Antibacterial activities of leaves extracts of *Desmodium gangeticum* (L.) DC. (Kyae me hpo)

Aye Aye Myat [1], Su Su Hlaing [2], Thu Mon Htun [3], Hnin Hnin Htun [4]

University of Medical Technology[1], University of Pharmacy[2], Mandalay, University of Medicine 2, Yangon[3], University of Traditional Medicine[4], Mandalay

INTRODUCTION

- Medicinal plants are nature's gift to human beings for disease free healthy life
- Herbal medicine represents one of the most important fields of traditional system for preventive as well as the therapeutic aid for various ailments
- It is a valuable source of natural compounds for antimicrobial agents for maintaining diseases associated with pathogenic bacteria and fungi (Mutyala & Aniel 2016)

- *Desmodium gangeticum* (L.) DC. belonging to family Fabaceae is known as Kyae me hpo, grows wild in Myanmar (Kress *et al*. 2003)
- It have valuable source of natural compounds and traditionally used in therapeutic aid for various ailments
- widely used in the Indian Ayurveda medicine (Mutyala & Aniel 2016)
  - typhoid, piles, asthma and bronchitis (Niranjan & Tewari 2008)
  - tonic, febrifuge, digestive, anti emetic, in inflammatory conditions of the chest and in various other inflammatory conditions (Rathi *et al.* 2004)

- *D. gangeticum* (L.) DC. - possess antioxidant, anti-inflammatory, anti-emetic, anti-ulcer and cardio-protective effects (Gopalakrishnan & Rajameena 2012)
- Ashin Na Ga Thein (1968) - used in Myanmar traditional medicinal applications, for the treatment of cough, asthma and fever

- 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 & Kolhapure 2004)
- This situation has forced to search for new antimicrobial sources like medicinal plants (Doshi *et al*. 2011)
- Medicinal plants and their derived are rich in antibacterial compounds (Singh *et al*. 2016)

- Prevention of bacterial infections, using plant extracts, is highly desirable due to low cost, environmental friendliness, and effectiveness against certain bacteria, compared to antibiotics which might be harmful to the environment (Cheng *et al.* 2014)
- Present study were carried out on morphological characters, phytochemical constituents, physicochemical properties, elemental analysis and antibacterial activity of the leaves extract of *D. gangeticum* (L.) DC.

General Objective

- To study the antibacterial activity of various extracts from the leaves of *D. gangeticum* (L.) DC.

Specific Objectives

- To identify the morphological characters of *D. gangeticum* (L.) DC.
- To investigate the qualitative and quantitative analysis of leaves of *D. gangeticum* (L.) DC.
- To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaves extracts of *D. gangeticum* (L.) DC.

MATERIALS AND METHODS

Plant collection and identification

- The plant specimens were collected from Pyin Sar village, Pyin Oo Lwin Township, Mandalay Region
- The collected specimens were identified and classified according to Hooker (1885) & Dassanayake (1998)

Extraction

- Various extracts of the dried leaves of *D. gangeticum* (L.) DC. were done by percolation method

Phytochemical tests

- Preliminary phytochemical tests were carried out by the methods of Harbone (1998) and Raaman (2006)

Physicochemical properties

- Physicochemical properties were determined for the quality control parameter of medicinal purposes (WHO, 2011)

Elemental analysis

- Elemental concentration were analyzed by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF) and Atomic Absorption Spectrophotometer (AAS) methods

Determination of antimicrobial activity

- Antibacterial activity of petroleum ether, ethyl acetate, ethanol and aqueous extracts of *D. gangeticum* (L.) DC. were tested by determining the (MIC) and (MBC) using microdilution method with Resazurin (Sarker *et al.* 2007)
- Twelve concentrations (0. 12 to 250 mg/ml) of various extracts were tested in *vitro* antibacterial activity against four pathogenic bacterial strains
- Ciprofloxacin was used as positive control

- Test organisms used in this study were supplied from Upper Myanmar Public Health Laboratory, Mandalay and Biotechnology Research Department, Kyaukse
- Test organisms
    - *Enterococcus faecalis* ATCC 29212,
    - *Escherichia coli* ATCC 25922,
    - *Pseudomonas aeruginosa* ATCC 27853 and
    - *Staphylococcus aureus* ATCC 25923.
- Bacteria concentration - 5×10^5 $CFUml^{-1}$
- The antibacterial activity test was done at Medical Laboratory Technology Department, University of Medical Technology, Mandalay

RESULTS

- **Morphological characters**
- **Phytochemical constituents of leaves of *Desmodium gangeticum* (L.) DC**
- **Physio-chemical properties**
- **Elemental analysis**
- **Antibacterial activity**

Morphological Characters

- Habit - perennial shrubs
- Leaves - unifoliolate compound

  alternate

Figure 1. *Desmodium gangeticum* (L.) DC. (Kyae me hpo)

- Inflorescences - axillary and terminal racemes
- Flowers - pale green tinge with purple
- zygomorphic
- Calyx - 4-lobed; pale green
  densely pubescent
- Corolla - papilionaceous
- standard, wings, keels
- Stamens - 10, diadelphous
- Ovary - oblong, white pubescent
- Fruit - Pods 7 to 8 jointed
- Seeds - reniform, small, yellow

Figure 2. Inflorescences of *Desmodium gangeticum* (L.) DC. (Kyae me hpo)

