Ethanol extract of *Curcuma longa* leaf, a potential drug candidate against *Bacillus* species mediated infections

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**Abstract**

Several studies have exposed the antimicrobial and antioxidant activities of *Curcuma longa*, widely used in food industry as a colorant, among other functions. This study was implemented to examine the antimicrobial activity of *Curcuma longa* leaves against six *Bacillus* species. Antimicrobial activities of three different extracts of *Curcuma longa* leaves on *Bacillus* species were tested on the basis of disk diffusion method and the zone of inhibition was measured. The ethanol extract of the plant leaf produced maximum zone of inhibition against *Bacillus cereus* Xb21 (12.50mm) followed by *Paenibacillus* sp. BF38 (11.10mm), *Bacillus simplex* Xb17 (10.40mm), *Bacillus megaterium* Hb42 (9.15mm), *Paenibacillus* sp. L32 (8.00mm) with the exception of *Terrabacillus* sp. 3LF showed resistance, whereas the ethyl acetate and hexane extracts did not exhibit against all the tested bacteria. The chloroform extract of *Curcuma longa* leaves exhibited against only *Bacillus simplex* Xb17 (14.45mm) and *Paenibacillus* sp. BF38 (12.25mm).The MIC (minimum inhibitory concentration) values of ethanol extract against tested bacteria were almost 15.625 μg/10µl. The results suggest that the ethanol extract of *Curcuma longa* leaves could be considered as potentially effective antimicrobial agents against tested *Bacillus* species.

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Introduction

Bacillus species are a diverse group of bacteria widely spread in soil and the aquatic environment. This group of bacteria acclimatise easily to diverse territories caused by their ability to form spores and withstand a range of variable environmental conditions (Rahman et al., 2013; Hasan et al., 2013; Priest et al., 1988). The antimicrobial agent is that kills microorganisms are called as microbicidal or inhibits their growth are known as microbiostatic. Use of substances with antimicrobial properties is known to have been common practice for over 2000 years.

Turmeric (Curcuma longa), a perennial herb and member of the Zingiberaceae family that is cultivated extensively in South Asia and other countries with a tropical climate. The extracts of turmeric roots has traditionally been used as an insect repellent, antimicrobial (Rudrappa and Bais, 2008), antidiabetic (Mohmed et al., 2009), rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhea and colic inflammatory disorders (Villages et al., 2008). The most important compounds responsible for the antioxidant activity of turmeric are phenolic compounds, such as curcuminoid dyes and essential oils (Antunes et al., 2012). Traditionally, the leaves of C. longa extensively used in culinary preparation are aromatic and contain essential oil. C. longa leaves oil bestowed with medicinal values has been used for treatment of various ailments and many of its therapeutic properties have been experimentally validated including its antimicrobial activity (Apisariyakul et al., 1995; Tripathi et al., 2002). Therefore, much attention has been focused on application of plant derived antimicrobials to control pathogens in foods. Consequently, alternative additives are needed, which possess antimicrobial activity and cause no health problems (Parvin et al., 2013; Morshed et al., 2011; Fayzunnessa et al., 2011; Dorman and Deans, 2000). There are many literatures reporting the medicinal values of Curcuma longa, but there is little scientific evidence for further using this plant commercially. Therefore, an attempt was made to isolate curcuminoids from Curcuma longa leaves for their antimicrobial activities.

Materials and methods

Plant material and preparation of the plant extracts

Fresh leaves of Curcuma longa were collected from the local area of Kushtia district in Bangladesh. The sample was identified by Botanist. Fresh leaves were cleaned and cut into small pieces and air dried for 2 days. The dried sample was again dried in a hot air oven at 50°C for 24 hrs, then pulverized by a mechanical grinder and passed through a 20 mesh sieve. 10 g powdered was successively extracted with ethanol, ethyl acetate, chloroform, and hexane using a Soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature with mild shaking. All the extracts were filtered and concentrated at 35°C under reduced pressure. The filtered extracts were concentrated in rotary vacuum evaporator at below 50°C and stored at 4°C for further experiment.

