Antibacterial activities of leaves extracts of

*Dendrolobium triangulare*

(Retz.) Schindl.

(Lauk- min- mwe- bet)

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INTRODUCTION

- **Plants** are widely used in many *indigenous medicine* for therapeutic purposes.
- **Medicinal plants** have long played important roles in the treatment of diseases all over the world.
- These *medicinal plants* consider as a rich resources of *ingredients* which can be used in drug development and synthesis.
- Myanmar is abundant plant resources
- *Dendrolobium triangulare* (Retz.) Schindl. is used in Myanmar traditional medicinal applications, such as tonic and abscesses
- *D. triangulare* (Retz.) Schindl. has been found in numerous therapeutic applications in traditional medicines such as Myanmar traditional medicine, Chinese traditional medicine and Indian Ayurvedic medicine
Different parts of the plants are used in the Indian system of medicine as a cure for dysentery, in bronchial spasms and coughs (Ghosal & Mehta 1973)

In Chinese traditional medicine, the top portion of young leaf is chewing and swallowing with a little water to treat diarrhea

The root is taken for sore throat

A combination of root and leaf paste is applied for internal injury (Ma et al. 2011)
Plant and plant-based products have become crucial against various diseases or pathogen infections due to their lesser toxic and side effects (Su et al. 2015).

Increasing development of drug resistance in human pathogens as well as the appearance of side effect of synthetic drugs needs to develop new antimicrobial drugs from natural sources (Mondal et al. 2004).

This situation has forced to search for new natural sources like medicinal plants (Doshi et al. 2011).
Medicinal plants and their derived are rich in antibacterial compounds which could be an alternate way to combat bacterial diseases even against some bacteria which are becoming resistant to certain synthetic medicines (Singh et al. 2016)

Plant extracts is highly desirable due to low cost, environmental friendliness, and effectiveness against certain bacteria, compared to antibiotics, harmful to the environment
In the recent years, plant derived products are increasingly being sought out as medicinal products, nutraceuticals, cosmetics.

The present study

*Dendrolobium triangulare*

- morphological
- phytochemical constituents
- physicochemical properties
- elemental analysis
- antibacterial activity
to study the antibacterial activity of various leaves extracts of *Dendrolobium triangulare* (Retz.) Schindl.
Specific Objectives

- to identify the morphological characters of *Dendrolobium triangulare* (Retz.) Schindl.
- to determine phytochemical and physicochemical analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl.
- to determine the elemental analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl.
- to detect the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaves extracts of *Dendrolobium triangulare* (Retz.) Schindl.
Materials and Methods

- **Morphological studies** - Identified and classified according to Flora of British India & Checklist of Myanmar

- **Phytochemical tests** - Harbone (1998) and Raaman (2006) method

- **Physicochemical properties** - WHO (2011)

- **Elemental analysis** - Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF)
  - Atomic Absorption Spectrophotometer (AAS)
Antibacterial activity test

- aqueous, ethanol, ethyl acetate and petroleum ether extracts against four pathogenic bacterial strains
- Bacteria concentration - (5×10⁵ CFU/ml⁻¹)
- determining the minimum inhibitory concentration (MIC)
- minimum bactericidal concentration (MBC)
- using twelve concentrations (0.08 to 160 mg/ml) of various extracts were tested in vitro antibacterial activity
- by microdilution method with Resazurin (Sarker et al. 2007)
➢ Tetracycline hydrochloride was used as positive control

➢ Test organisms were

- *Enterococcus faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923
- *Bacillus cereus* ATCC 14579
**Habit** - Perennial erect shrubs stems and branches triquetrous densely appressed with grey hairs

