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Antibacterial, antioxidant, collagenase inhibition, urease inhibition activities and chemical composition of the essential oil and extracts of *Cinnamomum zeylanicum* bark.

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Abstract

Significant biological activities of bacterial infection like antibacterial, antioxidant, urease and collagenase inhibitory activities of the essential oil and various extracts of *Cinnamomum zeylanicum* were determined. The *in vitro* antibacterial activity against nine epidermal bacteria and 11 MRSA isolates was conducted. The essential oil of *C. zeylanicum* exhibited the activity against all bacteria with the Minimum Inhibitory Concentration values (MIC) ranging from 0.15 to 2.5 µl. The extracts showed declined antibacterial activity. The scanning electron microscopic (SEM) studies demonstrated potential detrimental effect of the essential oil on the morphology of tested bacteria. Antioxidant activity of all the samples was also determined and 20% activity was observed in essential oil. Collagenase inhibitory potential was upto 25% and 30% in aqueous and methanol extracts respectively while upto 35% in essential oil. None of the samples showed urease inhibitory activity. The chemical composition of hydrodistilled essential oil of *C. zeylanicum* was also analysed by GC-MS. A total of 13 compounds representing 100% of the oil were identified. Therefore, due to different chemical components present in the cinnamon essential oil, further investigation concerned with studied activities of this essential oil to replace the medicines or as supplement in diseases, is recommended.

Keywords: Cinnamomum zeylanicum, Antibacterial activity, Minimum inhibitory concentration, Essential oil, Collagenase, Urease, Antioxidant activity.

Introduction

Epidermis acts as a barrier to infection, however if its defenses are disrupted, a variety of micro-organisms can cause disease. Our body has a resident flora of micro-organisms, including bacteria, yeasts and mites. The presence of these micro-organisms is important in preventing foreign organisms from colonization. But these may prove harmful if enter into otherwise sterile sites, for example when skin integrity is breached. Most infections result from a break in the epidermis such as in surgery, ulcer, cuts, animal or insect bites or burns. When injury occurs the opportunistic microorganisms, invades and multiply leading to a delay in the healing process and finally infectious condition. Some of the microorganisms frequently isolated from wound infection include Staphylococci, Pseudomonads, Proteus species, Bacilli, Candida species and Klebsiella amongst others. Eradication of these pathogens leads to rapid wound healing.

Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem. The resistance of the organisms increased due to indiscriminate use of commercial antibacterial drugs commonly used for the treatment of infectious diseases. In past few years there has been a gradual revival of interest in the use of medicinal plants in developed as well as in developing countries because plant derived therapeutics have been reported to be safe and without side-effects. This development of microbial resistance has led the researchers to search the antibacterial activity of medicinal plants. Therefore, there has been a growing interest in research concerning alternative natural antibacterial agents, including the extracts and essential oils from various species of edible and medicinal plants, herbs, and spices that are relatively less damaging to human health.

Cinnamomum zeylanicum is a medicinal plant belonging to family Lauraceae that is also used as a source of food. It is a tropical evergreen tree, native to Sri Lanka and the Malabar Coast of India. In India, Southeast Asia, United States and in the European countries, cinnamon is used for flavouring foods, beverages, boiled beef, pickles, chutneys and ketchup. Medicinally, cinnamon is used to stimulate the circulation, relieves spasms and helps to control high blood pressure, bleeding and indigestion. It has been used for aromatherapy treatment of diarrhea, stomach upset, against respiratory ailments and externally as a skin antiseptic and rubefacient [1,2, 3]. Cinnamaldehyde has been reported to be the major constituent of cinnamon. Earlier studies suggested that antibacterial activity of cinnamon was probably due to their major component cinnamaldehyde and their properties could be multiple

Bacterial infection leads to stress of the host. It is a challenge which needs to be coped. The production of ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) is increased during the stress imposed by host's defense system. The ROS-RNS detoxifying systems are considered essential for virulence of bacteria. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress [4, 5]. Modulation of stress can be considered one of the factors controlling pathogenicity or virulence of bacteria. .

Among the various factors responsible for pathogenesis some enzymes like Collagenases and Urease play crucial roles. Collagenases act as invasions, released by pathogen and locally damaging host cells, facilitating growth and spread of the pathogen. Collagenases are members of the matrix mettaloproteinases (MMP) family of proteolytic enzymes. It breaks down collagen, the framework of muscles. Urease hydrolyses urea, releasing ammonia, which neutralizes acids of stomach and thus enables survival and initial colonization of the pathogen. Most of the urease is found in bacterial cytoplasm, although upto 10%appears on the surface. The present work is the first attempt to examine the collagenase and urease inhibitory activity of *C. zeylanicum* with an aim to search a therapy having dual mode of action of killing bacteria as well as regulating virulence factor.

