Original Research Article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED INDIAN TRADITIONAL MEDICINAL PLANTS

ABSTRACT

Antibacterial and antifungal activities are the universal supporting activities for other therapeutic activities like anti-inflammatory, wound healing etc. The traditional medicinal plants Pongamia pinnata and Macaranga peltata selected. The selected plants different extracts (Ethanol, Chloroform, Petroleum ether, Ethyl acetate and Methanol) were prepared and preliminary phytochemical screening was performed. The antibacterial and antifungal activity was performed by Agar well method by using Ciprofloxacin and Erythromycin standards. In this study we come to conclusion that the selected medicinal plants alcohol extract is having significant antibacterial and antifungal activity.

Key words: Pongamia, Macaranga, Antibacterial, Antifungal etc.

INTRODUCTION

Medicinal plants are used for curing innumerable diseases. In terms of medicinal uses mixture of constituents found in extracts of plants are more effective than isolated compounds. Many herbs in nature possess tissue regenerating property as they possess pharmacologically active compound in minute quantity along with energy boosting molecules such as carbohydrates, lipids and proteins.

The plants possess various therapeutic activities which should be brought to the notice of the scientific field for the systematic evaluation. Hence an attempt was made to select the plants possessing antibacterial and antifungal activity. Traditionally Pongamia pinnata using for antiseptic activity in animals but not to humans due to uncomfortable odor

In the present study Mecaranga peltata leaves and Pongamia pinnata seeds selected since scientifically antibacterial extracts and principles are not explored and standardized for the proposed activity. Both plants are traditional medicinal plants possessing anti-inflammatory, antioxidant other relevant activities.

Singh RK et al. [1] reported, the anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rat using carrageenan, bradykinin, PGE induced models and inflammation intensity measured by production of inflammatory molecules histamine and 5-HT. The result

indicates that all extracts of seed (ethanol, petroleum ether, chloroform and acetone) shown anti-inflammatory activity when administered intra-peritoneally. (i.p)

Kumar P et al. [2] reported, the *Pongamia pinnata* flower and flower buds were having antibacterial and antifungal activity. Flower extracts shown higher antibacterial activity against Staphylococcus aureus and .Klebsiella. pneumoniae as compared to pod extract. Flower extract shown higher antifungal activity than pod extract

Rani MS et al. [3] reported, antibacterial activity of *Pongamia pinnata* on pathogens of clinical isolates. The seed extract of *Pongamia pinnata* with methanol and ethanol solvent at 100µg/ml concentration showed significant antibacterial activity on selected (Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris, Micrococcusluteus, Klebsiella pneumonia, Staphylococcus aureus) in clinical isolates

Kage DN et al. [4] reported, isolation of karajachromene from the seeds of *Pongamia pinnata*. Karajachromene at doses 25mg/kg & 50mg/kg shown 40.48% & 59.6% inhibition of paw oedema respectively as compared to standard diclofenac sodium (63.01%) at 10mg/kg body weight. He concluded that karajachromene exhibits anti-inflammatory reaction. The seed oil extracted using n-hexane for 20hr and after storage for 15days in cold condition the karajachromene crystals sedimented at the bottom test tubes

Dwivedi D et al. [5] reported, wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. The results confirm that *Pongamia pinnata* having potent significant wound healing activity. The results confirmed by changes in wound contraction, increased tensile strength, increased hydroxyproline and hexosamine content, modulation of pro inflammatory and anti-inflammatory cytokine, moderate antimicrobial activity and in vivo antioxidant activity.

Nehete M et al. [6] reported, the antioxidant, antimicrobial and wound healing potential of *macaranga peltata* bark extracts. The study confirmed that *Macaranga peltata* bark methanol extract having antimicrobial, antioxidant and wound healing activity

Badarudheen R et al. [7] reported, antibacterial activity of *Macaranga peltata.* The comparison of zone of inhibition in study indicates that methanolic extract of stem and leaves has better antibacterial activity than the acetone and petroleum ether fruit extract.

Verma M et al. [8] reported, antibacterial and antifungal potentials of *macaranga peltata.* The leaf and stem bark samples collected, shade dried and powdered. The methanol extracts

of these samples were obtained by soxhlet extraction method. The yield obtained from leaf was 47% and from stem bark was 30%. Both the extracts proved moderate anti-bacterial activity, among them leaf extracts showed better anti-bacterial activity than the stem bark extract against both gram-positive and gram-negative bacteria.

