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

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# 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 developing countries

- In the year 2010, diarrhoea and gastroenteritis of presumed infectious origin - $4^{th}$ 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

↓

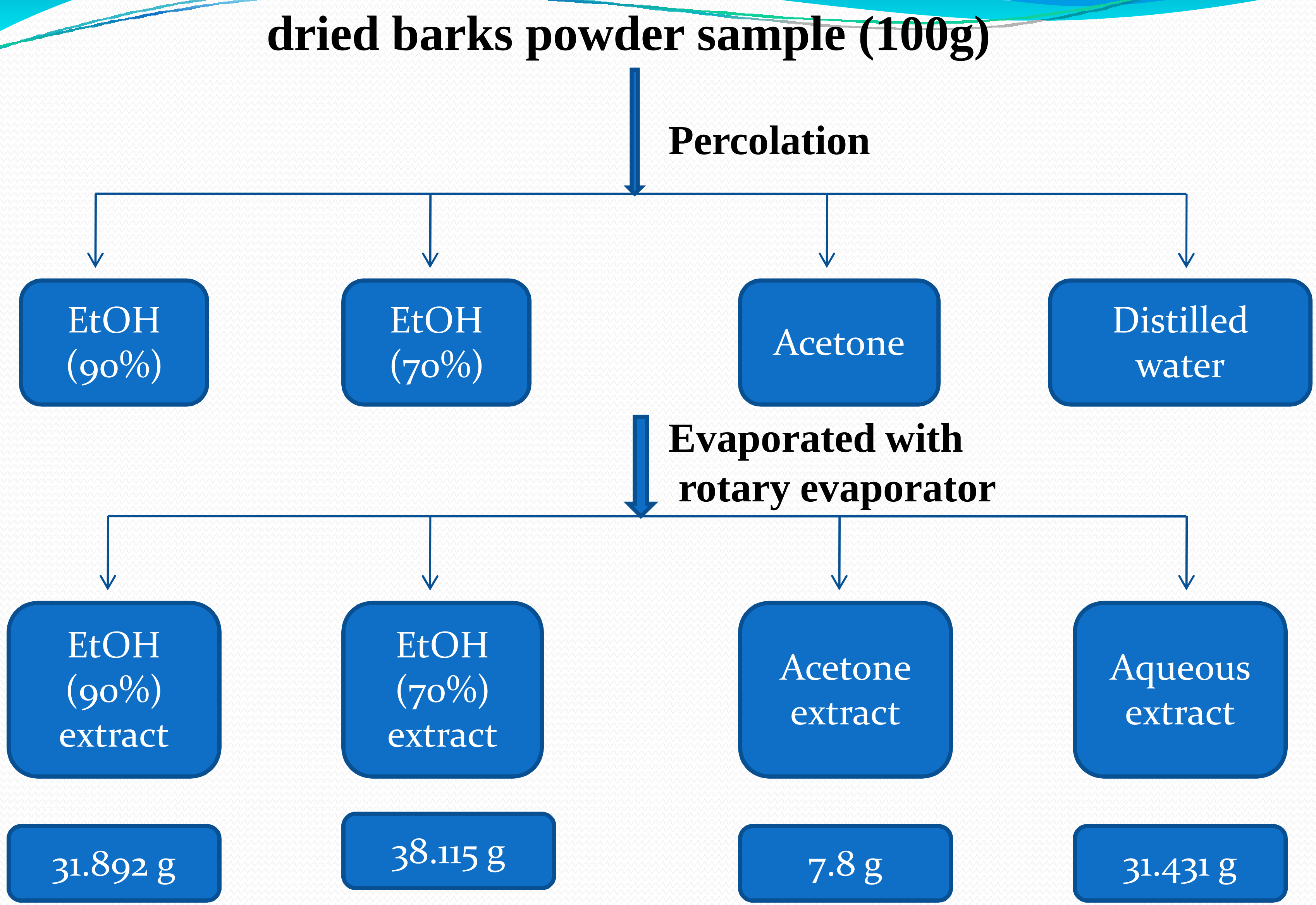
Air-dried

↓

Crushed & powdered

↓

Extracted with solvents (90% and 70% EtOH, Acetone, distilled water)


dried barks powder sample (100g)
Percolation
EtOH (90%)
EtOH (70%)
Acetone
Distilled water
Evaporated with rotary evaporator
EtOH (90%) extract
EtOH (70%) extract
Acetone extract
Aqueous extract
31.892 g
38.115 g
7.8 g
31.431 g

