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Cinnamomum verum (true cinnamon) leaf essential oil as an effective therapeutic alternative against oral and non-oral biofilm infections: A brief review.

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Abstract

Medicinal plants play a major role as an alternative therapeutic agents for various disease conditions including cardiac and hepatic diseases, microbial infections and non-communicable disease such as diabetes mellitus. With the excessive use of synthetic antimicrobial drugs, micro-organisms become more virulent and resistant to available antimicrobial therapeutic agents. Further, the majority (around 60%-80%) of human microbial infections are biofilm associated infections and various resistance mechanisms of biofilms make it more difficult to eradicate or treat biofilms using available antimicrobial therapeutics. Biofilm structure acts as a physical barrier and prevent penetration of antimicrobial agents towards the biofilm core. Currently, scientists pay their attention to invent novel effective antimicrobial agents with less side effects for these biofilm infections. Phytochemicals have identified as a potential alternative antimicrobial strategy in biofilm control and eradication. *Cinnamomum verum* is a native Sri Lankan medicinal plant that has been widely used as a culinary spice, exhibits many medicinal benefits especially activity against microbial infectious diseases. Essential oils extracted from leaf and bark of *C. verum* have been used as a safe and effective antimicrobial agents against various infections for centuries. This review analyses the available scientific literature evidences on appositeness of true cinnamon leaf essential oil as an alternative antimicrobial strategy to control microbial biofilm infections with medical importance.

Keywords: *Cinnamomum verum,* Leaf Essential Oil, Biofilms, Infections, Natural antimicrobial agent

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Medicinal plants

Anti-microbial resistance is an emerging problem in health care facilities in modern world [1]. Resistance of organisms against available antimicrobials has become a major problem, especially when it comes to infections in immunocompromised patients. Such resistance has resulted in a dramatic increase in the incidence of systemic and opportunistic microbial infections and mortality, which leads to an increasing need for the development of effective new antimicrobial therapeutic drugs. Methicillin-resistant *Staphylococcus aureus* (MRSA), a common causative agent of nosocomial infections, and which is exhibiting a resistance to vancomycin [2], *Pseudomonas aeruginosa* [3], *Streptococcus pneumoniae*, a common respiratory pathogen [4], *Mycobacterium tuberculosis* [5], causative agent of tuberculosis; and virulent strains of *Escherichia coli* [6] are few examples for bacterial pathogens with multidrug resistance. Azole group antifungal drugs are widely used as a treatment of Candidiasis since they cause few side effects in human body. Resistance to azoles arises during long-term exposure to the drug as well as low-level prophylactic treatment regimens [7].

Due to this increasing multiresistance of etiological agents, many scientists pay their attention to develop new antimicrobial remedies to combat these microorganisms based on medicinal plants derivatives with less side effects, lower toxicity and decreased development of microbial resistance [8].

Though plants have provided western medicine with an abundance of medicines and treatments for a variety of infections, species used in traditional medicines continue to be reliable sources for the discovery of useful antimicrobial compounds. As a result, hundreds of plants worldwide are used in traditional medicine as treatments for bacterial and fungal infections. But, among these vast range of plants, very few of these have subjected to *in vitro* screening [9].

Further, natural herbal antimicrobial products are not necessarily safer than synthetic antibiotics, some patients prefer to use herbal medicines due to various reasons. Thus, healthcare professionals should be aware on the available evidence for herbal antibiotics in order to ensure safe and effective use of those herbal medicines [10].

Cinnamon species

There are different types of Cinnamon species all over the world. Among them, true cinnamon/ ceylon cinnamon/ *Cinnamomum verum* is native to Sri Lanka and belongs to the family Lauraceae.

C.cassia (Chinese Cinnamon), *C. burmannii* (Indonesian cinnamon), *C. loureiroi* (Vietnamese cinnamon) and *C. citriodorum* (Malabar cinnamon) are other major cinnamon species spread in various regions of the world [11].

