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 abcesses
- *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

General Objective

- 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)

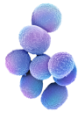
❖ **Antibacterial activity test -**

- aqueous, ethanol, ethyl acetate and petroleum ether extracts against four pathogenic bacterial strains
- Bacteria concentration - (5×10^5 $CFUml^{-1}$)
- 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

Results

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

Phytochemical constituents of leaves of *Dendrolobium triangulare* (Retz.) Schindl.

No.	Phytochemical Test	Extract	Test reagents	Observation	Results	Reference
1.	Alkaloids	1%HCL	- Wagner's reagent- Dragendorff's reagent, - Mayer's reagent	no colour change	–	
2.	Flavonoids	EtOH	Conc: HCl+Mg	brown	+	
3.	Glycoside	H_2O	Chloroform + 10% ammonia	white ppt	+	
4.	Phenolic compounds	H_2O	5% $FeCl_3$	dark green	+	
5.	Polyphenols	EtOH	10% $FeCl_3$+ 1%$K_3[Fe(CN)_6]$	dark blue	+	Raaman (2006)
6.	Phytosterols	EtOH	Acetic anhydride+ Conc: H_2SO_4	no colour change	–	
7.	Saponins	H_2O	Distilled water	more form	+	
8.	Reducing sugar	H_2O	Fehling A+B	no red ppt	–	
9.	Amino acid	H_2O	Ninhydrin	pale purple ppt	+	
10.	Carbohydrates	H_2O	Naphthol+ Conc: H_2SO_4	red ring	+	
11.	Tannins	H_2O	lead acetate	white ppt	+	
12.	Acid/Base/ Neutral	H_2O	Bromocresol green	green	acid	Harborne (1998)
13.	Cyanogenetic substance	H_2O	Na pictrate paper + conc; H_2SO_4	colour change	–	

Physicochemical properties of leaves of *Dendrolobium triangulare* (Retz.) Schindl.

No.	Physicochemical properties	Results	Reference
1.	**pH value**	**6.43**	Harborne (1998)
2.	**Ash value**		
	Total ash value	**6.8 %**	
	Acid insoluble ash	**3.35%**	
	Water soluble ash	**89.2%**	
3.	**Extractive value**		
	Water soluble extract	**20%**	
	Ethanol soluble extract	**9%**	
	Ethyl acetate soluble extract	**1.88%**	
	Petroleum ether soluble extract	**0.76%**	

Elemental analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl. by EDXRF

No.	Elements	Quantity determined percentage (%)
1.	Potassium	1.122
2.	Calcium	0.940
3.	Sulfur	0.160

Elemental analysis of leaves of *Dendrolobium triangulare* (Retz.) Schindl. by EDXRF

No.	Elements	Quantity determined percentage (%)
1.	Iron	0.012
2.	Manganese	0.006
3.	Zinc	0.001
4.	Copper	0.001

Heavy metal analysis of the leaves of *Dendrolobium triangulare* (Retz.) Schindl. By AAS

No.	Elements	Quantity determined percentage (%)
1.	Cadmium (ppm)	ND (not detected)
2.	Lead (ppm)	ND (not detected)

Antibacterial activity

- antibacterial activity - Microdilution method (Sarker *et al*. 2007)
- Extracts - aqueous, ethanolic, ethyl acetate, petroleum ether
- *Test Microorganisms*
  - *Eschericha coli*
  - *Enterococcus faecalis*
  - *Staphylococcus aureus*
  - *Bacillus cereus*