**Leaves** - trifoliate compound alternate; stipules long-pointed leaflets obovate-oblong subcoriaceous

*Figure 1. Habit*
**Inflorescence** - axillary racemes, 15- to 20- flowered

**Flowers** - White, about 0.8 cm zygomorphic

**Calyx** - 5-lobed; green

**Corolla** - white, glabrous

**Stamens** - 10, diadelphous, white

**Ovary** - oblongoid, green

**Pods** - inflated, curved

**Seeds** - reniform, small, green
<table>
<thead>
<tr>
<th>No.</th>
<th>Phytochemical Test</th>
<th>Extract</th>
<th>Test reagents</th>
<th>Observation</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>1%HCL</td>
<td>- Wagner's reagent- Dragendorff's reagent, - Mayer's reagent</td>
<td>no colour change</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>EtOH</td>
<td>Conc: HCl+Mg</td>
<td>brown</td>
<td>+</td>
<td>Raaman (2006)</td>
</tr>
<tr>
<td>3.</td>
<td>Glycoside</td>
<td>H_2_O</td>
<td>Chloroform + 10% ammonia</td>
<td>white ppt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Phenolic compounds</td>
<td>H_2_O</td>
<td>5% FeCl_3</td>
<td>dark green</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Polyphenols</td>
<td>EtOH</td>
<td>10% FeCl_3+ 1%K_3[Fe(CN)_6]</td>
<td>dark blue</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Saponins</td>
<td>H_2_O</td>
<td>Distilled water</td>
<td>more form</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Reducing sugar</td>
<td>H_2_O</td>
<td>Fehling A+B</td>
<td>no red ppt</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Amino acid</td>
<td>H_2_O</td>
<td>Ninhydrin</td>
<td>pale purple ppt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Carbohydrates</td>
<td>H_2_O</td>
<td>Naphthol+ Conc: H_2_SO_4</td>
<td>red ring</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Tannins</td>
<td>H_2_O</td>
<td>lead acetate</td>
<td>white ppt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Cyanogenetic substance</td>
<td>H_2_O</td>
<td>Na pictrate paper + conc; H_2_SO_4</td>
<td>colour change</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>
### Physicochemical properties of leaves of *Dendrolobium triangulare* (Retz.) Schindl.

<table>
<thead>
<tr>
<th>No.</th>
<th>Physicochemical properties</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH value</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Ash value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total ash value</td>
<td>6.8 %</td>
<td>Harborne (1998)</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>3.35%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>89.2%</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Extractive value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble extract</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanol soluble extract</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate soluble extract</td>
<td>1.88%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petroleum ether soluble extract</td>
<td>0.76%</td>
<td></td>
</tr>
</tbody>
</table>
Elemental analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl. by EDXRF

<table>
<thead>
<tr>
<th>No.</th>
<th>Elements</th>
<th>Quantity determined percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Potassium</td>
<td>1.122</td>
</tr>
<tr>
<td>2.</td>
<td>Calcium</td>
<td>0.940</td>
</tr>
<tr>
<td>3.</td>
<td>Sulfur</td>
<td>0.160</td>
</tr>
</tbody>
</table>
Elemental analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl. by EDXRF

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<th>No.</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Iron</td>
<td>0.012</td>
</tr>
<tr>
<td>2.</td>
<td>Manganese</td>
<td>0.006</td>
</tr>
<tr>
<td>3.</td>
<td>Zinc</td>
<td>0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Copper</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Heavy metal analysis of the leaves of *Dendrolobium triangulare* (Retz.) Schindl. By AAS

<table>
<thead>
<tr>
<th>No.</th>
<th>Elements</th>
<th>Quantity determined percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cadmium (ppm)</td>
<td>ND (not detected)</td>
</tr>
<tr>
<td>2.</td>
<td>Lead (ppm)</td>
<td>ND (not detected)</td>
</tr>
</tbody>
</table>
Antibacterial activity

- antibacterial activity - Microdilution method (Sarker et al. 2007)
- Extracts - aqueous, ethanolic, ethyl acetate, petroleum ether