The main two varieties of cinnamon are Ceylon or true cinnamon (*Cinnamon verum*) and cassia (*Cinnamom cassia*). *C. verum* is a native Sri Lankan plant and can be also found in tropical

Asia [12] and exotic to several African countries, such as Comoros, Ghana, Madagascar, Nigeria, Seychelles, Tanzania, and Uganda.

Though all of them belongs to same genus, *Cinnamomum,* they are exhibiting different physical and chemical properties.

The cinnamon tree (*C. verum*) grows in moist well-drained soils and rarely reaches more than 15 meters in height. The thick simple leaves have smooth margins and are usually oval; the veins are prominent and roughly parallel to each other. Young leaves are red/pink and mature to a deep green. The small bisexual flowers are greenish to yellow, with characteristic aroma and are borne in clusters.

True Cinnamon/ Ceylon Cinnamon/ *Cinnamomum verum*.

Other than culinary uses, in Ayurveda medicine, Cinnamon is considered a remedy for various disease conditions including respiratory, gastro-intestinal, endocrinal and gynecological ailments [13, 14]. Almost every part of the cinnamon tree, specially the bark and leaves have some medicinal or culinary use. The essential oils obtained from barks and leaves, vary significantly in chemical composition, which suggests that they might vary in their pharmacological effects as well [15].

Chemical composition

Cinnamon oil is extracted by leaves or bark of Cinnamon tree. Based on the part of the tree used for extraction, chemical composition of the extracted oil is vastly varying. Both cinnamon leaf and bark essential oils contain cinnamaldehyde and Eugenol as their key constituents but the leaf oil has higher levels of eugenol, and the bark oil has higher levels of cinnamaldehyde. Both contain trace amounts of 43 other chemical compounds including bicyclogermacrene, α-phellanderene, ß-carryophyllene, aromadendrene, p-cymene α-copaene, α-amorphene and 1,8-cineole [16,17, 18].

Oral microbiota

The oral cavity is considered a complex ecosystem with different ecological conditions, distribution of nutrients and different atmospheres, which confers different habitats to the oral microorganisms colonizing it. The resident microbiota of the oral cavity is diverse and can harbor many species of bacteria, mycoplasmas, fungi and viruses. However, both resident and transient colonizers may undergo variations depending on the individual's diet, immune conditions, saliva flow, treatments with antibiotic therapy, as well as dental treatments [19]. Due to the polymicrobial character of the oral ecosystem, local infections are usually caused by different types of microorganisms and even the interaction of more than one species, such as dental caries, periodontal disease and other fungal infections [20, 21]. Among these oral colonizers, *Streptococcus spp.* are found in both mucous membranes

and dental biofilms. These include: *Streptococcus gordonii*, *Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus mutans* and *Streptococcus sanguinis* [22]. The dental biofilm is a complex, polymicrobial structure that favors the survival of microorganisms that contribute, due to its relation of mutualism, allowing the expression of genes in order to favor communication between them, the evasion of the immune system, as well as the production of a specialized habitat for each microorganism present [22].

Biofilm formation

Biofilms are surface attached microbial communities. These communities are embedded in an extracellular matrix with host origin or microbial origin [23, 24]. These biofilms can be either monospecies biofilms which involve one microbial species or multi-species/polymicrobial biofilms. The vast majority of human microbiota exist as multispecies biofilms [25]. Biofilm formation is a complex and dynamic process and it considered to occur in four main stages. First the microbial cell adhere to the biotic or abiotic surface (initial attachment). As soon as planktonic microbial cells attach to a surface, there is a rapid alteration in the gene expression responsible for exo-polysaccharide (EPS)/extracellular polymeric substances production and maturation [26]. Then the attached microorganisms undergo irreversible attachment and form microcolonies on attached surfaces followed by biofilm maturation and dispersion of microbial cells which may then further colonize new areas [23, 27]. Even though the basic structure and development of microbial biofilms were well studied, the exact underlying processes of the transition of pathogenic microorganisms from planktonic to surface attached biofilm cells is still not well defined [27]. Importantly, sessile and planktonic microorganisms display distinct characteristics between these two states.

