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Essential oils of plants and their action against biofilm producing food borne pathogens

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Abstract
Food spoilage bacteria with potential pathogenicity have posed a significant threat to the food processing sector. One of the most notable properties of pathogens is to form biofilm that leads to increased resistance against conventional methods of treatment. Many scientific data highlight the promising potential of using essential oil as anti-biofilm agent against the food industry as it is a plant based product having novel therapeutic components. The current literature suggests that the anti-virulence properties of natural products are prioritised over their antibacterial activities. The focus of this study is on biofilm, quorum sensing mechanisms of food-borne pathogens, as well as the use of essential oils as anti-biofilm agents against some of the major food borne pathogens. The study suggests that essential oil has effects on biofilm producing microorganisms and their eradication.

Keywords: essential oil of plants, food pathogens, anti-biofilm, anti-virulence

Introduction
The use of plant-based products as natural medicines has piqued interest in recent decades. Essential oils are mixture of large and diverse class of terpenoids and phenolic compounds isolated from aromatic plants has come into light during the last few years for their biological properties like antifungal, antibacterial, anti-oxidant and antibiofilm [1]. Also, Essential oil is being studied for utilization in the food industry as an antimicrobial, antibiofilm, sanitizers and food preservatives. Many studies reported the uses of essential oil over food borne microorganisms as antibiofilm agents.
Biofilms are the association of microorganisms with extracellular polymeric substances adhering to different surfaces [2]. Biofilm forms a protective barrier while it fights against the adverse environmental circumstances. Biofilms implies major challenges for food industry since microorganisms can remain attached to various surfaces such as stainless steel, thermowares, polypropylene, glass etc. and on food products for long [3]. Biofilms have become a major problem for a wide range of industrial activities such as food processing, sea food, brewing, dairy processing, meat and poultry processing [4]. The most studied biofilm forming organisms in the food industry are *Bacillus* spp, *Pseudomonas* spp, *E. coli*, *Lactobacillus* spp, *Enterococcus* spp, *Staphylcoccus aureus*, *Salmonella* spp which are found to form matrices on many surfaces and equipment in food production [5]. In this study, the reported experiments on the use of essential oil as anti-biofilm agents against the food borne pathogens are reviewed. The methods of extraction of essential oils and the elucidation of their chemical constituents are also highlighted. This study is focused on the effect of microbial biofilm in the food industry and use of essential oil in the food industry as eco-friendly alternative of preservation.

Essential oil producing plants and the chemical composition of the oils
Essential oils are volatile, natural aromatic oily liquids with complex compositions that are extracted from different parts of plants such as leaves, peels, barks, flowers, buds, seeds etc. Essential oils have been known to possess antioxidant and antimicrobial activities, and also can act as natural additives in foods and food products. There is increasing attention on using essential oil in packaging material. Plant essential oil is usually the complex mixture of polar and non-polar compounds [6]. The general constituents of essential oil are terpenes, aromatic compounds, and terpenoids [7]. Compounds are divided into two major groups- terpenes hydrocarbons and oxygenated compounds. Terpenes hydrocarbons are made from isoprene units (5 carbon units, C_5), consists of monoterpenes (C_{10}), and sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}) etc. the general formula for terpenes is $(C_5H_8)n$ [9]. The sesquiterpenes are larger in molecules but similar to monoterpenes. The diterpenes, triterpenes and tetraterpenes are low in concentration [7]. Oxygenated compounds are the combination of C, H and O and derived from terpenes so they are termed as terpenoids.

Methods of extraction of essential oils
Essential oil can be extracted from several plants with different parts by various extraction methods. Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to damage the quality of essential oils. This results in the loss in bioactivity and nature characteristics.

