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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS: EXPLORATION ON MECHANISM OF BIOACTIVITY

Siti Nur Ashakirin [1], Minaketan Tripathy[*1, 2], Umesh Kumar Patil [3] and Abu Bakar Abdul Majeed [1, 2]

Laboratory of Fundamentals of Pharmaceutics [1], Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.
Pharmaceutical and Life Sciences Communities of Research [2], Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor Darul Ehsan, Malaysia.
Divisions of Natural Product Research and Pharmacognosy [3], Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar - 470003, Madhya Pradesh, India.

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Correspondence to Author:
Minaketan Tripathy

Laboratory of Fundamentals of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

E-mail: minaketan@puncakalam.uitm.edu.my

ABSTRACT: Eessential oils are known to possess antimicrobial activity. This review article explains the antimicrobial reaction, application, effects, and characteristics of cinnamaldehyde. Amongst the different essential oils, Cinnamaldehyde is observed to be the most effective antimicrobial agent. The current studies on cinnamaldehyde in the field of microbiology have been highlighted. This review explains the different methods, those are used for antimicrobial evaluation. Additionally, the anti-physiologic effect of antimicrobial agents using microbial respiration rate is also discussed.

INTRODUCTION: Antimicrobial potential is a capability to destroy microorganisms or suppress their multiplication and growth. It can be in the form of heat, radiation or a chemical that is able to destroy microorganisms those might cause infections of different nature. The antimicrobial activities are determined and compared by different types of test such as serial dilution (for example; MIC) and agar plates (for example; MBC) method. Both methods usually use bacteria *Escherichia coli* (*E. coli*) as a representative gram negative candidate so to identify the antimicrobial potential.

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E. coli is a common experimental bacterium to test many antimicrobial agents. *E. coli* bacteria normally live in the intestines of healthy human and animals. Most varieties of *E. coli* are harmless or may cause relatively mild diarrhoea.

However, in some strains, such as *E. coli* O157:H7 may cause severe abdominal cramps, bloody diarrhoea and vomiting. It can be transmitted via contaminated water or food especially raw vegetables and undercooked ground beef. Healthy adults usually recover from *E. coli* O157:H7 infection within a week, but paediatric and geriatric individual have a greater risk of developing a life-threatening form of kidney failure called haemolytic uremic syndrome. Consequently, inhibition of bacteria growth was considered necessary to prevent infection. The growth of bacteria *E. coli* can be effectively inhibited by cinnamaldehyde from cinnamon bark [1].

Cinnamon is a spice obtained from the inner bark of several trees from the genus Cinnamomum that is used in both sweet and savoury foods. Examples of Cinnamomum species was indicated in **Table 2** with the references of previous studies. Cinnamaldehyde is the organic compound that gives cinnamon its flavour and odour (also known as Cinnamic aldehyde) [2]. This organic compound was crucial to inhibit bacteria growth. Though some of the cinnamomum species have been rigirously tested in term of their chemical composition, application as bacteriacidal, anti-microbial, anticancer and also for food technology application, there are still extensive study in various field on cinnamaldehyde yet to be done.

Furthermore, the purity between cinamaldehyde (CIN) and trans-cinnamaldehyde (TC) was slightly different. The trans-cinnamaldehyde is more pure consisting <99% than cinnamaldehyde (unsaturated cinnamaldehyde) 98%. Trans - cinnamaldehyde synonyms as (E) - Cinnamaldehyde, 3-Phenyl-2-propenal, 3- Phenylacrolein, 3- Phenylpropenal; Cinnamyl aldehyde, or Cinnamic aldehyde. The solubility of this compound in water was low. So, it needs to undergo emulsification (o/w) to stabilize it.

Nanoemulsions (NEs) can be defined as a fine oil-in-water dispersions, having droplets covering the size range of 100–600 nm. Nanoemulsion emerged from extensive nanotechnology studies for a novel drug delivery system (NDDS) [3, 4]. Besides, nanoemulsions also known as nano sized or submicron emulsions (SME), consists of a systems with at least two nearly immiscible fluids dispersed one into another in the form of droplets with diameter lower than one micrometre [3]. The preparation of nanoemulsion consisting of two methods; the first method is low energy method (PIT, EIP, Spontaneous emulsification) and the second method is high energy method (HSH, HPH, Ultrasonic).

The purpose of this article is to provide an overview of the recently published data on the antimicrobial activity of essential oil cinnamaldehyde that is considered suitable for application in numerous food industry fields especially as antimicrobial agent [5-7]. Cinnamaldehyde should undergo nanoemulsion process of method to trigger antimicrobial activity and stabilized cinnamaldehyde effectively which is also discussed in this article.