On the other hand, oral biofilm formation slightly differ from the formation process of other non-oral biofilm. Pellicles are formed on oral surfaces by adsorbing salivary proteins and glycoproteins. This surface conditioning film is termed the acquired enamel pellicle. The major components of pellicle are salivary glycoproteins, phosphor proteins, lipids and other host molecules. After the pellicle formation, reversibly attached acidogenic micro-organisms including *Streptococcus mutans* [28] undergo adhesin-receptor mediated stronger irreversible attachment within a short period of time. Oral bacteria generally possess more than one type of adhesin molecules on their surface and can participate in multiple interactions both with host surface molecules and similar receptors on other microorganisms. The dental biofilm pioneer species modify local ecological conditions and further promotes colonization by other species that may lead to diseases, such as caries and periodontal diseases [29]. Among these secondary colonizers, *Candida* species is considered as a major contributor.

Biofilm infections

Available statistics reveal that the majority (60-80%) of human infectious diseases including chronic wound infections, endocarditis, cystic fibrosis, dental caries, periodontitis, oral thrush [30], rhinosinusitis, osteomyelitis, meningitis, renal infections, and medical device related infections including infections in contact lenses, mechanical heart valves, central venous and urinary catheters, peritoneal dialysis catheters, pacemakers and prosthetic joints. [26, 31] are biofilm associated infections. These biofilms producing microbial species are more resistant to routine antibiotics and host defense mechanisms, which enables microorganisms to survive in harsh conditions *in-vivo*. The difficulty in treating and controlling biofilm associated infections causes the prolonged hospitalization, reduction of the efficacy of antimicrobial therapies as well as poor recovery of the patients and even deaths with systemic infections.

Biofilms and their resistance

The resistance of biofilms to antimicrobial agents is often attributed to the failure of these agents to penetrate the biofilm matrix. Drug access into the biofilms is also assisted by the presence of water channels in the biofilm structure. Nevertheless, biofilm extracellular matrix components could reduce access to such an extent that cells lying deep within a biofilm escape exposure. This would occur through adsorption or neutralization of antimicrobial agent and would depend on the thickness of the biofilm and on the chemical nature of both the antimicrobial agent and the extracellular matrix material. For example, fluoroquinolones penetrate *P. aeruginosa* biofilms readily, whereas penetration by positively charged aminoglycosides is decreased. Similarly, fluconazole penetrates single-species *Candida* biofilms more rapidly than flucytosine. Further, rates of drug diffusion through biofilms of *Candida glabrata* or *Candida krusei* are faster than those through biofilms of *Candida parapsilosis* or *Candida tropicalis*, while diffusion of antimicrobial drugs through mixed-species biofilms of *C. albicans* and *S. epidermidis* is significantly slow [32, 33].

Since biofilms exhibit greater resistance to antimicrobials compared to suspension grown planktonic cells [34], different types of strategies are used in control and treatment of biofilm infections. Traditional methods of biofilm removal include mechanical removal of oral biofilms or the use of synthetic and natural antimicrobial agents [35]. Due to lack of availability, harmful side effects and polymicrobial resistance, many studies were conducted by many scientists to find new effective alternative antimicrobial strategies in order to control these biofilm infections. As a result of those studies, many plants and phytochemicals were identified as a potential anti-biofilm remedies [9].

Cinnamon oil as an antimicrobial agent

Essential oils are mixtures of various chemical compounds originating from the secondary metabolism of plant species, constituting a rich source of biologically active compounds. Thus, the novel antifungal drug discovery based on natural herbal products is exhibiting a great success [36].

Some publish evidence revealed the potential antimicrobial activity of essential oils extracted from Cinnamon plant. For example, Nir *et al.* in 2000 investigated the effect of cinnamon extract (Chinese cinnamon/*Cinnamonum cassia*) against *Helicobacter pylori*, Azzouz et al., (1982) showed the effect of Chinese cinnamon against seven mycotoxigenic molds *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium spp.* M46, *P. roqueforti*, *P. patulum*, and *P. citrinum* and observed growth inhibition of those molds [37, 38].