Bijesh K et al. [9] reported, isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of Staphylococcus aureus. The antimicrobial effect of methanol extract of *M. peltata* leaves may be due to the individual activity or synergistic activity of these identified phytochemical compounds. The following compounds identified by LCMS techniques, compounds are shikmic acid, Musennin, Rhamnetin, Lupeol acetate, Corilagin and Quercetrin.

Subrahmanyam VM et al. [10] reported, antibacterial and antifungal potentials of *macaranga peltata*. The results showed that leaf yields 47% methanolic extract and 30% methanolic extract obtained from stem bark. In the study also concluded that leaf extract having better antibacterial activity against gram positive microbes than gram negative microbes.

METHODOLOGY

Collection and Authentification of Plants:

The Macaranga peltata leaves were collected from Paneer, Deralakatte areas in Mangalore. The Pongamia pinnata seeds were purchased from Pioneer Agro industry, Coimbatore, Tamilnadu. The collected plant materials were subjected to authentication at Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore and the voucher specimen (4717-18/28.0/.2021) was deposited

Extraction: The authentified seeds and leaves were dried in hot air oven, powder and sieved to obtain coarse powder. The powders were subjected to maceration with Ethanol, Chloroform, Petroleum ether, Ethyl acetate and methanol respectively. Filtered and dried to obtain the extract and preserved in desiccator.

Preliminary phytochemical screening for Phytocompounds: The extracts were subjected to preliminary phytochemical screening to know the presence of Phytocompounds such as Alkaloids, Carbohydrates, Tannins, Proteins, Saponins, Steroids, Tri terpenoids, Flavonoids and fixed oils.

Test for Alkaloids:

- Dragendroff's Test: The extract sample was dissolved in chloroform and Dragendroff's reagent was added, Reddish brown colour precipitate obtained indicates presence of alkaloids(Positive result)
- Mayer's Test: The extract sample was dissolved in chloroform and Mayer's reagent was added, Cream colour precipitate.
- Hager's Test: The extract sample was dissolved in chloroform and Hager's reagent was added, yellow colour precipitate.
- Wagner's Test: The extract sample was dissolved in chloroform and Dragendroff's reagent was added, Reddish brown colour precipitate.

Carbohydrates:

- Fehling's Test: To 1 mL of Fehling's solution A added 1 mL of Fehling solution B , Added 2 mL of the plant extract solution, mixed well and boil for 45minutes. The red precipitate formed at the bottom of test tube.
- Molisch's Test: To 2 ml extract solution in a test tube, added 1 drop of Molisch's reagent (10% α-naphthol in ethanol) than added 1-2 mL of conc. H_2SO_4 down the side of the test tube, it formed a layer at the bottom of the tube.

Tannins:

- Fecl2 Test: To the 2ml of extract sample added few drops of ferric chloride formed brakish green precipitate (Tannins Positive)
- Lead acetate Test: To the 2ml of extract sample added few drops of 10% lead acetate solution formed white precipitate

Protein's test:

- Biuret test: To the 2ml of extract sample added few drops of copper sulphate solution and sodium hydroxide solution formed violet color(Proteins Positive)
- Millon's Test: To the 2ml of extract sample added few drops of Millons reagent, Heat gently and sodium nitrite solution added leads to formation of red precipitate

Saponin Glycosides:

- Foam Test: Shaken the 2ml of extract solution allowed to stand for 15 min foam retained indicated saponins present

- Haemolysis Test: To the 2ml of extract solution added few drops of blood, haemolysis of RBC and liberation of haemoglobin shows tannins present

Steroids and Triterpenoids:

- Liebermann Burchard Test: To the 2mgof extract dissolved in acetic anhydride , boiled, cooled the 1ml of concentrated H_2So_4 added along the sides of test tube, Pink colour indicates the presence of Triterpenoids(Green colour for steroids)
- Salkowski Test: To the 2mgof extract dissolved in 1ml of concentrated H_2So_4 yellow color formation indicates presence of Triterpenoids(Red colour for steroids)

Flavonoid:

- Shinoda Test: To the 2ml of extract solution, added Magnesium turnings and concentrated HCL drop wise magenta colour indicates presence of Flavonoids

Fixed oil and Fat:

- **Solubility Test**
  - **i. Non Polar solvent-** Fixed oils are freely soluble in chloroform and diethyl ether.
  - **ii. Polar Solvent -** Fixed oils are insoluble in water
- Translucent spot test: Put a spot of 2ml of extract solution on filter paper, residue remained after drying indicates fixed oils present.
- The extracts were subjected to Antibacterial (Staphylococcus aureus, Escherichia coli, Streptococcus Pneumonia and P acro nerous) and antifungal(Candida albicans) activity by Agar well diffusion method. The bacteria's were grown and preserved in the nutrient broth at 4°C for future use.

