



In Vitro Antimicrobial Activities of *Cinnamomum Iners* Leaf and Bark Extracts Against Pathogens of Food Borne Diseases



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Submission: 📅 March 31, 2018; Published: 📅 May 10, 2018

Abstract

In general, food borne diseases may arise from the consumption of contaminated food due to microbials. *Cinnamomum iners* Reinw. ex Blume is belongs to the family of *Lauraceae* which used as traditional herb to cure fever and digestive problem. In this study, we evaluated the antimicrobial activity of leaf and bark extracted with ethanol, acetone and water. Two different concentrations (15 and 30µg) were used to compare the antimicrobial activity using *in vitro* disc diffusion zone. Five bacterial strains such *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and three fungal strains namely *Trichophyton rubrum*, *Aspergillus fumigatus* and *Candida albicans* were used. Bark extracts of *C.iners* exhibited higher inhibition zone against bacterial strains. Our present study concludes that bark extracts could be potential source to inhibit wide range of micro organisms than leaf extracts of *C.iners*.

Keywords: Antimicrobial; *Cinnamomum iners*; Bark extract; *In vitro* disc diffusion

Introduction

Plant secondary metabolites exhibit several biological applications and becoming great attention, recently [1]. People utilize enormous therapeutic medicinal plants for various human ailments and proved as novel resource of bioactive compounds as antimicrobial agent for more than centuries [2]. In general, the antimicrobial agent is a chemical or physical agent which control or even kill the microorganisms. And also, it disturbs the growth and reproduction of microorganisms such as bacteria, fungi, parasites and viruses. Of them, fungi like *Aspergillus fumigatus* and *Candida albicans* have capability to cause life-threatening systemic infections [3,4]. Moreover, bacteria causes following poultry diseases such as, *Escherichia coli* infections, salmonellosis, paratyphoid infections, fowl cholera, *Anatipestifer* infections, *Mycoplasma*, necrotic enteritis, cholangiohepatitis in broiler chickens, gangrenous dermatitis, botulism, avian tuberculosis. The fungi, *Aspergillus fumigatus* causes Aspergillosis (Fungal Pneumonia) in poultry while Orthomyxo virus produce Avian influenza. Several causative agents such as, *Mycoplasma synoviae*, *Staphylococcus aureus*, *E.coli* and certain reoviruses are causes infectious synovitis. Respiratory viruses and *E. coli* induce *Mycoplasma gallicepticum*. Aspergillosis is an acute or chronic respiratory disease, *Aspergillus*

Granulomatous Dermatitis as a postvaccinal complication which observed in growing broiler breeders, Aflatoxicosis, Candidiasis, Fusariotoxicoses. Omphalitis (navel infection) is characterized with reddening and tissue oedema in the umbilical region [5].

Furthermor, diarrhea associated with *E. coli* take place in young piglets within a few days of birth through well after weaning. Occasional cases of septicemia are attributable to *E. coli* [6]. Colibacillosis is a disease caused by the bacterium *E. coli*, which normally resides in the lower intestines of most warm blooded mammals, including dogs. It is a common disease in poultry and systemic infection occurs when large numbers of Avian Pathogenic *E. Coli* (APEC) gain access to the bloodstream from the respiratory tract or intestine [7,8]. Bacteremia progresses to septicemia and death, or the infection extends to serosal surfaces, pericardium, joints, and other organs. Infected cattle seem to be the most frequent source of infection, although buffalos, goats, sheep and camels can also pass on the bacteria [9].

Milk production is associated with agriculture for the reason that farmers nurture the live stocks in villages. Though dairying associated with agriculture, milk is consumed by only rich sector

of people and consumption of milk is a night mare for poor section of people and hence results in undernourishment or malnutrition. India is the largest producer of milk and accounts for more than 13% of milk production [10]. Milk is an essential part of daily diet for the growing children and expectant mothers. Milk is a major constituent of the diet; its quality assurance is considered essential to the welfare of a community. Milk is nutritious food for human beings, also serves as a good medium for the growth of many microorganisms *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Microbacterium*, *Propionibacterium*, *Micrococcus*, *coliforms*, *Proteus*, *Pseudomonas*, *Bacillus* especially *E.coli*, *S. typhi*, *Paeruginosa* and *S.aureus* [11,12].