Similarly Suksrikarm (1987) reported the antimicrobial activity of Cinnamon oil against many microbial species including *Lactobacillus* spp., *Bacillus thermoacidurans*, *Salmonella* spp., *Corynebacterium michiganense*, *Pseudomonas striafaciens*, *Clostridium botulinum*, *Alternaria* spp., *Aspergillus spp.*, *Canninghamella spp.*, *Fusarium spp.*, *Mucor spp.*, and *Penicillium spp.* [39]

Further, Singh *et al.,* (2007) showed the antifungal effect of Cinnamon oil against pathogenic fungi, *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Aspergillus terreus, Fusarium moniliforme, Fusarium graminearum, Penicillium citrinum* and *Penicillium viridicatum* [16].

Anti-Bacterial activity of Cinnamon oil against major oral pathogens in caries and periodontal diseases, *Streptococcus mutans*, *S. mitis*, *S. salivarius*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* was studied by Zainal-Abidin Z. *et al.,* (2013). According to authors, cinnamaldehyde was the major component of cinnamon oil (82.5% in relative amount) and cinnamon oil showed antibacterial activity against the tested bacteria (MIC 0.21 - 0.63 mg/mL and 0.8 – 0.15 mg/mL respectively). Importantly, they observed cell membrane changes following 2h exposure to the *C. verum* oil. This finding suggested cinnamon bark oil as a potential therapeutic agent in preventing bacterial-related oral diseases [40].

Oral microbial biofilms are causative agent for cariogenic oral plaques and different chemical and physical approaches are employed in controlling of oral biofilms. Among them, effect of *C. verum* essential oil on oral plaques is well documented. Wiwattanarattanabut K. *et al.*, (2017) examined the antimicrobial and anti-plaque effects of *C. verum* bark essential oil using oral biofilm forming *Streptococcus mutans* KPSK2 and clinical strain *Lactobacillus casei.* They observed a significant reduction of established biofilm biomasses with the treatment. According to their findings, the preventive effect of true cinnamon oil was in dose- and exposure time-dependent. Further at least 50% of the biofilm mass was reduced when the biofilm was treated with oil at the MIC for an hour [41].

Filoche *et al.* conducted a study in 2005 on Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. In their study they used Cinnamon oil as an anti-microbial agent. According to their observations, Cinnamon exhibited the greatest antimicrobial potency (1.25–2.5 mg/ml) against cariogenic *Streptococcus mutans* and *Lactobacillus plantarum in-vitro* biofilms [42].

Study conducted by Moon et al. in 2011 demonstrated a high antibacterial activity of eugenol against some oral bacterial species including *S. gordonii* and *Porphyromonas gingivalis*, moderate antibacterial activity on *S. mutans*, *Fusobacterium nucleatum*, and *Prevotella* as well as weak antibacterial activity on *S. sobrinus*, *S. sanguinis* and *S. anginosus* etc [43].

Mode of antimicrobial action of true Cinnamon leaf oil

Phenolic compound eugenol belongs to the group of phenylpropanes is the major active component of *C. verum* leaf EO which is responsible for its bioactivity. There are few hypotheses developed by various scientists to explain the mode of antimicrobial action of eugenol. Schmidt *et al.* in 2007 researched on the anti-*Candida* activity of *C. verum* leaf oil and according to them, the antifungal activity is caused by cytochrome P-450 mediated conversion of eugenol into quinone methide. This cytotoxic Quinone methide deplete the levels of intacellular glutathione (GSH) and react with cellular macromolecular components which cause eventual cell apoptosis [44].

Linalool is another component of *C. verum* leaf EO, which contains in small amounts. This linalool can be contributing in controlling virulence mechanisms of *Candida* spp. including inhibition of germ tubes and transition to the hyphal form [44].

Another review conducted by Marchese *et al.* in 2017 identified several other modes of actions of Cinnamon leaf EO on different bacterial species. Eugenol can interfere with cell membrane integrity and nonspecific permeability by altering the membrane fatty acids and disrupt the plasma membrane of bacterial cells. Further, eugenol can inhibit bacterial enzymes of some bacterial species such as *Escherichia coli* and *Bacillus subtilis* [45].