Table 1. Phytochemical constituents of leaves of *Desmodium gangeticum* (L.) DC

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	–	Raaman (2006)
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	+	
6.	**Phytosterols***	EtOH	Acetic anhydride+ Conc: H_2SO_4	green	+	
7.	**Saponins**	H_2O	Distilled water	no stable foam	–	
8.	**Reducing sugar**	H_2O	Fehling A+B	pale 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	–	

(+) present (–) absent *Terpenoids present

Table 2. Physico-chemical properties of leaves of *Desmodium gangeticum*

No.	Physico-chemical Parameters	Quantity determined percentage *D. gangeticum*
1	pH	5.95
2	Total ash	6.9 %
3	Acid insoluble ash	1.7 %
4	Water soluble ash	90.05 %
5	Water soluble matter	26.28 %
6	Ethanol soluble matter	5.38 %
7	Ethyl acetate soluble matter	2.27 %
8	Pet-ether soluble matter	1.54 %

Table 3 Percentage of macroelements of the leaves of *D. gangeticum* (L.) DC. by using EDXRF

No.	Elements	Quantity determined percentage (%)
1.	Potassium	1.123
2.	Calcium	0.706
3.	Sulfur	0.194

Table 4. Percentage of microelements of the leaves of *D. gangeticum* (L.) DC. by using EDXRF

No.	Elements	Quantity determined percentage (%)
1.	Iron	0.009
2.	Manganese	0.003
3.	Zinc	0.001
4.	Copper	0.001

Table 5. Heavy metal analysis of the leaves of *D.gangeticum* (L.) DC. by using AAS

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

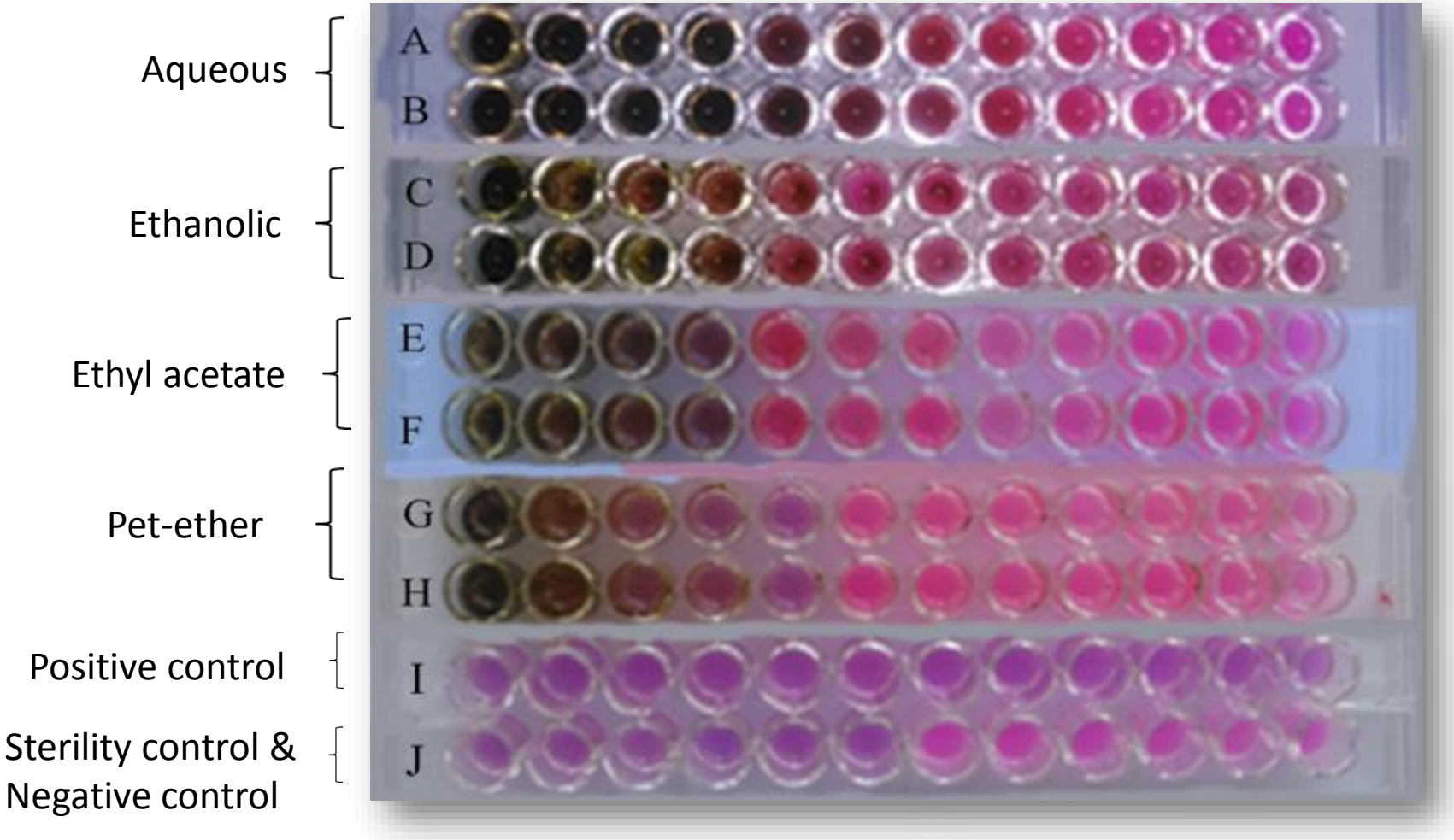

Figure 3. MIC of aqueous, ethanolic, ethyl acetate, pet-ether leaves extracts against *Enterococcus faecalis*