Distillation
Steam distillation-Steam distillation is the most widely used method for plant essential oil extraction. Basically, the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and break down of cell structure of plant material. A new process design and operation for steam distillation of essential oils to increase oil and reduce the loss of polar compounds in wastewater was also reported [6]. The system consists of a packed bed of the plant materials, only steam passes through it and the boiling is not mixed with plant materials. Thus, the process

requires the minimum amount of steam in the process and the amount of water in the distilled is reduced. Then the water soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent [6].

Hydro distillation–HD has become the standard method of essential oil extractions from plant material such as flower or wood, which is often used to isolate non water- soluble natural products with high boiling points. The process involves the complete immersion of plant material in water, followed by boiling. This method protects the oils extracted acts as a barrier to prevent it from overheating. The steam and essential oil vapour are condensed to an aqueous fraction. The advantage of the techniques is that the required material can be distilled at a temperature below 100º C.

Hydro diffusion–Hydro diffusion extraction is a type of stream distillation in which the plant material has been dried [9]. For hydro distillation, steam is applied from the top of plant material, operated under low pressure or vacuum and reduces the steam temperature to below 100ºC. Hydro diffusion method is superior to steam distillation because of a shorter processing time and higher oil with less steam used. HD and innovative microwave hydro diffusion and gravity (MHG) methods were compared for their effectiveness in the isolation of essential oil from rosemary leaves (*R. officinalis*) [10]. The MHG method exhibits the excellent advantages over traditional alternatives.

Solvent extraction- Conventional solvent extraction is applied for delicate or liquid to liquid material and those who are not tolerant to the heat of steam distillation. Different solvents including acetone, hexane, petroleum ether, methanol or ethanol can be used for extraction [11]. In general, the solvent is mixed with the plant material and then heated to evaporate following by filtration, then the filtrate is concentrated by solvent evaporation. From the concentration, it is then mixed with pure solvent to extract the oil and distilled at low temperatures. However, this method is time consuming and expensive [12].

Soxhlet apparatus extraction–The soxhlet extraction is continuous solid/ liquid extraction process. The solid material which is placed in the thimble containing solid and it allows liquid to pass through it act as a filter. Organic solvent is then heated in boiling flask due to which the vapour generated arises to the condenser. The process is repeated until all the material is extracted out from the solid. It is easy to handle and flexible time taking system [13].

Cold pressing extraction- The cold pressing procedure is safe and effect than hot pressing method since the hot pressing method is done by using high temperature which could adversely affect the some of the bioactive compounds of the essential oil. In cold pressing, the purity and quality of the essential oil is preserved [14]. This method involves the preservation of the valuable compounds like phytosterol, tocopherols in some extracted oil. Because of these qualities, there is growing global demand for this cold pressing method.

Advanced techniques

Microwave assisted extraction (MAE)-MAE is one of the most innovative techniques for isolating vegetables oil from oilseeds. Pre-treatment of oil seeds are done in the microwave oven. It was found that microwave pre-treatment to oil extraction from avocado resulted in 97% yield [15].

Ultrasonic assisted extraction-UAE is a new innovative technique which makes use of ultrasonic sound waves. This method involves an innovative way of increasing extracted oil yield by making plant cell wall thinner. It was observed that UAE has consumed 30 minutes and solvent extraction has taken 12 hours. So, UAE is less time consuming and high yield production than solvent extraction [12]

Super critical fluid–Conventional methods including solvent extraction and steam distillation have long time preparation and required large amount of organic volatile compounds Therefore, a super critical fluid has been considered as an advanced medium for essential oil extraction [16]. The most useful super critical fluid is CO_2 which under high pressure conditions turns into an inert liquid.

Microbial biofilm formation

Biofilm are the microbial clusters of cells attached to different surfaces with the attachment of free-floating microorganisms to a surface. Biofilm can form association with the abiotic or biotic environment with the help of self-produced matrix which is slime like substances called as extracellular polymeric substances (EPS) matrix. Biofilm formation can also be influenced by external factors such as chemical growth factors, enzyme activities, and other physical growth etc. [17]. EPS acts as a barrier against the external environmental factors. The composition of EPS varies among different classes of microorganisms depending on their cellular composition such as exopolysaccharide, nucleus and other protein components such as Tap A, fibrous protein Tas A etc. [18]. Biofilm forming microorganisms interact within cell to cell by chemicals which enables them to communicate with the external environment.