Types and Nature of an Essential Oil: Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from natural sources, usually plants. They are not oils in a strict sense, but often exhibit similar physico chemical properties as that of oils. Essential oils often have an odour and also known as ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it contains the "essence of" the plant's fragrance.

The characteristic fragrance of the plant from which it is derived [8]. The term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by a given living organism. Studies have shown that certain essential oils may have the ability to prevent the transmission of some drug-resistant strains of pathogen. Typically, essential oils are highly complex mixtures of often hundreds of individual aroma compounds [5].

TABLE 1: ESSENTIAL OILS ARE DERIVED FROM VARIOUS SECTIONS OF PLANTS

Berries	Leaves	Flowers
All Spice	Basil	Chamomile
Juniper	Bay Leaf	Clary Sage
Seeds	Cinnamon	Clove
Almond	Common Sage	Geranium
Anise	Eucalyptus	Hyssop
Celery	Lemon Grass	Jasmine
Cumin	Melaleuca	Lavender
Nutmeg Oil	Oregano	Manuka
Bark	Patchouli	Marjoram
Cassia	Peppermint	Orange
Cinnamon	Pine	Rose
Sassafras	Rosemary	Yiang-Yiang
Wood	Spearmint	Peel
Camphor	Tea Tree	Bergamot
Cedar	Thyme	Grapefruit
Rosewood	Wintergreen	Lemon
Sandalwood	Resin	Lime
Rhizome	Frankincense	Orange
Ginger	Myrrh	Tangerine
		Root
		Valerian

Toxicity of Essential Oils: Cinnamaldehyde is considered to have low toxicity, yet reported to cause skin irritation to humans [9]. Other conceivable toxin found in the bark of cinnamon is coumarins which cause poisonous effect as compared to that of cinnamaldehyde. The level of coumarin found in 'cinnamon' extracts and powders has concerned the German Federal Institute for Risk Assessment (BfR). Coumarin is a substance discovered regularly in numerous plants particularly in *C. cassia* plant in high concentration and this compound is the reason of skin irritation. This coumarin compound is a natural substance comes under the benzopyrone chemical class, a dull crystalline substance in the standard state.

The skin irritation relating cinnamaldehyde at high dosages has been reported by Cocchiara *et al*., 2005 based on experiments in animal models of mice, gunea pigs, and rabbits. Other essential oil from plant, for example, tea tree oil induced membrane-toxicity owing to the presence of monoterpenoid components [10]. These components can effectively diffuse through the cell-membrane and initiate biological reactions resulting membrane-toxicity. In addition, cinnamaldehyde (*C. aromaticum*) can act as a grain protectant because of its fumigant and antifeedant properties [11]. The microbial membrane-toxicity of cinnamaldehyde against a few microbes has been demonstrated by means of SEM imaging, pointing towards structural distortions of the cell envelope [12].

Toxicity of cinnamaldehyde is studied using the parameter LD_{50} that describes the concentration, capable of reducing fifty percent population of test organic entity / microorganisms [9].

Mechanism Action of Essential Oils: Consumers' preference for fewer chemicals in food has led to research on potential use of natural antimicrobials in food for controlling spoilage and pathogenic microorganisms. The antimicrobial effects of essential oils have been studied earlier. The study indicated the antimicrobial activity of cinnamaldehyde against enteric pathogens could be useful in food processing and preservation. The work of Domadia *et al.,* (2007) suggested that, although cinnamaldehyde got its instability issue, still can be a promising agent with antibacterial potential [13]. The efficient of antimicrobial activities of any components, can be maintained using a suitable delivery systems based on biphasic dosage forms [14].

Mechanism Reaction:

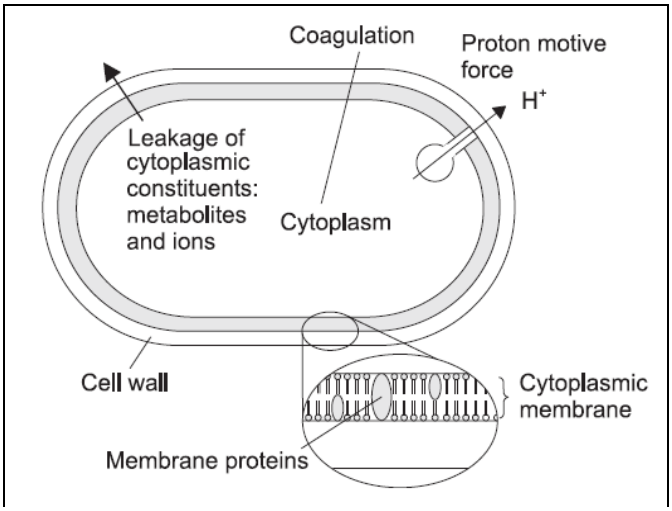

FIG. 1: MECHANISM OF MICROBIAL SITE OF ACTION

It has been demonstrated that the antimicrobial activity of the EOs causing structural and functional depletion to the bacterial vital organelles [15] as seen from the **Fig. 1**.