Formation of cytotoxic intracellular Reactive Oxygen Species (ROS) is another antibacterial mechanism of action of eugenol containing *C. verum* leaf EO which causes lipid peroxidation and increases cell permeability by damaging the cell envelope [45, 46].

Cytotoxicity of *C. verum* Oil

Cinnamon has been used as a spice and as a traditional herbal medicine for centuries in many parts of the world. The available *in vitro* laboratory and animal *in vivo* experimental evidence suggest that cinnamon has anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hypo-lipemic, and immunomodulatory effects etc. Further, modern scientists researched on the cytotoxic effects of cinnamon oil to ensure safe use of this important phytochemical agent.

Unlu M. *et al.*, (2010) investigated the antimicrobial activity of the oil in order to

evaluate its efficacy against 21 bacterial species (ATCC) and 4 *Candida* species (ATCC) including, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis, Candida albicans, Candida parapsilosis* and *Candida krusei* using disc diffusion and minimum inhibitory concentration methods. They noticed significant antimicrobial effect of *C. verum* oil. The cytotoxic and apoptotic effects of the essential oil on fibroblasts were examined by MTT assay and acridine orange/ethidium bromide staining. They observed a strong cytotoxicity with less than 20 µg/mL IC_{50} values for their experiments. Importantly, cytotoxic effect on cancer cell lines were significantly higher than effect on normal cell line. Their study showed the potential antimicrobial and anticarcinogenic properties of the essential oil of Cinnamon, indicating the possibilities of its potential use as remedies for the topical treatment of infections and neoplasms [47].

Another study conducted by Singh *et al.,* (2009) analyzed the cytotoxic effect of an aqueous Cinnamon extract (ACE) from the bark of *C. verum* on various human as well as mouse cell lines and primary cells. As they found, ACE was cytotoxic to cancerous cells at concentrations just above 0.16mg/mL (containing 1.28 µM cinnamaldehyde). At a critical concentration of 1.28mg/mL (containing 10.24 µM cinnamaldehyde), ACE treatment resulted in 35-85% growth inhibition of the majority of the cancerous cells which indicates that ACE had a significant inhibitory effect on the majority of cancer cells and thus may prove to be a chemotherapeutic agent [48].

El-Meleigy *et al.,* (2010) conducted a research to study the cytotoxicity of four essential oils including cinnamon oil. They could not observe any deleterious effect on two human cells namely, MRCs (lung normal cell) and CRL-1539 (colon normal cell) when cells subjected to low concentration of essential oils from 1-10 µL/mL, while by increasing concentration to 25 µg/mL the number of surviving cells was decreased. At 100 µL/mL, the percentages of surviving cells were 61% for cinnamon that was the maximum post-exposure survival rate for oils tested [49].

A study conducted by Assadollahi *et al.,* (2013) determining the effects of aqueous true Cinnamon extract on HL-60 cells as a model for Acute promyelocytic leukemia (APL) .According to their observations, Cinnamon extract inhibited the growth of HL-60 cells as correlated with concentration and time. After 72 h of exposure, the growth of HL-60 cells with 0.01 mg/L Cinnamon extract, was inhibited by 90.1%. Further as a conclusion, they introduce true cinnamon extract as a single drug or besides other medications for treating promyelocytic leukemia [50].

A recent study published by Wijesinghe *et al*., (2020) used *Galleria mallonella* larvae experiment model for determination of *in-vivo* toxicity of *C. verum* leaf EO and they found that *C. verum* leaf oil does not exhibit any toxic effect on experimental model at any concentration tested ranging from 0.5 to 1000 mg/mL [18].

Conclusion

C. verum essential oil is a potential alternative antimicrobial strategy for oral and non-oral infection control. Though *C. verum* leaf oil is identified as a potential non-toxic, effective microbial biofilm control strategy, there are lack of published data to support the observations, specially anti-*Candida* biofilm effect, *ex-vivo* and *in-vivo* toxicity of *C. verum* leaf oil. Since *Candida* is the major etiological fungal species for acidogenic oral plaque formation, it is very important to conduct research on this aspect as well.

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