Bacillus species used

Six test strains viz. Bacillus megaterium Hb42, Bacillus simplex Xb17, Terribacillus sp. 3LF, Bacillus cereus Hb21, Paenibacillus sp. L32, and Paenibacillus sp. BP38 were employed for the present study. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

In Vitro Antibacterial activity assay

In vitro antibacterial activities of the test samples were carried out by disc diffusion method (Barry, 1976; Hasan et al., 2013). The solutions of known concentration (μg/10μl) of test samples were prepared by dissolving exact amount of samples into measured volume of different solvent. The sample discs were made by soaking the sterilized filter paper discs (6mm diameter) with known volume of the test samples. Then the extract soaked discs were placed carefully over the nutrient agar media spreading with bacterial strains using the sterilized forceps. The prepared plates were incubated at 37°C overnight.
After the incubation, the zones of inhibition were visualized surrounding the discs. The antibacterial activities were assessed by measuring the zone of inhibition in millimeter (mm) against the test organisms. Standard Neomycin (30 μg/μl) was used as positive control for comparison of the antibacterial activity. The experiment was carried out in triplicate and read the mean value.

**Determination of minimum inhibitory concentration (MIC)**

It was described in details previous study on this test. Minimum inhibitory concentrations (MIC) of the ethanol, ethyl acetate, chloroform and hexane extracts were determined using serial dilutions (500 μg/10µl to 62.5 μg/10µl) of different extracts in ethanol, ethyl acetate, chloroform and hexane solvent against six tested microorganisms by agar well diffusion method (Hasan et al., 2013; Reiner, 1982). Freshly grown bacterial strains 100 μl (10^6 cells/ml) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (10.0 to 1000.00 μl) of the stock extract (1 mg/ml) and antibiotic respectively and incubated for 24 hrs at 37°C. Presence of turbidity denoted presence of microorganism in the test tube after the period of incubation, whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

**Data analysis**

All data were measured average value of three replicates and standard error (±). Results were subjected to ANOVA using Statistical Packages for Social Sciences (SPSS version 20.0) and the means separated using least significant differences (LSD) at 5% level of significance.

**Results**

The results of antimicrobial activity of ethanol, ethyl acetate, chloroform and hexane extracts of Curcuma longa leaves against different Bacillus species are presented on the Table 1.

**Table 1.** Determination of antimicrobial activity of ethanol, ethyl acetate, chloroform and hexane extracts of Curcuma longa leaf against different bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Zone of inhibition (mm)</th>
<th>Leaf extracts of Curcuma longa (500μg/10µl)</th>
<th>Standard (30μg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>EA</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Bacillus megaterium Hb42</td>
<td>9.15±0.23*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus simplex Xb17</td>
<td>10.40±0.14*</td>
<td>-</td>
<td>14.45±0.08</td>
</tr>
<tr>
<td>Terribacillus sp. 3LF</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus cereus Xb21</td>
<td>12.50±0.30*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paenibacillus sp. I32</td>
<td>8.00±0.00*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paenibacillus sp. BF38</td>
<td>11.10±0.46*</td>
<td>-</td>
<td>12.25±0.17</td>
</tr>
</tbody>
</table>

Data were measured in mm and represented as mean ± SD of triplicate. EA: Ethyl acetate. (*) indicates significance value P<0.005.

**Table 2.** Determination of minimum inhibitory concentration (MIC) of different leaf extracts of Curcuma longa.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Minimum inhibitory concentration(MIC)</th>
<th>Organic extracts of Curcuma longa leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>EA</td>
</tr>
<tr>
<td>Bacillus megaterium Hb42</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus simplex Xb17</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>Terribacillus sp. 3LF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus cereus Xb21</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>Paenibacillus sp. I32</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>Paenibacillus sp. BF38</td>
<td>125</td>
<td>-</td>
</tr>
</tbody>
</table>