Antibacterial and Antifungal activity: Agar well diffusion method

The microbial cultures were subcultured one day before performing the antimicrobial activity. The nutrient agar media was prepared by dissolving the nutrient agar powder (24grams) Himedia in 1000ml distil water. The agar solution media was cooled to 50^oC and inoculated with the Bacterial samples. The agar solutions containing bacteria were poured to the Petridishes and allowed for solidification. The wells were created in solidified agar media using borer.

The Standards (Ciprofloxacin 10µg/ml, Erythromycin 10µg/ml), Blank (DMSO) and test samples of plants extracts (Macaranga peltata 100mg/ml and Pongamia pinnata100mg/ml)

were prepared. 0.1ml of Sample solutions, Blank and Standards were dropped to agar wells in petriplates with 100mm diameter using 1ml graduated pipette and Incubated in BOD incubator(SIPLAB, Serve well Instruments Pvt Ltd) for 24 hours at 37^{o}C. The zone of inhibition was measured and compared with blank and standards to know the bactericidal activity of selected medicinal plant extracts. The same procedure was used for antifungal activity and standard Fluconazole was used for the present study.

RESULTS AND DISCUSSION

The Pongamia pinnata seed extracts and Macaranga peltata leaves extracts were descripted as follows:

PONGAMIA PINNATA

- **Description of Ethanol extract:**
- Nature: Semisolid paste
- Colour: Yellowish brown
- Odor: Oily odor(Fixed oil smell)
- After complete drying oily nature retained
- % yield: 10.8
- **Description of Chloroform extract:**
- Nature: Oily extract/Oil
- Colour: Slightly dark yellow colour
- Odor: Acrid oily odour
- % yield: 21.72
- **Description of Petroleum ether extract:**
- Nature: Viscous Oily extract/Fixed Oil
- Colour: Light yellow colour
- Odor: Oily odor
- % yield: 21.64
- **Description of Ethyl acetate extract:**
- Nature: Jelly liquid/Viscous liquid
- Colour: Yellow cream red colour
- Odor: Characteristic odor
- % yield: 21.12%
- **Description of Methanol extract:**

- ❖ Nature: Jelly Solid
- ❖ Colour: Reddish brown colour
- ❖ Odor: Bitter Characteristic odor(Astringent odor)
- ❖ % yield: 10.12

1. MACARANGA PELTATA

- ❖ **Description of Ethanol extract:**
- Nature: Amorphous solid
- Colour: Bark blackish green colour
- Odor: Characteristic
- % yield: 24.16
- ❖ **Description of Chloroform extract:**
- Nature: Amorphous solid
- Colour: light greenish yellow color
- Odor: characteristic odor
- % yield: 10.35
- ❖ **Description of Petroleum ether extract:**
- Nature: Amorphous solid
- Colour: yellowish brown colour
- Odor: Characteristic
- % yield: 10.15
- ❖ **Description of Ethyl acetate extract:**
- Nature: Amorphous solid
- Colour: Light yellow colour
- Odor: Characteristic
- % yield: 14.64
- ❖ **Description of Methanol extract:**
- ❖ Nature: Amorphous Solid
- ❖ Colour: Reddish brown colour
- ❖ Odor: Characteristic Astringent odor
- ❖ % yield: 16.36

Table 1: PERCENTAGE YIELD OF EXTRACT

SL NO	SOLVENT USED FOR EXTRACTION	PERCENTAGE YIELD OF EXTRACT (GRAM EXTRACT/ 100GRAM POWDER)	
		PONGAMIA PINNATA SEED	MACARANGA PELTATA LEAF
01	Ethanol	10.8	24.16
02	Chloroform	21.72	10.35
03	Petroleum Ether	21.64	10.15
04	Ethyl acetate	21.12	14.64
05	Methanol	10.12	16.36

