



In Vitro Antibacterial Activity of *Cinnamomum zeylanicum* and Screening of Its Bioactive Compounds

Al-Zubaidi Sura

Medical Biotechnology
Department, College of
Biotechnology, Al-Qasim
Green University, Babylon,
51013, Iraq

Abstract:

Background: *Cinnamomum zeylanicum* is a plant containing antimicrobial properties in the extracts. It is the presence of compounds like cinnamaldehyde that makes it possess an antibacterial effect and it does act as such to bacteria.

Materials and Methods: Cinnamon sticks of *Cinnamomum zeylanicum* were dried and ground in a mortar and pestle at room temperature level. Three cold extractions (25 0 C) and 72 hours were carried out to macerate the plant samples in order to obtain ethanol extracts of 80% (v/v) concentration. Extraction of volatile bioactive chemicals through GC-MS. GC is then employed to determine the component constituents of the essential oils and the molecular mass of those constituents is determined by subsidizing Mass Spectrometry (MS) using fragmentation.

Results: The components of findings were fifteen in the case of *C. zeylanicum* following a GC-MS analysis. These are; 9,12-octadecadienoic acid (Z,Z)-, Cinnamic alcohol, PALmitic acid vinyl ester, Caryophyllene, Trans -2-decenal, Eugenol, D-limonene, Hexadecanoic acid, ethyl ester, 6-Methyl-5-hepten-2-one, Benzaldehyde, Cinnamic aldehyde, Phenyl ethyl acetate and 6-Methyl-5-hepten Enterococcus faecalis (26.09±0.43) was demonstrated to have a strong efficacy on the metabolites of *Cinnamomum zeylanicum*.

Keywords: Antibacterial Activity, Bioactive Compounds, *Cinnamomum zeylanicum*

Introduction

Cinnamon is a plant that belongs to Lauraceae family; it is an annual plant that is of extreme economic value and it also takes very long period of time before the fruit is produced. The by-products of cinnamon are twigs and leaves, but the

greatest effect is the cinnamon bark on the trunk and beside. The product is also refined to give essential oils and oleoresins that are extensively used in a large number of other markets, including the food, beverage, tobacco, cosmetics and medicine markets [1, 2]. The chemicals found in



Corresponding Author: Al-Zubaidi Sura, Nursing (Instructor Doctor, Medical Biotechnology Department, College of Biotechnology, Al-Qasim Green University, Babylon, 51013, Iraq



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cinnamon lower cholesterol, blood pressure and tumors. It is also reputed to possess antiviral, antifungal, antibacterial, antioxidant and tumor. The antioxidants may also be found in cinnamon as it contains high amount of chemicals like tannins, calcium oxalate, eugenol, safrole, and sinamaldehyde [3]. Cinnamomum cinnamon bark essential oil (Steam distilled) was acquired through steam distillation. The cinnamon tree leaf and the bark contain flavonoids, saponins, and essential oils. Cinnamaldehyde and linalool are some of the chemicals that have been found to possess antioxidants. The primary compositional structure of cinnamon essential oil is cinnamaldehyde (30.72 percent), eugenol (17.62 percent), and coumarin (13.3 percent). Antioxidants stabilize the free radicals by preventing the development of chain reactions of free radicals and compensating the deficit of electrons that the free radicals experience. The oxidation process is made manageable by the use of antioxidants to prevent its occurrence at all times. In addition, antioxidants are also able to neutralize, detoxify and prevent the actions of free radicals. When the cinnamon bark extract has a value lower than 50 ppm 2, this is a sign that it possesses an extremely strong antioxidant effect [4-6]. 2, 2-diphenyl-1-picrylhydrazyl/ (DPPH) is one of the guidelines when in search of antioxidant active properties. When the DPPH free radicals (purple) are reacted with the antioxidant-containing plant extracts, it leads to the formation of 1.1-diphenyl-2-picrylhydrazyl (yellow), a compound with several useful biological applications.