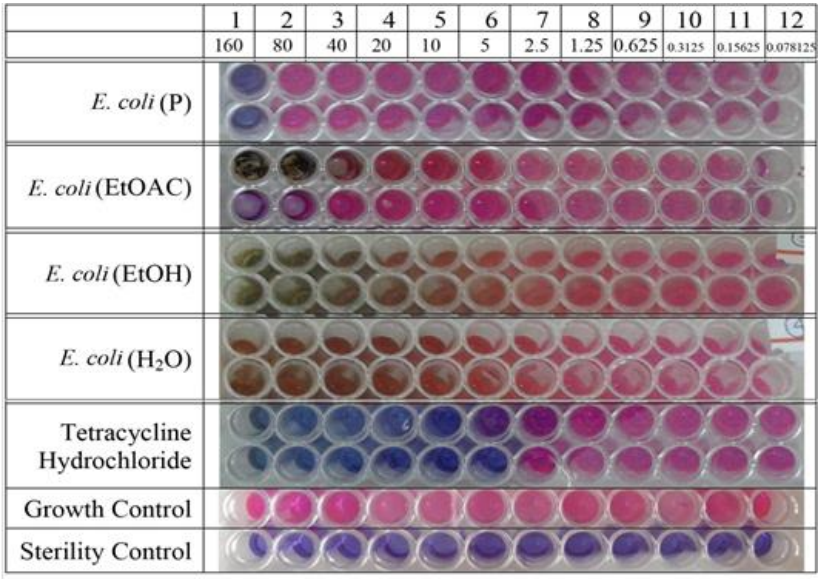


Figure 4. Minimum inhibitory concentration of plant extract against *E. coli*

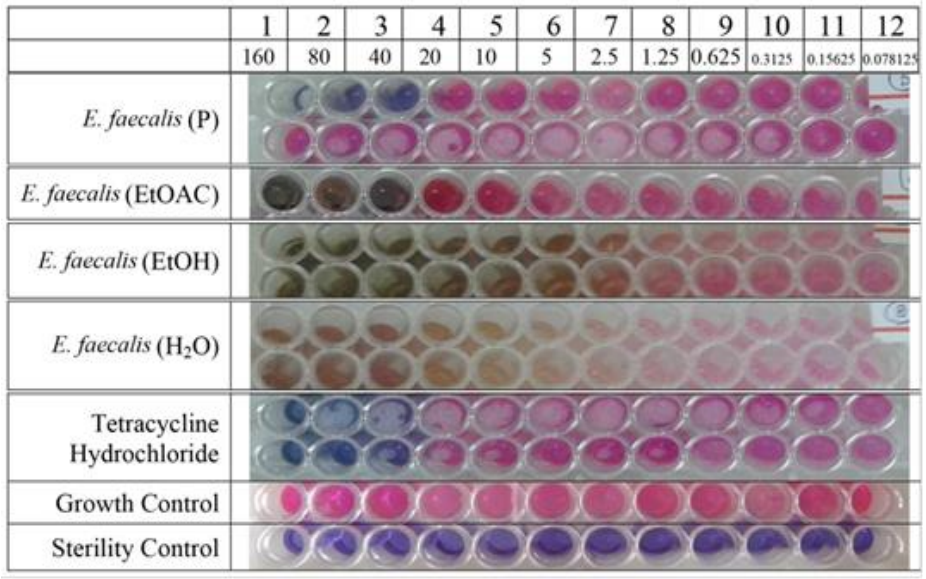


Figure 5. Minimum inhibitory concentration of plant extract against *E. faecalis*

