



REGULAR ARTICLE

ANTIBACTERIAL ACTIVITY OF COMMERCIAL AND WILD CINNAMON SPECIES

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SUMMARY

Butanolic extracts of two different *Cinnamon* species (*C. zeylanicum*; Commercial variety and *C. flexuosus*; Wild variety), were investigated for their *in vitro* antibacterial activity. The antibacterial activity against plant and human pathogenic bacteria was evaluated on the basis of Inhibition Zones (IZs) measurement by "Disc diffusion method". Maximum inhibition was shown by *C. zeylanicum* against gram positive bacterium-*Staphylococcus aureus*. *C. flexuosus* extract also showed inhibition but was inactive against *Klebsiella pneumoniae*. *C. flexuosus* extract was more active than *C. zeylanicum* as indicated by the results. The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values for both the seeds (in µg/ml) were also determined. The lowest value was obtained for *Staphylococcus aureus* [MIC-76.25 (*C. f.*) 78.5(*C. z.*) MBC- 11 2.6(*C. f.*) 98.35(*C. z.*)] thus this bacteria is most inhibited by the extract, whereas *Bacillus subtilis* was least inhibited as indicated by MIC[(98.50 (*C. f.*) 96.2 (*C. z.*)] and MBC [169.50 (*C. f.*) 151.2 (*C. z.*)] values.

Key words: Antibacterial, *C. zeylanicum*, *C. flexuosus*, MIC, MBC

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1. Introduction

Plants are the invaluable sources of pharmaceutical products that have drawn the attention of ethnopharmacologist from around the world. A number of scientific investigations have highlighted the importance and contribution of so many plants families namely Umbelliferae, Lauraceae, Leguminosae, Cupressaceae, etc. used as medicinal plants. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Search for new plants with potential antimicrobial properties has been reviewed owing to the side effects associated with the traditional antibiotics (Olade Rangel, 2005; Brown, 1995; Saxena et al., 1999; Abu-Shanab et al., 2004).

Cinnamon has been known from remote antiquity. In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhea and other problems of the digestive system.^[26] Cinnamon is high in antioxidant

activity.^{[27][28]} The essential oil of cinnamon also has antimicrobial properties,^[29] which can aid in the preservation of certain foods.^[30] (Allkin et al, 1983; Ambasta et al., 1986; Anonymous, 2006; Markham et al., 1994; Rotter et al., 1990). Mahmood et al. (1999) reported an in vitro antibacterial activity of two essential oil viz., *C. zeylanicum* and *C. flexuosus*, *C. martini* and *M. arvensis* and their constituents against certain Gram negative and Gram positive organisms. Dean and Ritchie (1987) tested fifty essential oils indifferent concentration for their antibacterial activity against 25 genera of bacteria.

The present investigation was designed to investigate the antibacterial properties of the butanolic extract from the seeds against human and plant pathogenic bacteria.

2. Materials and Methods

Plant materials and alcoholic extracts

The bark of plant *C. zeylanicum* (Commercial variety) and *C. flexuosus* (Wild variety) were collected from Plant breeding Department and fields of Krishi Vishwavidyalaya, Raipur, in April 2010. The plant materials were further identified by the Head, Dept. of Plant Pathology, R. D. University, Jabalpur. Dried and milled plant materials were extracted sequentially with petroleum ether and methanol. Methanol extracts produced using 500g of plant material was vacuum dried and was further partitioned with n-hexane, CHCl_3 , BuOH to give a total of four fractions. The butanol extract was evaporated to dryness in vacuum resulting in 150g of brownish yellow mass that was stored at low temperature for further study.

Microorganism used

Five human and plant pathogenic bacteria- *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis* were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. *Streptococcus sp.* was obtained from Chandraker pathology laboratory, Jabalpur. The Disc diffusion method outlined by the National Committee for Clinical Laboratory standards (NCCLS, 1993) was used with 40 $\mu\text{g}/\text{ml}$ Gentamycin sulphate disc as reference antibiotics. Zone size was measured after the incubation of 37°C for 24 hrs.