Some of the oldest known traditional spice species are ceylon cinnamon or real cinnamon which is the same species of the family Lauraceae. *C. zeylanicum* is also a component of a number of other folk medicines against diseases such as diabetes, neuralgia, dyspnea, leucorrhoea, rheumatism, wound, toothache, and eye inflammation using the inner bark to make medicines to treat indigestion, flatulence and prevention of flu [6, 7]. Most of the diseases have also been also shown to have their progression halted by the recently *C. zeylanicum* and extracts

of the plant as well. To a large extent, this is owed to anti-inflammatory, anti-bacterial, antioxidant, anti-tyrosinase, anti-cancer, anti-mutagenic and anti-diabetic qualities of the chemicals found in *C. zeylanicum*. More specifically, ceylon cinnamon is one of the very few plants, which may be found in the contemporary pharmaceutical industry in powder, ointment, oils, and pills [8]. It is fortunately analogous in the aspect of the look of numerous Cinnamon types. The wrong information on bioactive substances and the functionality of *C. zeylanicum* may be spread in the event of the mix up with the other types. It is with this consideration that we felt it pertinent to elaborate on the morphological aspect, antioxidant, traditional uses, phytochemical composition and pharmacological activities of *C. zeylanicum* in order to avoid any potential implication of such similarities [9, 10] and to fill the knowledge gap in the folkloric as well as the medicinal applications of the plant.

Material and Methods

Cinnamomum zeylanicum Extraction.

Cinnamomum zeylanicum sticks were dried and ground in room temperature to powder. In the example of the ethanol extracts of the various plant samples, 80 per cent (v/v) ethanol was extracted by using maceration in three consecutive cold extractions (25 o C) on 72h. With regards to aqueous extracts, the extra fraction of the plant samples were moistened with distilled water after 24h. The whole filtrate was dried by putting it on a rotary evaporator at 500 o C.

GC-MS Component Analysis

This can be done through the volatile bioactive compounds through the GC- MS whereby the components of the essential oils are separated using GC (Gas Chromatography) and the molecular weight of the different components is determined using MS (Mass Spectrometry) by applying the mass fragmentation. As a vapour of an organic substance is forced through the ionization chamber of a mass spectrometer, high-energy electrons will be launched on this

substance and cast high-energy electrons and cast electrons of the substance.

Antimicrobial Activity

Microorganisms and Well Diffusion Agar Method.

Bacillus subtilis, *Enterococcus faecalis*, *Escherichia coli* and *Streptococcus pyogenes* were the bacterial strains that were lyophilized and sub-cultured under the sterile condition at 37 C in the present study. The culture of the stock was then sub-cultured in slant nutrient agar and prepared by washing the culture several times in sterile ringer solution. The changes in the optical density of the suspension at 630 nm reached a microbial suspension of 0.5 McFarland standard or 1.5108 CFU/mL. The 6 mm filter paper discs that were covered with 20 0 07.5 ml of extract were streaked throughout the dish of the Petri containers holding the microbial suspension and laid on the infected plates. Then, they were incubated at 24 hours in 37 C o. The centimetres of the diameter of the filter paper discs were measured. Subsequently, the surface was prepared with some wells of the medium (diameter 6mm) and 20 uL of the extract were added to them. The diameter of the wells and the surrounding inhibition zones were measured using millimeters and the temperature of the petri dishes were kept at 37 o C with a duration of 24 hours.

Statistical Analysis

The data were all run with the assistance of an SPSS-based one-way analysis of variance (ANOVA). The average was used to represent the findings and the standard deviation and the experiments were repeated three times.