## Determination of antimicrobial activity

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

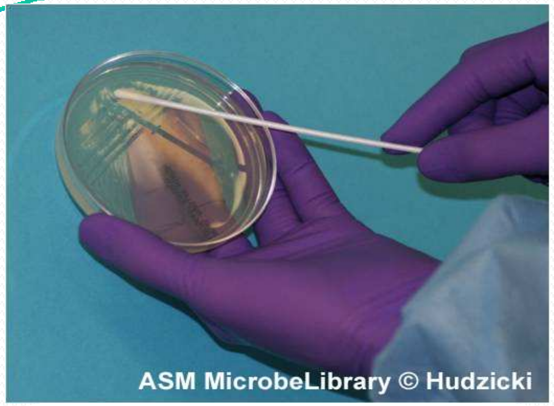
## Bacteria used

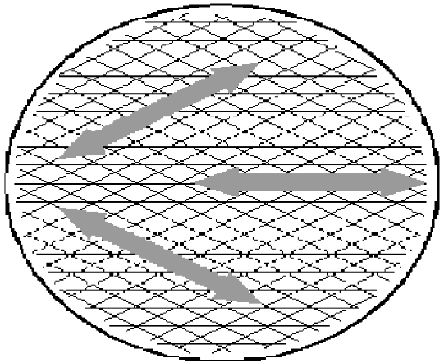
- *Shigella dysenteriae* (DMR, CM)
- *Shigella flexneri* (DMR, CM)
- *Salmonella typhi* (DMR, CM)
- Chloramphenicol sensitive strain of *Escherichia coli* (DMR, CM)
- Amikacin sensitive strain of *Escherichia coli* (ATCC 25922)

