ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *Alpinia Calcarata* Roscoe. - A VALUABLE MEDICINAL PLANT

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ABSTRACT
The present study was designed to investigate the anti-microbial activity of four solvent extracts (Petroleum ether, Dichloromethane, Acetone and Methanol) of rhizome of *Alpinia calcarata* Rosce. The rhizome of this plant is an important antimicrobial agent and a digestive stimulant. The plant extracts showed considerable activity against Ten tested strains viz., *Pseudomonas aeroginosa*, *Escherichia coli*, *Enterobacter aerogens*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Vibrio cholerae*, *Salmonella paratyphi*, *Klebsiella pneumoniae* and *Proteus vulgaris* by using agar disc diffusion assay. This study reveals that *Alpinia calcarata* has antimicrobial activity against gram positive and gram negative bacteria. Methanol extract showed inhibition against eight strains and showed highest inhibition zone (15±0.3) against *Bacillus subtilis*. The minimum inhibitory concentration (MIC) of the *Alpinia calcarata* was ranging from 0.39 mg mL\(^{-1}\) to 0.65 mg mL\(^{-1}\).

Keywords: Disc diffusion assay, Organic extracts, Rhizome, Gram positive, Gram negative, minimum inhibitory concentration.

INTRODUCTION
From ancient time many medicinal plants had been used as antimicrobial agents (Mahesh and satheesh, 2008). In recent years researchers have focused on the higher plants for the search of antimicrobial substances (Trakranrungsie *et al.* 2004; Udomkusonsri *et al.* 2007). Medicinal plants have been a major sources of cure for human diseases since time immemorial. It is no wonder that one-fourth of world population i.e. 1.42 billion people are dependent on traditional medicines for the treatment of various ailments (Reddy 2004). This practice continues inspite of advances in the field of allopathy during 20\(^{th}\) century. Thus plants still remain one of the major sources of drug in the modern as well as traditional system of medicine throughout the world. Many plants contain microbial inhibitors (Voravuthinkuchai 2006) and they are used as traditional medicines and as sources of wide range of substances which can be useful to treat infectious or other serious diseases (Yineger H, 2009). Zingiberaceae as one of the largest

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families of the plant kingdom include 53 genera and over 1300 species (Kai Larsen, K 1980) and well known for a number of medicinal properties (Kumar et al. 2006, Prajapathi et al. 2005). *Alpinia* is the largest genus of the family with more than 200 species. Albuquerque (2004) recently detected a broad spectrum of antibacterial and antifungal compounds in this genus (Ibrahim, 2009). *Alpinia calcarata* commonly known as snap ginger is an important cultivated medicinal crop of India and it is known for its antimicrobial activity (George et al., 1949, Robinson et al., 2009). It is widely distributed in warmer part of Asia and India (Kritikar 2003). It is a well known hailed official drug throughout the country and also as a holistic gift of nature with varied medicinal, culinary and cosmetic uses. This species has various therapeutic activities, viz. thermogenic, aromatic, nervin tonic, stimulant, revulsive, carminative, stomachic, disinfectant, aphrodisiac, expectorant, broncho-dilator, antifungal, febrifuge, tonic and many more (Warrier et al. 1993). The rhizomes of *Alpinia calcarata* are anti-inflammatory (Asolkar, et al. 1992).

**MATERIALS AND METHODS**

**Collection of Plant Material and Extraction**

The rhizomes were collected in the month of December 2012 from the botanical garden of St. Joseph’s College, Mannanam, Kottayam, Kerala and a voucher specimen has been deposited in Rapinat Herbarium, Tiruchirappalli for future reference (Fig.1).

**Fig.1. Alpinia calcarata - inflorescence**

The rhizomes were washed thoroughly in tap water, air – dried, powdered and stored in an air – tight container for further use. 80 g of powdered rhizomes were soaked in 300 ml of Petroleum ether, Dichloromethane, Acetone and Methanol separately in Soxhlet apparatus for 48h at 31⁰ C until complete extraction of materials. Extracts were filtered using a buckner funnel using Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume into 50ml. The concentrated extract was stored in pre-weighed screw capped bottle and kept in refrigerator at 4⁰C.

**Bacterial Strains**

The antibacterial activities of the plant extracts were studied against three pure cultures of gram positive bacteria viz., *Bacillus subtilis* MTCC # 441 (BS), *Staphylococcus aureus* MTCC # 3163 (SA), *Streptococcus faecalis* MTCC # 459 (SF) and seven pure cultures of gram negative viz., *Escherichia coli* MTCC # 119 (EC), *Enterobacter aerogenes* MTCC # 2990 (EA), *Klebsiella pneumoniae* MTCC # 3040 (KP), *Pseudomonas*
aeruginosa MTCC # 2474 (PA), Salmonella paratyphi MTCC # 734 (SP), Vibrio cholerae ATCC # 14104 (VC), Proteus vulgaris # MTCC 1771 (PV). The purity and viability of cultures were checked by culturing on nutrient agar slants and incubated at 37°C for 24 h. The isolates were subcultured in nutrient broth and stored in 4°C.