Materials and Methods

Plant material

C. zeylanicum bark was procured from local market of Kurukshetra, Haryana, India. The plant species was confirmed by a botanist and a voucher specimen (GC-411) was preserved at Wild Life Institute of India Dehradun. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and ground to powder.

Chemicals

Cow urine sealed bottles from counter of Divya Pharmaceuticals were purchased. Before evaluation of antibacterial activity, cow urine was tested for presence of other pathogens microscopically as well as in broth culture. All other chemicals were of analytical grade and purchased from Hi- media Pvt Ltd Mumbai and Sigma Aldrich.

Preparation of essential oil and Plant Extracts

Air-dried *C. zeylanicum* bark was pulverized into powdered form. The powder of sample (250g) was subjected to hydrodistillation for 4 h in a Clevenger-type apparatus to obtain the essential oil. The distillated oil was dried over anhydrous sodium sulfate and preserved in a sealed dark vial at 4 C until further analysis (0.68% w/w). Plant material (10gm) was extracted with 100ml each methanol, water and cow urine by hot extraction method.

Antibacterial Studies

Microbial strains

The *in vitro* antibacterial activity of the essential oil and extracts from *C.zeylanicum* bark was tested against nine epidermal infection causing bacteria. Five Gram-positive strains were *Staphylococcus aureus* (MTCC 3160 and NCDC 109), *Staphylococcus epidermidis* (MTCC 435 and MTCC 3086), *Staphylococcus hominis* (MTCC 4435) and four Gram-negative bacteria were *Pseudomonas* aeruginosa (MTCC 7453 and MTCC 424), Klebsiella pneumonia (MTCC 4030) and Proteus vulgaris (MTCC 426). The bacteria were procured from Institute of Microbial Technology (IMTECH) Chandigarh and National Dairy Research Institute (NDRI) Karnal. 11 MRSA isolates from human nasal secretion were also used in the study. The strains were cultured at 37^0C on nutrient agar medium in aerobic conditions for 24 hrs.

Determination of Zone of Inhibition, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration

Agar well diffusion method was carried out by allowing perforation of various extracts and essential oil dissolved in DMSO at a concentration of 30mg/ml and 100µl/ml respectively. Petri plates containing 30 ml nutrient agar medium were kept for the solidification before inoculating 10^6 CFU/ml microorganism, desired numbers of wells of uniform diameter of 8mm were made after solidification, using sterile aluminium borer. After incubation for 24 h at 37 ^{0}C the plates were observed and the antibacterial activity was evaluated by measuring zone of inhibition (diameter mm). The tests were conducted in triplicate. Ciprofloxacin (10.0 µg/ml) was used as positive control. The negative control was 10% DMSO.

MIC of extracts was determined by microdilution technique as described by the National Committee for Clinical Laboratories standards (2000) [6]. The bacteria inoculums were prepared in 5 ml nutrient broth and incubated at 37^0C. The final inoculums were of approximately 10^6 CFU/ml (Mcfarland standard) [7]. Controls with 0.5 ml of culture medium without the samples and other without microorganisms were used in the tests. Tubes were incubated at 37^0C for 24 h. The activity was measured as a function of turbidity at 660 nm. Lack of turbidity was further confirmed by pouring suspension aliquot of 0.1 ml into pre-sterilized Petri dishes with nutrient agar medium. The tests were conducted in triplicate.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of compound required to kill a particular bacterium. Directly after the MIC was determined, a sample was taken from each test tube that exhibited no increase in OD_{600} (no bacterial growth) and was streaked onto freshly prepared agar plates. After incubation for 24 h at 37 ^{0}C the plates were inspected for growth of the bacteria. The MBC was determined to be the concentration at which no bacterial growth was observed.