TABLE 1: MECHANISM ACTION OF CINNAMOMUM SPECIES

Mechanism of action	Studies
Cell wall penetration	Mode of action against *Candida albicans* [16]
	Brief review; trans-cinnamaldehyde inhibit the growth of *E. coli* and *S. typhimirium* without disintegrating the OM or intracellular ATP [17]
	Efficient action towards *Salmonella* spp. [18]
	Reducing MIC and effective against *Staphylococcus* [19]
Binding with vital organelles	Changes in membrane fatty acid composition [20]
	Inhibit and binding to protein FtsZ in *Bacillus* spp. [13]

Cell Wall Penetration: Generally, Gram-negative bacteria are more resistant to EOs than Gram-positive bacteria [1, 17, 21]. Before examining the effects of EOs on bacteria, it is important to consider the structural differences of the cell walls of Gram-positive and Gram-negative bacteria. Approximately 90% – 95% of the cell wall of

Gram-positive bacteria consists of peptidoglycan, to which other molecules, such as teichoic acid and proteins, are linked. The structure of the bacterial cell wall allows hydrophobic molecules to easily penetrate the cells and act upon both cell wall and cytoplasm. The phenolic compounds, present in the EOs, generally show antimicrobial activity against Gram-positive bacteria. The cell wall of Gram-negative bacteria (example *E. coli*) is more complex. It has a peptidoglycan layer that is 2-3 nm thick, which is thinner than the cell wall of Gram-positive bacteria, and is approximately 20% of the dry weight of the cell.

An outer membrane (OM) lies outside the thin peptidoglycan layer. The peptidoglycan and OM are firmly linked by Braun's lipoprotein; that is covalently bound to the peptidoglycan and is embedded in the OM. The presence of an OM is one of the features that differentiate Gram-negative from Gram-positive bacteria. It is composed of a double layer of phospholipids that is linked to inner membrane by lipopolysaccharides (LPS). The peptidoglycan layer is covered by an OM that contains various proteins as well as LPS [23].

Binding with Vital Organelles: Bacteria's cell membrane which consists of fatty acid is one of the important organelle for all bacteria. So, the changes in membrane fatty acids composition usually occur when the microbial cells binds to sub lethal concentration of cinnamaldehyde in growing media as response of the stress condition [18].

Effects upon Respiration: It is essential for bacteria to undergo respiration process like any other living organisms. In certain cases bacteria used oxygen to respire, as humans and other animals do, but certain situations bacteria use one or more different molecules as a final electron acceptor for respiration. The aim of respiration is to provide the cell with the appropriate molecules for creating energy in the form of adenosine triphosphate (ATP). ATP is the energy currency of cells, allowing important cellular processes to proceed. In fact, the respiration in bacteria is basically having two types aerobic and anaerobic which may be obligate or facultative which lead to the further classification. Inhibition of cell respiration towards bacteria was determined by using an essential oil also as antimicrobial agent. Investigation was done by using TTO, shows the reducing growth of *Escherichia coli* cause by inhibition of glucose-dependent respiration and triggers the leakage of K^+ ion [22]. Unfortunately, the experimental study of respiration effects on bacteria has not been very famous among the researcher.

Testing of Antimicrobial Activity of Essential Oil:

TABLE 2: TEST METHOD USED TO MEASURE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL ESPECIALLY CINNAMALDEHYDE

Purpose	Test method	
Screening for antibacterial activity	Disk diffusion Agar well	
Determination of strength of antibacterial properties	Agar dilution method Broth dilution (MIC and MBC)	Visible growth Optical density / turbidity Absorbance Colorimetric Conductance/ impedance Visible count
Determination of rapidity and duration of antibacterial activity	Time-kill analysis/survival curves	
Observation of physical effects of antibacterial activity	Scanning electron microscopy	

Zone of Inhibition (ZOI), Microbial Inhibition Concentration (MIC) and Microbial Bactericidal Concentration (MBC):

Zone of Inhibition (ZOI): MIC and MBC data obtained from cinnamon essential oil with regard to inhibition zones analysis, using disk diffusion method. The disc-diffusion method is the frequently utilized technical method for antimicrobial screenings of EOs. Antimicrobial activity is generally evaluated by this method for preliminary studies. In this method, a paper disc soaked with the EO is placed on the inoculated surface of an agar plate and the zone of microbial inhibition is measured. Diverse parameters in this test could influence the result, such as the volume of EO on the paper discs, the thickness of the agar layer and the solvent [15]. As indicated by the research method Ali and colleagues, 2005 [23]; the Kirby Bauer disk diffusion test was used to test activity of bioactive compound on microbes *H. pylori* which gives the significant trend of result.