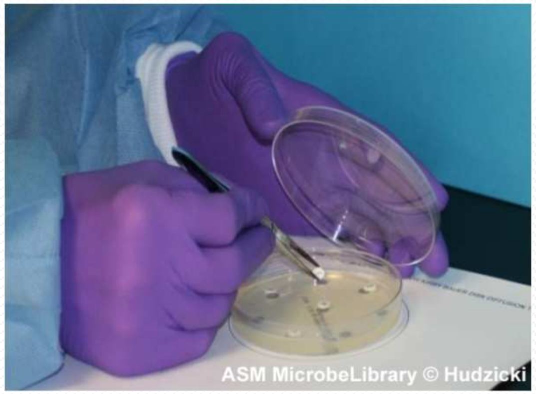
## Standard antibiotic discs

- Ceftriaxone 30 μg
- Chloramphenicol 30 μg
- Amikacin 30 μg

# Antimicrobial susceptibility testing



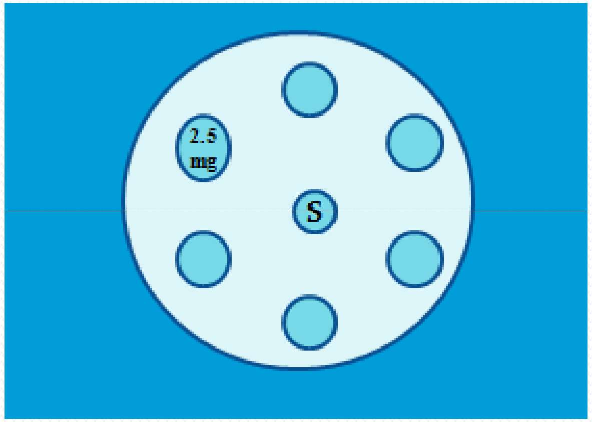



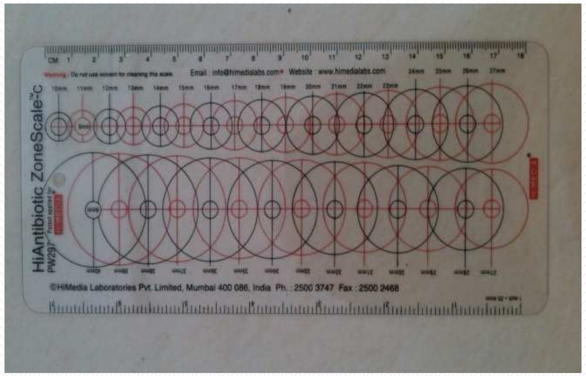



1. The test pathogens were seeded over the Mueller-Hinton agar (MHA) plates with a sterile swab

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

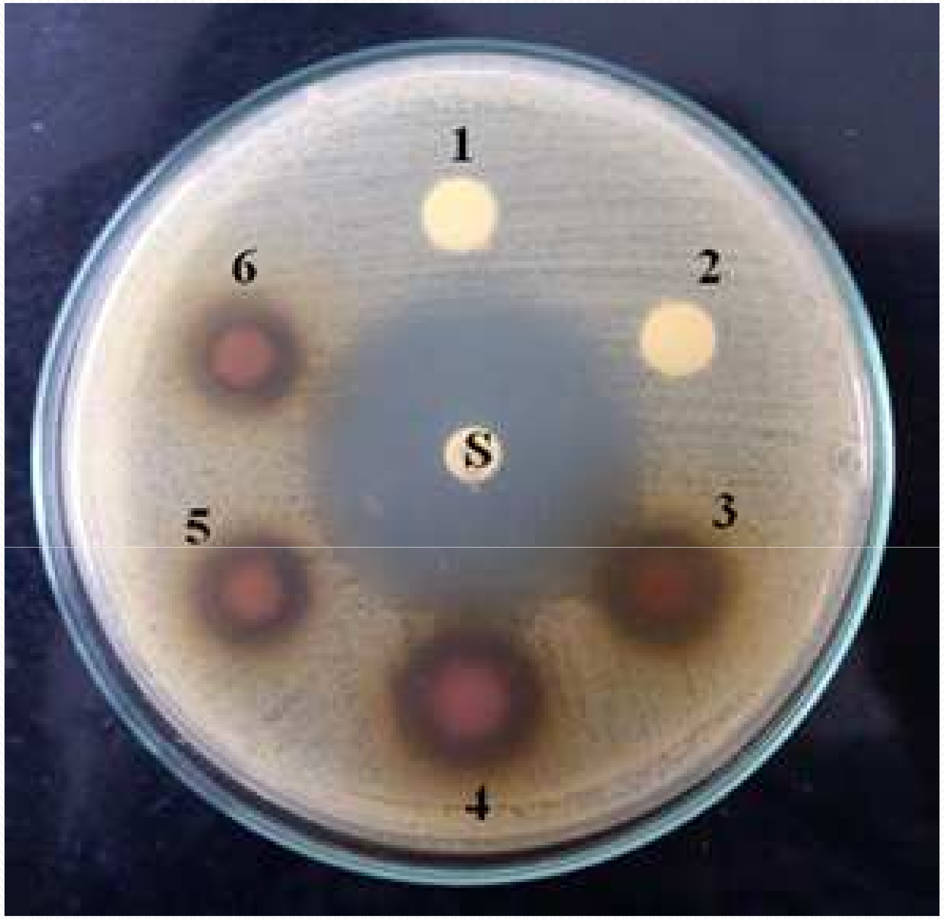
| No | Type of compound | Extract | Results |
|---|---|---|---|
| 1. | Alkaloid | 10% HCl | + |
| 2. | Carbohydrates | $H_2O$ | + |
| 3. | Glycosides | $H_2O$ | + |
| 4. | Phenolic compounds | $H_2O$ | + |
| 5. | Amino acids | $H_2O$ | + |
| 6. | Saponins | $H_2O$ | + |
| 7. | Starch | $H_2O$ | + |
| 8. | Tannins | $H_2O$ | + |
| 9. | Flavonoids | EtOH | + |
| 10. | Steroids | Pet ether | + |
| 11. | Reducing sugars | $H_2O$ | + |
| 12. | Terpenoids | Ethyl Acetate | + |
| 13. | Cyanogenic Glycosides | $H_2O$ | - |