Results and Discussion

Gas chromatography-mass spectrometry (GC-MS) is one of the potent techniques of determining the existence of chemical elements in the medicinal plants. It is a method of isolating chemicals present in plants that are then reacted on to electrons in order to determine their mass and fragmentation pattern. These parameters are compared with a set of known compounds database in order to detect them. Such a process can be used by scientific community to establish the phytochemicals that give the conventional medical efficacies of the plant by confirming the therapeutic efficacy of a plant. Consequently, the present paper has assessed the antibacterial and the chemical contents of *Cinnamomum zeylanicum*. As illustrated in Table 1, the sixteen chemical compounds detected in *C. zeylanicum* by the GC-MS are shown. They are: 9,12-Octadecadienoic acid (Z,Z)-, Cinnamic alcohol, Palmitic acid vinyl ester, Caryophyllene, Trans -2-decenal, Eugenol, D-limonene, Hexadecanoic acid, ethyl ester, 0 -Copaene.

Table 1. *Cinnamomum zeylanicum* bioactive secondary metabolites by using GC-MS technique.

No.	Compound	Molecular Formula	No.	Compound	Molecular Formula
1.	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	9.	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂
2.	Cinnamic alcohol	C ₉ H ₁₀ O	10.	β-Pinene	C ₁₀ H ₁₆
3.	Alpha terpineol	C ₁₀ H ₁₈ O	11.	6-Methyl-5-hepten-2-one	C ₈ H ₁₄ O
4.	Palmitic acid vinyl ester	C ₁₈ H ₃₄ O ₂	12.	Benzaldehyde	C ₇ H ₆ O
5.	Caryophyllene	C ₁₅ H ₂₄	13.	Cinnamic aldehyde	C ₉ H ₈ O
6.	Trans -2-decenal	C ₁₀ H ₁₈ O	14.	Phenyl ethyl acetate	C ₁₀ H ₁₂ O ₂
7.	Eugenol	C ₁₀ H ₁₂ O ₂	15.	α-Copaene	C ₁₅ H ₂₄
8.	D-limonene	C ₁₀ H ₁₆			

Bioactive chemicals of *C. zeylanicum* are available in some of the parts of the plant including the buds, leaves, fruits, bark, flowers, root bark, oils. Furthermore, *C. zeylanicum* has the property of the volatility of chemicals, majority of which are antioxidants. In addition, most of the chemicals are derived using tannins, phenylpropanoids, cinnamyl, hydrolyzed phenol and terpenoid. Outside, benzyl benzoate, eugenol, linalool, and eugenyl acetate are the most prevalent antioxidants in the species of *C. zeylanicum*. Cinnamaldehyde and transcinnamaldehyde are the most important bioactive components of *C. zeylanicum* as regards to anti-tyrosinase action. Its primary ingredient is the compound cinnamaldehyde that contributes spiciness and aroma to *C. zeylanicum*. Aroma and essence compounds are usually extracted when the *C. zeylanicum* is used because it has therapeutic value and abundance of bioactive compounds. Recent uses of cinnamon include medicine where it organizes the metabolite pro-

anthocyanidins in form of cinnamon [11-13] which inhibit inflammatory substances as well as assist various pathways including insulin delivery. Bioactive molecules that are present in essential oils can also kill unwanted microorganisms including microorganisms that lead to food poisoning and food spoilage. On Tri-Comparison of the two kinds of extracts (methanol and Ethyl acetate fraction) with two standard antibiotics RF-Rifampicin and CRO-Ceftraxone against *Enterococcus faecalis* 26.09±0.43, 17.83±0.26, 33.00±0.57 and 29.45±0.49 respectively. Although it was recorded 21.08±0.34, 12.47±0.21, 29.07±0.54 and 25.00±0.43 with *Escherichia coli*. Simultaneously record 25.00±0.41, 19.06±0.30, 31.25±0.51 and 27.04±0.45 *Streptococcus pyogenes*. Whereas, *Bacillus subtilis* were recorded as 16.09±0.27, 20.35±0.32, 25.00±0.40 and 21.49±0.31. *Cinnamomum zeylanicum* metabolites showed a high level of activity against *Enterococcus faecalis* (26.09±0.43).