Antibacterial activity

The antibacterial activity was determined by 'Disc Diffusion Method' (NCCLS, 1993). Three to five bacterial colonies from each agar plate were lifted with a sterile loop and transferred into a tube containing 5ml of nutrient broth. The turbidity of each bacterial suspension was adjusted to reach an optical comparison of a 0.5 Macfarland Standard, resulting in a suspension containing approximately 1×10^8 cfu/ml. The test solution was prepared by dissolving butanol extract in 20% MeOH. The final concentration of solution so obtained was 100 $\mu\text{g}/\text{ml}$. Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure

was repeated by streaking two more times, rotating the plates approximately 60° each time to ensure even distribution of the inoculum. After allowing the inoculum to dry at room temperature, the disc loaded with the extract of desired concentrations were placed on the agar plates. The positive control was prepared using Gentamycine sulphate solution of concentration 40 $\mu\text{g}/\text{ml}$. The plates were then incubated at 37°C for 18-24 hrs. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter was measured.

The MIC and MBC was determined by 'Well Assay Method' (Holtz et al., 2002). The solutions for MIC determination were prepared by two fold serially decrease dilution up to 65.25 $\mu\text{g}/\text{ml}$ and that for MBC from 285.25 $\mu\text{g}/\text{ml}$ to 105.25 $\mu\text{g}/\text{ml}$. The wells of 6mm diameter were bored in the agar plates. Each well was loaded with the extract of different concentration. Microbial growth was determined macroscopically and recorded after 18-24 hrs of incubation at 37°C. The MIC was determined as the lowest dilution corresponding to absence of IZ, whereas the lowest dilution that yields no growth was considered as the MBC.

3. Results and Discussion

Nowadays more attention has been directed towards solvent and aqueous extracts of various parts of popular plant species containing biologically active compounds. Several workers are investigating extracts of natural products for chemotherapeutic benefits. In the present investigation butanolic extracts of plant seeds of *C. flexuosus* and *C. zeylanicum* were assessed for their antibacterial activity against some bacteria by using Disc diffusion method. As given in Table-I among the Six selected bacteria, maximum zone of inhibition was formed by butanolic seeds extract of *C. zeylanicum* against *Staphylococcus aureus* followed by *Streptococcus species*, while very low inhibition was shown by *Shigella sonii* and *K.pneumoniae* at a concentration of 100 $\mu\text{g}/\text{ml}$ (Table-I). The butanolic extract of *C. flexuosus* exhibited lower antibacterial potential than *C. zeylanicum*. *Staphylococcus aureus* was inhibited the most followed by

Streptococcus sp. and *E.coli*, while no zone of inhibition was observed for *K. pneumoniae*. Least antibacterial activity was recorded against the gram-positive bacteria *Bacillus subtilis* by both the seeds extract.

In contrast to above obtain results Mohammed (2003) reported the antibacterial activity of alcoholic extract of *Lathyrus odoratus* flowers. The isolated antibacterial compound was effective against *Bacillus subtilis* and *E.coli*. Similarly, Ali et al. (1999) had screened and tested hexane and

methanol extracts of sixteen plants of the family Caesalpiniaceae and Lauraceae and tested for their antibacterial activity exhibited by the isolated phytochemicals. They observed methanol extracts of all the examined plants to exhibit strong growth inhibition against tested bacteria than hexane extracts. Pistelli et al. (2002) reported that the gram-positive bacteria *Staphylococcus aureus* was more sensitive only to n-hexane extract of the aerial parts of *Astragalus verrucosus* in comparison with other solvent extracts.