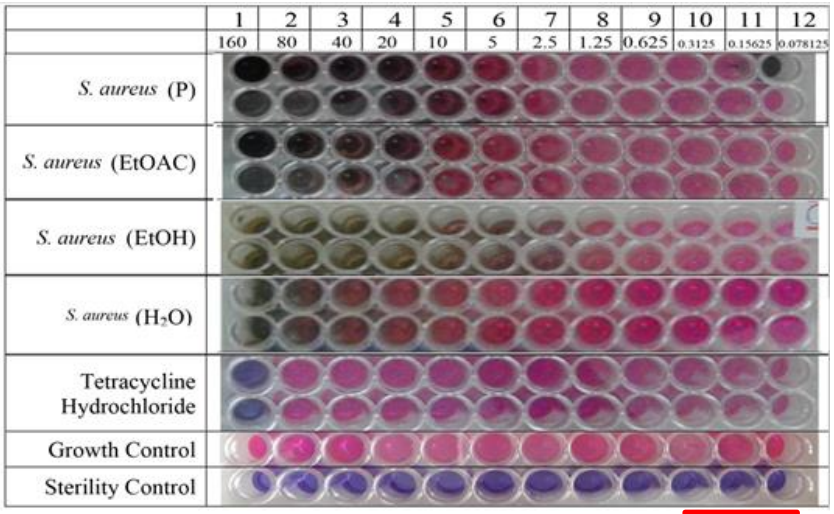


Figure 6. Minimum inhibitory concentration of plant extract against *S. aureus*

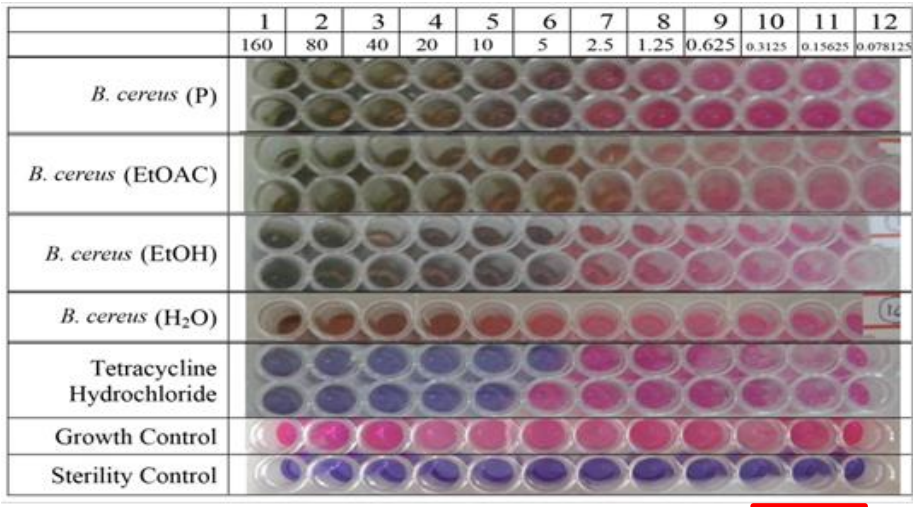


Figure 7. Minimum inhibitory concentration of plant extract against *B. cereus*