**(+) = present**
**(-) = absent**

# Antimicrobial activities of different extracts of *Anacardium occidentale* L. barks

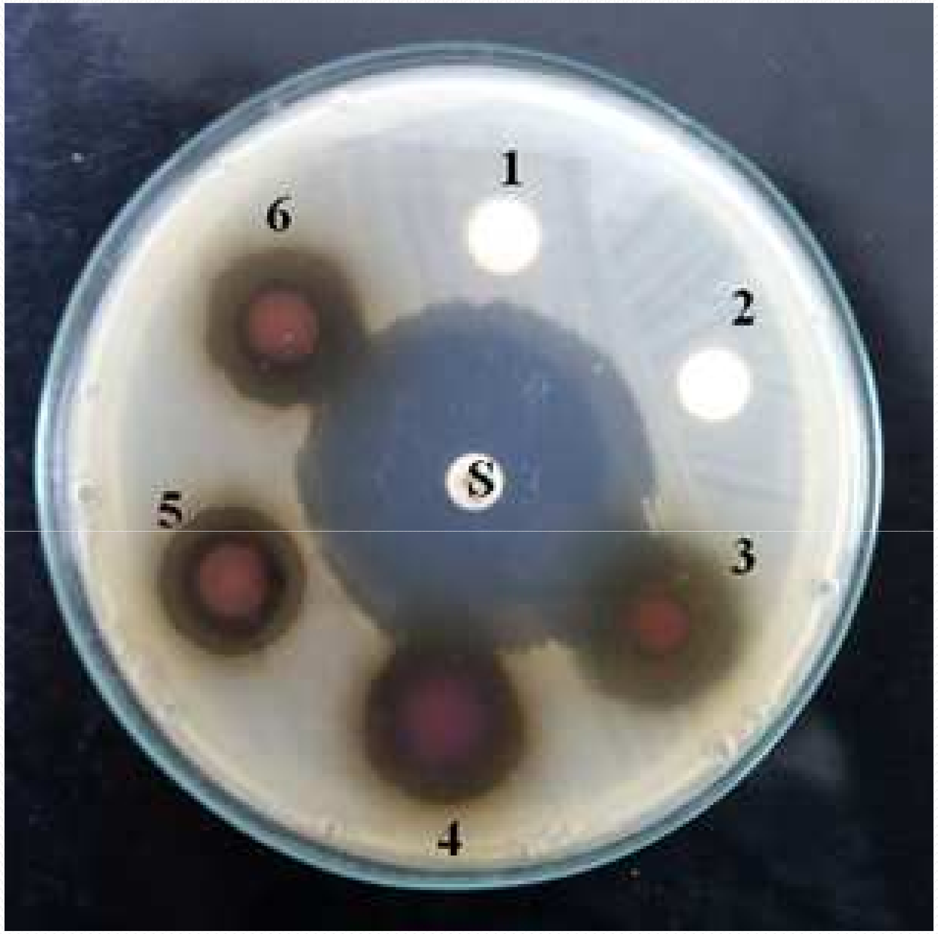
| Test organisms | | Diameter of inhibition zone (mm) of different extracts of *Anacardium occidentale* L. barks | | | |
|---|---|---|---|---|---|
| | | Ethanol (90%) extract | Ethanol (70%) extract | Acetone extract | Aqueous extract |
| *Shigella flexneri* | | 12 mm | 12 mm | 12 mm | 13 mm |
| *Shigella dysenteriae* | | 14 mm | 15 mm | 15 mm | 16 mm |
| *Salmonella typhi* | | 14 mm | 14 mm | 14 mm | 16 mm |
| *Escherichia coli* | CK | 9 mm | - | - | 9 mm |
| | AK | - | - | - | 9 mm |

**CK = Chloramphenicol 30 µg**
**AK = Amikacin 30 µg**
**Disc - 8 mm in diameter**




1 = Acetone
2 = Ethanol (70%)
3 = Aqueous extract
4 = Acetone extract
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
S = Ceftriaxone 30 μg