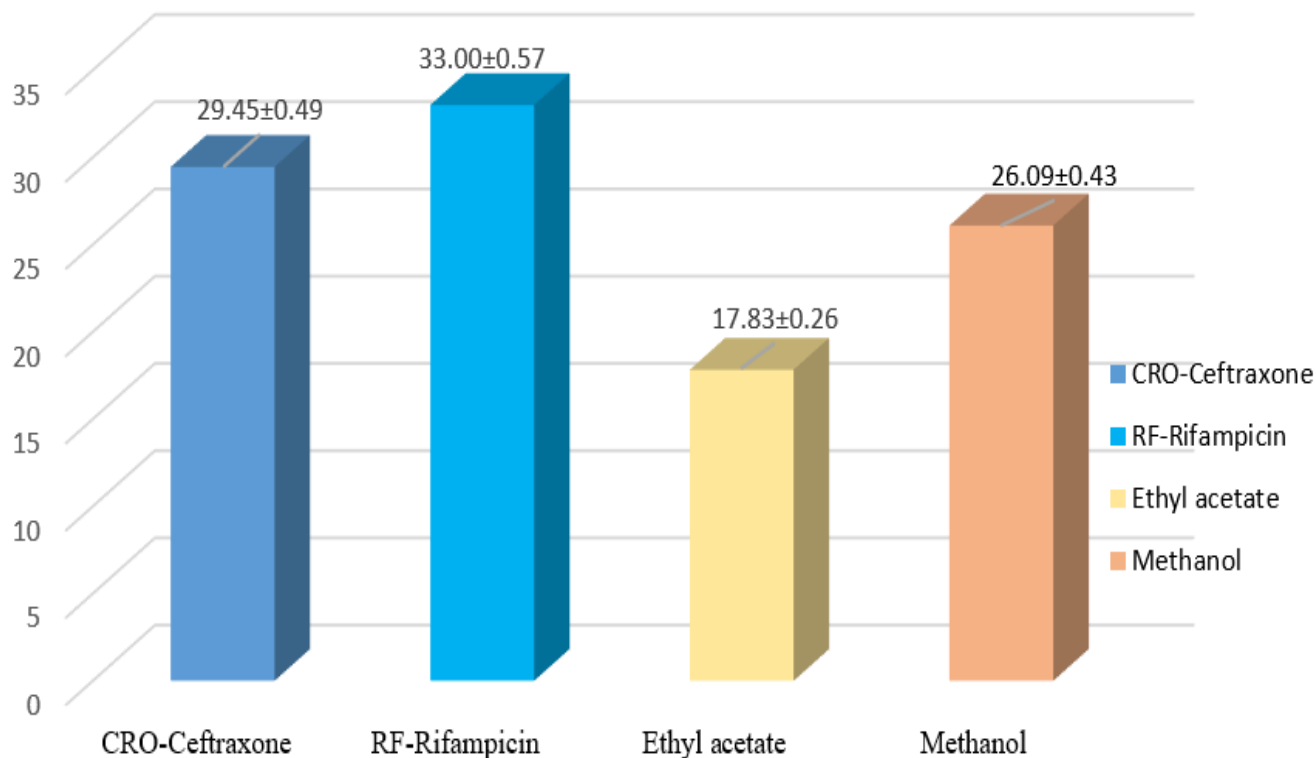


Figure 1. Bioactive metabolites of *Cinnamomum zeylanicum* and standard antibiotics RF-Rifampicin, and CRO-Ceftraxone against *Enterococcus faecalis*