Table 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACTS:

SL NO	CHEMICAL TEST	PONGAMIA PINNATA SEED					MACARANGA PELTATA LEAF				
		Ethanol extract	Chloroform extract	Petroleum ether extract	Ethyl acetate extract	Methanol extract	Ethanol extract	Chloroform extract	Petroleum ether extract	Ethyl acetate extract	Methanol extract
01	**Alkaloids:**										
	• Dragendroff's Test	-	+	+	+	-	-	+	+	+	-
	• Mayer's Test	-	+	+	+	-	-	+	+	+	-
	• Hager's Test	-	+	+	+	-	-	+	+	+	-
	• Wagner's Test	-	+	+	+	-	-	+	+	+	-
02	**Carbohydrates:**										
	• Fehling's Test	+	-	-	-	+	+	+	+	+	+
	• Molisch's Test	+	-	-	-	+	+				+
03	**Tannins:**										
	• Fecl2 Test	+	-	-	-	+	+	+	-	-	+
	• Lead acetate Test	+	-	-	-	+	+	+	-	-	+
04	**Protein's test**										
	• Biuret test	+	+	-	-	+	+	+	-	-	+
	• Millon's Test	+	+	-	-	+	+	+	-	-	+
05	**Saponin Glycosides**										
	• Foam Test	-	+	+	+	-	-	-	-	-	-
	• Haemolysis Test	-	+	+	+	-	-	-	-	-	-

06	**Steroids and Triterpenoids**										
	• Liebermann Burchard Test	-	+	+	+	-	-	+	+	+	-
	• Salkowski Test	-	+	+	+	-	-	+	+	+	-
07	**Flavonoid Test**										
	• Shinoda Test	+	-	-	-	+	+	+	-	-	+
	• Conc HCL	+	-	-	-	+	+	+	-	-	+
	• Alkali Test	+	-	-	-	+	+	+	-	-	+
08	**Fixed oil and Fat**										
	• **Solubility Test**										
	iii. Non Polar solvent										
	a. Chloroform	+	+	+	+	+	-	+	-	-	-
	b. Diethyl ether	+	+	+	+	+	-	+	-	-	-
	iv. Polar Solvent										
	a. Water	--	--	--	--	--	+	-	-	-	+
	• Translucent spot test	+	+	+	+	+	-	+	-	-	-