**Antimicrobial activity of different extracts of barks on *Shigella flexneri***

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2 = Ethanol (70%)
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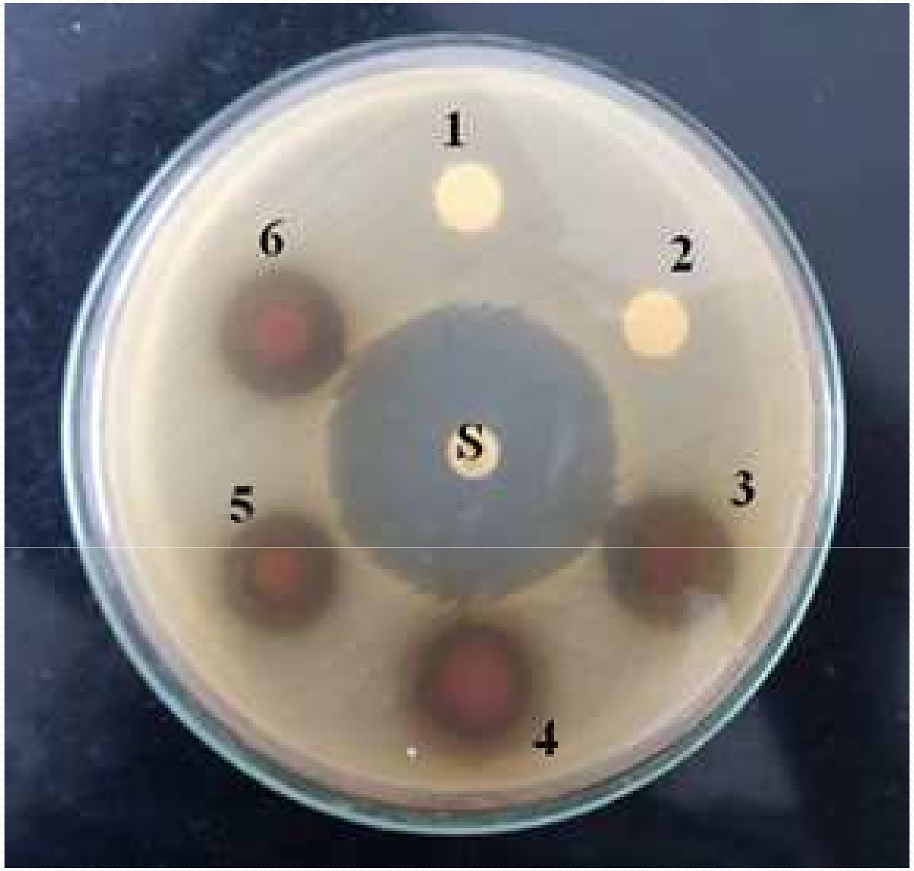
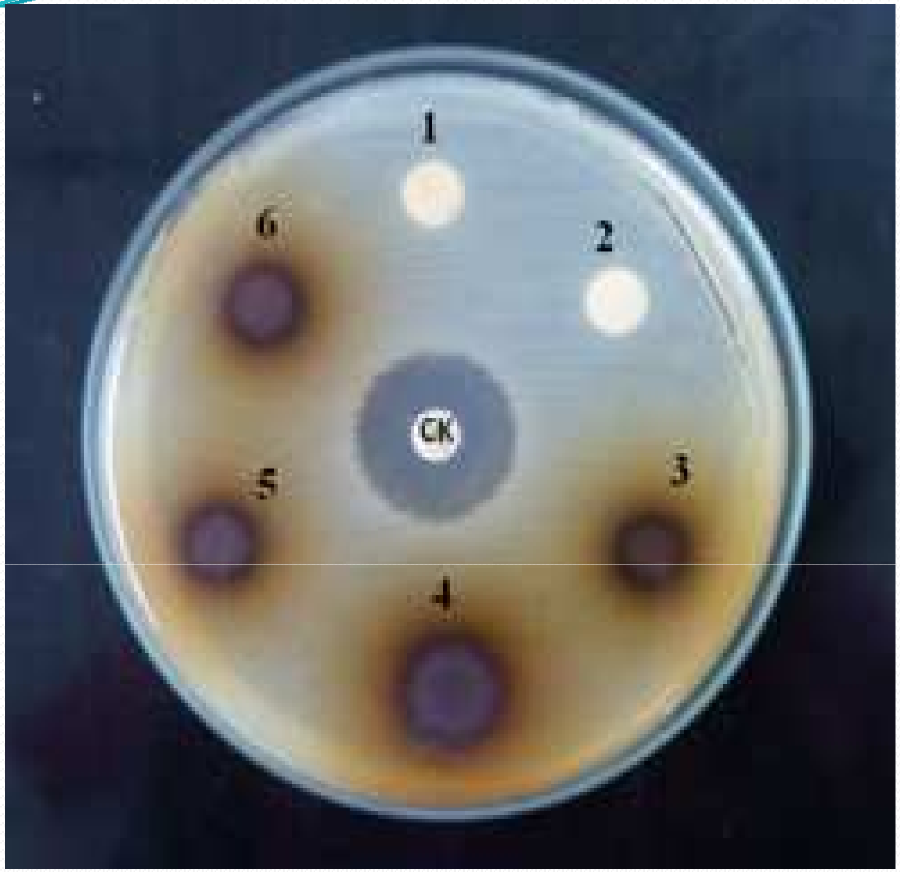
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
S = Ceftriaxone 30 μg