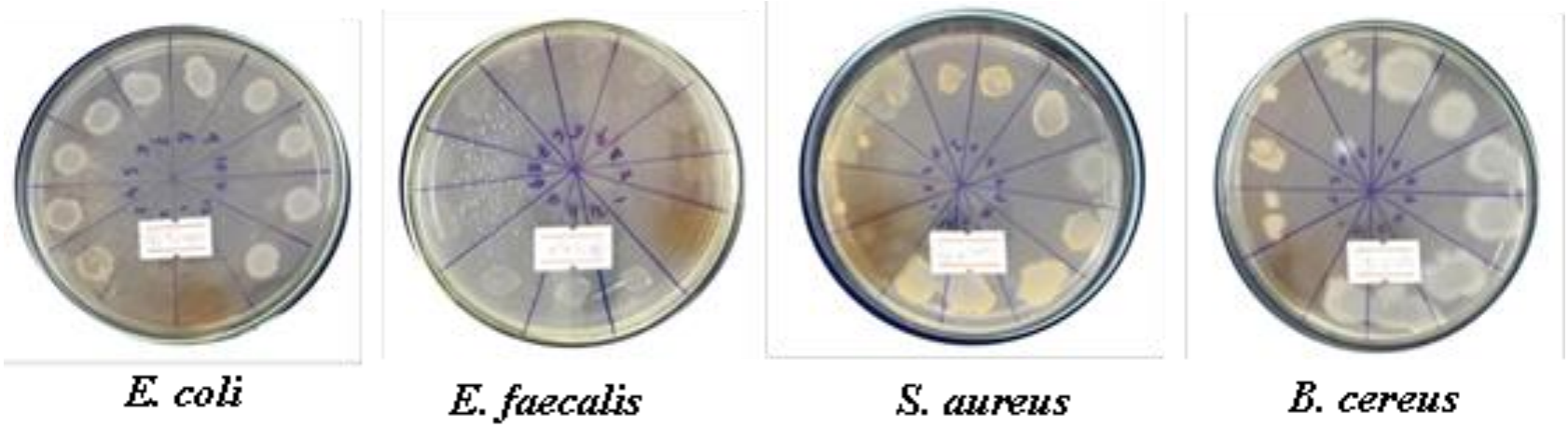


Figure 8 MBC of aqueous extracts against test organisms

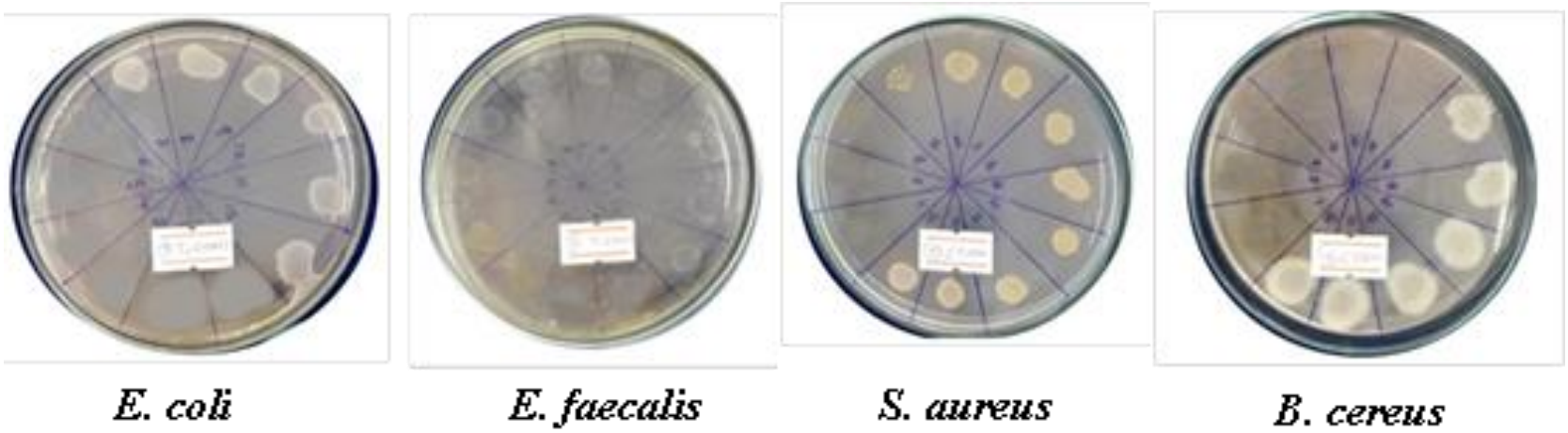
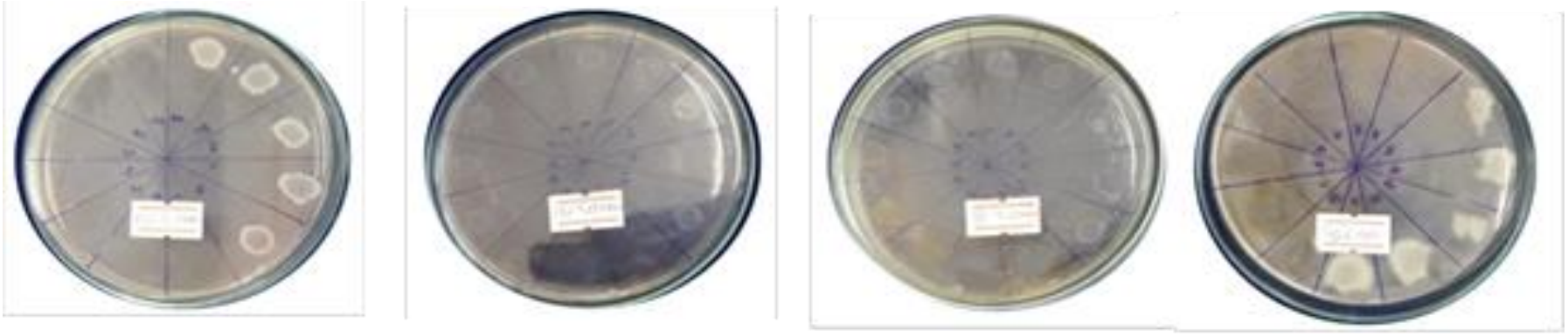
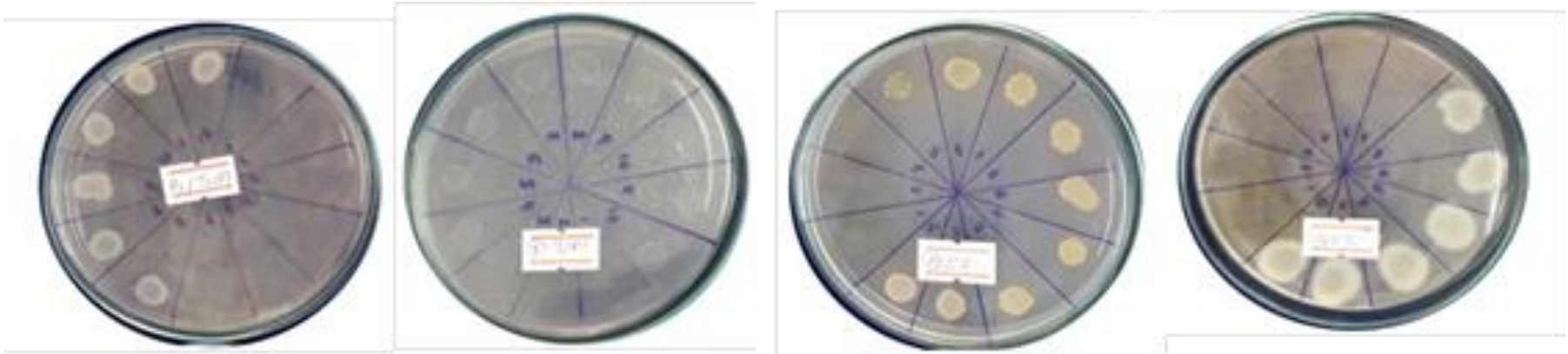


