IJPSR (2014), Vol. 5, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 April, 2014; received in revised form, 14 June, 2014; accepted, 17 July, 2014; published 01 November, 2014

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST FOOD-BORNE PATHOGENIC BACTERIA

Asma Afroz Rowsni, Kamrul Islam, Md. Murad Khan and Md. Shahidul Kabir*

Department of Microbiology, Stamford University, Bangladesh.

Keywords:

Antimicrobial Activity, Disc Diffusion Assay, Essential Oils, Food Borne Pathogens

Correspondence to Author:

Md. Shahidul Kabir

Associate Professor,
Department of Microbiology,
Stamford University Bangladesh,
51 Siddeswari Road, Dhaka,
Post code 1217, Bangladesh.

E-mail: mskabir@yahoo.com

ABSTRACT: The increasing resistance of microbes to conventional drugs has promoted the scientists to search for new biocides with broad activity. Plant derived essential oils (EOs) have been known to show antimicrobial activity against specific pathogens and therefore could be considered as alternative antimicrobial agents in controlling pathogens. The present study was done to investigate the antimicrobial activity of soybean, rice, mustard, olive, coconut and castor oil against 24 isolates of 6 different types of food borne pathogens. Antibacterial activities of essential oils were determined using agar disc diffusion assay. All EOs showed antibacterial activity against *Escherichia coli*, *Pseudomonas* sp., *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella* spp., and *Salmonella* spp. *Staphylococcus aureus* was found more sensitive to soybean and mustard oils compared to other Gram negative bacteria. Castor oil was effective against most of the bacteria namely *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella* spp. and *Salmonella* spp. The antibacterial activity of EOs could be used for treatment of bacterial infection and preservation of food.

INTRODUCTION: The increasing resistance of microorganisms against a number of antimicrobial drugs has led the scientists to search for alternatives among aromatic plant extracts or essential oils (EOs) as their antimicrobial activity has long been recognized and tested against different pathogenic microorganisms [1, 2]. EOs are generally collected from aromatic plants of temperate or warm country where they constitute an important part of the traditional pharmacopoeia [3]. As a source of natural microbial agents, EOs are becoming more popular due to their wide applications as food preservative, complementary medicine and therapeutic agents[4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(11).4876-79

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4876-79

Antimicrobial compounds of plants are synthesized constitutively or can be synthesized as a mechanism of self-defence in response to a particular infection. Vegetables, spices and fruits containing high level of EOs are excellent sources of natural compounds with antimicrobial activity against microorganisms of agricultural and health interest[5]. Although the main target of the EOs is bacterial cell membrane, their exact mechanism of antimicrobial activity is poorly understood [6].The mechanism of actions of EOs depends on their chemical composition and attribute to a cascade of reactions involving the whole bacterial cells [7].

The cell wall of Gram negative bacteria are more resistance to the EOs and their components as the entrance of hydrophobic EOs is more readily hindered by Gram negative bacteria as compared to the Gram positive bacteria[8]. EOs are complex mixtures of volatile components containing biosynthetically related groups[9]. Chemical components such as terpenoids including monoterpenes, sesquiterpenes and their oxygenated derivatives present in plant derived EOs can easily diffuse across the cell membrane to induce biological reactions [10]. Chemical analysis of EOs has shown that their composition varies significantly from one plant species to another. Even the chemical composition of EOs from a single plant species can vary significantly depending on their different geographical sources [11]. In addition, EOs have low mammalian toxicity, easy to obtain and degrade quickly in water and soil that make them environmental friendly [12].The aim of this study is to investigate the antimicrobial activity of six essential oils against six different types of pathogenic bacteria isolated from food.

METHODS AND MATERIALS:

Essential oils:

Commonly used natural oils were purchase in sealed containers from different shops in Dhaka city. Essential oils used in this study were: soybean, rice, mustard, olive, coconut and castor oil.

Test organisms:

Six types of pathogenic bacteria used in this study include: *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus Vibrio cholerae, Klebsiella* spp., and *Salmonella* spp. All of these bacteria species were previously isolated from the food samples and preserved in the Department of Microbiology, Stamford University Bangladesh. Four isolates from each type of bacteria were included in this study. Bacterial isolates were subcultured at least twice from the stock on Nutrient agar (Himedia Laboratories Ltd., India) to prepare a fresh culture before using them in the assay.

Preparation of inoculum:

Freshly subcultured bacteria were inoculated in Trypticase soya broth and incubated at 37°C for 2-4 hour to match the turbidity to that of 0.5 Mac Ferland standard. Inoculum was spread onto Mueller Hinton Agar, MHA (Himedia Laboratories Ltd., India) using sterile cotton swabs. Plates were allowed to air dry for 15 minutes. MHA was supplemented with 0.5% (v/v) Tween-20 (Sigma, UK) to facilitate smooth diffusion of oil in disc diffusion assay.

Disc diffusion assay:

Filter paper discs (6mm) were placed aseptically on to the MHA plates using forceps and needle. Aliquots (20 µl) of six oil samples were placed on each disc separately. Commercially available Gentamicin discs (10µg) (Oxoid, UK) were used as control. All plates were incubated at 37^0C for 24 hours and the diameters of zones of inhibition (mm) were measured on the agar surface.

Statistical analysis:

All the experimental results were performed in triplicate and the results were expressed as mean ± standard deviation (SD) for 4 isolates of every type of bacterium. Calculation was done using Microsoft Excel 2010 software.