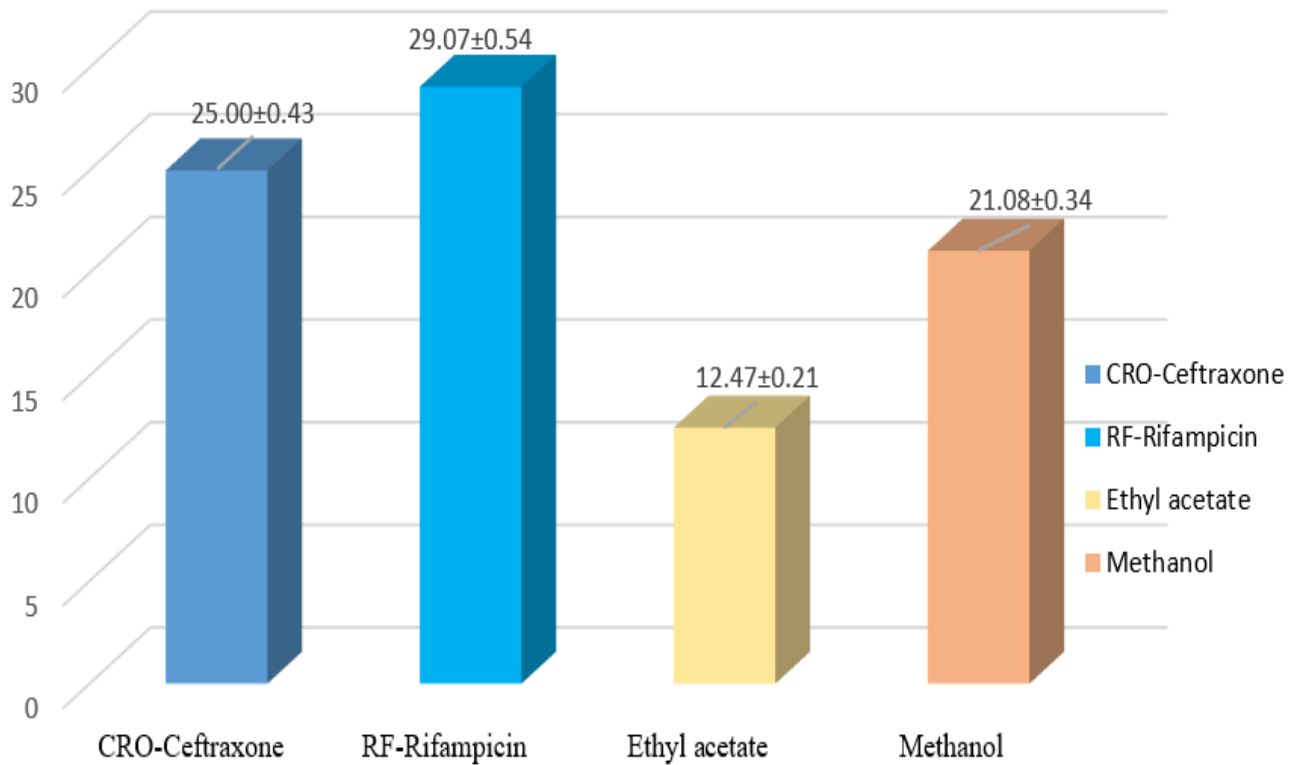


Figure 2. Bioactive metabolites of *Cinnamomum zeylanicum* and standard antibiotics RF-Rifampicin, and CRO-Ceftraxone against *Escherichia coli*