Biofilm producing food pathogens

Microbial biofilm by pathogens leads to severe contamination problems in food industry and food processing that directly affect the human health life. The food processing methods are suitable environment for biofilms to form on food contact surfaces. Species for biofilm formation depends on the attachment suitable surface's characteristics, food nutrient components, environmental conditions. Surface properties such as hydrophobicity, surface roughness, electrostatic charging impact the biofilm formation and thus affect the overall hygienic conditions of the surfaces [19]. It was reported that through the food processing, biofilm forming microorganisms contaminate them which results in food spoilage, possible disease outbreak and economic loss [20].

The most reported biofilm forming microorganisms in food industries are *Bacillus, Staphylococcus, Listeria, Aeromonas, Campylobacter, Salmonella, Pseudomonas* etc. [19].

Table 1 gives an idea how different pathogens affect the food industry with potential threat to human health.

Table 1: Food borne pathogens and their effect in foods processing

Pathogens	Characteristics	Contaminated food	Harmful spoilage effects	References
Bacillus cereus	Gram positive	Dairy products, rice, vegetables, meat	Diarrhea and vomiting symptoms	[21]
Camphlyobacter	Gram negative	Poultry, unpasteurized milk	Diarrhea, fever, stomach cramp, nausea,	[22]

jejuni			vomiting	
Esherichia coli	Gram negative	Raw meat and fruits, vegetables	Diarrhea outbreaks and haemolytic uremic syndrome	[23]
Listeria monocytogenes	Gram positive	Dairy products, meat, cheese, ice cream, frozen and ready to cook food items, poultry	Listeriosis in elders, pregnant women and immune patients	[24]
Salmonella enteric	Gram negative	Poutry meat, fish,	Gastroenteritis or septicaemia	[25]
Staphylococcus aureus	Gram positive	Meat products, egg, dairy products, bakery products	Methicillin resistance, vomiting	[26]
Pseudomonas spp	Gram negative	Low acid dairy products, fruits and vegetables	Blue colouration on fresh cheese	[27]
Geobacillus stearothermophilus	Gram positive	Dried dairy products	Production of acids or enzyme	[28]
Anoxybacillus flavithermus	Gram positive	Dried milk powder	Poor hygiene	[29]
Pectinatus spp	Gram negative	Beer and brewery	Rapid cell growth makes beer turbid and smells like rotten eggs due to production of sulphur compounds.	[30]
C. violaceum	Gram negative	Poultry, fresh vegetables, salads	Abdominal pain, immune disease, nausea, vomiting	[29]
Clostridium perfringes	Gram positive	Raw meat and poultry	Diarrhea, vomiting	[31]
Aeromonas hydrophilia	Gram negative	Fish, sea food, ready to eat fish items	Gastroenteritis, abdominal cramps	[32]

Mechanism of inhibition of biofilm by essential oil

The essential oils can act as potent antibiofilm agents by inhibiting the intercellular communication systems [33]. Essential oils can kill biofilm cells by the alternation of their cytoplasmic constituents. Moreover, there are reports which indicated that the due to the absence of outer layer of gram positive bacteria direct interaction of EOs is possible, due to which inactivation of the bacterial enzymes take place [34]. It is also found that the effect of essential oil on the biofilm also depends on the composition and structure of the biofilm as well as the constituents of Eos [33]. The EOs can stop the biofilm forming by blocking the quorum sensing mechanism, inhibiting the transcription and mutation of the genes [35]. EOs could also cause the cell apoptosis by reducing the oxidation in the cells [36]. The most commonly studied organism was *Chromobacterium violaceum* because its pigment production is regulated by QS and for the study the paper disc test is applied. When the production of AHL molecule is inhibited, colourless halos are produced which signifies inhibition of communication among biofilm forming cells [37].