Bacterial contamination of raw milk can originate from different sources of animals such as air, milking equipment, feed, soil, feces and grass [13]. Milk is spoiled by a wide range of microorganisms some of which are pathogenic and are responsible for milk borne diseases [14,15]. Not only raw milk, also pasteurized milk, concentrated milk, dried milk, cottage cheese, yoghurt, buttermilk, cream cheeses are also spoiled by microbes like bacteria and fungi. Common contaminating yeasts of cheeses include *Candida spp.*, *Kluyveromyces marxianus*, *Geotrichum candidum*, *Debaryomyces hansenii*, and *Pichia* spp. [16]. Ternstrom et al. [17] found that five taxa of psychrotrophic *Pseudomonas* spp. were involved in the spoilage of raw and pasteurized milk. Brucellosis is one classical example of milk borne infection. *Brucella* spp being transmitted from goats to humans either through direct contact or through the milk of the infected animal. *Brucella* is responsible for a type of granulomatous hepatitis or an acute febrile illness in human. Tuberculosis is another disease which can be transmitted through raw milk [18]. *Candida* species are considered as pathogens because of their versatility and ability to survive in various anatomical sites [19]. *Candida* species found on normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina [20], and are responsible for various clinical manifestations from mucocutaneous overgrowth to blood stream infections [21].

Cinnamomum iners is an evergreen tree belonging to the family *Lauraceae*. According to the Agro forestry Tree Database, *C. iners* is commonly found in India, Myanmar, Thailand, Malaysia, Indonesia and Southern Philippines. The major bioactive compounds of this plant are saponins, terpenes, cinnamic aldehyde and eugenol [22]. Medicinal properties of *C. iners* that include its antiplasmodial, cytotoxicity, amylase inhibitor, antinociceptive and anti-inflammatory, anti-diarrheal, carminative activity reported by ethnobotanical reports [23- 26]. With the synonym of wild cinnamon, the bark of this plant was used as a substitute for cinnamon in various parts of Malaysia and Thailand. Even this plant has multiple therapeutic uses, only very few reports are available [27]. The plant leaves were used to cure fever, digestive problem, headache and minor wounds [28]. The bark is used as detoxifying agent by taking as a tea [22]. In our present study, the antimicrobial activities of both leaves and bark extracts of *C.iners* against pathogens of food borne diseases.

Materials and Methods

Collection of plant material

Matured leaf and bark of *C. iners* was collected in Kumarapuram, Kanyakumari District. The voucher specimen was numbered and deposited in St.Xavier's College Herbarium (XCH), Palayamkottai. Then plant material was washed and shade dried in room temperature. The dried plant material was coarsely powdered and stored in an air tight container for extract preparation.

Preparation of plant extracts

10gms of the dried powdered plant materials were soaked (1:6 w/v) separately with 60ml of each of the solvents viz. acetone, ethanol and water in a soxhlet apparatus for 48hr (according to the boiling point of each solvents used) until complete extraction of the materials. At the end of 48hr each extract was filtered through What man No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. All the extracts were stored at 4 °C in air tight bottles for further studies.

Microbial cultures

Test bacteria: Five bacterial species were tested and collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (Punjab), India. The bacteria include viz Gram negative *Escherichia coli* (MTCC 1195), *Staphylococcus aureus* (MTCC 96) *Serratia marcescens* (MTCC 8780), *Klebsiella pneumoniae* (MTCC 2405) and *Pseudomonas aeruginosa* (MTCC 424).

Test Fungi: The fungal moulds consist of *Trichophyton rubrum* (MTCC 296), *Aspergillus fumigatus* (MTCC 2550) and *Candida albicans* (MTCC 227) were also collected from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, Punjab, India.

In vitro antimicrobial activities

Disc diffusion assay as described by Rosoanaivo and Ratsmanaga-Urverg and Rabe and Van Staden [29- 31] was used for anti-bacterial activity. Sterile double distilled water and Chloramphenicol (10mg/disc) were used as positive reference control to determine the sensitivity of plant extract on each bacterial species. The antifungal activity of various extracts of *Cinnamomum iners* were tested by disc diffusion method [32]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation.

The filter paper discs (What man No. 1 diameter) impregnated with 15 and 30µg/ml concentrations of the extracts were placed on test organism-seeded plates. DMSO (2%) was used to dissolve the extracts before application on test organism-seeded plates. Nystatin (10µg/disc) used as positive control and the activity was determined after 72hrs of incubation at 28 °C. The diameters of the inhibition zones were measured with a caliper. All experiments were carried out in triplicate and their results are expressed as mean±SE (n=3). The statistical analyses were performed by one way ANOVA with SPSS 17.0 software (Chicago, USA) and relationships were considered to be statistically significant when P<0.05.

Result

Table 1: *Aspergillus fumigatus* and *Candida albicans*.