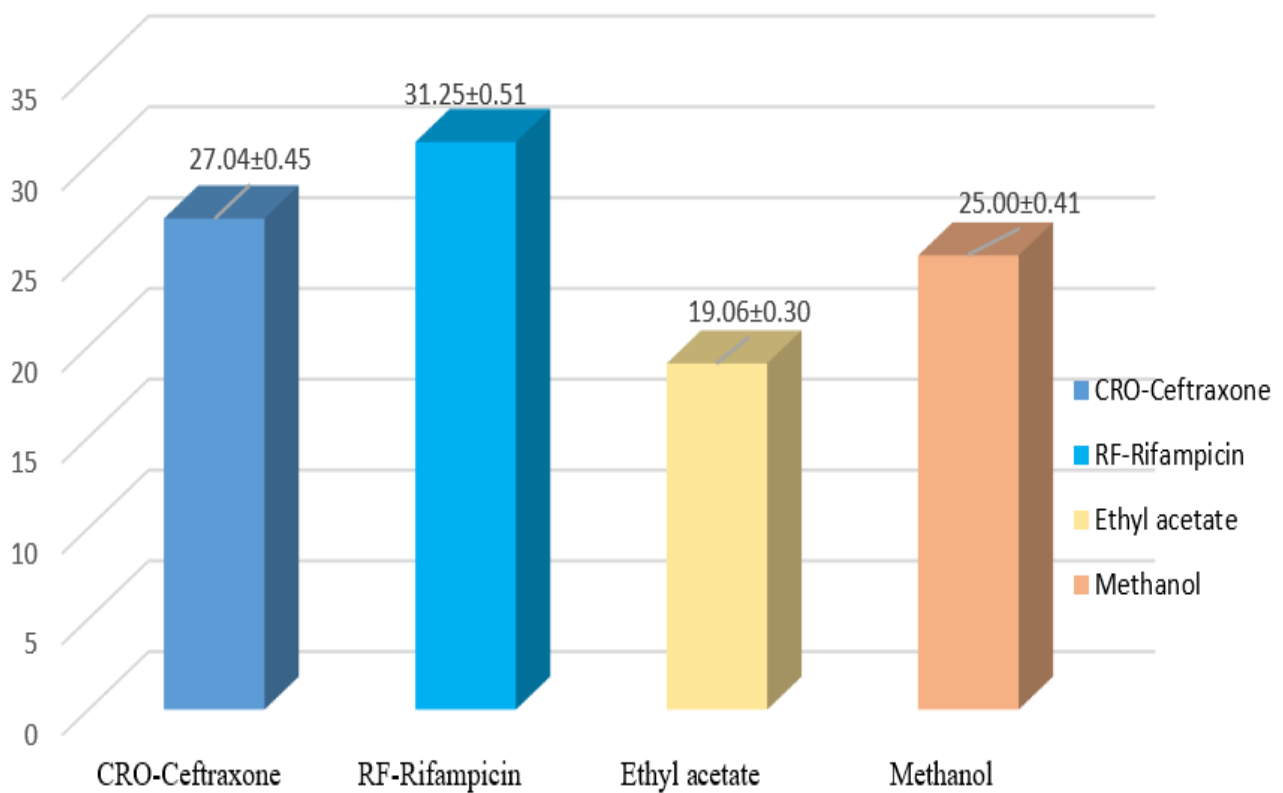


Figure 3. Bioactive metabolites of *Cinnamomum zeylanicum* and standard antibiotics RF-Rifampicin, and CRO-Ceftraxone against *Streptococcus pyogenes*