Scanning electron microscope (SEM) observations

Scanning Electron Microscopy has been widely used in microbiology to measure the changes in morphology of bacteria. This work also validates the use of SEM to differentiate between treated and untreated cells. To determine the efficacy of the shortlisted sample and morphological changes of the bacteria, SEM observations were performed on the tested bacteria. The method of SEM was modified from Kockro method [8]. The bacterial cells were incubated for 24 h in nutrient broth at 37 C. The suspension was divided into two portions. To one portion was added suitable concentration (MIC) of the essential oil. The other portion was left untreated as a control. The bacterial sample was washed gently with 50mM phosphate buffer solution (pH 7.2) fixed with 2.5% glutaraldehyde and 2% Osmium tetroxide. The specimen was dehydrated using sequential exposure per ethanol concentration ranging from 30% to 100%. After dehydration, the specimen was dried with CO_2.Finally the specimen was sputter coated with gold in an ion coater for 2 min, followed by microscopic examinations (Zeiss 950 DSM).

Antioxidant activitiy

1,2-diphenyl 1 -picryl hydrazil (DPPH) assay is very convenient for the screening of large number of samples of different polarity because of its high throughput. It evaluates the ability of antioxidants to scavenge free radicals. The free radical scavenging activity of essential oil and extracts was assayed at different concentrations from 10-100 µg/ml. The absorbance was measured at 517 nm and activities were compared with L-ascorbic acid as standard. The free radical scavenging activity of each solution was then calculated as percent inhibition. Antioxidant activities of test compounds or extracts were expressed as IC_{50}, defined as the concentration of the test material required to scavenge a 50% of initial DPPH concentration.

Inhibition (%) = 100 (A blank-A sample)/Ablank

Gelatin digestion assay for Collagenase inhibition activity

Collagenases belong to matrix metalloproteinases (MMP) family of proteolytic enzymes which are involved in the degradation of triple helical region of native collagen. In order to determine the collagenase inhibitory activity of *C. Zeylanicum* agarose solution (1%) was prepared in collagenase buffer (50mM tris- HCL, 10 mM $CaCl_2$, 0.15M NaCl, 7.8 pH) with 0.15% porceine gelatin (Sigma Aldrich, Cat. G-2500) and allowed to solidify in wells of 6 well plate (3ml/well) for 1h at room temperature. Different concentrations of Essential oil and extracts (30 µl) were incubated with 50 µl of bacterial collagenase-1 (0.1mg/ml) in 50 µl of collagenase buffer for 30 min. The reaction mixture (10 µl) was loaded onto paper disks placed on gelatin-agarose gel and incubated for 18h at 37^0C. The degree of gelatin digestion in agarose gel was visualized by Coomassie Blue staining after removal of the paper disks. Following destaining, the area of light translucent zone over blue background was determined to estimate gelatinase activity.

Assay for urease inhibition

Ureases are the enzymes that catalyze the conversion of urea to ammonia, which reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield a blue green dye (indophenol) with λ_{max} 625 nm. The intensity of the color is proportional to the concentration of ammonia produced by the urea. To determine the urease inhibitory activity 0.1 ml of a sample solution was incubated for 5 min. with 0.2ml of a Jack bean urease solution and 1.2 ml of phosphate buffer pH 8.2 at 37^0C in test tubes. After a preincubation, 0.5 ml (66mM) of urea was added to the reaction mixture, and the whole incubated for 20 min. Urease activity was determined by measuring the ammonia produced with the indophenols method described by Weatherburn [9]. Briefly, 1 ml each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and an alkaline reagent (1% w/v NaOH and 0.075% active chloride NaOCl) were added to each test tube. The increase in absorbance at 640nm was measured after 30 min, all reactions being performed in triplicate in a final volume of 4 ml. Thiourea was used as the standard inhibitor of urease.

Percent inhibition (%) : 100-(OD_{sample}/$OD_{control}$) 100.

GC-MS analysis

Among all the samples tested for various biological activities, essential oil was found to possess highest activity. The components of essential oil from *C.zeylanicum* bark was analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 µl of extracted sample was injected in GC-MS equipped with a split injector and a PE Auto system XL gas chromatograph interfaced with a Turbo-mass spectrometric mass selective detector system. The MS was operated in the EI mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 ml/min. The analytical column connected to the system was an Rtx-5 capillary column (length-60m 0.25mm i.d., 0.25 µm film thickness). The column head pressure was adjusted to 196.6 kPa. Column temperature programmed from 100˚C (2 min) to 200˚C at 10˚C/min and from 200˚ to 300˚C at 15˚C/min with hold time 5 and 22 min respectively. A solvent delay of 6 min was selected. The injector temperature was set at 270 C. The GC-MS interface was maintained at 280 C. The MS was operated in the ACQ mode scanning from m/z 40 to 600.0. In the full scan mode, electron ionization (EI) mass spectra in the range of 40–600 (m/z) were recorded at electron energy of 70 eV. Compound identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley or by comparing the RT with those of authentic standards available.