- pink – growth, blue - inhibition of growth

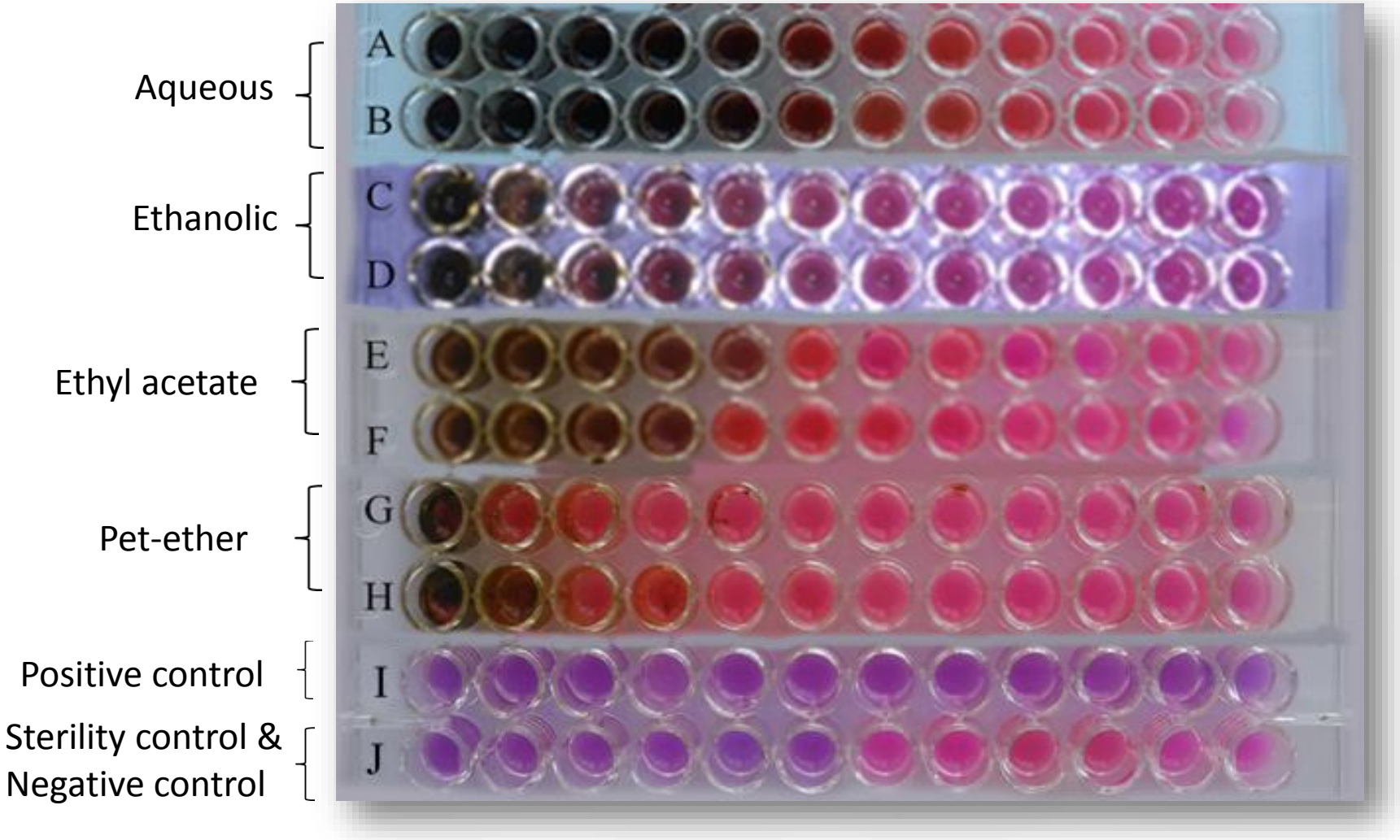

Figure 4. MIC of aqueous, ethanolic, ethyl acetate, pet-ether extracts leaves against *E.coli*