(+) = Result positive; (--)= Result Negative

Table 3 : ANTIBACTIAL ACTIVITY OF PONGAMIA PINNATA SEED EXTRACTS AND MECARANGA PELTATA LEAF EXTRACTS

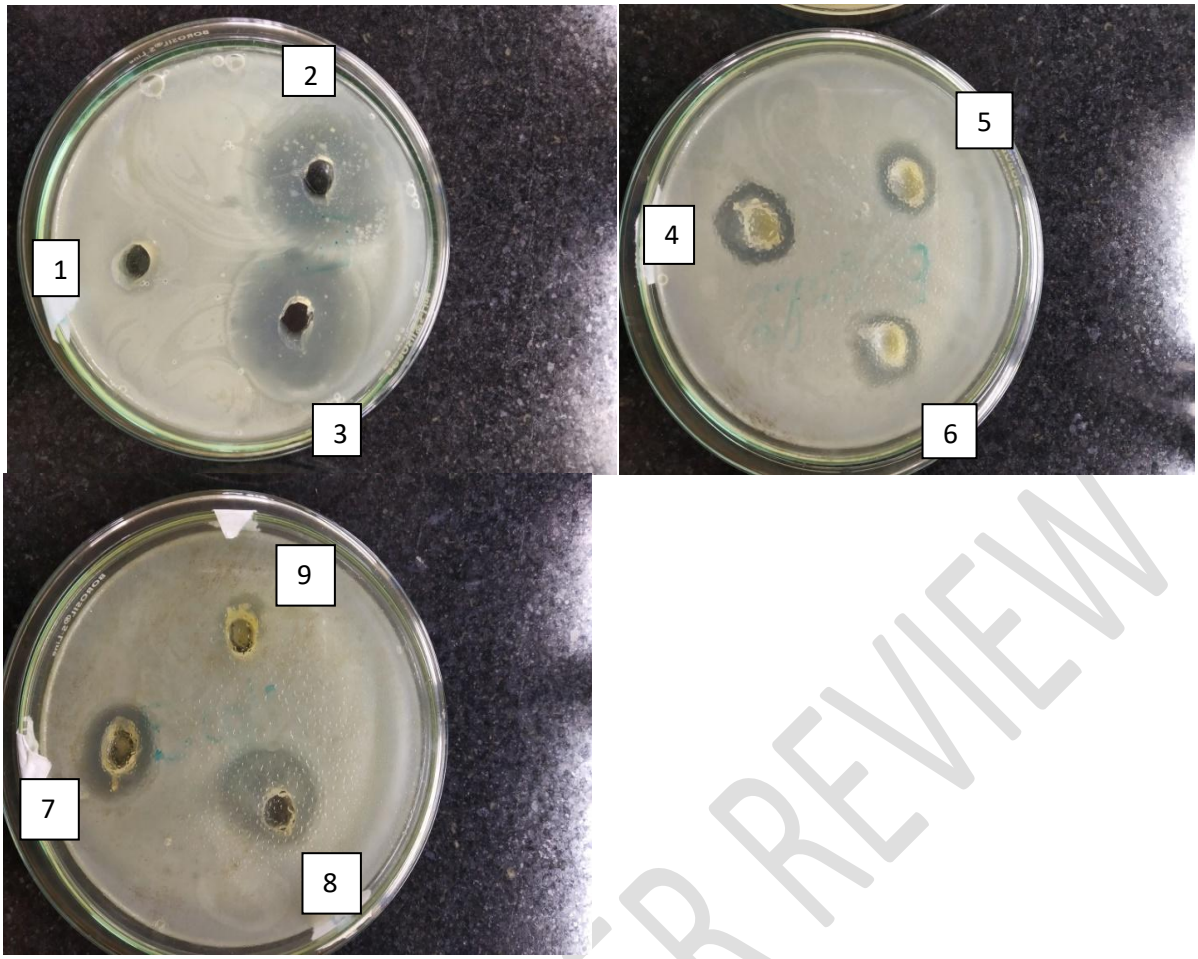
SL NO	ZONE OF INHIBITION OF *PONGAMIA PINNATA* EXTRACTS					ZONE OF INHIBITION OF *MECARANGA PELTATA* LEAF EXTRACTS			
	Samples/ Standards	S aureus	E coli	S pneumonia	P acro nerous	S aureus	E coli	S pneumonia	P acro nerous
01	Negative Control: (DMSO)	10 ± 0.5	12.5 ± 0.5	11.5 ± 0.5	10 ± 0.5	10 ± 0.5	10 ±0.5	10 ± 0.5	12.0 ± 0.5
02	Positive Control 01: Ciprofloxacin 0.1 ml(100µg) of Extract(1mg/1ml)	30 ± 0.2	39 ± 0.3	25.8 ± 0.4	31 ± 0.2	30 ± 0.2	30 ±0.4	20 ± 0.7	25.0 ± 0.2
03	Positive Control 02: Erythromycin 0.1 ml(100µg) of Extract(1mg/1ml)	34 ± 0.7	35 ± 0.5	30 ± 1	31 ± 0.5	32.5 ± 0.3	35 ± 0.5	24 ± 0.2	33.5 ± 0.3
04	Alcohol Extract 0.1 ml(10mg) of Extract(100mg/ml)	**23.5 ± 0.5**	19 ± 0.5	18 ± 0.5	19 ± 0.2	18 ± 0.3	19 ± 1	**19.5 ± 0. 4**	**17.5 ± 0.5**
05	Chloroform Extract 0.1 ml(10mg) of Extract(100mg/ml)	19 ± 0.1	18 ± 0.5	16 ± 0.4	19 ± 0.4	20 ± 0.2	14 ± 0.5	11.5 ± 0.5	17.0 ± 0.4
06	Petroleum Ether 0.1 ml(10mg) of Extract(100mg/ml)	12 ± 0.2	20 ± 0.3	11.5 ± 0.3	12.5 ± 0.5	17 ± 0.3	12.5 ± 0.5	11 ± 0.5	11.0 ± 0.5
07	Ethyl Acetate Extract 0.1 ml(10mg) of Extract(100mg/ml)	15.5 ± 0.5	15 ± 0.4	11.5 ± 0.5	13 ± 0.3	18 ± 0.4	12 ± 0.1	13 ± 0.2	12.0 ± 0.2
08	Methanol Extract 0.1 ml(10mg) of Extract(100mg/ml)	14.5 ± 0.4	**25.5 ± 0.5**	10 ± 0.2	11.0 ± 0.1	19 ± 0.5	19 ± 0.3	14 ± 1	12.0 ± 0.2

