

# The Antimicrobial activity of some essential oils against some selected food borne pathogens

Reham A. El Fayoumy\*, Mohamed I. Abou-Dobara\*, Phillip Pendleton#, Amira A. EL-Fallal\* and Ahmed K.A. EL-Sayed\*.

\*: Botany and Microbiology Department, Faculty of Science, Damietta University, New Damietta City, Egypt.

#: School of Chemistry Engineering, The University of Adelaide, SA 5005, Australia.

**Abstract**— Essential oils (EO) are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant. Essential oils are considered to be secondary metabolites and important for plant defence as they often possess antimicrobial properties. The active compounds of essential oils can be divided into four groups according to their chemical structure: terpenes such as lemon EO, terpenoids such as jasmine EO, phenyl propenes such as vanilla EO, and others such as mustard EO which has Allyl isothiocyanate (AITC) as active compound. Mustard EO showed the highest and strongest antimicrobial activity against all tested pathogens (*Bacillus subtilis*, *E. coli* ATCC25922, *Aspergillus flavus* and *Rhizopus stolonifer*) with biggest inhibition zone diameter for *R. stolonifer* 20 mm, 16 mm for *A. flavus*, 15 mm for *E. coli* and 10 mm for *B. subtilis* following by Lemon EO which caused 12 mm for *A. flavus*, 10 mm for *E. coli*, 5 mm for *B. subtilis* and no inhibition effect for *R. stolonifer*. Jasmine EO has antimicrobial activity against all the tested bacteria and *A. flavus* while Vanilla EO was the weakest in the inhibition effect against the tested pathogens. *R. stolonifer* was most sensitive pathogen to mustard EO only and resistant to others, but *B. subtilis* was the most resistant pathogen to the four tested essential oils.

**keywords**— Essential oils, antimicrobial activity, food borne bacteria, food borne fungi.

## 1 INTRODUCTION

Foodborne disease is any illness resulting from the food spoilage or contaminated food by pathogenic bacteria, viruses, parasites or fungi [1]. Food safety is a known problem worldwide, affecting hundreds of millions of people that suffer from contaminated or spoilage food. Food can get easily spoilage if it was not preserved good enough to protect it from growing food borne pathogens. Food preservation involves preventing the growth of food borne microorganisms that lead to food spoilage or food contamination just like bacteria, fungi, or other micro-organisms by a number of techniques [2]. Many processes designed to preserve food will involve a number of food preservation methods, Maintaining or creating nutritional value, texture and flavour is an important aspect of food preservation. There are many traditional techniques just like: Drying, Cooling, Freezing, Boiling, Heating, Salting, sugaring, smoking, pickling, lye, canning, jelling, jugging and burial. Most of traditional techniques to preserve food do not have any side effects but most of time not enough to preserve food for long time or protect it from dangerous pathogens in addition to most of these techniques make changes in taste or texture or colours of foods. Other industrial or modern techniques just like; pasteurization, vacuum packing, artificial food additives, modified atmosphere, non-thermal plasma, hurdle technology and high pressure food preservation [2], each one of these technique is cost the

economy so much or if it was cheap just like pasteurization was not enough to preserve all types of food or preserve it for long time and can't deal with dangerous food borne pathogens. In addition to some methods of food preservation are known to create carcinogens. Also the modern methods to preserve food by using chemical antimicrobial compounds or radiation to preserve food are very dangerous to human's health. There are certain harmful effects of using chemicals for preservation such as; Sulfites are common preservatives used in various fruits, may have side effects in form of headaches, palpitations, allergies, and even cancer. Nitrates and Nitrites are used as curing agents in meat products. it gets converted into nitrous acid when consumed and is suspected of causing stomach cancer. Benzoates are used in foods as antimicrobial preservatives, and have been suspected to cause allergies, asthma and skin rashes. Sorbates/sorbic acid are added to foods as antimicrobial preservatives. Reactions to sorbates are rare but have included reports of urticaria and contact dermatitis [3]. Also a nuclear radiation when used for preservation does not make foods radioactive, but may cause changes in food color or texture. Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant [4]. Essential oils have been used for centuries in medicine, perfumery, cosmetic, and have been added to