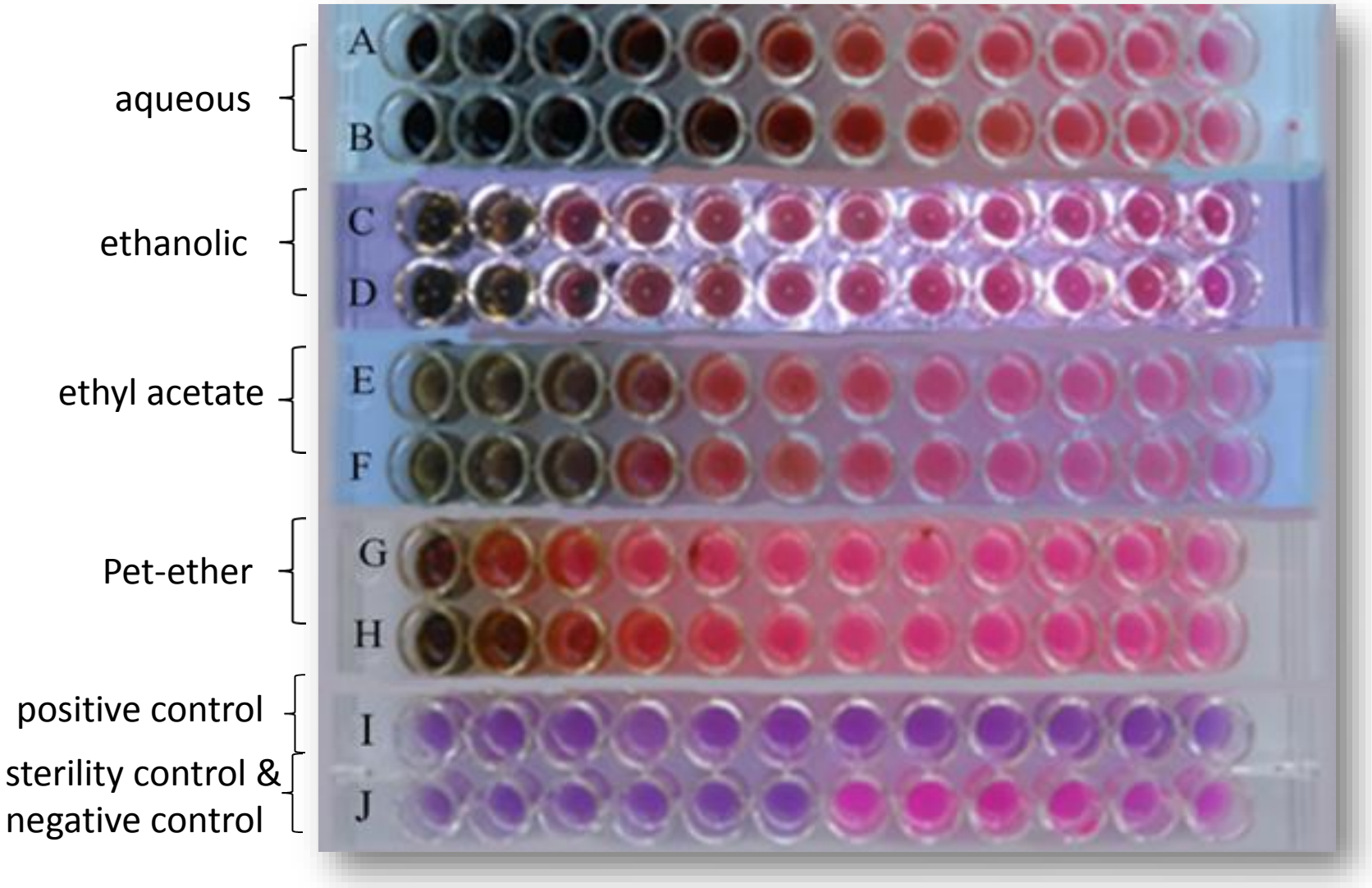

Figure 5. MIC of aqueous, ethanolic, ethyl acetate, pet-ether leaves extracts against *Pseudomonas aeruginosa*