Table 2 lists out different types of essential oils and their chemical constituents that can inhibit biofilm forming food pathogens.

Table 2: Essential oils and some food- borne pathogens that are inhibited by them

Essential oil	Plant source	Main components	Test pathogens	Reference
Lavender oil	*Lavandula angustifolia*	Camphor, caryophyllene, eucalyptol, lavendulyl, limonene, linalyl acetate, transocimene, terpinen-4-ol	*S. aureus E.coli*	[38]
Chamomile oil	*Matricaria chamomilla*	(E)-β- farnesene α- bisabolol oxide A	*Lactobacillus spp*	[36]
Melissa essential oil or lemon balm	*Melissa officinalis*	Limonene, β-caryophyllene oxide,	*S. aureus E.coli*	[38]
Ocimum Gratissimum oil	*Ocimum gratissimum*	Eugenol, 1, 8- cineole	*S. aureus E.coli*	[35]
Oregano oil	*Origanum vulgare*	Carvacol, thymol, terpinene	*S. aureus*	[39]
Satureja thymbra oil	*Satureja thymbra*	Carvacol, thymol, p- cymene	*Lactobacillus fermentum, Salmonella enteric, Listeria monocytogenes*	[40]
Thyme oil	*Thymus vulgaris*	Eucalyptol, camphor	*A. hydrophila*	[41]
Cassia oil	*Cinnamomum aromaticum*	Cinnamaldehyde, eugenol, linalool	*P. aeruginosa P. putida S. aureus*	[42]
Lemon grass oil	*Cymbopogon Citrates*	Myrcene	*Aeromonas hydrophilia*	[41]
Clove oil	*Syzygium aromaticum*	Eugenol, eugenyl acetate, caryophllene	*P. aeroginosa, C. violaceum, Esherichia coli*	[43]
Tree tea oil	*Melaleuca alternifolia*	Terpinen -4- ol, 1, 8- cineole, p – cymene.	*S. aureus, P. aeroginosa*	[45]
Basil oil	*Ocimum basilicum*	Basil methyl chavicol	*Aeromonas hydrophila*	[41]
True cinnamon tree oil	*Cinnamomum zeylanicum*	Cinnnamyl acetatr, trans- alpha- bergamotene, caryophyllene oxide	*S. enteric*	[5]
Wild Thyme oil	*Thymba capitata*	Carvacrol, γ-terpinene, p – cymene, linalool, borneol	*S. enteric*	[46]
Tree tea oil, lemon oil	*Melaleuca alternifolia, Cymbopogon Citrates*	Terpinen -4- ol, 1,8- cineole, p – cymene, mycrene	*Camphlyobacter jejuni*	[47]

Conclusion and future perspectives

Biofilm formation of pathogenic bacteria on food contact surfaces represents big challenges to the food industry. The existence of biofilms along food manufacture surfaces can lead to financial loss and serious health issues. The removal of biofilm is difficult from food contact surfaces as it serves a good substratum for biofilm growth. Further, the biofilm formation leads to food contamination, food spoilage and outbreaks of food borne diseases. Food companies rely on the chemical sanitizers and disinfectants such as sodium hydroxide or sodium hyprochloride solutions as well as other physical methods such as hot stream washing of

equipment which are not so far effective. As per the reported studies suggests that various efforts are being made worldwide to study the various aspects of biofilm formation and their quorum sensing mechanisms and their active inhibiting agents. Among many strategies, one of the cost effective and ecofriendly methods can be the application of essential oil in the food industry as it is a plant based natural product.

In this study, the effect of essential oil and their chemical constituents as the antibiofilm agents against some of the food borne pathogens are reviewed. So far investigation suggests that the EOs and their components have shown effective inhibition against the food borne pathogens and can represent as solution to the problem.

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