ISSN: 2278-2648

International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP |Vol.13 | Issue 4 | Oct - Dec -2024

www.ijrpp.com

DOI :https://doi.org/10.61096/ijrpp.v13.iss4.2024.705-713

Research

Invitro Antioxidant activity, Anti-inflammatory and Antimicrobial activity of *Wrightia tinctoria*.

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Published on: 23 Dec 2024

Published by:
Dr .N. Sriram Publications

Abstract

Wrightia tinctoria R. Br. belongs to the family Apocynaceae, commonly called Sweet Indrajao, Pala Indigo Plant, and Dyer's Oleander. “Jaundice curative tree” in south India. Sweet Indrajao is a small, deciduous tree with a light gray, scaly, smooth bark. Native to India and Burma, Wrightia is named after a Scottish physician and botanist, William Wright (1740–1827). Sweet Indrajao is called dhudi (Hindi) because of its preservative nature. The juice of the tender leaves is used effectively in jaundice. Crushed fresh leaves, when filled in the cavity of a decayed tooth, relieve toothache. In the Siddha system of medicine, it is used for psoriasis and other skin diseases. In the current investigation,WTET extract has shown anti-inflammatory and antioxidant properties. WTET Oil extract has shown significant in vitro antioxidant activity by DPPH assay in the current investigation. At 100 mg/ml WTET, the average inhibition percentage is 80.5%, with the IC_{50} value found to be 86.1861 mg/ml compared to the standard ascorbic acid IC_{50} value of 33.7334 mg/ml. Further investigation of in vitro anti-inflammatory activity by the protein denaturation method compared to the standard. At 300 mg/ml, WTET has an average inhibition percentage of 47.6%, with the IC_{50} value found to be 326.2789 mg/ml compared to the standard IC_{50} value of 121.29 mg/ml. Overall investigation results, such as WTET, had good antioxidant and moderate anti-inflammatory activity. WT ET also investigate with *invitro* antimicrobial activity against human pathogenic microbes causes skin infection as a result significant compared to standard

Keywords:*Wrightia tinctoria,* antioxidant, anti-inflammatory and antimicrobial, DPPH assay.

INTRODUCTION

Wrightia tinctoria R. Br., a member of the Apocynaceae family, is commonly referred to as Sweet Indrajao, Pala Indigo Plant, or Dyer's Oleander. In southern India, it is known as the "jaundice curative tree." This