Antibacterial activity
A modified agar diffusion method was adopted to determine antibacterial activity. Around 6mm diameter of discs of whatman filter paper (No.1) were impregnated with 10µl of crude extracts using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2h and were placed at equidistance in each of the plates using a sterile forceps. The reference antibiotic (RA) discs containing Ciproflaxin were prepared at appropriate concentrations to serve as the positive controls for gram positive and gram negative bacteria respectively. Each plate then was poured with melt soft agar containing 105 CFU/ml of the bacteria. The air dried discs were placed on inoculated agar plates. The plates were incubated at 37°C for 24 hr. The area of the zone of inhibition was measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.

Minimum inhibitory Concentration (MIC)
Bacterial strains that were sensitive to rhizome extracts of dried Alpinia calcarata by the agar disc diffusion method were further tested to determine minimum inhibitory concentrations (MICs). The Minimum inhibitory Concentrations of the methanol extract was tested against the Bacillus subtilis MTCC # 441 (BS), Staphylococcus aureus MTCC # 3163 (SA), Streptococcus faecalis MTCC # 459 (SF), Escherichia coli MTCC # 119 (EC), Klebsiella pneumoniae MTCC # 3040 (KP), Salmonella paratyphi MTCC # 734 (SP), Vibrio cholerae ATCC # 14104 (VC), Proteus vulgaris # MTCC 1771 (PV) cultures in a 96 – well microtiter plate. The tested sample was first of all dissolved in DMSO (stock - 200mg mL⁻¹) and the solution obtained was added to NBPG (Nutrient broth containing 0.05% phenol red and supplemented with 10% glucose) to a final concentration of 4 mg mL⁻¹(0.1 mL of stock solution to 5mL of NBPG) for each crude extract. 100µL of each concentration was added in a well containing 95 µL of NBPG and 5 µL of standard inoculums. The plates were covered with a sterile plate scale, and incubated at 35°C for 24h. The assay was repeated twice. Microbial growth was determined by observing the change of colour in the wells (red when there is no growth and yellow when there is growth). The lowest concentration that does not permit any visible growth when compared to the control (no colour change) was considered as the Minimum inhibitory Concentration (MIC).

RESULTS AND DISCUSSION
Petroleum ether, Dichloromethane, Acetone, methanol extracts of the rhizome powder were screened for their antibacterial effects. Methanolic extracts of rhizomes showed a wide spectrum of activity against test bacteria (Table.1).
Table 1. Antimicrobial profile of different extracts of rhizomes of *Alpinia calcarata* against human pathogenic bacteria (Zones if inhibition in mm)

<table>
<thead>
<tr>
<th>Extraction</th>
<th><em>B. subtilis</em></th>
<th><em>S. aureus</em></th>
<th><em>S. faecalis</em></th>
<th><em>E. coli</em></th>
<th><em>E. aerogenes</em></th>
<th><em>K. pneumoniae</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>S. paratyphi</em></th>
<th><em>V. cholera</em></th>
<th><em>P. vulgaris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>7 ± 0.3</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Acetone</td>
<td>11 ± 0.2</td>
<td>R</td>
<td>R</td>
<td>8 ± 0.4</td>
<td>14 ± 0.3</td>
<td>10 ± 0.3</td>
<td>R</td>
<td>13 ± 0.6</td>
<td>9 ± 0.4</td>
<td>7 ± 0.3</td>
</tr>
<tr>
<td>Methanol</td>
<td>15 ± 0.3</td>
<td>10 ± 0.3</td>
<td>10 ± 0.4</td>
<td>12 ± 0.4</td>
<td>R</td>
<td>8 ± 0.6</td>
<td>R</td>
<td>7 ± 0.5</td>
<td>9 ± 0.3</td>
<td>10 ± 0.4</td>
</tr>
<tr>
<td>Reference</td>
<td>16 ± 0.5</td>
<td>11 ± 0.5</td>
<td>15 ± 0.5</td>
<td>-</td>
<td>11 ± 0.4</td>
<td>-</td>
<td>17 ± 0.2</td>
<td>15 ± 0.4</td>
<td>14 ± 0.6</td>
<td>7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean of three trials ± standard error. R = resistance to the extract.

This suggests that methanol is a good solvent for extracting bioactive compounds from *Alpinia calcarata* extract against bacteria. The diameter of inhibition zone for each of the organisms was compared with standard antibiotic. It was noted that the inhibition zone of the organisms to be either less than or greater than or equal to standard antibiotic. Higher inhibition zone of (15 ± 0.3 mm) was recorded for the methanol extracts of rhizomes against *Bacillus subtilis*. Methanol extracts have MIC values of 0.39mg mL⁻¹ for *Bacillus subtilis* (Fig. 3 & 4).

CONCLUSION

Antimicrobial properties are useful tools in the control of microorganisms especially in the treatment of infections and food spoilage. Based on these results, we may conclude that *Alpinia calcarata* showed antibacterial activity against all tested organisms and had large inhibition against *B.subtilis*. The promissory extracts of *Alpinia calcarata* open the possibility of finding new clinically effective antimicrobial compounds. The MIC is a helpful parameter used to assess bacteriostatic activity under similar conditions.
Fig. 3. MIC plate shows the antibacterial effects of the methanolic rhizome extract of *Alpinia calcarata*

Fig. 4. MIC values of the methanolic rhizome extract of *Alpinia calcarata*
BIBLIOGRAPHY