Values are mean (in mm) ± Standard Deviation, DMSO-Dimethyl sulphoxide

Table 4 : ANTIFUNGAL ACTIVITY OF PONGAMIA PINNATA SEED EXTRACTS AND MECARANGA PELTATA LEAF EXTRACTS*(CANDIDA ALBICANS)*

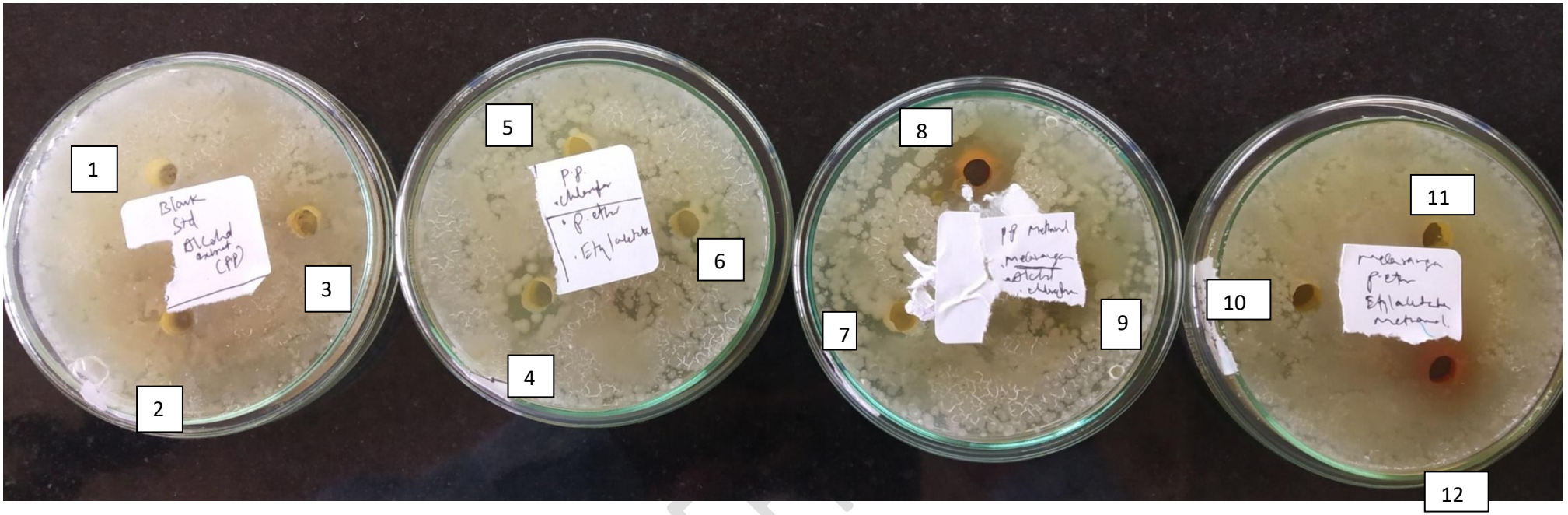
SL NO	*PONGAMIA PINNATA* EXTRACTS	ZONE OF INHIBITION against Candida albicans	*MECARANGA PELTATA* EXTRACTS	ZONE OF INHIBITION against Candida albicans
01	Negative Control (DMSO)	10 ±00	Blank (DMSO)	10 ±00
02	Positive Control Fluconazole 0.1 ml(100µg) of Extract(1mg/1ml)	20 ± 00	Standard 01 Fluconazole (10µg/ml)	20 ± 00
03	Alcohol Extract 0.1 ml(10mg) of Extract(100mg/ml)	16 ± 0.4	Alcohol Extract (100mg/ml)	22.5 ± 0.5
04	Chloroform Extract 0.1 ml(10mg) of Extract(100mg/ml)	17.5 ± 0.2	Chloroform Extract (100mg/ml)	21.5 ± 0.1
05	Petroleum Ether 0.1 ml(10mg) of Extract(100mg/ml)	14 ± 0.5	Petroleum Ether Extract(100mg/ml)	12 ± 0.5
06	Ethyl Acetate Extract 0.1 ml(10mg) of Extract(100mg/ml)	17.0 ± 0.4	Ethyl Acetate Extract(100mg/ml)	17.0 ± 0.2
07	Methanol Extract 0.1 ml(10mg) of Extract(100mg/ml)	19 ± 0.5	Methanol Extract (100mg/ml)	**26.5 ± 0.1**