foods as part of spices or herbs. Essential oils are considered to be secondary metabolites and important for plant defence as they often possess antimicrobial properties [5]. The antibacterial properties of secondary metabolites were first evaluated using essential oil vapours by De la Croix in 1881 [6]. Since then, essential oils or their components have been shown to not only possess broad-range antibacterial properties [7], but also antiparasitic [8], insecticidal [9], antiviral [10], antifungal [11], and antioxidant [12] properties. The increasing negative consumer perception of the synthetic preservatives has required a return to natural preservatives [13]. Although the food industry primarily uses essential oils as flavourings, they represent an interesting source of natural antimicrobials for food preservation. However, application of essential oils as food preservatives requires detailed knowledge about their properties, i.e., the minimum inhibitory concentration (MIC), the range of target organisms, the mode of action, and the effect of food matrix components on their antimicrobial properties. Essential oil constituents are a diverse family of low molecular weight organic compounds with large differences in antimicrobial activity. The active compounds can be divided into four groups according to their chemical structure: terpenes, terpenoids, phenyl propenes, and others. Because of the side effects of using synthetic compounds for food preservation or preservation via radiation on human's health and other preservative ways which expensive and have bad feedback on the economy, it shows a growing interest about the replacement of synthetic preservatives with natural, effective and nontoxic antimicrobial compounds such as essential oils (EOs) of spices and herbs, for food preservation. Many essential oils containing antimicrobial agent just like thymol (thyme), eugenol (clove), cinnamic aldehyde (cinnamon), allyl isothiocyanate (mustard), camphor (rosemary), carvacrol (oregano) [14] and citral isomers (lemongrass) promise antimicrobial effects [16], with oregano, clove, cinnamon, rosemary, thyme, sage, and vanillin showing particular antimicrobial effects on Gram-positive bacteria [15]. Mustard oil has been found to be potent for a broad spectrum of spoilage and pathogenic microorganism both in vitro [17,18]. The health benefits of mustard essential oil can be attributed to its properties as a stimulant, irritant, appetizer, antibacterial, antifungal, insect repellent, hair vitalizer, cordial, diaphoretic, antirheumatic and tonic substance. Mustard oil has had contradictory reputations in different parts of the world over the years. There, it is used as an edible oil and is considered very healthy. Allyl isothiocyanate (AITC) is an antimicrobial effective compound of mustard essential oil. Its chemical structure is  $C_4H_5NS$ . This compound is derived from the

glucosinolates (sinigrin). The health benefits of vanilla essential oil can be attributed to its properties as an antioxidant, aphrodisiac, anticarcinogenic, antibacterial, antifungal, febrifuge, antidepressant, sedative, tranquilizing and relaxing substance. The essential oil of Vanilla is extracted by solvent extraction of a resinous substance obtained from fermented vanilla beans. These beans come from vanilla plants, which bears the scientific name *Vanilla Planifolia*. Its bioactive compound which has a great antibacterial and antifungal is vanillin which belong to phenylpropanoids group [13,19]. The health benefits of jasmine essential oil can be attributed to its properties as an antidepressant, antimicrobial, aphrodisiac, antispasmodic, cicatrisant, expectorant, galactagogue, emmenagogue, parturient, sedative and uterine substance. Jasmine essential oil is extracted from the flowers of Jasmine, and it has various scientific names including *Jasminum Grandiflora* (Royal Jasmine) and *Jasminum Officinale* (Common Jasmine). The oil is extracted mainly from the latter variety. The main effective compound in jasmine essential oil is linalool, which belongs to monoterpenoids and has a very good antiseptic and disinfectant properties [20]. Lemons are one of the most popular citrus fruits in the world, and are widely used for culinary purposes, since they are a good source of vitamins and aid in digestion. It also adds a pleasant taste and aroma to food. The health benefits of lemon oil can be attributed to its stimulating, calming, carminative, anti-infection, astringent, detoxifying, antiseptic, disinfectant, sleep inducing, and antifungal properties. The effective antimicrobial compound in Lemon essential oil is limonene which belong to terpenes (monoterpenes) group [21]. In our research we will try to test the antimicrobial activity of four essential oils and compare their abilities to inhibit some gram positive foodborne bacteria, some gram negative foodborne bacteria and some foodborne fungi by some microbiological assays.

## 2 Materials and Methods

### 2.1 Materials

The tested pure essential oils: Lemon, Jasmine, Vanilla and Mustard were purchased from Karama Company at Cairo, Egypt. Ethanol (99.9 % grade) and sodium chloride, each of these chemicals was purchased from Sigma Aldrich, Australia, and used without further purification. The media used for the microbial growth were nutrient broth, nutrient agar, DOX broth and DOX agar. All of these media also were purchased from Sigma Aldrich, Australia.

### 2.2 Tested Bacterial and Fungal strains

The tests were performed against *Bacillus subtilis* as Gram positive foodborne bacteria, *E. coli* ATCC 25922 as Gram negative foodborne bacteria and two fungal strains (*Aspergillus flavus* and *Rhizopus stolonifer*). All strains were from Hetzel building – school of Health Science – University of South Australia – Adelaide- Australia.

