); some medicinal plants also produce multidrug resistance inhibitors which enhance the activities of antibiotics against multidrug resistant bacteria pathogens (Eze, Oruche, & Eze, 2013). This finding prompted efforts in screening of crude extracts for synergistic interaction with standard antibiotics against resistant bacteria which would pave the way for possible isolation of multidrug resistance inhibitors of plant origin.

During the last years the interest in the recovery, recycling and upgrading of residues from aromatic plants processing has been very important. These plants produce large volume of wastes both solid and liquid, which represent a disposal and potentially environmental pollution problem. Nowadays, the biological potential of various plant waste materials is the focus of numerous studies. This trend includes not only the examination of fruits and plant products such as wine, olives, beetroot, tomato, garlic, and pomegranate (Cioffi et al., 2010; Kallel et al., 2014; Sagdic et al., 2011; Vulic et al., 2012), but also aromatic plants (Gavarić et al., 2015; Pogačar, Klančnik, Bucar, Langerholc, & Možina, 2015; Timasheva & Gorbunova, 2014). These investigations also include their antibacterial activity, considering the fact that waste material extracts represent the valuable source of different phenolic compounds. These studies are important, both in terms of economy and ecology, in order to elucidate the way to exploit post-distillation waste material of aromatic plants and other plant waste materials more efficiently.

Essential oils are valuable natural molecules which are widely used in cosmetics agricultural, and food industries. For most medicinal and aromatic plants, essential oil contents are only up to 1%, which means that there will be 99% of residues after extraction of essential oils. Containing rich secondary metabolites, the residues could be potential sources of bioactive compounds, e.g. natural antioxidants, antimicrobial and antiviral which, in recent years, have been increasingly sought after in cosmetic, food and pharmaceutical industries (Zhao, 2014).

Aromatic plants such as anise seeds and star anise have a long traditional use in both folk and conventional medicine and in the pharmaceutical industry (Shojaii & Fard, 2012). Aromatic and medicinal plants are found to be a rich source of various phenolic compounds, particularly phenolic acids and secondary metabolites that are expected to be the cause of its biological activity. In recent years, numerous studies confirm various bioactivities of essential oils. The lack of works on the complete utilization of plants has necessitated the more detailed study of the biochemical composition of raw materials and the development of complex processing technologies which must provide the most exhaustive extraction of biologically active substances with different spectra of activity. Essential oils in plants usually composes a small portion of all the biologically active components accumulated inside a plant, so waste residues obtained after essential oil extraction contain valuable organic components (extractive substances) of interest. The liquid and solid waste residues formed in the process of distillation are not utilized. Liquid wastes are distillation water (distillation fraction water) obtained in the steam distillation of volatile organic components. In the essential oil production, distillation water is a distillation process waste and amounts more than 70% of the weight of processed raw materials; it is not recycled in technological process, but disposed into sewers. However, distillation water contains a variety of biologically active components, which are so necessary in the pharmaceutical, perfume and cosmetics, and food industries. It is known that some components are water-soluble. For this reason, it is possible to say that distillation water is a saturated aqueous extract (Timasheva & Gorbunova, 2014).

The objective of this study was to evaluate the bioactivity of aniseeds and star anise, waste liquid material that remains after the essential oil production. Such residual materials are often disposed of and may present an environmental problem. Their high phenolic content and the potential to provide an economically feasible source of natural antimicrobials are unused. Hence, the present study was conducted to study out the antibacterial activity of aniseeds and star anise waste residues (post-distillation waste material) extracts, against the locally isolated microorganisms from patients having infectious diseases in our country and the ability of waste extracts to act synergistically with conventional antibiotics to better manage resistant bacterial infectious diseases.

2. Materials and methods

2.1. Plant formulations preparation

Aniseeds and Star anise (fruits) were bought from a commercial source in Cairo, Egypt, and then divided into three groups; the first was left without irradiation and considered as control, while the second and the third groups were exposed to gamma irradiation at dose levels of 10 and 30 kGy. Irradiation was performed in the National Center for Radiation Research and Technology (NCRRT) Nasr City, Cairo, Egypt, at dose rate 3.49269 kGy/h using the “Indian Gamma Chamber 4000 A” with a 60Co source.

The post-distillation waste liquid residues remaining after the recovery of essential oils were prepared after hydro-distillation of the aniseeds and star anise in a Clevenger-type apparatus (SenthilKumar, Swaminathan, & Kumar, 2009). The essential oil was collected, and the post-distillation waste liquid residue filtered and then freeze dried and dissolved in methanol for further testing as post-distillation extract, aniseeds waste residue extract (ASWRE) and star anise waste residue extract (SAWRE).

2.2. Bacterial strains and antibiotic susceptibility test

The clinical isolates used were collected from patients hospitalized in various departments of the Arab Contractors Medical Center, Cairo, Egypt. 100 isolates belonging to two Gram positive bacteria (*Staphylococcus* spp. (25) and *Streptococcus* spp. (13)) and four Gram negative bacteria (*Escherichia coli* (17), *Klebsiella* spp. (12), *Acinetobacter* spp. (18) and *Pseudomonas* spp. (15)), were obtained over 6 months period from various clinical specimens (urine, sputum, prostatic secretion, wound, tip of endo-trachial tube, diabetic foot, blood, tip of urinary catheter, cerebrospinal fluid).

All clinical isolates were screened for antibiotic resistance using Kirby-Baurer disc diffusion technique (Cheesbrough, 2000) using 18 different antibiotics that affect cell wall, protein synthesis and DNA (penicillin (P), Amoxicillin/Clavulanate (AMC), Vancomycin (VA), Amoxicillin (AX), Ampicillin/sulbactam (SAM), Nitrofurantoin (F), Aztreonam (ATM), Cefoperazone (CEP), Chloramphenicol (C), Clindamycin (DA), Gentamicin (CN), Tetracyclin (TE), Erythromycin (E), Ofloxacin (OFX), Norfloxacin (NOR), Cephradine (CE), Ciprofloxacin (CIP), Trimethoprime/Sulfamethoxazole (SXT)).

The inhibition zones in mm were interpreted as sensitive, intermediate and resistant using the interpretation chart supplied by antibiotic discs manufactures (Bioanalyse, Turkey). At least one resistant isolate from each of the tested organisms, which was resistant to more than two of the above antibiotics, was chosen and biochemically identified to the species level for further study.

2.3. HPLC of the phenolic profile of ASWRE and SAWRE

Phenolic compounds in ASWRE and SAWRE were determined by HPLC according to the method of Goupy, Hugues, Biovin, and Amiot (1999) at Agricultural Researches Center, Food Technology