Table-I: Antibacterial activity of the Butanolic extract (100µg/ml) of seeds of *C. flexuosus* and *C. zeylanicum*

| Bacteria | Zone of inhibition (in mm) | | |
|--|----------------------------|--------------|----------------|
| | <i>C.f</i> | <i>C. z</i> | S ^b |
| Gram positive | | | |
| <i>Bacillus subtilis</i> (MTCC-1789) | 6.66 ± 0.08 | 6.76 ± 0.26 | 33 ± 0.04 |
| <i>Staphylococcus aureus</i> (MTCC-737) | 19.83 ± 0.44 | 31.0 ± 0.57 | 28 ± 0.02 |
| <i>Streptococcus species</i> | 18 ± 0.57 | 27.50 ± 0.50 | 40 ± 0.11 |
| Gram negative | | | |
| <i>Escherichia coli</i> (MTCC-443) | 10.07 ± 0.52 | 13.66 ± 0.33 | 38 ± 0.50 |
| <i>Klebsiella pneumoniae</i> (MTCC-2405) | - | 9.33 ± 0.44 | 38 ± 0.02 |
| <i>Shigella sonii</i> * | 6.60 ± 0.04 | 6.68 ± 0.08 | 32 ± 0.02 |

Values are means ± SEM. of three replicate analysis; - = not active against tested micro-organism; S = Standard: Gentamycin sulphate (40µg/ml); *C.f* = *C. flexuosus*; *C. z* = *C. zeylanicum*.

Table II: MIC and MBC Results (in µg/ml) against bacteria

| Bacteria | MIC | | MBC | |
|------------------------------|------------|-------------|------------|------------|
| | <i>C.f</i> | <i>C. z</i> | <i>C.f</i> | <i>C.z</i> |
| <i>Shigella sonii</i> * | N.T. | 90.0 | N.T. | 110.0 |
| <i>Escherichia coli</i> | 90.5 | 89.6 | 142.5 | 135.3 |
| <i>Klebsiella pneumoniae</i> | N.T. | 95.3 | N.T. | 142.50 |
| <i>Staphylococcus aureus</i> | 76.25 | 78.5 | 112.6 | 98.35 |
| <i>Streptococcus species</i> | 85.5 | 82.0 | 122.4 | 118.5 |
| <i>Bacillus subtilis</i> | 98.50 | 96.2 | 169.50 | 151.2 |

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, *C.f* = *C. flexuosus*; *C.z* = *C. zeylanicum*, NT = Not tested

The antibacterial activity of the extract and their potency were quantitatively assessed by determining the MIC and MBC values respectively (Table-II). It was considered that if the extract displayed MIC less than 100µg/ml, the antimicrobial activity was good; from 100 to 500µg/ml the antimicrobial activity was moderate; from 500 to 1000µg/ml the antimicrobial activity

was weak; over 1000µg/ml the extract was considered to be inactive. The *Cinnamon sp.* i.e. *C. flexuosus* and *C. zeylanicum* shows MIC values below 100µg/ml, therefore present a good activity against the selected bacteria. However, the MIC value comes out to be maximum for *B.subtilis* for both species, therefore this bacteria is least effective. The lowest value comes out for *Staphylococcus*

aureus which shows that the seed extracts are more effective against *Staphylococcus aureus*. The results of MIC were in accordance with the MBC values, where the maximum value was obtained for *B.subtilis* and minimum for *Staphylococcus aureus*.

In contrast Leite *et al.* (2006) investigated the antibacterial activities of various organic and aqueous extracts of leaves of *Indigofera suffruticosa* (Fabaceae) and found that only the aqueous extract showed strong inhibitory activity against the gram positive bacteria *Staphylococcus aureus* with a MIC of 5000µg/ml. Mandal *et al.* (2005) isolated acasiaside A and B, two acylated bisglycoside saponins isolated from *Acacia auriculiformis* which posses MIC values of 700µg/ml to inhibit the growth of *Bacillus megaterium*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Doughari (2006) conducted studies on *Tamarindus indica* to evaluate antimicrobial activities of extracts of the stem, bark and leaves. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and organic extracts on gram negative and gram positive. The lowest MIC and MBC were demonstrated against *Salmonella paratyphi*, *Bacillus subtilis* and *Salmonella typhi* and the highest MIC and MBC were exhibited against *Staphylococcus aureus*.

From the above studies it can be concluded that the antimicrobial activity of the selected seeds extract would be helpful in treating various kinds of diseases. Crude extracts and their mechanism of interaction with different active fractions of the plants are needs to explore. The bioactive compounds from *C. flexuous* and *C. zeylanicum* seeds can be used as antibacterial after further studies.

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