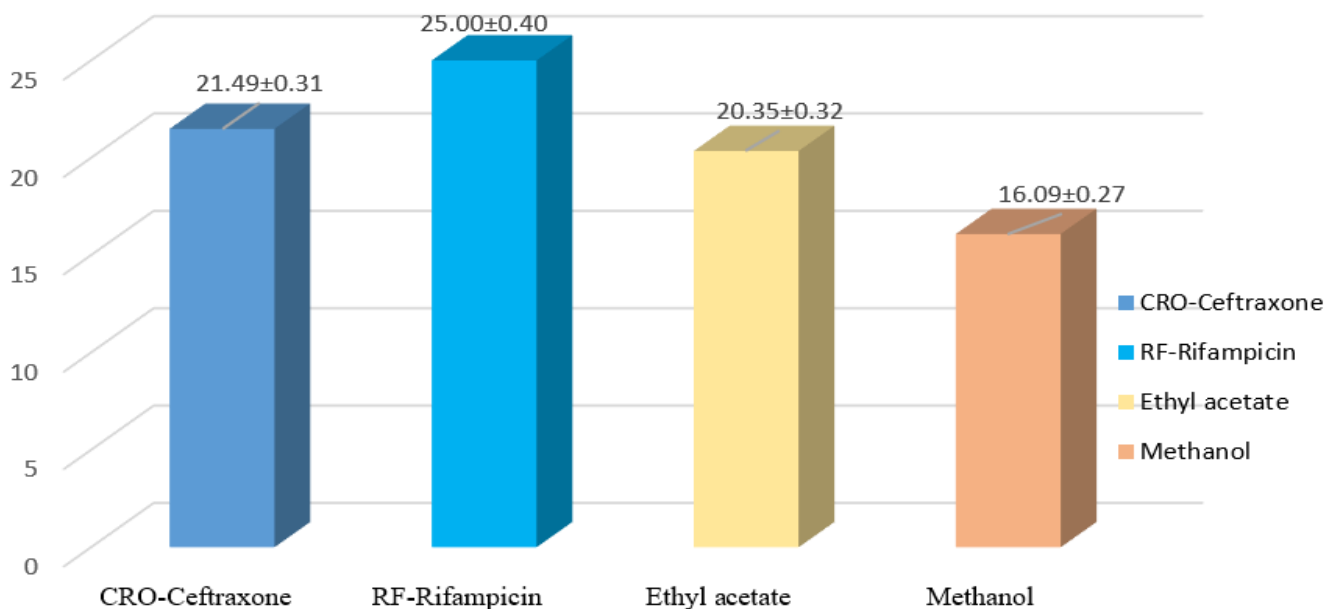


Figure 4. Bioactive metabolites of *Cinnamomum zeylanicum* and standard antibiotics RF-Rifampicin, and CRO-Ceftraxone against *Bacillus subtilis*

The antimicrobial action of the essential oil of *C. zeylanicum* was tested on disc diffusion agar, well diffusion agar, MIC, and MBC of various kinds of pathogenic Gram positive (e.g. *L. innovua*, *S. aureus* and *B. cereus*) and Gram negative bacteria (e.g. *S. typhi*, *P. aeruginosa* and *E. coli*). The results of Disc/well diffusion agar test showed that it was type-dependent; type [14, 15], it possessed the greatest inhibitory zone against *L. innocua* compared to *E. coli*. To a larger extent, the well diffusion agar had a larger area of inhibition or antibacterial action as compared to disc diffusion agar assay. Contrary to the well diffusion agar method where the essential oil is subjected to the direct physical contact between the bacteria and the essential oil, the disc diffusion agar test is performed by the level at which the antimicrobial agent diffuses through the disc surfaces to the media. As Gram-negative bacteria have a double membrane that makes them antimicrobial-resistant [16-19], it is unsurprising that the decrease in the essential oil concentration inhibited or killed Gram-positive ones. This is attributed to the fact that Gram-positive bacteria have single layer mucopeptide in cell membranes. It was found that the Gram-negative bacteria bore a more complicated lipopolysaccharide and phospholipid layer in the cell membranes which resulted in a significantly slower diffusion rate of the lipophilic

antimicrobial chemicals present in the essential oils. Researchers have made similar findings. The antimicrobial activity of the *C. zeylanicum* essential oil has been linked to compound cinnamaldehyde, which is the major chemical compound of the oil. This is believed to occur by inhibiting the formation of significant bacterial enzymes, or irreversibly ruining the walls of Gram-negative and Gram-positive bacteria and fungi. Therefore, the high level of cinnamaldehyde of essential oil of *C. zeylanicum* may be the reason of its high antibacterial activity. Mechanism of Action of the Antimicrobial Essential Oil. As a result of the appearance of sunken and deformed cells, it is possible to conclude that the essential oil of *C. zeylanicum* can harm the *E. coli* cell membrane and discharge the inner chemicals. Actually, the essential oil produced a significant amount of cell damage and lysis in the *E. coli* cells. Cells of *L. innocua* that were subjected to the essential oil also had disturbed stressed wall morphology. The deformation of the cell surfaces caused by currents of the oil on the surfaces of the cells caused wrinkles and abnormal shapes of the treated *L. innocua* cells. Anti-*E. coli* and anti-*L. innocua* effect of essential oils of *C. zeylanicum* on cell wall [20]. The oil of *C. zeylanicum* required appears to modify the membrane