Values are mean (in mm) ± Standard Deviation, DMSO-Dimethyl sulphoxide



1. Negative Control 2. Positive Control 01(Ciprofloxacin) 3. Positive Control 01(Erythromycin)	4. Chloroform extract 5. Petroleum ether extract 6. Ethyl acetate extract	7. Methanol extract 8. Methanol extract 9. Negative Control

Fig 1 : ANTIBACTERIAL ACTIVITY OF PONGAMIA PINNATA EXTRACTS ON E COLI



1. Negative control 2. Standard 3. Pongamia pinnata ethanol extract	4. Pongamia pinnata chloroform extract 5. Pongamia pinnata petroleum ether extract 6. Pongamia pinnata ethyl acetate extract	7. Pongamia pinnata methanol extract 8. Mecaranga peltata alcohol extract 9. Mecaranga peltata chloroform extract	10. Mecaranga peltata petroleum ether extract 11. Mecaranga peltata ethyl acetate extract 12. Mecaranga peltata methanol extract

Fig 2: ANTIFUNGAL ACTIVITY OF PONGAMIA PINNATA SEED AND MACARANGA PELTATA LEAF EXTRACTS

Discussions:

1. In the study percentage extractive yield of *Pongamia pinnata* is as follows: chloroform extract 21.72%, petroleum ether extract 21.64%, ethyl acetate extract 21.64% and very low ethanol 10.8% and methanol extract 10.12% yield obtained.
2. *Mecaranga peltata* found to contain polar compounds as it is shown by extractive yields i.e. ethanol 24.16%, methanol 16.36% extract yields
3. The preliminary phytochemical screening shown *Pongamia pinnata* seed extracts contain non-polar compounds mainly fatty acids and Macaranga leaf contains polar compounds
4. The highest zone of inhibition reported by sample extracts for selected microorganisms is as follows *Pongamia pinnata* ethanol extract against S.aureus(23.5±0.5), *Pongamia pinnata* methanol extract aginst E.coli(25.5±0.5), Macaranga ethanol extract aginst S.pneumonia(19.5±0.4) and P.acro nerous (17.5±0.5) respectively
5. The highest antifungal activity is reported by *Macaranga peltata* methanol extract against candida albicans.

Conclusions

❖ **Antibacterial activity:**

1. *Pongamia pinnata* alcoholic extract shown significant anti-bacterial activity against *S. aureus*, whereas other extracts(Chloroform, Petroleum ether, Ethyl acetate, methanol) of *Macaranga peltata* shown significant anti-bacterial activity against *S. aureus*
2. *P. pinnata* methanol extract shown good bactericidal activity against *E. coli*
3. The ethanolic extracts of both plants shown prominent bactericidal activity compared to all other solvent extracts
4. The descending order of antibacterial activity: Ethanolic extract> Chloroform extract> Methanol extract> Petroleum ether= Ethyl acetate
5. All the extracts shown very less antibacterial activity against *Streptococcus Pneumonia* (S Pneumonia resistant to selected plant extracts)
6. Selected Second Standard erythromycin shown better bactericidal activity than Ciprofloxacin and many clear zone of inhibition were noticed in erythromycin treated wells
7. Against S. aureus P. pinnata alcoholic extract and M. peltata Chloroform, P. ether, Ethyl acetate and methanol extracts shown significant bactericidal activity

8. S. Pneumonia is found to be slightly resistant to selected standard antibiotics (Ciprofloxacin and Erythromycin)
9. All the extracts of both plants shown very less activity against P. acro nerous
10. The S. aureus and E coli infections can be well treated with the selected medicinal plants extracts

❖ **Antifungal activity:**

1. The standard Fluconazole and sample extracts have shown antifungal activity compared to blank.
2. Macaranga peltata leaf methanol extract, Ethanolic extract and chloroform extract have shown better antifungal activity compared to selected dose of standard Fluconazole(10µg/ml)
3. All the extracts of Pongamia pinnata shown less antifungal activity than standard.

4. COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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