**Antimicrobial activity of different extracts of barks on *Shigella dysenteriae***



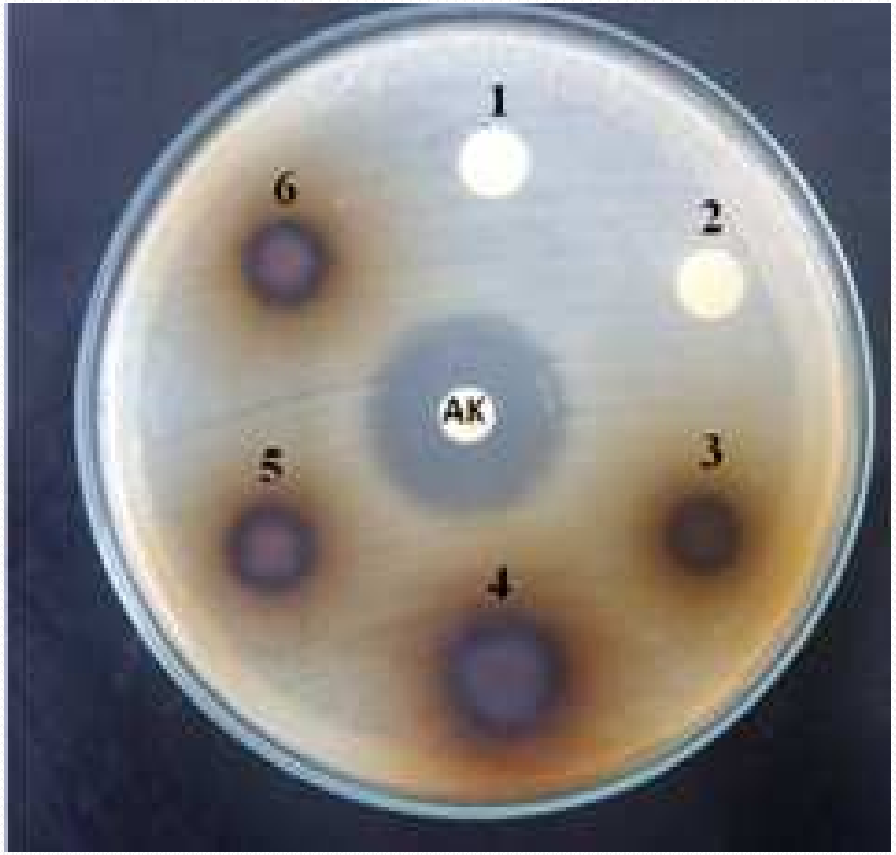

1 = Acetone
2 = Ethanol (70%)
3 = Aqueous extract
4 = Acetone extract
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
S = Ceftriaxone 30 μg

**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***




1 = Acetone
2 = Ethanol (70%)
3 = Aqueous extract
4 = Acetone extract
5 = Ethanolic (90%) extract
6 = Ethanolic (70%) extract
AK = Amikacin 30 μg

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

# 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


Adeniyi, B.A., Odelola, H.A and Oso, B.A. (1996) Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). *African Journal of Medical Sciences*, 255: p.221-224.