Demonstrating that ZOI gives significant result of antibacterial activity of cinnamaldehyde / trans-cinnamaldehyde by observing the diameter of ZOI (mm) on the plates [24]. Really there are alternate disk methods which infrequently used called disk volatilization method and would not be discussed in this article [1].

Microbial Inhibition Concentration (MIC) and Microbial Bactericidal Concentration (MBC): Also, the minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It likewise can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent and incubated at 30-37 °C for 48 hours (2 days) [8]. Usually the growth inhibition test was measured at OD_{630} values of cultures and the lowest concentration that completely inhibited was taken as MIC [25]. The MBC is distinguished by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥99.9%. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC.

Since the MBC test uses colony-forming units (CFU) as a proxy measure of bacterial viability, it can be confounded by antibacterial agents cause aggregation of bacterial cells. Examples of antibacterial agents which do this include flavonoids and peptides. From the result of research by Becerril *et al*., 2007 [26] showed that the cinnamaldehyde in the broth dilution method were less efficient than in the vapour diffusion assay. Previous reports indicated the rapid inhibitions of energy metabolism of some tested organism upon the exposure of bactericidal concentration of cinnamaldehyde [27]. Also Cinnamaldehyde produced significant inhibitory effect when used alone without combination of others antimicrobial substances [28], further indicating that strong antimicrobial effect against various microorganism that tested by MIC.

The above observation was supported by the work of Unlu *et al*., 2010 [29] while evaluating antimicrobial activity against 21 bacteria and 4 Candida species, using disc diffusion and MIC methods. Recently, the improved method of combined effects are evaluated using the indices of fractional inhibitory concentration (FIC) and Effect of the Combination (EC) jointly revealing synergistic effects of antibacterial activities [30].

Respiration of Bacteria: Some microorganisms are autotrophs; these are microscopic organisms that obtain carbon from carbon dioxide. They receive their energy from distinctive sources. Organisms that uutilizes light to obtain their energy are known as Photoautotrophs (blue-green algae). Chemoautotrophs are bacteria that receive their energy from inorganic compounds like hydrogen sulphide and use energy to run the cell activities. The remaining microorganisms are heterotrophs, those acquire carbon by ingesting organic molecules from decaying organisms or by living on or in another organism known as a host. Generally the structure of microorganisms exclude the ribosomes, prokaryotes lack organelles.

As prokaryotic cell with emphasis on microbes *E. coli* the selective respiratory quinones known as lipid-soluble components of membrane bound electron-transport chain enable the survival in stress condition [31]. Otherwise, to get energy from molecules, for example, sugars, microbes must uutilize fermentation or cellular respiration. These are methods that uutilize the energy as a part of molecules to make adenosine triphosphate, known as ATP. This is uutilized for development, movement, temperature regulation, and numerous different methods that require energy. Fermentation, the procedure most microbes utilize, generally takes place in an anaerobic environment. Microbes produce unique by products like alcohol, carbon dioxide, lactic acid, formic acid and acetic acid.

A few types of microscopic organisms experience the process of cellular respiration. This is a more complex process that does not require oxygen, and the energy of the organic molecules is more proficient in the production of ATP. Carbon dioxide and water are the by-products most typicallyy produced. Besides, the inhibition of glucose-dependent respiration and the stimulation leakage of intracellular K^+ cause by the tea tree oil (TTO) concentration effects at the minimum inhibitory level [22, 32]. Unfortunately, the research of

the respiration test towards cinnamaldehyde as the strongest antimicrobial agent was not been studied.

CONCLUSION: Cinnamaldehyde, their constituents and other essential oils basically show antibacterial activity against food borne pathogen *in vitro* and to a lesser extent in foods. The relationship between EOs and food constituents require more study before these substances can be reliable and can be used in commercial application. Further studies are needed to explore the potential not only for food products but also for medicinal applications. Particularly the anti-physiological effect of essential oils might be of interest for researchers in the future days to come.

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