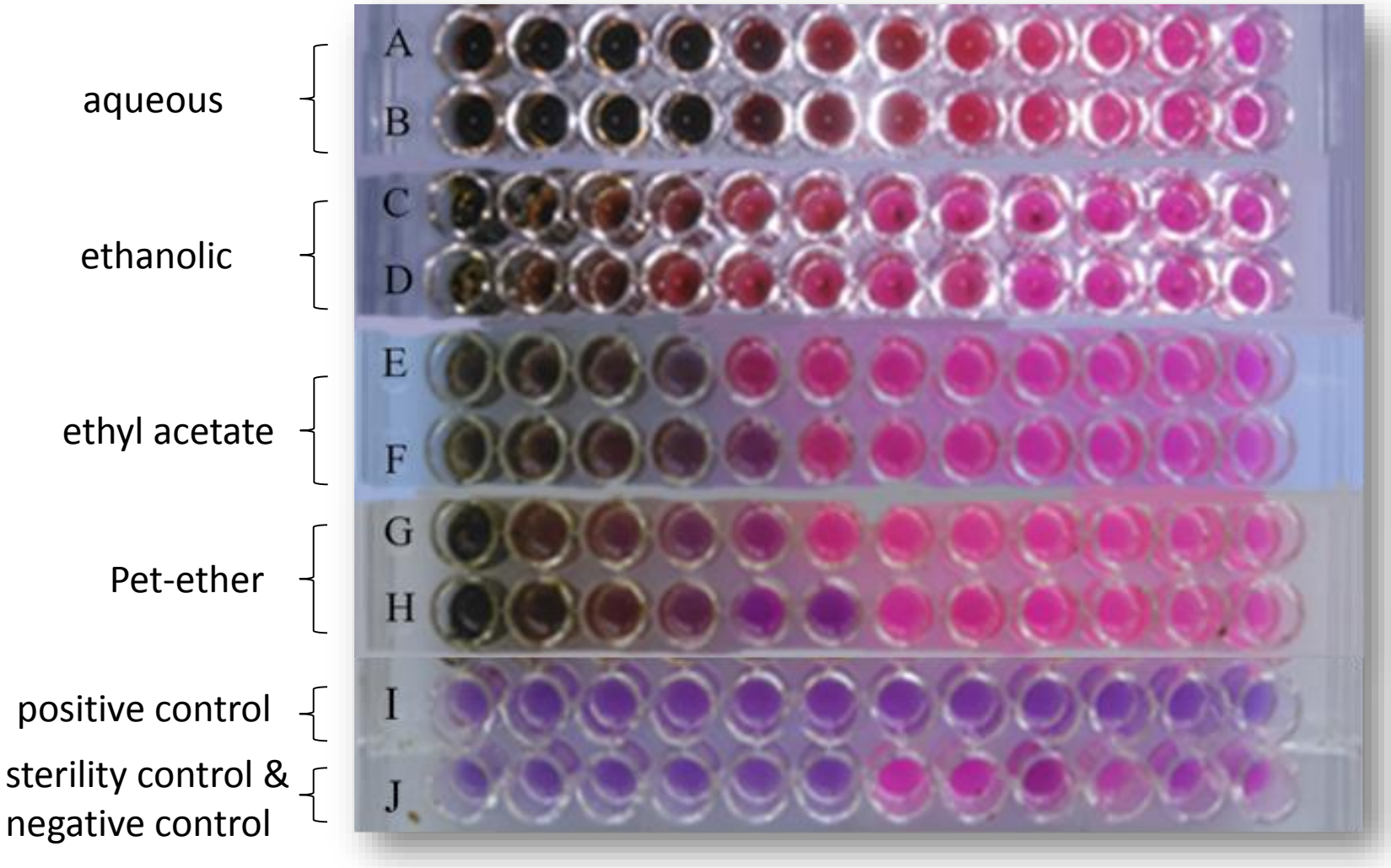

Figure 6. MIC of aqueous, ethanolic, ethyl acetate, pet-ether leaves extracts against *Staphylococcus aureus*