Samples (µg/ml)	<i>Escherichia Coli</i> (cm)	<i>Staphylococcus Aureus</i> (cm)	<i>Serratia Marcescens</i> (cm)	<i>Klebsiella Pneumoniae</i> (cm)	<i>Pseudomonas Aeruginosa</i> (cm)
LEAF (cm)					
Acetone 15	0.70±0.00	0.93±0.06	-	-	-
Acetone 30	0.76±0.03	0.96±0.03	0.73±0.03	-	-
Ethanol 15	-	-	0.70±0.00	-	-
Ethanol 30	0.76±0.03	-	0.76±0.03	-	-
Water 15	-	-	-	-	-
Water 30	-	-	-	-	-
BARK					
Acetone 15	0.93±0.08	0.83±0.03	-	0.76±0.03	-
Acetone 30	0.93±0.03	0.83±0.03	0.73±0.03	0.80±0.05	-
Ethanol 15	0.73±0.03	0.70±0.00	-	0.76±0.03	-
Ethanol 30	0.86±0.03	1.00±0.00	-	0.83±0.03	-
Water 15	-	0.76±0.03	-	-	-
Water 30	0.86±0.03	0.90±0.05	0.76±0.03	0.73±0.03	0.76±0.06

In the present study, both leaf and bark extracts of *C.iners* were prepared in various organic solvents and screened for antibacterial and anti fungal activity with two different concentrations (15 and 30µg). The results were summarized in the (Table 1). The inhibitory activities of the *C.iners* extracts were compared with the standards. Leaf acetone extract (30µg) showed maximum inhibition zone against *S.aureus* (0.93±0.06cm) than *E.coli* (0.76±0.00cm). On the other hand, leaf ethanol extract (30µg) showed minimum antibacterial activity (0.76±0.03cm) than ethanol extract (15µg) against *S.marcescens* (0.70±0.00cm) and *E.coli* (0.76±0.03cm). There was no antibacterial activity shown by different leaf extracts against *K.pneumoniae*, *Paeruginosa*. No significant activity was found in leaf water extract (15 and 30µg).

The bark acetone extract (30µg) of *C.iners* showed maximum inhibitory activity against *E.coli* (0.93±0.03 cm) than *S. aureus* (0.83±0.03cm) and *S. pneumoniae* (0.80±0.05). Bark ethanol extract (30µg) showed maximum inhibitory zone against *S.aureus* (1.00±0.00 cm) than *E.coli* (0.86±0.03cm) and *K. pneumoniae* (0.83±0.03cm). Furthermore, bark water extract showed maximum inhibitory against *S.aureus* (0.90±0.05cm) and minimum antibacterial activity against *K. pneumoniae* (0.73±0.03cm). No effective inhibitory activity was shown against any of the tested fungal microorganisms (*Trichophyton rubrum*, *Aspergillus fumigatus* and *Candida albicans*).

Discussion

Plants have the capability in order to resist disease by low molecular weight compounds like secondary metabolites such as alkaloids, coumarins, isoflavonoids, polyacetylenes, quinones, tannins and terpenes which proved antimicrobial activities. Plants have unlimited capacity to synthesize aromatic secondary metabolites. Most of them are phenols or their oxygen-substituted derivatives [33]. Moreover, traditional medicine practitioners' uses

water mostly to prepare plant extracts whereas, organic solvents have been found to give more consistent antimicrobial activity when compared to water extracts [34]. In several previous studies, methanol, ethanol, and water were used frequently to prepare plant extracts for biological activities like antimicrobial activity [34- 38].

Antimicrobials of plant origin have enormous therapeutic potential. There is a long history of providing the novel therapeutics from plant-derived antimicrobials [39]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phyto chemicals and are used to cure the disease in herbal and homeopathic medicines [40]. These are non-nutritive substances and have protective or disease preventive property [41]. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds [42].

There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In previous studies, Sheng Yang et al. [43] reported that *Cinnamomum osmophloeum* (*Lauraceae*) possesses significant anti fungal activity because of Cinnamaldehyde, the major compound in the leaf. With advances in phytochemical techniques, several active principles of many medicinal plants have been isolated and introduced as valuable drug in modern systems of medicine. *C.iners* methanolic extracts possessed anti-microbial activity against gram positive and negative pathogenic bacteria [44]. *C.zeylanicum* is most sensitive in controlling the growth of *B.pseudomallei*, *S.aureus*, *K. pneumoniae*, and *S. pneumoniae*. And, it is found to be very effective against the multidrug resistant human pathogen *B. pseudomallei* that cause melioidosis.

Alkaloids are reported for anti bactericidal effects in earlier studies [46-48]. Cinnamaldehyde is an aromatic aldehyde and main component of bark extract of cinnamon (*Cinnamomum verum*) and are proved to be active against many pathogenic bacteria [49,50]. The stem bark of C.tamala has antibacterial potential [51]. Leaves and barks of cinnamon have aromatic, astringent, stimulant and carminative activities [52]. In conclusion, both leaf and bark extract of *C.iners* studied here which provide preliminary idea that this is potentially rich in antimicrobial compounds. As per previous report [53], High Performance Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GCMS) will be carried out to screen bio-chemical constituents present in the bark and leaf extract which responsible for the antimicrobial activity in our further studies.

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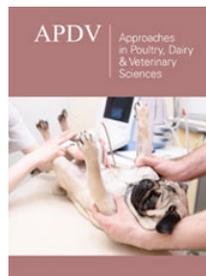
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