RESULTS AND DISCUSSION:

In this study essential oils were tested against different food borne pathogens including both Gram-positive and Gram-negative bacteria. Most of the authors highlighted the antimicrobial activity of plant derived non-edible essential oils but this study emphasized on the antimicrobial activity of essential oils which are usually consumed as edible oils.

TABLE 1: ANTIMICROBIAL ACTIVITY OF EDIBLE ESSENTIAL OILS AGAINST FOODBORNE PATHOGENS

	Zone of inhibition (mm±SD)					
	Escherichia coli	***Pseudomonas* spp.**	***Staphylococcus aureus***	***Vibrio cholerae***	***Klebsiella* spp.**	***Salmonella* spp.**
Soybean	7.67±0.58	7.33±0.58	6.67±0.58	7.33±1.53	8.33±2.08	8.67±2.08
Rice	7.67±1.15	7.33±1.53	7.33±0.58	6.67±1.15	6.33±0.58	6.67±1.15
Mustard	7.33±1.15	7.33±1.53	6.33±0.58	7.00±1.00	8.33±0.58	8.00±2.00
Olive	6.67±0.58	8.33±2.52	7.33±1.15	7.33±1.53	7.33±1.53	8.33±1.15
Coconut	7.33±0.58	8.00±2.00	6.67±0.58	6.00±0.00	6.33±0.58	6.33±0.58
Castor	7.67±0.58	7.67±2.08	8.67±3.06	10.67±1.15	8.67±2.52	9.00±3.00
Gentamicin	12.33±7.09	16.00±3..61	18.00±5.00	19.33±3.51	17.00±6.00	16.67±3.21

All bacterial strains were found to show some susceptibility to each essential oil when tested by disc diffusion method (**Table 1**).

The diameter of the zones of inhibition varied depending on essential oils and bacterial species used. Castor oil showed greater zone of inhibition against *Staphylococcus aureus* (8.67±3.06 mm), *Vibrio cholera* (10.67±1.15 mm), *Klebsiella* spp. (8.67±2.52 mm) and *Salmonella* spp. (9.00±3.00 mm) as compared to other oils tested. Rice and olive oils showed the higher activity against *Escherichia coli* (7.67±1.15 mm) and *Pseudomonas* spp. (8.33±2.52 mm), respectively compared to other oils. Rice and coconut oil showed shorter zones of inhibition against *Klebsiella* spp. (6.33±0.58mm) and *Escherichia coli* (6.00±0.00 mm), respectively when compared to other bacterial isolates. Soybean (6.67±0.58 mm) and mustard (6.33±0.58 mm) oils produced smaller sizes of zones against *Staphylococcus aureus* relative to other bacteria studied. Olive (6.67±0.58mm) and castor oils (7.67±0.58 mm) showed smaller zone sizes against *Escherichia coli* as compared to the other bacteria.

Escherichia coli was found to be equally sensitive to soybean, rice, mustard, olive, coconut and castor oils used in this study. Sensitivity of *E. Coli* has been demonstrated towards carvacrol, cinnamaldehyde, eugenol, guaiacol, thymoloregano, thyme, and rosemary oils [13]. The antimicrobial activity of oregano and thyme has also been reported against *E. coli* O157:H7 [14]. In this study olive oil and coconut oils were found most microbiocidal against *Pseudomonas* spp. while other oils were found less active. The fennel and anise oils were found active against *Pseudomonas* spp. at concentration above 1% while 0.6% caraway oil was bactericidal to *Pseudomonas* strain [15, 16]. *Vibrio cholerae* showed highest zone of inhibition towards castor oil. It was reported that *Coriandrum sativam* essential oil has the antimicrobial activity against *Vibrio cholera* [17]. The same authors also showed bactericidal activity of *Coriandrum sativam* essential oil against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi*. In this study *Klebsiella* spp., *Staphylococcus aureus* and *Salmonella* spp. were found sensitive to all six essential oils. Among them *Salmonella* spp. showed highest sensitivity towards soybean, olive and castor oils.

The antibacterial activities of EOs are poorly understood. In most of the cases it was found that EOs affect and damage bacterial cell membrane [6, 7, 18]. Therefore Gram negative bacteria demonstrate higher resistant against EOs due to the presence of outer membrane [7]. Consequently they may destroy the beneficial members of the intestine such as, *Bifidobacterium* spp. and *Lactobacillus* spp. However, some researchers have reported on the resistance of such bacteria which indicates the need for further research. Carvacrol, cinnamaldehyde, citral and thymol were reported as most effective against *Salmonella enterica* [13]. It has been reported that vetiver has the lowest MIC (0.008% v/v) against *Staphylococcus aureus* [15].

CONCLUSIONS: All essential oils included in this study possess good antimicrobial activity against common food borne pathogens. The tested essential oils can thus be consumed in context of the health benefit as they inhibit the growth of food borne pathogens. EOs can also be used in different concentrations for the preservation of fish, meat, fruits and vegetables.

ACKNOWLEDGMENTS: This work has been supported by the Department of Microbiology, Stamford University Bangladesh.

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How to cite this article:
Rowsni AA, Islam K, Md. Khan M and Md. Kabir S: Antimicrobial Activity of Essential Oils against Food-Borne Pathogenic Bacteria. Int J Pharm Sci Res 2014; 5(11): 4876-79.doi: 10.13040/IJPSR.0975-8232.5 (11).4876-79.