Statistical analysis

The results of various studies were analyzed using analysis of variance (ANOVA) with Statistical Analysis System (SAS) Version

9 (SAS Institute, Cary, NC, USA) [10]. Significant differences among means from triplicate analyses ($p < 0.05$) were determined by Duncan's Multiple Range Test. All the tests were carried out in triplicate and the results were expressed as mean ± standard deviation (SD).

Results and Discussion

Antibacterial activity and Scanning Electron Microscopy

The *in vitro* antibacterial potential of oil and various extracts against the employed microorganisms was quantitatively assessed by the presence/absence of inhibition zones, MIC and MBC values. Essential oil showed good *in vitro* antibacterial activity against all the tested bacteria including Gram-positive and Gram-negative bacteria with diameter zones of inhibition 21-40mm along with MIC ranging from 0.15 to 2.5 µl. Gram positive bacterium *S.aureus* (NCDC 109) was found to be most sensitive with an inhibition zone of 40mm. whereas minimum zone of inhibition (21.33mm) was reported for *S.aureus* (MTCC 3160). Essential oil also showed strong inhibitory effects against MRSA isolates with zones ranging from 26-35mm. MRSA I and MRSA IX were found to be resistant. Among the gram negative bacteria *P.aeruginosa* (MTCC 424) was most susceptible having an inhibition zone of 32.66 mm. The most resistant bacteria was *K.pneumoniae* (26.33mm). (Table 1and Table 2)

Table 1. Diameter of Zone of Inhibition (mm)

Microorganism	Extracts			Essential Oil	Ciprofloxacin
	Aqueous	Methanol	Cowurine		
S.epidermidis (1435)	-	14.33±0.57	-	36.66±1.52	31±1
S.epidermidis (3086)	-	15.66±0.57	-	29.33±0.57	29.33±1.52
S.aureus (109)	-	19±1	-	40±1	30±1
S.aureus(3160)	-	11.33±1.15	-	21.33±0.57	26.66±0.57
S.hominis(4435)	-	-	-	24.77±1.52	25.66±0.57
MRSA I	-	-	-	-	-
MRSA II	-	-	-	35.33±0.57	-
MRSA III	-	11.66±0.57	-	35.66±1.15	13.66±0.57
MRSA IV	-	-	-	32±1	18±2
MRSA V	-	-	-	31±1.54	-
MRSA VI	-	-	-	33.33±1.15	13.66±1.15
MRSA VII	-	-	-	31±1	-
MRSA VIII	-	-	-	26±1	-
MRSA IX	-	-	-	30.33±0.57	-
MRSA X	-	-	-	32±0	-
MRSA XI	-	-	-	-	-
K.pneumoniae(4030)	-	-	-	26.33±1.52	27±1.73
P.vulgaris(426)	-	-	-	29.33±0.57	28±0
P.aeruginosa(424)	-	-	-	32.66±0.57	30±1
P.aeruginosa(7453)	-	-	-	29.33±1.15	32±1

Diameter of inhibition zones (mm) including the diameter of well (8 mm). Values are means of three replicates (±SD). Ciprofloxacin was tested at 10µg/ml.

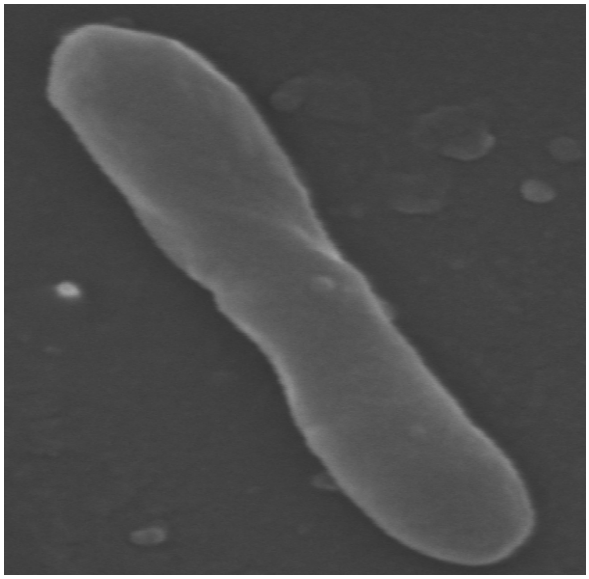
Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Microorganism	Methanol Extract mg/ml		Essential oil (µl/ml)		Ciprofloxacin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
S.epidermidis (1435)	15	30	0.7	1.5	0.31	0.15
S.epidermidis (3086)	15	30	1.5	1.5	2.5	1.25
S.aureus (109)	7.5	15	0.7	1.5	2.5	1.25
S.aureus(3160)	30	30	0.7	1.5	1.25	0.62
S.hominis(4435)	-	-	1.5	3.1	1.25	0.62
MRSA I	-	-	-	-	-	-
MRSA II	-	-	3.1	1.5	-	-
MRSA III	30	30	3.1	1.5	1.25	0.7
MRSA IV	-	-	6.2	3.1	2.5	1.25
MRSA V	-	-	6.2	3.1	-	-
MRSA VI	-	-	3.1	3.1	2.5	1.25
MRSA VII	-	-	6.2	3.1	-	-
MRSA VIII	-	-	12.5	6.2	-	-
MRSA IX	-	-	3.1	1.5	-	-
MRSA X	-	-	3.1	3.1	-	-
MRSA XI	-	-	-	-	-	-
K.pneumoniae(4030)	-	-	1.5	3.1	1.25	0.62
P.vulgaris(426)	-	-	1.5	1.5	0.15	0.15
P.aeruginosa(424)	-	-	1.5	3.1	2.5	1.25
P.aeruginosa(7453)	-	-	1.5	3.1	0.62	0.31