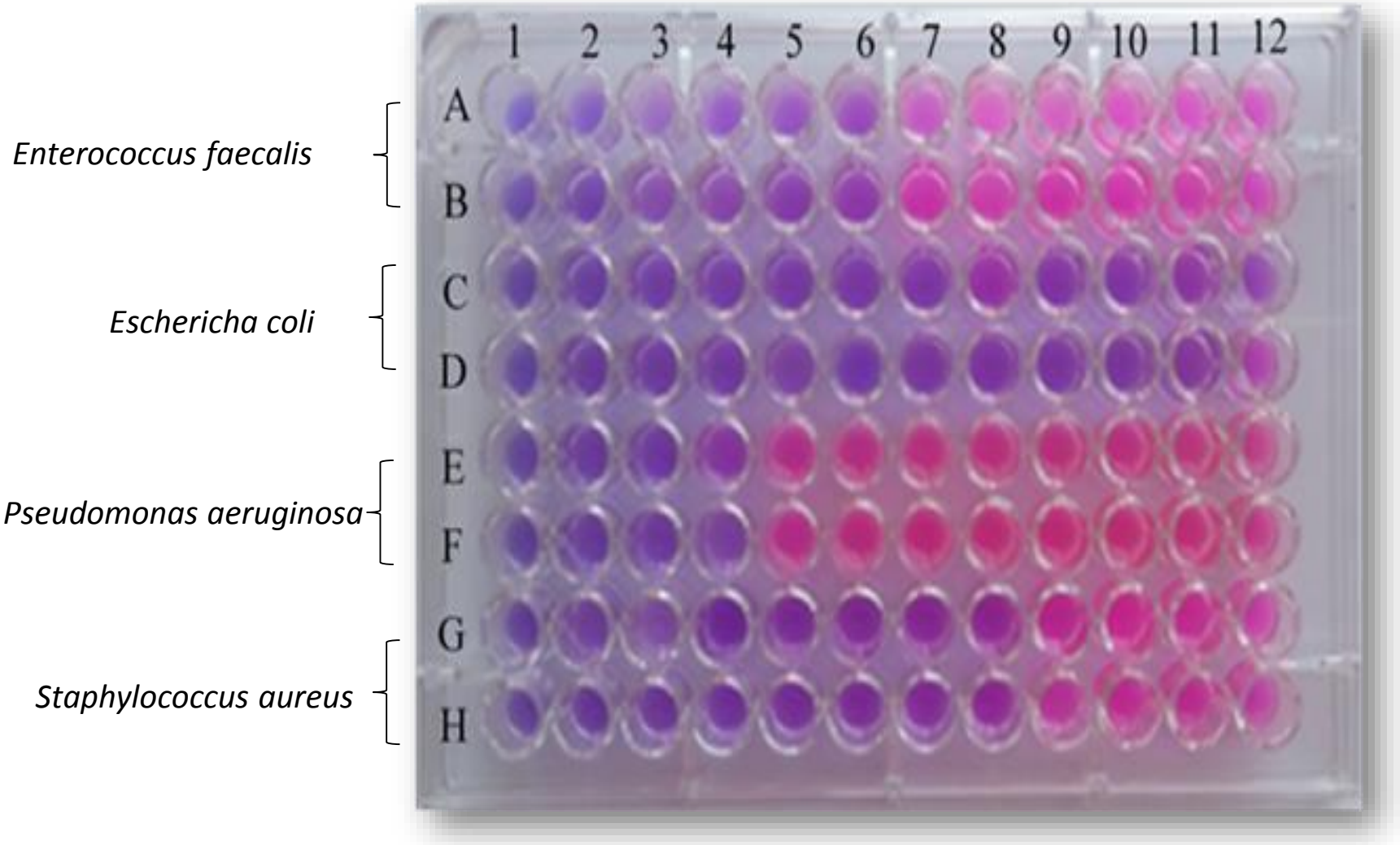

Figure 7. MIC of Antibiotic (Ciprofloxacin) against test organisms

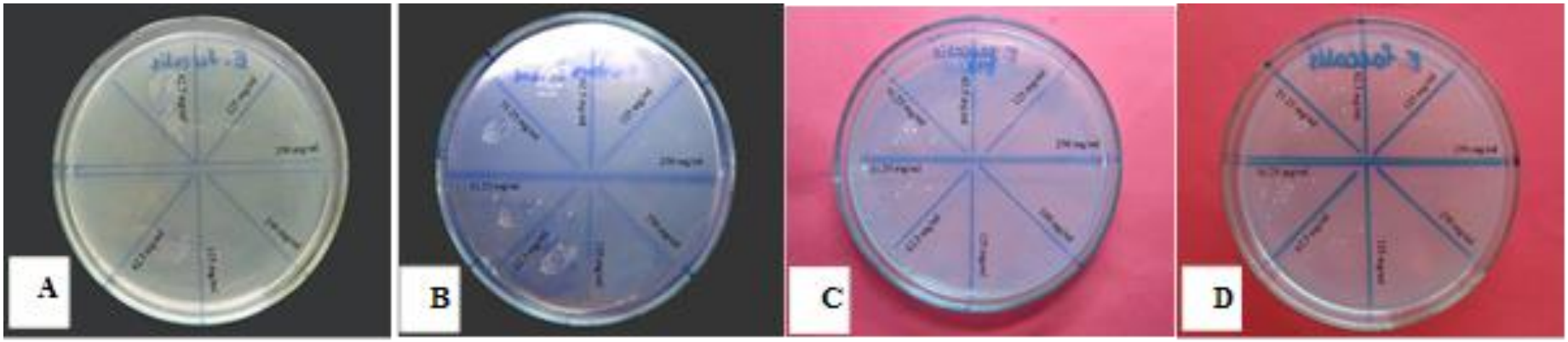

Figure 8. MBC of (A) aqueous, (B) ethanolic, (C) ethyl acetate and (D) pet-ether extracts against *E. faecalis*

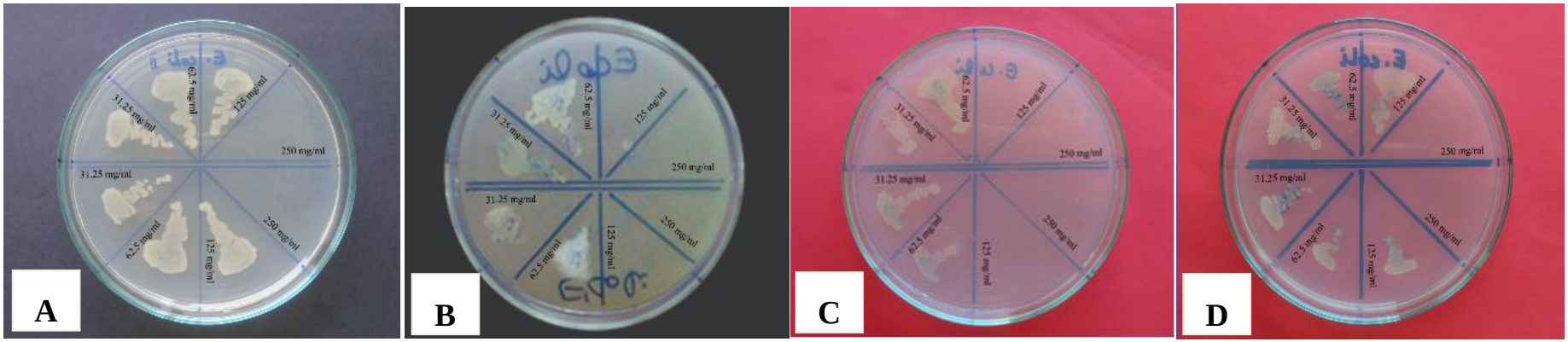

Figure 9. MBC of (A) aqueous, (B) ethanolic, (C) ethyl acetate and (D) pet-ether extracts against *E. coli*

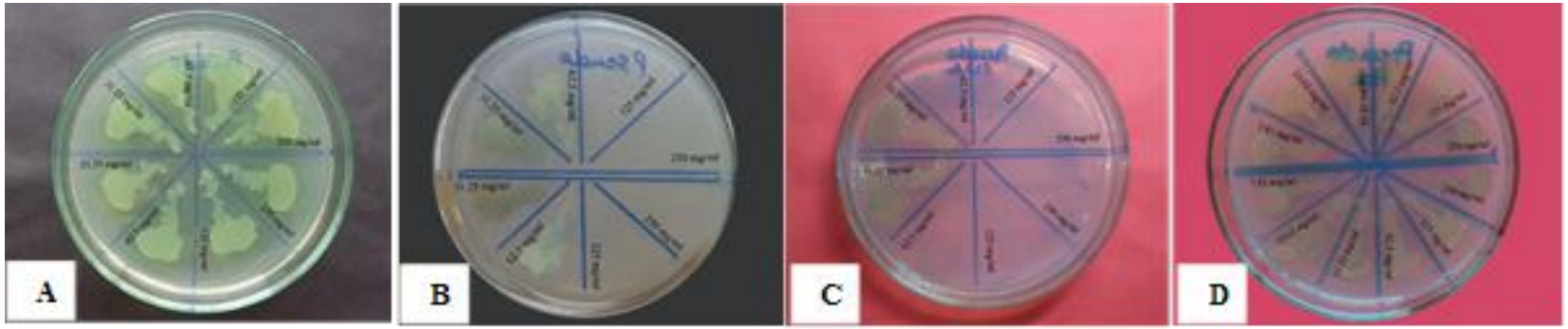

Figure 10. MBC of (A) aqueous, (B) ethanolic, (C) ethyl acetate, (D) pet-ether extracts against *P. aeruginosa*

