ANTIMICROBIAL ACTIVITY OF ANACARDIUM OCCIDENTALE L. (သီဟိုသီဟိုသီဟိုသီဟိုဠဠ) (THIHO- THAYET) BARKS ON BACTERIA CAUSING COMMON GASTROINTESTINAL INFECTION AND ITS PHYTOCHEMICAL CONSTITUENTS

Aye Thida Htun¹, May Thandar Htun¹, Khaing Win Htun⁴, Su Su Yee², Saw Hla Myint³, Khin Phyu Phyu¹ and Yi Yi Myint¹

1. Department of Medical Research (Upper Myanmar)
2. University of Pharmacy (Yangon)
3. University of Yangon
4. 1000- Bedded General Hospital, Nay Pyi Taw
INTRODUCTION
Plants - selected and used empirically as drugs for centuries, with this knowledge and accumulated practice passing from generation to generation (Taylor et al., 2001)

Medicinal plants – various activities, then a rich source of antimicrobial agents (Mashesh and Satish, 2008)

The effects of plant extracts on bacteria - studied by a large number of researchers in different parts of the world (Ateb and Erdourul, 2003)

In addition to resistance problem, antibiotics - associated with adverse effects including hypersensitivity reactions, bone marrow depression, liver disease and kidney disease (Al-Barl et al., 2006)
Since antiquity, man - used plants to treat common infectious diseases and some of these traditional medicines still form part of the habitual treatment of various illnesses.

Today, various types of infections.

Among them, gastrointestinal infection - very common and frequent causes of morbidity and mortality in developing countries.

In the year 2010, diarrhoea and gastroenteritis of presumed infectious origin - 4th of the leading causes of morbidity (MOH, 2012).
Diarrhoea remains the second leading cause of death among children under five globally.

Nearly one in five child deaths – about 1.5 million each year, is due to diarrhoea (WHO, 2009).

In 2008, high diarrhoea morbidity was seen in Chin, Kayah, Rakhine, Shan (East) and Mon State.

Yangon, Mandalay, Bago (West) and Mgway Division had low diarrhoea morbidity with high sanitary latrine coverage (MOH, 2010).
**Anacardium occidentale** L. (Thiho-thayet) - one of the common plant used in herbal medicine and reported to have variety of activities

- infusion of stem bark and leaves of the plant - used as a remedy for tooth ache, sore gums
- leaves, stem and bark extracts - utilized widely for the treatment of diarrhoea, dysentery, colonic pain, diabetes, urinary disorders, asthma, eczema, dyspepsia and venereal diseases
- also easily available and used orally by Myanmar people
In Myanmar, phytochemical analysis and antimicrobial activity of *Anacardium occidentale* L. leaves - documented previously

- no studies - carried out on *Anacardium occidentale* L. barks for antimicrobial activity

- present study- aimed to evaluate the antimicrobial activity of barks extracts of *Anacardium occidentale* L. on bacteria causing gastrointestinal infection its phytochemical constituents
OBJECTIVES
Objectives

- To conduct the preliminary phytochemical tests *Anacardium occidentale* L. barks

- To determine the antibacterial activity of different extracts of *Anacardium occidentale* L. barks on bacteria causing gastrointestinal infection
MATERIALS AND METHODS
Botanical studies of *Anacardium occidentale* L.

**Collection**

- *Anacardium occidentale* L. barks - from Thar-yar-gone Village, Bago Township, Bago Division

**Classification and identification**

- Fresh specimens of vegetative and floral parts used for classification and identification (Hooker (1879), Backer (1965) and Dassanayoke (1983)) at Department of Pharmacognosy, University of Pharmacy (Yangon)
Phytochemical analysis of *Anacardium occidentale* L. barks

- Qualitative analysis - investigated by the quality control method for WHO (1998) and Maung Tin-Wa (1972)
Extraction of barks

- Plant material
- Air-dried
- Crushed & powdered
- Extracted with solvents (90% and 70% EtOH, Acetone, distilled water)
dried barks powder sample (100g)

Percolation

- EtOH (90%) extract: 31.892 g
- EtOH (70%) extract: 38.115 g
- Acetone extract: 7.8 g
- Distilled water extract: 31.431 g

Evaporated with rotary evaporator
Determination of antimicrobial activity

- Determined by agar disc diffusion technique according to modified Kirby and Bauer method (WHO, 2003)

Bacteria used

- *Shigella dysenteriae* (DMR, CM)
- *Shigella flexneri* (DMR, CM)
- *Salmonella typhi* (DMR, CM)
- Chloramphenicol sensitive strain of *Escherichia coli* (DMR, CM)
- Amikacin sensitive strain of *Escherichia coli* (ATCC 25922)
Standard antibiotic discs

- Ceftriaxone 30 µg
- Chloramphenicol 30 µg
- Amikacin 30 µg
Antimicrobial susceptibility testing

1. The test pathogens were seeded over the Mueller-Hinton agar (MHA) plates with a sterile swab.

2. The Mueller Hinton plate were swabbed over the entire surface of the medium 3 times, rotating the plate 60° after each application.