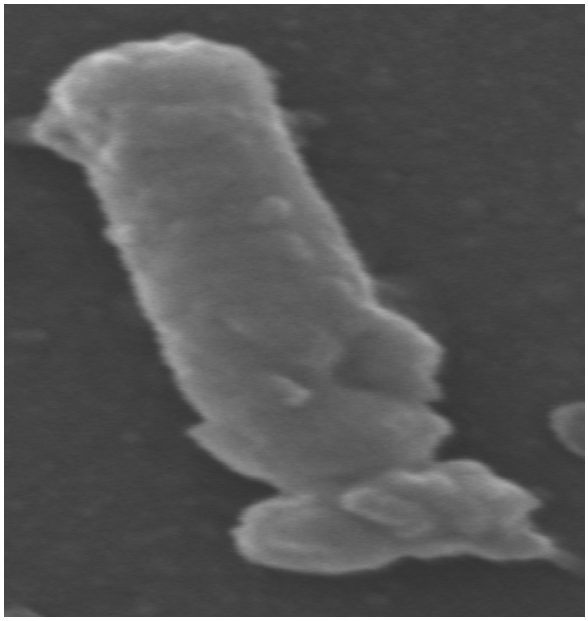
Whereas among the three extracts only methanol extract was found to exhibit inhibitory effect against four gram positive bacteria except *S.hominis*. The diameter of zones of inhibition was found in the range of 11-19mm and MIC ranging from 7.5-30mg/ml. Only

MRSA III isolate showed little sensitivity to methanol extract, all other isolates were found to be resistant. All gram negative bacteria were resistant to the extracts. Aqueous and cowurine extracts showed no activity. Control treatments (DMSO) did not show inhibitory effect on any of the tested microorganisms. The antibacterial activity of positive control (Ciprofloxacin) ranged from 25 to 32mm.

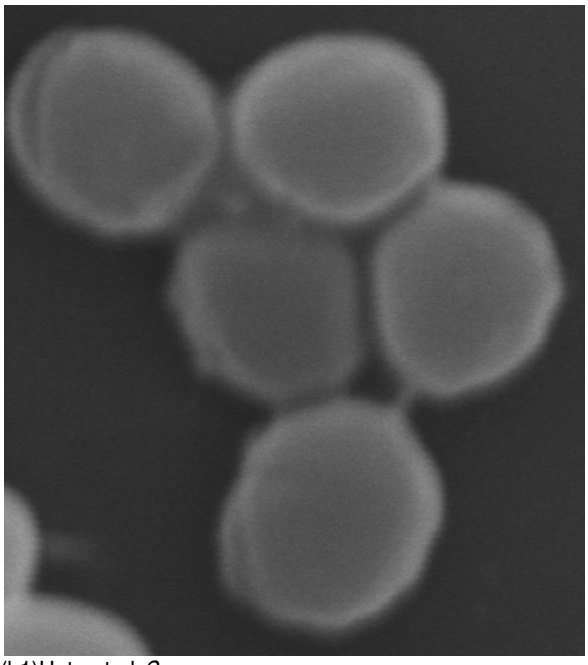
The antibacterial activity of the essential oil from *C.zeylanicum* bark can be attributed to the presence of high concentration of trans-Cinnamaldehyde (80.10%) [11]. In addition, the components in lower amounts like various terpenes may also contribute to antibacterial activity of the oil, involving probably some type of synergism with other active compounds. Terpenes may be related to lipophilic action that increases permeability and loss of cellular components. The synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their high antibacterial activity as compared to other extract samples.