## 2.3 Methods

### 2.3.1 Bacterial and fungal cultures preparation

Cultures of the two tested bacteria strains were prepared by streaking frozen culture stocks on to nutrient agar media and left to grow until visible colonies appeared (24 h, 37 °C for both bacteria). One colony of each strain was then picked to start primary seed cultures in sterile nutrient broth (5 mL, 16 h, and 37 °C) in a shaker incubator (180 rpm) to make future assays. The two strains fungi were prepared by streaking frozen culture stocks on to DOX agar media and left to grow until visible colonies appeared (5 days, 30 °C) to make also future assays.

### 2.3.2 the antimicrobial activity of tested essential oils by agar diffusion disk assay

A disk diffusion assay is typically used to determine the relative antimicrobial efficiency of the four tested essential oils (Lemon, Jasmine, vanilla and Mustard) against *Bacillus subtilis*, *E. coli* ATCC 25922 as examples of food borne bacteria and *Aspergillus flavus*, *Rhizopus stolonifer* as examples of food borne fungi [22]. In this study, 1 ml of the 16 h seed bacterial culture was added to 10 ml of sterilized Nutrient broth (NB) media and incubated at 37 °C with 150 rpm agitation until cultures reached mid-exponential growth phase: (OD600 = 0.5). These were then diluted with sterile physiological saline solution [PS; 0.85% (w/v) sodium chloride] until the turbidity of each suspension was adjusted to match 0.5 McFarland standard (OD600 = 0.132), corresponding to a bacterial density of about 10<sup>8</sup> CFU/ml [23]. The assay was performed by streaking 0.1 ml of each bacterial suspension onto Nutrient agar plate (90mm) with a sterile cotton swab to obtain uniform growth. For fungi strains case, mold stock inoculum suspensions were pre-

pared from 7-day cultures grown on Dox agar, the inoculum were prepared by removing the sporulated fungi from the agar plate with a loop and suspending them in 10 ml of sterile water. The fungal suspensions were filtered once through a sterile gauze to remove hyphae and adjusted spectrophotometrically at a 530-nm wavelength to optical densities that ranged from 0.09 to 0.3 [24]. (DOX) agar plates (90 mm) were inoculated with 0.1 ml of the molds stock inoculum and simultaneously in three directions with a sterilized cotton swab to get uniform growth. The inoculated agar was allowed to dry for 15 to 30 min. wells were made in the inoculated agar medium (Nutrient agar and DOX agar) then filled with 200 µL of each essential oil. The essential oils were used diluted 1:10 (v/v) with analytical grade ethanol to overcome the poor solubility of the essential oils in water which make obstruction for diffusion essential oils through the agar and show its antimicrobial effect on the tested pathogens [25]. Control well was filled with 200 µL of ethanol, then incubated the tested bacteria at 37 °C for for 16 h and tested fungi at 30 °C for 5 days. After incubation, the zones of inhibition were measured. Studies were performed in triplicate, and the diameters of the inhibition zones were averaged.

## 2.4 Statical analysis

All measurements were made in triplicate and the data evaluated as mean ± standard deviation including statistical analysis using the one-way analysis of variance (ANOVA) statistical analysis package in MS Excel.

## 3 Results and Discussion

The results below presents the preliminary measures taken to confirm that Mustard essential oil has highest antimicrobial activity among tested essential oils (Lemon, Jasmine and Vanilla) which have different effective compounds (AITC in mustard essential oil (EO), lemonen in lemon essential oil (EO), linalool in Jasmine essential oil (EO) and vanillin in Vanilla essential oil (EO)) against some tested food borne pathogens (*Bacillus subtilis* as Gram positive foodborne bacteria, *E. coli* ATCC 25922 as Gram negative foodborne bacteria and two fungal strains (*Aspergillus flavus* and *Rhizopus stolonifer*). These microbes have been found as spoilage pathogens in some food consumed in Middle Eastern countries and caused some outbreaks in other places around the world.

### 3.1 the antimicrobial activity of tested essential oils by agar diffusion disk assay

SPSS analysis reveals that high significant effect ( $P < 0.001$ ) of type of essential oils on the antimicrobial activity. In this assay we used agar diffusion disk assay to assessment and

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**Corresponding Author:** Reham A. El Fayoumy, Botany and Microbiology Department, Faculty of Science, Damietta University, Egypt  
Tel: + 201064838628,  
e-mail: reham\_ahmad64@yahoo.com

pared from 7-day cultures grown on Dox agar, the inoculum were prepared by removing the sporulated fungi from