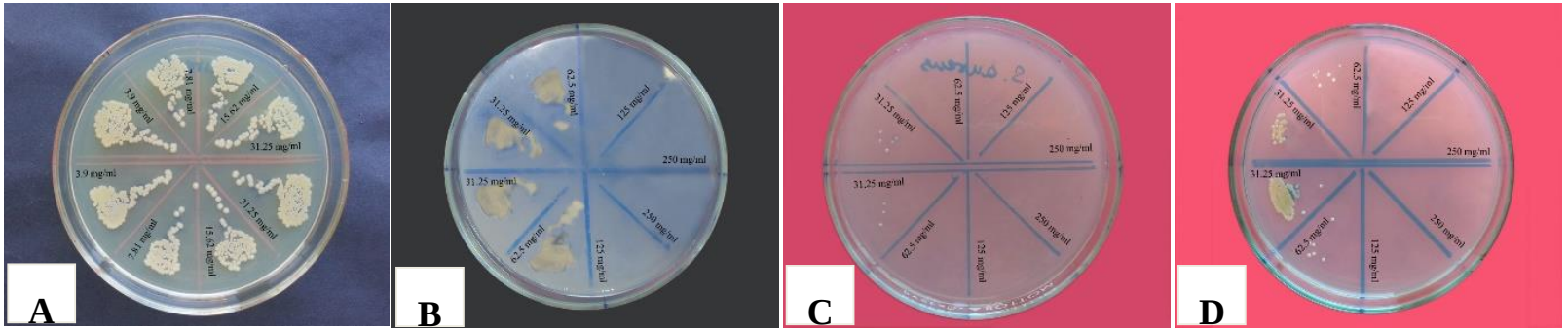

Figure 11. MBC of (A) aqueous, (B) ethanolic, (C) ethyl acetate and (D) pet-ether extracts against *S. aureus*

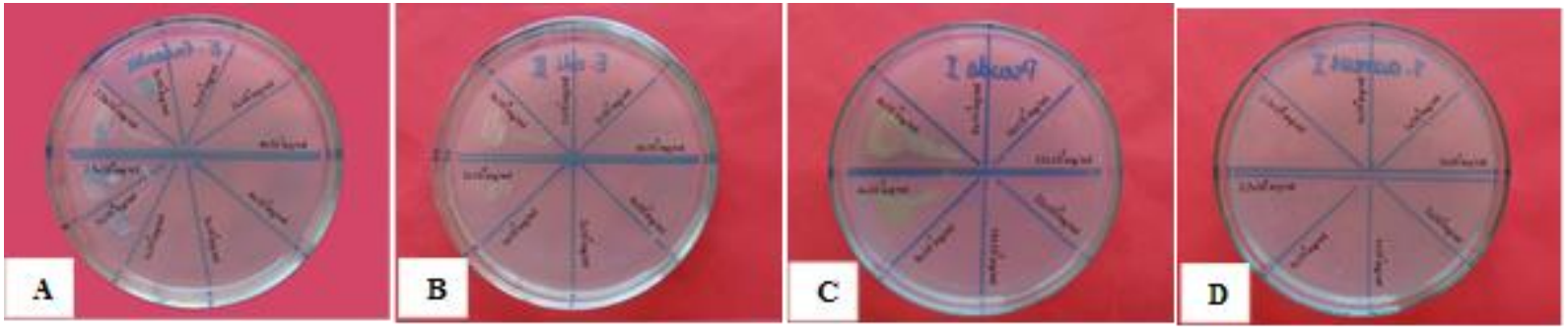

Figure 12. MBC of Antibiotic (Ciprofloxacin) against test organisms

Table 3. Antibacterial activity of MIC and MBC values for various leaf extracts from *Desmodium gangeticum* (L.) DC.

Tested Microorganisms	Aqueous extract		Ethanolic extract		Ethyl acetate extract		Pet-ether extract		Ciprofloxacin	
	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})
Enterococcus faecalis ATCC 29212	7.81	125	62.5	125	31.25	62.5	15.62	125	1×10^{-3}	1×10^{-3}
Staphylococcus aureus ATCC 25923	15.62	62.5	62.5	125	31.25	62.5	15.62	125	2.5×10^{-4}	5×10^{-4}
Pseudomonas aeruginosa ATCC 27853	31.25	>250	125	125	62.5	62.5	250	>250	4×10^{-3}	8×10^{-3}
Escherichia coli ATCC 25922	15.62	250	125	125	31.25	125	125	125	5×10^{-4}	1×10^{-3}