small, deciduous tree features a light gray, scaly smooth bark and is indigenous to India and Burma. The species is named in honor of the Scottish physician and botanist William Wright (1740-1827). In Hindi, it is called dhudi, reflecting its preservative properties. The juice extracted from its young leaves is effectively utilized in the treatment of jaundice. Additionally, crushed fresh leaves placed in the cavity of a decayed tooth can alleviate toothache. Within the Siddha system of medicine, Wrightia tinctoria is employed for treating psoriasis and various skin ailments (Srivastava, R., 2014). Traditionally, this plant is recognized for its significant medicinal properties, being used to address a range of health issues, including severe conditions such as AIDS and cancer (Dixit, A., et al., 2014). Wt exhibits a broad range of pharmacological effects, including antimicrobial, anti-psoriatic, anti-diarrheal, anti-helminthic, antioxidant, anti-cancer, anti-inflammatory, analgesic, anti-diabetic, diuretic, hepatoprotective, and anti-ulcer activities. In the Ayurvedic tradition, the mechanisms of action for Wt are characterized by properties such as tikta, kashaya, rooksha, sita, and katu (Oviya, I.R., et al 2015). Phytochemical analyses have identified the presence of various compounds, including alkaloids, triterpenoids, steroids, flavonoids, phenolics, carbohydrates, and lipids. The plant demonstrates analgesic, anti-inflammatory, antipyretic properties and is effective in treating psoriasis. Toxicological assessments indicate that this plant is safe and well-tolerated, supporting its application in traditional medicine for anticancer purposes, as well as for a variety of other conditions, including snake and scorpion bites, renal issues, and menstrual disorders (Daulat, C.A. and Pradip, D.T., 2023). Furthermore, Wt has been shown to offer protection against vascular and hepatic damage and possesses antioxidant properties, positioning these herbs as promising candidates for the management of cardiometabolic disorders (Jamshed, H., et al 2019).The total phenolic content of Wrightia tinctoria bark extract (WTBM) was determined to be 30.3 mg of gallic acid equivalent per gram of dry weight. The IC50 values for DPPH and ABTS radical scavenging activities were recorded at 72.2 ± 2.8 µg/ml and 45.16 ± 1.95 µg/ml, respectively. High-Performance Liquid Chromatography (HPLC) analysis identified the presence of gallic acid, rutin, and quercetin in WTBM. These results indicated that WTBM effectively inhibited the proliferation of breast cancer cells and induced apoptosis, highlighting the potential chemopreventive properties of W. tinctoria bark (Fatima, N., et al 2016). Extracts and fractions of W. tinctoria demonstrated selective cytotoxicity towards cancer cells, primarily through apoptosis, while exhibiting lower toxicity to non-cancerous cells, suggesting their development as safer chemopreventive agents (Chaudhary, S., et al 2015). Additionally, the extracts displayed potential antibacterial activity against the tested organisms. Among the three solvents evaluated, the ethanol extract of the leaves exhibited the largest inhibition zones. Specifically, the ethanol extract of Wrightia tinctoria showed maximum inhibition zones of 29 mm against Escherichia coli, 24 mm against Bacillus subtilis, 30 mm against Staphylococcus aureus, and 24 mm against Pseudomonas aeruginosa. Preliminary phytochemical analysis of Wrightia tinctoria revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins (Vedhanarayanan, P., et al 2013).*Wrightia tinctoria* leaves possessed potent antimicrobial properties against plant pathogenic bacteria suggesting that the active principles may be useful in the control of plant disease(Shankar, S.R., et 2010). Based on the above-studied work, the present work is to prepare the ethanolic extract of Wrightia tinctoria oil and investigate their potential pharmacological explanations for their in vitro anti-inflammatory activity compared with standard albumin denaturation. Futherinvestiagate antimicrobial and antifungal activity compared with standard against skin infection.

MATERIAL AND METHODS

Collection of samples

Wrightia tinctoria oil collected from Yazhini biotech laboratory, Srivilliputhur, Tamilnadu for this investigation.

Method of preparation of samples

10 gram of Fresh leaf ***Wrightia tinctoria*** oil is grind with 100 ml of water, ethanol and methanol separately for 5 hours under reflux condenser in water bath, cool and filter. The filtrate is evaporated under vacuum to get aqueous and ethanolic extract

Pharmacological evaluation

In vitro Antioxidant activity

WTET extract investigated for in vitro antioxidant activity by DPPH assay for the estimation of anti-oxidant potential of WTET aqueous extract.WTET also tested by DPPH assay.

Determination of DPPH radical scavenging activity

Antioxidant activity in the sample WTET were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Brand-Williams et al., 1995). 100µL of SC extract was taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. Read the plate on Elisa plate reader at 490nm.

Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

Radical scavenging activity was calculated by the following equation

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of testsample) / (Absorbance of control)] x 100.

Table 1: WT ETOIL *Invitro* antioxidants activity by DPPH assay

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)	IC_{50} (mg/ml)
1	100 mg	0.29	0.07	75%		
2		0.29	0.06	79%		
3		0.29	0.05	82 %	**80.5%**	
4		0.29	0.06	79%		
5		0.29	0.05	82%		
6		0.29	0.04	86%		
1	200 mg	0.29	0.09	68%		
2		0.29	0.05	82%		
3		0.29	0.08	72 %	**75.8%**	
4		0.29	0.06	79%		86.1861 mg/ml
5		0.29	0.07	75%		
6		0.29	0.06	79%		
1	300 mg	0.29	0.05	82%		
2		0.29	0.06	79%		
3		0.29	0.07	75 %	**78%**	
4		0.29	0.06	79%		
5		0.29	0.08	72%		
6		0.29	0.05	82%		
Invitro anti-oxidant activity compared to Standard vitamin C						
1	100 mg	0.29	0.03	89%	**91%**	
2		0.29	0.02	93%		
3		0.29	0.03	93%		