structure in a fashion that it interacts with the structure and significantly alters its lipid content and the number of overall and distinct surface areas that it has. Besides, it might potentially reach the deeper layers of the cells, where it might potentially speed up their demise. *C. zeylanicum* contains many phenolic chemicals. These are quenching radicals whose structure i.e. reactive benzene rings are directly associated with those activities occurring in the biological systems. The predominant phenolic components of the *Cinnamomum zeylanicum* essential oils are cinnamaldehyde, eugenol, carvacrol, cinnamic acetate and thymol. The *C. zeylanicum* is able to treat hyperlipidemia, which is one of the properties of phenolic chemicals. This can be explained by the fact that the production of cholesterol is reduced and inhibition of the lipid peroxidation occurs. The *C. zeylanicum* was reported to possess the best antioxidant activity using the bark when compared to the other components of trees like leaves and flowers that have been used in treatment. The antioxidant properties of the essential oils seem to be the best in comparison with other plant extracts such as leaves and bark. Most types of biomolecules can react with peroxynitrite (ONOO⁻), as it breaks down to yield NO₂ radicals and OH radicals. These radicals have the ability to destroy the brain, skin, heart, lungs and blood vessels through oxidative stress. One of the cinnamon-derived active oils, eugenol, was also discovered to inhibit the damage of peroxynitrite, in laboratory experiments. On the other hand, the concentration of eugenol in the oil differs between Cinnamon variety to cinnamon variety with most active one being *C. zeylanicum*. Thus the pure peroxynitrite inhibitory activities of cinnamon oil extracts rich in eugenol can be categorized as a spice, which inhibits the action of radicals NO₂ and OH, based on their pure peroxynitrite inhibitory activities. Moreover, extracts of various organs of trees, a few in vitro and in vivo experiments have been conducted on the assessment of antioxidant effects of *C. Zeylanicum*. It has been disclosed in various researches that there are overall antioxidant level and the positive outcomes of such a level that

include the reduction of the quantity of the blood lipid peroxide, the improvement of the activity of the hepatic antioxidant enzymes, and the minimization of the risks of inflammatory diseases and infertility in men. These antioxidants are healthy and have long been utilized as a main ingredient and a preservative in the food industry where it prevents the foaming of fatty food and gives richness of taste. The capacity to manufacture less lethal foods, one with fewer health determinants as well as less synthetic contents in the manufacturing process and packaging is a major challenge of most a food company currently. The antioxidant properties of plants particularly *C. zeylanicum* have attracted the interest of both the producer and consumer. The possibility of the natural substances in terms of health benefit is tremendous due to the antioxidant qualities. In addition the natural molecules can be employed to substitute the synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *C. zeylanicum*, which is utilized as an antioxidant in food, has also been found to boost the antioxidant enzymes activity, react to reactive oxygen species (ROS), and lower malondialdehyde, a naturally occurring compound in food when high amount of oxidative stress is present. A researched article indicated that the antioxidant abilities of the *C. zeylanicum* extracts are not influenced by irradiation that is one of the present food preservative strategies. This means that the *C. zeylanicum* compounds can also survive harsh conditions of processing as well. This proves the fact that it can be used as a food preservative. Besides, *C. zeylanicum* is a nutraceutical that is utilized in pharmaceutical sector [21]. It has aroma properties that can be applied in the aroma industries that manufacture perfumes and drugs as well as edibles.

Conclusion

The data which have been presented above allow concluding that *C. zeylanicum* is an effective plant with regard to the illness prevention as it is highly concentrated with antibacterial activity. It is also evident that the molecules differ with the type of

plant, under which they are collected and mode of analysis employed to describe those characteristics even when most of the bioactive compounds that enable such functional attribute have been isolated and identified. More potent antibacterial chemicals will be inevitably found in *C. zeylanicum*. Though it is now being utilized as a spice and in traditional medicine, *C. zeylanicum* may one day become the building blocks of the chemicals in the human clinical trials in determining their effectiveness in preventing and curing various illnesses.

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