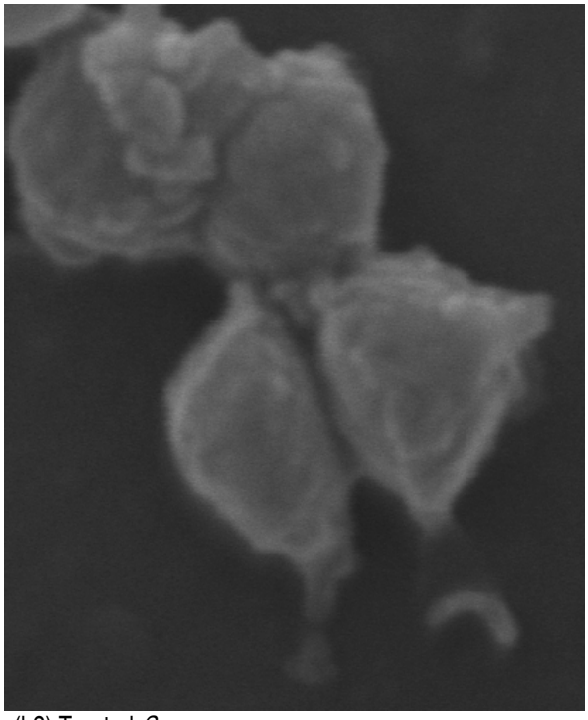
(a1)Untreated *P. aeruginosa*



(a2)Treated *P. aeruginosa*



(b1)Untreated *S.aureus*



(b2) Treated *S.aureus*

Figure-1

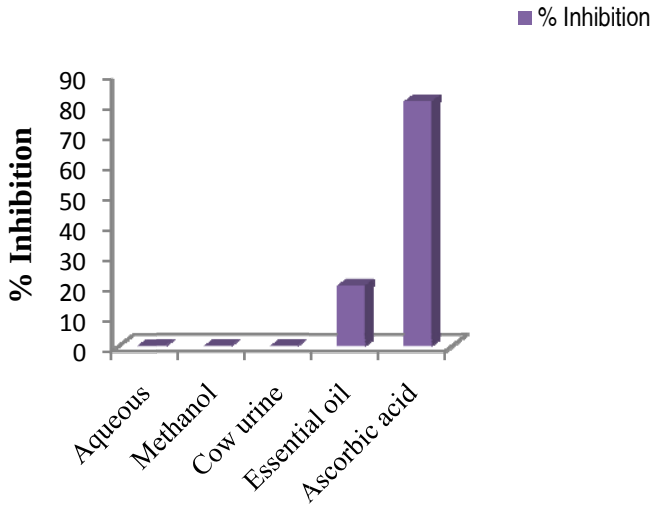


Figure-2 Antioxidant activity

Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical has been widely used to test the free radical scavenging ability of various natural products [12]. DPPH is a free radical that keeps its stability in aqueous or ethanolic solutions. It accepts an electron or hydrogen ion to become a stable diamagnetic molecule [13]. In the present study, antioxidant activity of three extracts and essential oil of *C.zeylanicum* was assayed by DPPH. The extracts exhibited no activity whereas 20% activity was exhibited by the essential oil (Fig-2). El- Baroty *et al* reported 15% antioxidant activity in essential oil of cinnamon supporting our findings.

The present study reveals the first SEM report on morphological alterations of S. aureus (NCDC 109) and P.aeruginosa (MTCC 424) cells treated with C.zeylanicum oil. The observations revealed the physical damage and considerable morphological alteration to the tested microorganisms. Fig. 1 shows the SEM images of treated and control samples for two selected bacterial species. These images directly illustrate the detrimental effects of the essential oil on the tested bacteria. The control cells in the absence of the oil were intact and showed a smooth surface (A1, B1), in contrast, bacterial cells treated with the essential oil at MIC values underwent considerable damage (A2, B2). The SEM images showed that morphological alterations in bacterial cells might be due to the lysis of outer membrane followed by the loss of cellular electron dense material on surface of the treated cells, resulting in the release of inner cell material.

