Antibacterial activity of freeze dried encapsulated garlic oleoresin powder

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Received on: 11-04-2012; Revised on: 18-05-2012; Accepted on: 24-06-2012

ABSTRACT

Antibacterial effect of freeze dried encapsulated garlic oleoresin powder, were studied on Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Proteus spp. Antibacterial activity of freeze dried encapsulated garlic oleoresin powder extract by well diffusion method was characterized by inhibition zones. The maximum zone of inhibition (14 mm) was observed against S. aureus at 20% garlic oleoresin concentration. The inhibition zones possess various significant inhibitory effects against the tested organism. The minimum inhibitory concentration (MIC) was tested in all the pathogens. It was assessed that the aqueous extract exhibits better inhibitory activity (MIC) of about 32 (µg/ml) in most of the tested pathogens. The result also indicates that traditional medicinal application and pharmacological effects of antibacterial activity.

Key words: Bacillus subtilis, Encapsulation, Escherichia coli, Minimum inhibitory concentration, Pseudomonas aeruginosa, Staphylococcus aureus,

INTRODUCTION

Garlic (Allium sativum L.), belongs to the Liliaceae family and is a common food spice used widely in many parts of the world. According to the traditional Indian medication Ayurveda, Garlic is one of the most effective antimicrobial herbs, as it has anti-bacterial, anti-fungal, anti-viral and antiseptic properties. It is used in treatment of the diseases like running cold, saliva formation, chronic bronchitis, respiratory problems, whooping cough, bronchitic asthma, influenza, chronic diarrhea, pulmonary tuberculosis, rheumatism, impotence, etc. It can also fight infection, reduce cholesterol, protect against heart diseases and stroke, control diabetes, and prevent cancer. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Allium vegetables, particularly garlic exhibit a broad antibiotic activity against both Gram positive and Gram negative bacteria.

Oleoresins are the substances responsible for most of the spice flavours. Garlic oleoresin is the dark brown extract which is very reactive and unstable in light, temperature and oxygen is a disadvantage. Its sensitivity can be overcome by effective encapsulation. Encapsulation protects the oleoresin against such destructive changes, and also converts it into a free-flowing powder. Freeze drying is the dehydration process typically used to extend shelf life of the product, entrap aroma compounds into defined porous structures and reduce weight for easy handling and transportation. Hence in the present study it is evaluated the antibacterial effect of the freeze dried encapsulated garlic (Allium sativum L.) oleoresin powder against tested organisms.

MATERIALS AND METHODS

Core and wall materials
The core material used for the encapsulation process was garlic oleoresin, procured from Synthite Industries Ltd., Kerala - 682 311 and the wall material used for the coating of garlic oleoresin was maltodextrin, which was procured from Hi-media Laboratories Ltd., Mumbai, India.

Test Organisms
The five bacterial cultures of both Gram positive and Gram negative bacterial strains used for screening are Bacillus subtilis (MTCC 441), Pseudomonas syringae (ATCC 7386), Staphylococcus aureus (MTCC 29212), Escherichia coli (ATCC 25922), and Proteus spp. The stock cultures were maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in three ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 12 hours. The suspension was diluted with sterile distilled water to obtain approximately 10⁶ CFU/ml.

Production of encapsulated garlic oleoresin powder by Freeze drying technology
The feed emulsions were prepared based on a method[8] and pre-freezed in a deep freezer at (-20°C) for 3 days and further kept for sublimation drying under the temperature of -56°C and vacuum pressure of about -3 bar using a laboratory model (LYO055) freeze drier. The resultant encapsulated garlic oleoresin powders were packed in aluminium foil pouches and sealed air tight using the hand sealer. It was stored in a desiccator containing calcium chloride at 27°C to prevent moisture absorption.

Aqueous garlic powder preparation
The resultant freeze dried encapsulated garlic oleoresin powder from 10% and 20% garlic oleoresin concentration of about 10 mg was taken. The aqueous extracts were made by diluting the concentrated extract with appropriate volumes of sterile distilled water.