Al-Barl, M.A., Sayeed, M.A., Rahman, M.S and Mossadik, M.A. (2006) Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis* a novel species collected in Bangladesh. *Research Journal of Medicine and Medical Sciences*, 1: p.77-81.

Ateb, D.A. and Erdourul, O.T. (2003) Antimicrobial activities of various medicinal and commercial plant extracts. *Turkey Journal of Biology*, 27: p.157-162.

Bilcalho, B. (2001) Volatile compounds of cashew apple (*Anarcardium occidentale* L.). *Z. Naturforsch*, 56(1-2): p.35-39.

Khaing Myo Myo Su (2007) *Phytochemical investigation on the leaves of Anacardium occidentale L. (Thiho-thayet) for its antidiarrhoea and antidysenteric activities*. Ph. D. Thesis. Yangon University.

Mashesh, B and Satish, S. (2008) Antimicrobial activity of some important medicimal plants against plant and human pathogens. *World Journal of Agricultural Science*, 4(5): p.839-843.

Maung Tin-Wa (1972) Phytochemical screening: Methods and Procedure. *Phytochemical Bulletin of Botanical Society of America*. 5(3): p. 4- 10.
MOH (Ministry of Health) (2010) Morbidity and Mortality. In *Myanmar Health Statistics*. p. 52.

Taylor, J.L.S., Rabe, T., McGraw. L.J., Jager, A.K and Staden, J.V. (2001) Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulation*, 34: p.23-37.

Thinn Thinn Swe (1997) *A pharmacognostic study on Anacardium occidentale Linn.(Thiho-thayet)*. M.Sc. Thesis (Botany). University of Yangon.

WHO (1998) Quality Control methods for medicinal plant materials, Geneva. p. 28- 46.

WHO (2003) *Basic laboratory procedures in clinical bacteriology*. 2nd ed. Geneva. p. 103-119.

WHO (2009) *Diarrhoea: Why children are still dying and what can be done*. Geneva, p. 1.


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 $37^0$C for 3-4 hours to produce the growth turbidity

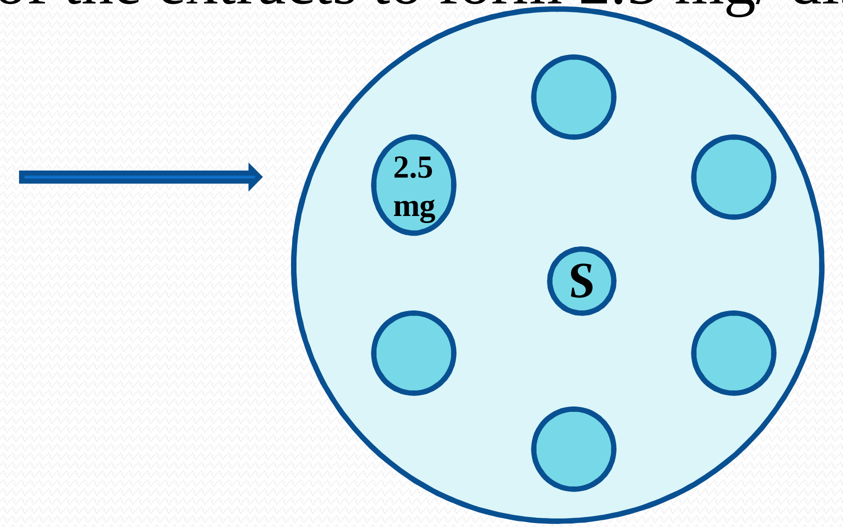
↓

Standardized density of bacterial suspension with 0.5% McFarland standard turbidity

## Preparation of dics 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