3. Filter paper discs were applied at equidistance by using a pair of sterile forceps.
4. Each disc were gently pressed down with sterile forceps to ensure even contact with the medium.

5. The plates were incubated at 37°C for 18-24 h.

6. After that, the plates were observed for a zone of inhibition.

7. The diameter of the inhibition zone were measured with antibiotic zone scale in mm.
RESULTS
Plant identification

- plant identified as *Anacardium occidentale* L. belonging to the family Anacardiaceae
# Phytochemical analysis

<table>
<thead>
<tr>
<th>No</th>
<th>Type of compound</th>
<th>Extract</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>10% HCl</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Amino acids</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Starch</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoids</td>
<td>EtOH</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Steroids</td>
<td>Pet ether</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Reducing sugars</td>
<td>H₂O</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Terpenoids</td>
<td>Ethyl Acetate</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Cyanogenic Glycosides</td>
<td>H₂O</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) = present  
(-) = absent
Antimicrobial activities of different extracts of *Anacardium occidentale* L. barks

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Diameter of inhibition zone (mm) of different extracts of <em>Anacardium occidentale</em> L. barks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol (90%) extract</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>12 mm</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>14 mm</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>14 mm</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>CK 9 mm</td>
</tr>
<tr>
<td></td>
<td>AK -</td>
</tr>
</tbody>
</table>

CK = Chloramphenicol 30 µg  
AK = Amikacin 30 µg  
Disc - 8 mm in diameter
Antimicrobial activity of different extracts of barks on *Shigella flexneri*
Antimicrobial activity of different extracts of barks on *Shigella dysenteriae*
Antimicrobial activity of different extracts of barks on *Salmonella typhi*
1 = Acetone
2 = Ethanol (70%)
3 = Aqueous extract
4 = Acetone extract
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
CK = Chloramphenicol 30 μg

Antimicrobial activity of different extracts of barks on chloramphenicol sensitive strain of *E.coli*
Antimicrobial activity of different extracts of barks on amikacin sensitive strain of *E. coli*

1 = Acetone
2 = Ethanol (70%)
3 = Aqueous extract
4 = Acetone extract
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
AK = Amikacin 30 µg
DISCUSSION
From the phytochemical investigation - alkaloids, flavonoids, glycosides, terpenoids, steroids, phenolic compounds, amino acids, tannin, carbohydrates and reducing sugars were significantly present.

Cyanoglycosides - absent

Regarding the medicinal value of *Anacardium occidentale* L., antibacterial properties may be due to tannin.
• The powdered extracted with solvents (ethanolic (90%), (70%), acetone and distilled water) by percolation

• The yield of 70% ethanol extract of barks - highest and acetone extract the lowest

• This may be due to the constituents of barks are more soluble in ethanol, less soluble in acetone

• The yield of different plant extracts may be - high by Soxhlet extraction but effective thermolabile constituents - degraded
Antimicrobial activities of different extracts (ethanol (90%), ethanol (70%), acetone and aqueous) of barks - on Shigella dysenteriae, Shigella flexneri, Salmonella typhi, chloramphenicol sensitive strain of Escherichia coli and amikacin sensitive strain of Escherichia coli.

different extract of barks - more effective against Shigella flexneri, Shigella dysenteriae and Salmonella typhi.

showed no significant activity on test strains of Escherichia coli.

present study revealed - crude extracts of Anacardium occidentale L. barks are not effective in the treatment of gastrointestinal infection caused by Escherichia coli.
• results obtained from this study - among the different plant extracts, aqueous extract of *Anacardium occidentale* L. barks was more effective for antimicrobial activity than others

• may be due to the active constituents of *Anacardium occidentale* L. barks extracts for antimicrobial activity more soluble in distilled water than other solvents
REFERENCES


THANK YOU
Preparation of medium

- Mueller-Hinton Agar plate prepared according to the procedure of the manufacturer’s recommendation

- After autoclaving, 25 mL of the media poured into 9 cm diameter petridishes and allowed to set at room temperature

- When the agar had solidified, the plates were dried at 50°C by placing them in the upright position in the incubator with the lids tilted

- The plates then labeled
Preparation of sub culture

3-5 colonies picked with a wire loop from the primary culture plate

inoculated into nutrient agar plate

incubated at 37°C for 18 hrs
Preparation of bacterial suspension

picked with a wire loop

introduced into test tube containing normal saline

incubated at 37°C for 3-4 hours to produce the growth turbidity

Standardized density of bacterial suspension with 0.5% McFarland standard turbidity
Preparation of discs for plant extracts

- Sterile paper discs of 8 mm diameter were impregnated with 25 µL of 100 mg/mL of the extracts to form 2.5 mg/disc

- Filter paper disc

- dried in the oven at 50°C to evaporate the solvent
Positive control

Commercially available antibiotic disc with known potency

Negative control

The same solvents employed to dissolve the plant extracts