Gelatin digestion assay for Collagenase Inhibitory activity

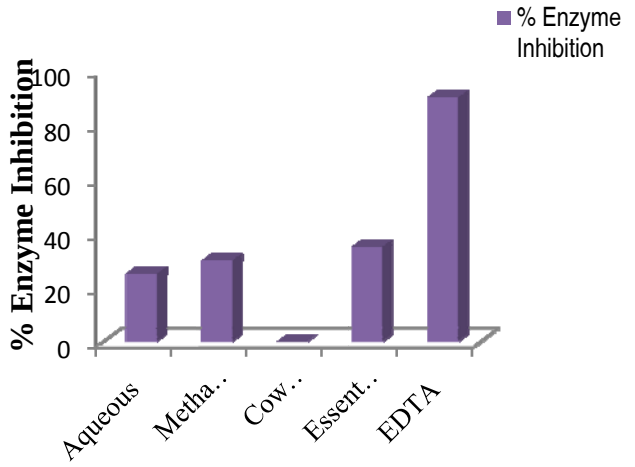


Figure-3 Gelatin digestion assay for Collagenase Inhibitory activity

For visual investigation of the inhibitory effect of *C.zeylanicum* on collagenase, an indirect assay was developed using bacterial collagenase. Following incubation of bacterial collagenase with different concentrations of *C.zeylanicum* extracts and oil, the remaining gelatinolytic activity was compared with initial enzyme activity represented by the control. The control group treated with reaction products of bacterial collagenase and collagenase buffer (pH 7.8) exhibited the highest gelatinolytic activity in the discrete zone, representing no enzyme inhibition. However gelatine digestion was clearly decreased following addition of 30mM/ml of

well known MMP inhibitor, EDTA . Area of clear zone was reduced by 30%. Similarly, gelatinolytic activity was decreased following dose dependent treatment of aqueous and methanol extract of *C.zeylanicum*. A modest reduction in gelatine digestion was observed with 20 mg/ml or higher concentrations of aqueous and methanol extracts of *C.zeylanicum,* representing inhibition of more than 25% and 30% of bacterial collagenase –I activity. Essential oil exhibited 35% collagenase inhibitory activity. Similar modest collagenase inhibitory activities (between 20–40% inhibition) were exhibited by lavender (31.06%), rose aqueous (26.39%), and bladderwrack (24.52%) [15].No activity was exhibited by cowurine extract (Fig-3).

Urease inhibitory activity

The urease inhibitory activity of the extracts and essential oil of *C. Zeylanicum* was determined by the method described by Weatherburn. However none of the samples exhibited urease inhibitory activity. Significant urease inhibitory activity i.e. 72 % was observed in the ethyl acetate fraction of roots of *Glycyrrhiza glabra* [15]. In another study ethanolic extracts of *Taraxacum officinale*, *Mentha longifolia* and methanolic extracts of *Achillea millefolium* and *Aristolachia bracteata* showed high urease inhibitory inhibitory potential [16].

Chemical composition of the essential oil

Among all samples studied essential oil showed best antibacterial, antioxidant and collagenase inhibitory activity, so further studies were carried with essential oil.

The essential oil was analyzed by GC–MS. The identified compounds of the sample are listed in Table 3 and Figure 4 according to their elution order. In total 13 constituents were tentatively identified, representing 100% of components in the essential oil. Results showed that cinnamaldehyde (80.10%), caryophyllene (5.91) , cinnamyl acetate (5.24%), linalool (3.28%) and eugenol (2.09%) were found to be the major compounds in the essential oil of *C.zeylanicum* bark. Although percentage composition of many terpenes was low but they cannot be ignored for their various significant biological activities.