Figure 9 MBC of ethanolic extracts against test organisms



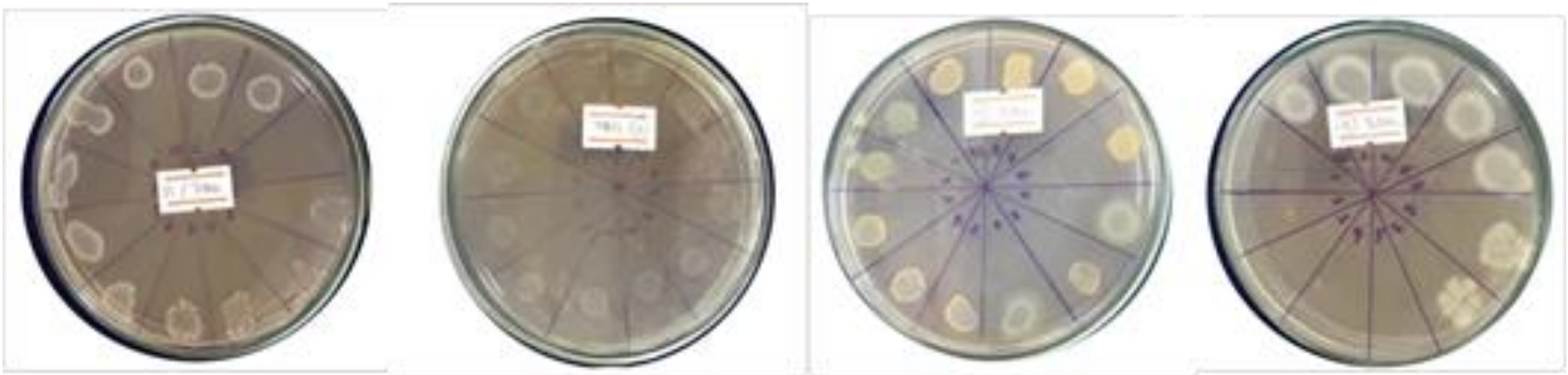
E. coli *E. faecalis* *S. aureus* *B. cereus*

Figure 10 MBC of ethyl acetate extracts against test organisms



E. coli *E. faecalis* *S. aureus* *B. cereus*

Figure 11 MBC of pet ether extracts against test organisms



E. coli *E. faecalis* *S. aureus.* *B. cereus*

Figure 12 MBC of Antibiotic (Tetracycline) against test organisms

Table 1 Antibacterial activity of MIC and MBC values

Tested Microorganisms	Aqueous extract		Ethanolic extract		Ethyl acetate extract		Pet-ether extract		Tetracycline Hydrochloride	
	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})
Escherichia coli **ATCC 25922**	**80**	**80**	**80**	**80**	80	160	160	160	4×10^{-3}	1.28×10^{-1}
Enterococcus faecalis **ATCC 29212**	10	160	5	80	40	80	40	40	3.2×10^{-2}	6.4×10^{-2}
Staphylococcus aureus **ATCC 25923**	160	160	10	40	20	80	20	40	1.28×10^{-1}	$>1.28\times10^{-1}$
Bacillus cereus **ATCC**	160	160	5	5	**2.5**	**2.5**	**2.5**	**5**	4×10^{-3}	8×10^{-3}