comparing of both antibacterial and antifungal activities of four different essential oils Mustard, Lemon, Jasmine and Vanilla essential oil against *B. subtilis* as example of food borne gram positive bacteria, *E. coli* ATCC25922 gram negative foodborne bacteria, *A. flavus* and *R. stolonifer* are food borne fungi. We used analytical grade ethanol to dilute the essential oils at concentration 1/10 (v/v), the inhibition effect was observed for each essential oil by measuring the diameter of inhibition zone in mm. Ethanol only was used as control in this assay. The antimicrobial activity for each essential oil was compared with each other by comparing the inhibition zone diameter. Fig. 1 showed that Mustard essential oil has the highest and strongest antimicrobial activity against all tested pathogens among other tested essential oils which has biggest inhibition zone diameter for *R. stolonifer* 20 mm following by Lemon essential oil. Although *R. stolonifer* was the most tested organism affected by mustard oil, but in the same time the other tested oils did not have any inhibition effect on it. Vanilla essential oil was the weakest in the inhibition effect against the tested pathogens, which has weak antimicrobial effect only against *A. flavus* but did not has any effect against the tested bacteria. Fig. 1 proved that Jasmine essential oil has antimicrobial activity against all the tested bacteria and *A. flavus* but not strong as Mustard or Lemon essential oil. From the data we found that *R. stolonifer* was most sensitive pathogen to mustard essential oil only and resistant to others, but *B. subtilis* was the most resistant pathogen to the four tested essential oils. By comparing the data; whatever Mustard and Lemon essential oil has good antimicrobial activity against the tested pathogen but the Mustard essential oil has the highest and strongest effect by comparing with Lemon essential oil which cause 20 mm inhibition zone diameter for *R. stolonifer*, 16 mm for *A. flavus*, 15 mm for *E. coli* and 10 mm for *B. subtilis*, but Lemon essential oil cause just 12 mm for *A. flavus*, 10 mm for *E. coli*, 5 mm for *B. subtilis* and no inhibition effect for *R. stolonifer*. In this investigation, the mustard essential oil has the strongest antimicrobial effect against all the tested food borne bacteria and fungi compared with other tested essential oils: Lemon, Jasmine and Vanilla essential oil. The difference of the antimicrobial activities between the tested essential oils, based on that each one of these oils has different effective antimicrobial compound, so they have different mode of action against each tested pathogen. The active compounds in essential oils can be divided into four basic groups according to their chemical structure: terpenes, terpenoids, phenyl propenes, and others [13]. Allyl isothiocyanate (AITC) which is belonging to others is the main bioactive compound in mustard essential oil was proved to be the

most efficient essential oil as an antimicrobial against all the tested food borne pathogens compared with other tested essential oils [14].

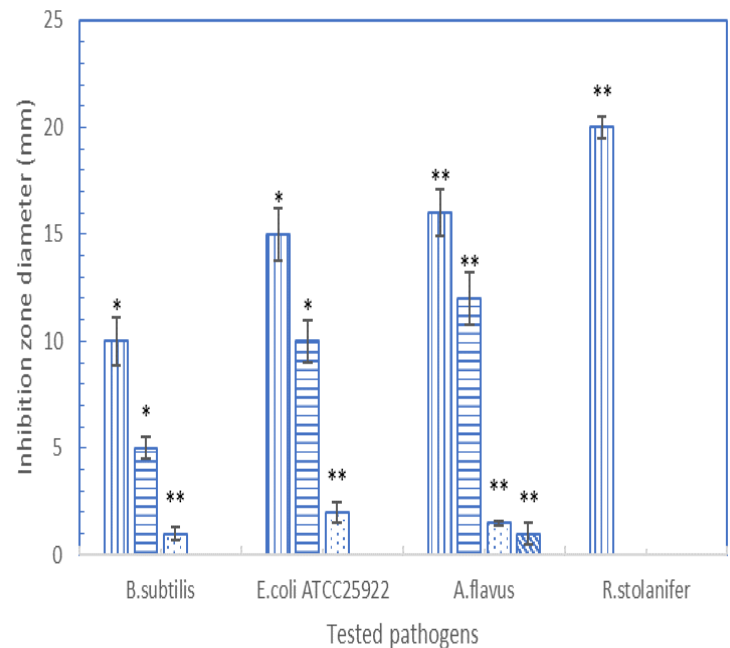


Fig. 1: Agar diffusion disk assay showing the antimicrobial activity of tested different essential oils against *B. subtilis*, *E. coli* ATCC25922, *A. flavus* and *R. stolonifer*.

For each figure: - Mustard EO, - Jasmine EO, - Lemon EO and - Vanilla EO

\*\* is high significant and \* is significant effect

## Conclusions

In this work we compared or screened the antimicrobial effect of four different essential oils (Jasmine, Lemon, Vanilla and Mustard oils) having different effective antimicrobial compounds against some selective food borne bacteria and fungi. Mustard oil showed the most effective essential oil that inhibit all the tested microbes which has the highest antimicrobial activity between the tested essential oils, so according to these results AITC the effective compound of Mustard oil was most effective against all tested food borne pathogens compared with other, so it can be used as natural food preservative enhancing food security and safety during storage and transportation.

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