Antibiotic sensitivity testing
The test microorganisms were also tested for their sensitivity against the antibiotics chloramphenicol by the disk diffusion method[9]. The cultures were enriched in sterile nutrient broth for 6-8 hours at 37°C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Using an ethanol dipped and flamed
for cephs, the antibiotic discs were aseptically placed over the seeded MHA plates sufficiently separated from each other to avoid overlapping of the inhibition zones. The plates were incubated at 37°C for 24 hours and the diameter of the inhibition zones was measured in mm. All the media used in the present investigation were obtained from Hi-media Laboratories Ltd., Mumbai, India.

Agar dilution susceptibility test
The agar dilution susceptibility test was performed based on modified method [4, 10] to determine the MIC. Extracts dissolved in sterilized DMSO (128 µg/ml concentration) were taken as standard stock. A series of two fold dilutions of each extract in the final concentration of 128, 64 and 32 µg/ml were prepared in MH agar. After solidification, the plates were spotted with 2 µl of overnight grown bacterial cultures approximately containing 1 × 10⁶ CFU/ml. The test was carried out. The plates were incubated overnight at 37°C. After 18 ~ 24 hours, the MIC was determined and the percentage of growth inhibition was calculated by,

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\text{Percentage of inhibition, } P = \frac{T - SC}{PC} \times 100
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Whereas, T - Test; SC- Solvent control; PC - Positive control

RESULTS AND DISCUSSION
The antibacterial activity of freeze dried encapsulated garlic oleoresin powder aqueous extract was reported in Table-1. Out of the five pathogens Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus Escherichia coli and Proteus spp showed aqueous extracts action against pathogens. It has exhibited different degrees of antibacterial activity ranging from 9 mm to 14 mm against studied. The antibacterial activity of the extracts showed in magnitude of inhibition patterns with standard positive control. Wherein the 10% and 20% of concentration of freeze dried encapsulated garlic oleoresin powder exhibited maximum zone of inhibition against Bacillus subtilis (11 mm); Pseudomonas aeruginosa (10 mm); Staphylococcus aureus (14 mm); Escherichia coli (12 mm) and Proteus spp (11 mm) in the present study. However, though aqueous extracts exhibited antibacterial activity against certain studied micro organism but it has significantly effect against S.aureus and Escherichia coli. The sensitivity of E.coli and S. aureus to a water extract of garlic agrees with earlier observations [10]. It was observed that there was a variation in the size of the inhibition zone among the different group of bacteria. This may be due to the lipid content of the membranes of the different groups of the micro organisms and the permeability of allin and other garlic constituents.

The minimum inhibitory concentrations of the aqueous extracts were determined using agar dilution assay. Most of the extracts displayed antibacterial activity against tested pathogens. The MIC value of the aqueous extracts were obtained from 32 – 128 µg/ml concentration, which has been illustrated in Table-2, whereby the aqueous extracts exhibit maximum activity of MIC 32 µg/ml concentration against S.aureus. It was also observed that the maximum activity of MIC occurs at 34mg/ml for B.subtilis and 32µg/ml for P. aeruginosa. The highest MIC value was found to be 128µg/ml against S.aureus and Proteus spp. Investigate organic solvent extract of the aerial part of garlic plants as it has been found that volatile metabolites also have an effect on bacterial growth [13]. Based on the experiment result from MIC studies and zone of inhibition studies, 20% freeze dried encapsulated garlic oleoresin powder is considered to be good inhibitors for antibacterial activity.

CONCLUSION
10% and 20% aqueous extract of freeze dried encapsulated garlic oleoresin powder is significantly different. The freeze dried encapsulated garlic oleoresin powder exhibit certain bio active compounds responsible for the anti bacterial activity. The further investigation of the therapeutically active and pharmacological effect constituents of garlic aqueous extract will be carried out.

ACKNOWLEDGEMENT
The Freeze drier equipment was funded by grants from DST-FIST to school of Bioengineering, SRM University, Kancheepuram. The garlic oleoresin supplied by M. Myson Ukken, M/s. Clarity extracts, Thrissur is also acknowledged.

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