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

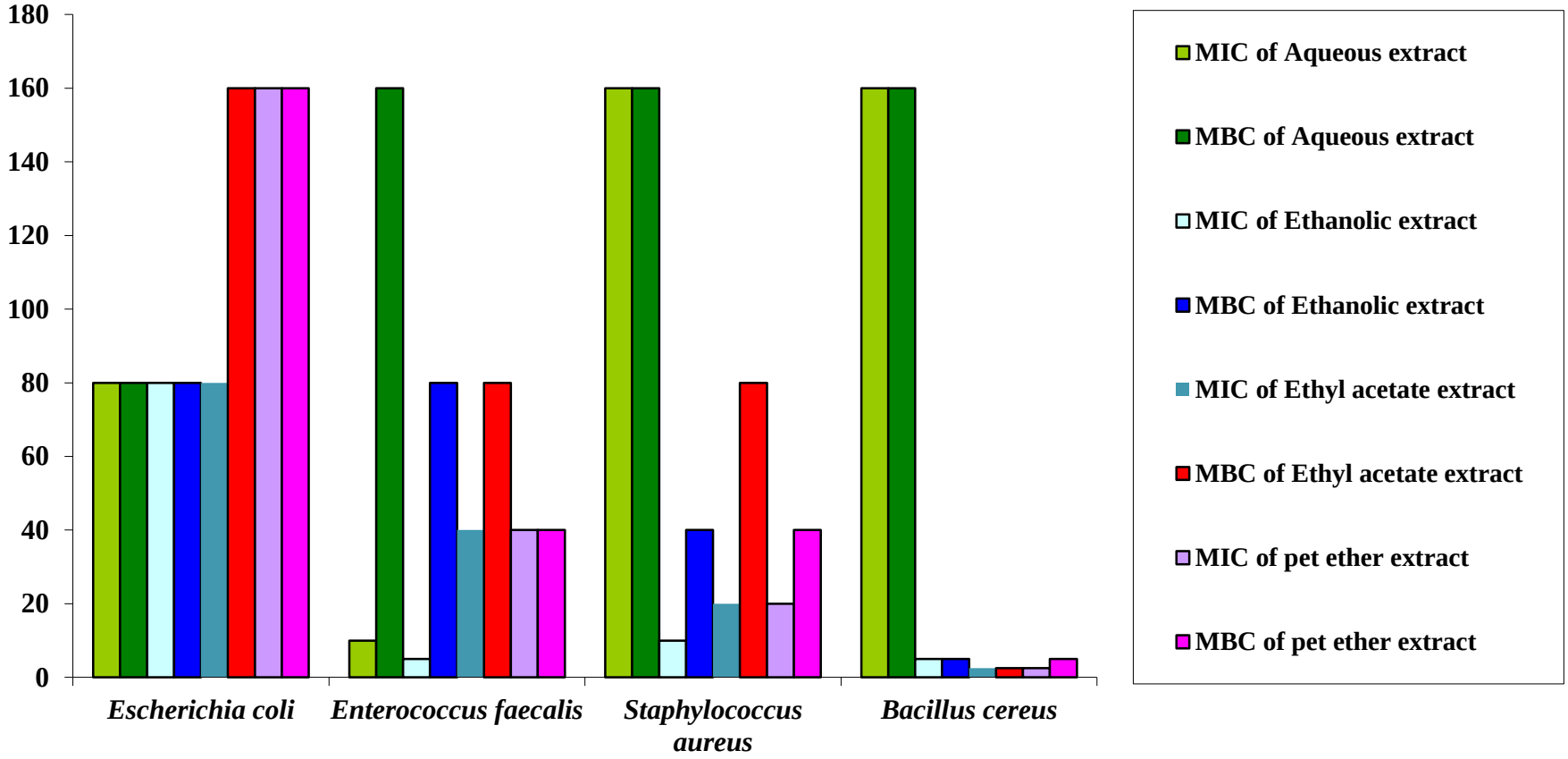


Figure 13 Antibacterial activity of MIC and MBC values

DISCUSSION AND CONCLUSION

- 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

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Thank you!