Antibacterial potentialities of the extract of *Curcuma amada* Roxb. against a panel of pathogenic bacteria

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**ABSTRACT**  This paper represents the antibacterial potentialities of the crude rhizomatous extract of *Curcuma amada* Roxb. First, the crude extract was taken condensed under reduced pressure and the assessed for antibacterial screening against some multi drug resistant pathogenic bacteria. It is found that the extract can inhibit the growth of this bacterium at 150 to 200 μg/ml of concentrations. Hence, this extract can be used as antibacterial medicine without any side effects.

**Keywords:** *Curcuma amada* Roxb., antibacterial potentialities, pathogenic bacteria.

**Introduction**
Nature cure is a constructive method of treatment which aims at removing the basic cause of disease through the rational use of the medicinal plants. It is not only a system of healing, but also a way of life among tribals, in tune with the internal vital forces or natural elements comprising the human body. This paper represents the antibacterial potentialities of *Curcuma amada* Roxb. against some multi drug resistant bacteria.

*C. amada* Roxb. is endemic to South Asia, which is found wild in many parts of Northeast and in the hills of South India. Cultivated for its edible rhizome, it has the flavour of green mango and is used as a vegetable. It is also used in preparation of pickles, chutney, etc., and in traditional and tribal medicines [1].

**Materials and methods**

**Collection of plants**
Whole plant of *C. amada* Roxb. was collected in the month of July 2014 from experimental garden of Department of Botany, University of Kalyani, and was identified in the Department of Botany, University of Kalyani, Nadia.

**Extraction and isolation**
2.5 kg shade dried rhizomes of *C. amada* Roxb. plant was powdered and extracted three times with 95% EtOH (each 500 ml, 48 h) at room temperature. The extract was evaporated under reduced pressure and a solid residual mass was obtained.

**Preparation of sample solution**
The test solution was prepared by dissolving the dark brown residual mass in few drops of propylene glycol and then diluting with sterile water in the concentration of 500 μg/ml to 100 μg/ml. Few drops of propylene glycol diluted with sterile water were used as control. All the dilutions were sterilized by filtration using membrane filter (0.02 μ pore size) [2].

**Microorganisms**
The extract was individually tested against a panel of pathogenic bacteria like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterococcus faecalis*, *Proteus mirabilis*, *Proteus vulgaris*, *Vibrio cholera* etc. All the bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference strains of bacteria were maintained on solid nutrient agar and liquid broth nutrient agar medium slants with a subculture period of 30 days.

**Preparation of McFarland standard**
The turbidity standard was prepared by mixing 0.5 ml of 1.75% (w/v) BaCl₂·2H₂O with 99.5 ml of 1% H₂SO₄, BaSO₄ (v/v). The standard was taken in screw cap test tube to compare the turbidity. The bacterial culture of selected strains were grown for 48-72 hours and subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 BaSO₄ turbidity standard 10⁸ Colony Forming Unit (CFU) per ml was achieved. These inoculates were used for seeding of the nutrient agar medium, LB medium respectively [3].
Disc diffusion assay

1 mg of each sample(s) including one of its acetylated derivatives was separately dissolved in 1 ml of propylene glycol and then the volume was adjusted to 10 ml by adding sterile water. The ultimate concentration reaches to $10^2 \mu g/ ml$ and sterilized by filtration (0.22 μm filter). From the solution of each concentrated sample(s) final concentrations were made from 500 μg/ ml to 100 μg/ ml by adding sterile double distilled water. The sterile paper discs (6 mm diameter) were saturated with 10 μl of the solution of the respective sample(s) at a concentration of 500 μg/ ml to 100 μg/ ml and placed on the inoculated agar of 10^8 cfu/ml. Antibacterial tests were then carried out by disc diffusion method using 100 μl of suspension containing 10^8 CFU/ml of bacteria on nutrient agar medium, LB medium respectively. Negative controls were prepared using propylene glycol. Gentamicin (10 μg/ disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 300 C, 370 C, 300 C and 280 C respectively for 48 h, 24 h, 48 h and 72 h. Antibacterial activity was evaluated by measuring the zone of inhibition and the diameters of these zones were measured in millimetres against the test organisms [4].

**Determination of Minimum inhibitory concentration**

The minimal inhibitory concentration (MIC) values were studied for the bacteria strains, being sensitive to the sample(s) or acetylated derivative in disc diffusion assay. The inoculates of the bacterial strains were prepared from 24-72 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extract was dissolved in sterile propylene glycol and first diluted to the highest concentration (500 μg/ml) to be tested, and then serial dilutions were made in order to obtain a concentration range from 500 to 100 μg/ml in 10 ml sterile test tubes containing nutrient broth and LB broth medium respectively. MIC values of the extract against bacterial strains were determined based on a micro well dilution method as previously described. The plate was covered with a sterile plate sealer and then incubated at appropriate temperatures for 24 - 72 h at respective incubating temperatures. Bacterial growth was determined by absorbance at 600 nm and confirmed by plating 10 μl samples, forming clear wells on nutrient agar medium or LB medium respectively. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. Each test in this study was repeated, at least, thrice [5].

**Statistical analysis**

The observed values were expressed as mean ± standard deviation. All the values reflected in the table are statistically analyzed by paired t test with control and found significantly different at 5% level ($P<0.05$)

**Results and discussions**

Antibacterial assay was also performed with the extract. Results in table number 1 indicate that this extract was positive in antibacterial assay and the MIC value was 150 μg/ml for the bacterium *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Proteus vulgaris* and 200 μg/ml for the bacterium *Acinetobacter baumannii*, *Enterococcus faecalis*, *Proteus mirabilis*, *Vibrio cholerae* respectively (Table 1).

### Table 1: Determination of antibacterial activity of the extract of *C. amada* Roxb.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Staphylococcus aureus</th>
<th>Staphylococcus epidermidis</th>
<th>Pseudomonas aeruginosa</th>
<th>Streptococcus pyogenes</th>
<th>Streptococcus agalactiae</th>
<th>Klebsiella pneumoniae</th>
<th>Acinetobacter baumannii</th>
<th>Citrobacter freundii</th>
<th>Enterococcus faecalis</th>
<th>Proteus mirabilis</th>
<th>Proteus vulgaris</th>
<th>Vibrio cholerae</th>
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<td>No growth</td>
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<tr>
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<td>350</td>
<td>5.2±0.3</td>
<td>4.1±0.3</td>
<td>4.3±0.1</td>
<td>5.2±0.4</td>
<td>4.9±0.1</td>
<td>4.3±0.4</td>
<td>5.2±0.4</td>
<td>5.1±0.4</td>
<td>4.3±0.3</td>
<td>4.7±0.1</td>
<td>4.9±0.3</td>
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<tr>
<td>300</td>
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<td>3.8±0.3</td>
<td>3.9±0.5</td>
<td>4.6±0.2</td>
<td>4.7±0.5</td>
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<td>4.7±0.3</td>
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<tr>
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<td>3.5±0.4</td>
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<td>2.9±0.1</td>
<td>2.9±0.1</td>
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<tr>
<td>150</td>
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<td>3.1±0.5</td>
<td>2.7±0.1</td>
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<tr>
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</tr>
</tbody>
</table>
The observed values were expressed as mean ± standard deviation. The control sets show no inhibition zone. All the values reflected in the table are statistically analyzed by paired t test with control and found significantly different at 5% level (P<0.05).
Hence, it may be cited that this extract have some antibacterial potentialities. This sample may be treated as a cost effective, eco-friendly, herbal antibiotic against the common human pathogenic bacteria.

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References