MIC of different organic extracts (values in µg /10µl).
The zone of inhibition was measured in millimetre. In accordance with concentration of 500 μg/μl, the highest zone of inhibition for ethanol extract was Bacillus cereus Xb21 (12.50±0.30 mm), followed by Paenibacillus sp. BF38 (Figure 2) (11.10±0.46 mm), Bacillus simplex (Xb17) (10.40±0.14 mm) (Figure 2), Bacillus megaterium (Hb42) (9.15±0.23 mm), Paenibacillus sp. L32 (8.00±0.00 mm), whereas no zone of inhibition was shown by Terribacillus sp. 3LF. It was found for chloroform extract, the zone of inhibition for Bacillus simplex Xb17 and Paenibacillus sp. BF38 were 14.45±0.08 mm and 12.25±0.17 mm respectively, however, no zone of inhibition was shown rest of others Bacillus species. It has been seen that Ethyl acetate and hexane extract did not produce the activity against all of the tested bacteria (Figure 1).

**Discussion**

In the present study, ethanol extract of Curcuma longa leaves showed significant antimicrobial activity against most of the tested organisms (p<0.005) except Terribacillus sp. 3LF. This is because of the fact that some essential oils contain active components that influence certain metabolic functions of microbial cells. Wilkins and Board suggested that antimicrobial activity of oils may be due to impairment of variety of enzymes systems that are involved in the production of energy or synthesis of structural components in the microbial cells (Wilkins and Board, 1989). The differences in the sensitivity of food borne pathogens may be due to differences in methods used in study (Parvin et al., 2013; Kumar et al., 1997).

![Fig. 1. The comparison of antimicrobial activity of Curcuma longa leaf extracts and standard antibiotic (Neomycin 30μg/μl) against Bacillus species. EA: Ethyl acetate, BM: Bacillus megaterium Hb42, BS: Bacillus simplex Xb17, TS: Terribacillus sp. 3LF, BC: Bacillus cereus Hb21, PSL32: Paenibacillus sp. L32, PSBF38: Paenibacillus sp. BF38.](image1)

![Fig. 2. Ethanol extract of Curcuma longa leaf produce zone of inhibition at different concentrations against Bacillus species (a. Bacillus simplex Xb17, and b. Paenibacillus sp. BF38).](image2)

The plant extracts were described with standard antibiotic i.e. neomycin (30 μg/μl) and negative control (only solvent absorbing disc). The negative control exhibited no activity against all tested bacteria. The standard antibiotic showed significant antimicrobial activity against most tested bacteria but did not produce antimicrobial activity against only Terribacillus sp. 3LF.

Among the tested extracts, the most active was ethanol extract, and chloroform, while no inhibitory action of ethyl acetate and hexane extract was observed. In comparison with the present study, it is supported that crude ethanol extracts of
Curcuminoids of *Curcuma longa* from the varieties of Kasur, Faziabad and Bannu areas the antibacterial activity against three *Bacillus species* and one Azotobacter resulted effective activity with Kasur variety than others and *B. subtilis* was the most sensitive to Curcuminoids and oils of turmeric (Naz et al., 2010).

Similar work on antibacterial studies using root extract of *C. igneus* on *P. aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella* sp. was reported (Nagarajan et al., 2011). Antimicrobial activities of different medicinal plants have been described worldwide by many researchers (Morshed et al., 2011; Samy, 2005; Palombo and Semple, 2001; Antunes et al., 2012; Rahman et al., 2013). Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria (Kumar et al., 1997; Luthra et al., 2001), rhizome extract of Acorus (Sabitha et al., 2003), leaf extract of *Mikania triangularis* (Cruz et al., 1996), root and leaf extract of *Withania somnifera* (Mahesh and Satish, 2008) have been reported.

**Conclusions**

In conclusion, the findings of the present study clearly indicate that the ethanol extract of *Curcuma longa* leaves possess the antimicrobial activity like that of the rhizome which has proved evidence for its antimicrobial potential. The ethanol might be a good solvent for extraction of *Curcuma longa* leaves. Further studies may recommend for the isolation of bioactive constituents and biological assay methods for the standard drug preparations.

**Acknowledgment**

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**References**


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