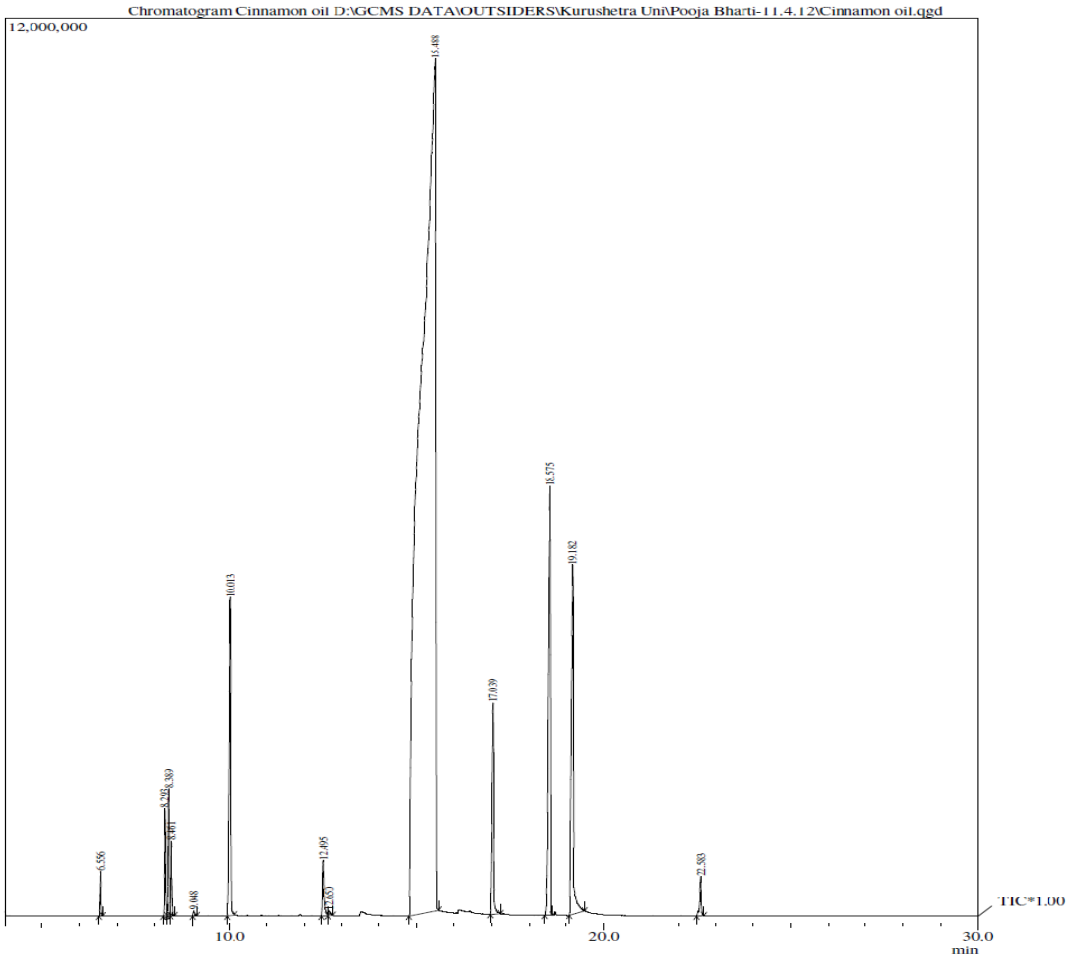


Figure- 4. GC-MS Chromatogram of Essential oil of *C.zeylanicum*.

The compounds were identified by comparison of retention indices and the mass spectrum with those of NIST library.

Table 3. Chemical composition (%) of the essential oil from *C.zeylanicum* bark.

S.No.	Compound	Retention Time	Peak Area (%)
1	-Pinene	6.556	0.27
2	P-Cymene	8.293	0.74
3	D-Limonene	8.389	0.87
4	1-8-Cineole	8.461	0.48
5	γ-Terpinene	9.048	0.04
6	Linalool	10.013	3.28
7	Terpeniol	12.495	0.57
8	Terpinyl isobutyrate	12.650	0.06
9	trans-Cinnamaldehyde	15.488	80.10
10	Eugenol	17.039	2.09
11	Caryophyllene	18.575	5.91
12	Cinnamyl acetate	19.182	5.24
13	Caryophyllene oxide	22.583	0.37

Conclusion

Medicinal plants can prove to be an efficient means to eradicate the pathogens by dual mode viz antibacterial activity and by inhibiting enzymes responsible for virulence.The essential oil of *C.zeylanicum* seems to possess multiple potential as antibacterial agent .It possesses both direct antibacterial activity and capacity in controlling the pathogenesis and spreading factors of bacterial infections. The results of present work reconfirm that the compounds like cinnaldehyde and eugenol are responsible for direct strong antibacterial and antioxidant activity. But the synergistic effect of various terpenes leading to lipophilic mode of action on the outer membrane of bacteria is also revealed. Any infection increases the level of oxidative stress in the host body. In the light of this study, it seems possible that cinnamon essential oil contributes to the subsiding bacterial infections as well as declining the oxidative stress. The moderate collagenenase inhibitory activity further enhances this oil's potential usage against epidermal bacterial infection. Our results showed that *C. zeylanicum* have probable applications in preventing the epidermal diseases. However further researches are necessary to use it as a lead compounds in drug designing.

Acknowledgement

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