Test Microorganisms

- Escherichia coli
- Enterococcus faecalis
- Staphylococcus aureus
- Bacillus cereus
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160</td>
<td>80</td>
<td>40</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>1.25</td>
<td>0.625</td>
<td>0.3125</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.** Minimum inhibitory concentration of plant extract against *E. coli*

**Figure 5.** Minimum inhibitory concentration of plant extract against *E. faecalis*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<td>1.25</td>
<td>0.625</td>
<td>0.3125</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6.** Minimum inhibitory concentration of plant extract against *S. aureus*

**Figure 7.** Minimum inhibitory concentration of plant extract against *B. cereus*
Figure 8  MBC of aqueous extracts against test organisms

Figure 9  MBC of ethanolic extracts against test organisms
Figure 10  MBC of ethyl acetate extracts against test organisms

Figure 11  MBC of pet ether extracts against test organisms
Figure 12  MBC of Antibiotic (Tetracycline) against test organisms
Table 1  Antibacterial activity of MIC and MBC values

<table>
<thead>
<tr>
<th>Tested Microorganisms</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Ethyl acetate extract</th>
<th>Pet-ether extract</th>
<th>Tetracycline Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg ml⁻¹)</td>
<td>MBC (mg ml⁻¹)</td>
<td>MIC (mg ml⁻¹)</td>
<td>MBC (mg ml⁻¹)</td>
<td>MIC (mg ml⁻¹)</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>ATCC 25922</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus faecalis</strong></td>
<td>10</td>
<td>160</td>
<td>5</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>ATCC 29212</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>160</td>
<td>160</td>
<td>10</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>ATCC 25923</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>160</td>
<td>160</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>ATCC</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration
Figure 13  Antibacterial activity of MIC and MBC values
Antibacterial activity of *Dendrolobium triangulare* (Retz.) Schindl. belonging to family Fabaceae used in traditional medicinal was demonstrated.

According to the preliminary phytochemical tests - flavonoids, glycosides, phenolic compounds, polyphenols, amino acid, carbohydrates, saponin and tannins were present.

Alkaloids and harmful cyanogenic substance were absent in this plants.

Phytoconstituents were responsible for therapeutic activities and pharmacological activities of medicinal plants.
Physicochemical results of pH, total ash, acid insoluble ash, water soluble ash

Various extractable values of water, ethanol, ethyl acetate and petroleum ether were investigated

 Accordance with this results
  - extractable value of aqueous is the most significant
  - petroleum ether is the least

Extractable matter indicate that *D. triangulare* possess the pharmaceutically important compounds

Physicochemical parameter present in this study may be useful for the quality control data which can be used as herbal medicine
- Macroelements - Potassium
  - Calcium
  - Sulphur

- Microelements - Iron
  - Manganese
  - Zinc
  - Copper

- The conformation test with AAS indicated that some heavy metals were absent in this plant and it may be used safely for long time
Antibacterial activity of all four plant extracts exhibit MIC and MBC values - 2.5 to 160 mg ml\(^{-1}\)

Extracts with high anti-bacterial activity against gram negative bacteria were aqueous and ethanolic extracts [MIC and MBC values at 80 mg ml\(^{-1}\)]

Most sensitive gram positive bacteria was the *Bacillus cereus* with ethanolic, pet-ether and ethyl acetate extracts [MIC and MBC values - 2.5 to 5 mg ml\(^{-1}\)]
- *D. triangulare* (Retz.) Schindl. has a long history of use in Asian countries in indigenous medicine

- The scientific investigation of this leaves extracts
  - several phytoconstituents
  - useful physicochemical data
  - various elements
  - potent antibacterial activities

- Therefore, this plant may takes part in the treatment of various disorder in traditional medicine


• Dr Moe Swe, Director General, Department of Traditional Medicine, Naypyidaw

• Dr. Thein Zaw Lin, Acting Rector, University of Traditional Medicine, Mandalay

• Dr Htet Htet Win (Assistant Director, Biotechnology Research Department, Kyaukse, for her kind helps in the antibacterial study