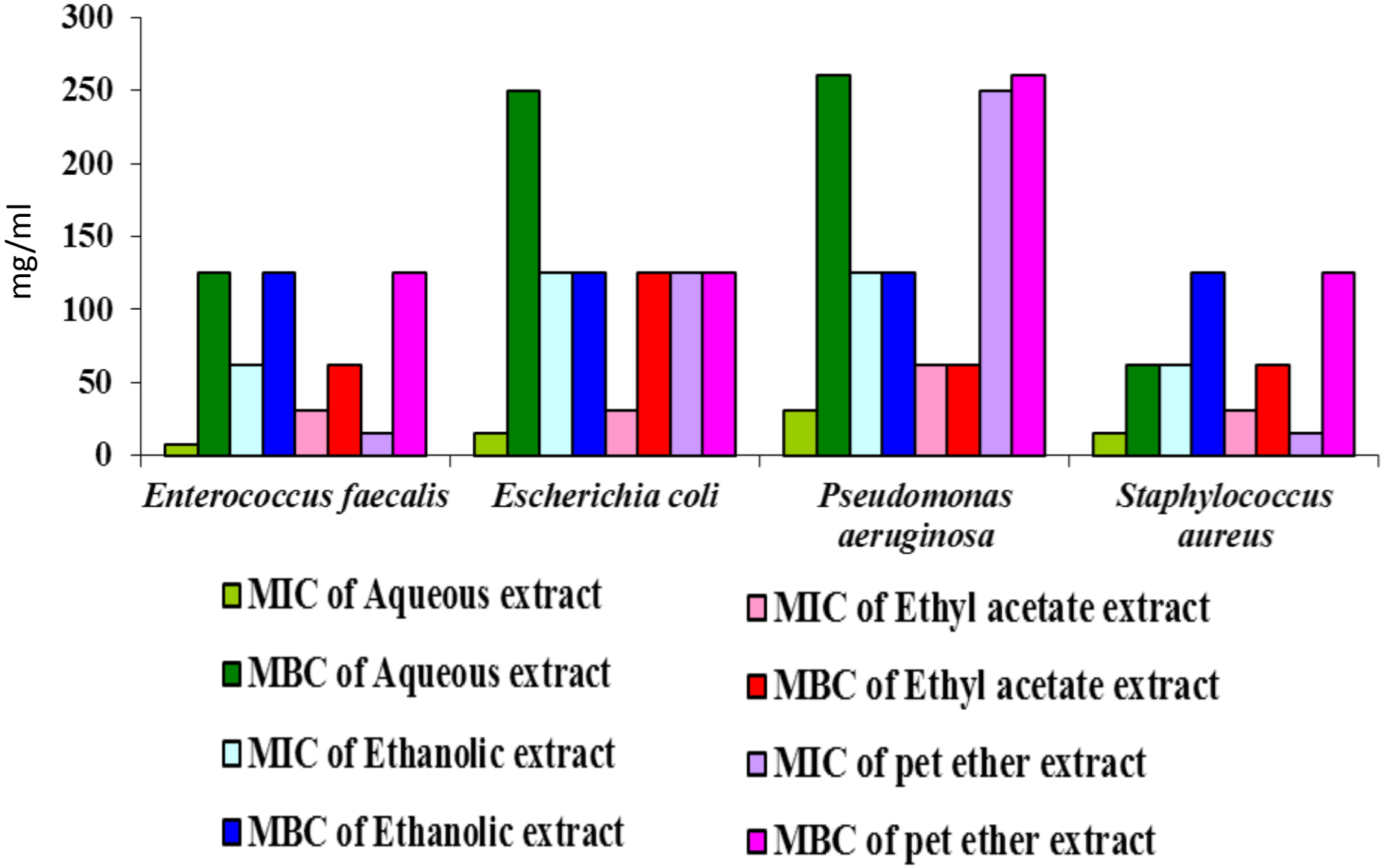

Figure 12. Antibacterial activity of MIC and MBC values for various leaf extracts

DISCUSSION AND CONCLUSION

- **The antibacterial activity of aqueous, ethanolic, ethyl acetate and petroleum ether extract of leaves of *D. gangeticum* (L.) DC. was determined by microdilution method with resazurin.**
- **Determinations of phytochemical constituents, physico-chemical properties, elemental analysis, heavy metal contents of *Desmodium gangeticum* (L.) DC. (Kyae me hpo) were studied**
- **Preliminary phytochemical analysis indicated that presence of flavonoids, glycosides, phenolic compounds, polyphenols, amino acid, carbohydrates, tannins, terpenoids, reducing sugar and the absence of alkaloids, saponin and harmful cyanogenic substance**

- **Ash values**
  - **Water soluble ash > Acid insoluble ash**
- **Extractable values**
  - **Water > Ethanol > Ethyl acetate > Petroleum ether fraction (least)**
- **showed that large amount of polar phytoconstituents were present in the leaves of this plant**
- **This properties are importance because compounds present in plant may have different solubility**

- **Elemental analysis was done by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrophotometer**
- **Macroelements**
  - **Potassium (Major element)**
  - **Calcium**
  - **Sulphur**
- **Microelements**
  - **Iron (most abundant)**

- **Leaves powder of these plants were analysed by Atomic Absorption Spectroscopy (AAS) to know the present or absent of heavy mental**
- **Toxic elements; lead and cadmium were not present in this species**
- **Antibacterial activities were used with microdilution method by using Resazurin (as an indicator)**
- **Resazurin indicated the detection of bacterial growth**

- **Twelve different concentrations crude extracts were tested for their antibacterial potential.**
  - ***Enterococcus faecalis***
  - ***Staphylococcus aureus***
  - ***Eschericha coli***
  - ***Pseudomonas aeruginosa***

- **MIC - 7.81 mg ml^{-1} to 250 mg ml^{-1}**
- **MBC - 62.5 mg ml^{-1} to >250 mg ml^{-1}**
- **For gram positive bacteria, aqueous extracts show more significant inhibition activity against like *Enterococcus faecalis* than *Staphylococcus aureus***
- **For gram negative bacteria, aqueous and ethyl acetate extracts showed more significant inhibition activity against *E. coli* than *P. aeruginosa***
- **Therefore, the leaves extract of *D. gangeticum* showed scientific evidence for the antibacterial activity and the therapeutic use of this plant in the traditional medicine.**

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many
Thanks!