Table 2: WTET OIL ANTIOXIDANT IC_{50} VALUE (mg/ml) Compared to STANDARD VIT C IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average (%)	IC_{50} (mg/ml)
1	100 mg	80.5%	
2	200 mg	75.8%	86.1861 mg/ml
3	300 mg	78%	
	Standard Ascorbic acid vitamin C		
1	50 mg	91%	33.7334 mg/ml
2	100 mg	87%	
3	150 mg	86%	
4	200 mg	92%	
5	250 mg	84%	

In-vitro Anti-inflammatory activity - Inhibition of albumin denaturation

The reaction mixture was prepared separately by mixing 0.5ml extract of WTET with 0.45 ml aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 ˚C for 20 min, then heat to 57 ˚C for 30 min. Cool the solution and transfer it to the 96 well plates and measure the absorbance at 660nm. Standard was used as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100 Where A control – Absorbance of reaction mixture except drug. A sample – absorbance of the reaction mixture with the Sample

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Table 3: WTET OIL Invitro anti-inflammatory activity

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)	IC_{50} (mg/ml)
1	100 mg	0.30	0.18	40%		
2		0.30	0.17	43%		
3		0.30	0.16	46 %	**45%**	
4		0.30	0.17	43%		
5		0.30	0.15	50%		
6		0.30	0.15	50%		
1	200 mg	0.30	0.16	46%		
2		0.30	0.15	50%		
3		0.30	0.16	46 %	**45%**	326.2789 mg/ml
4		0.30	0.18	40%		
5		0.30	0.17	43%		
6		0.30	0.16	46%		
1	300 mg	0.30	0.12	60%		
2		0.30	0.18	40%		
3		0.30	0.17	43 %	**47.6%**	
4		0.30	0.15	50%		
5		0.30	0.18	40%		
6		0.30	0.14	53%		
Invitro anti-inflammatory activity compared to Standard diclofenac sodium						
1	100 mg	0.30	0.06	80%	**78%**	
2		0.30	0.06	80%		
3		0.30	0.07	76%		

Table 4: WTETAnti-Inflammatory Activity Ic_{50} Value (Mg/Ml) Compared To Standard Diclofenac Sodium Ic_{50} Value (mg/ml)

S.NO	Concentration (mg)	Average (%)	IC_{50} (mg/ml)
1	100 mg	45%	
2	200 mg	45%	326.2789 mg/ml
3	300 mg	47.6%	
Standard Diclofenac sodium			
1	50 mg	91%	
2	100 mg	93%	
3	150 mg	85%	**121.29mg/ml**
4	200 mg	89%	
5	250 mg	88%	

Antibacterial activity

The study utilized the following bacterial strains: Staphylococcus aureus, Staphylococcus epidermidis and Candida albicans. In this study, the antibiotic streptomycin was used as a positive control. Onto sterile Petri dishes, nutritional agar medium is added in millilitre increments, left to set, and then disposed of. After ensuring a uniform layer of medium using a spreading rod, 100µL of broth produced from a particular bacterial strain was piped on top. This process was continued until the media had dried entirely. For the last twenty-four hours, the temperature of the Petri dishes has been maintained at 37˚C. Dimethyl sulfoxide was utilized as the negative control and streptomycin at a dosage of 1 mg/mL as the positive control.

Antifungal activity

Zone of inhibition assay were employed to assess antifungal efficacy. In summary, test solutions dissolved in DMSO were incorporated into sterile melted Sabouraud Dextrose Agar (SDA) at 45ºC in a 1:10 ratio, resulting in final concentrations of 1, 10, and 100 mg/ml. The mixture was thoroughly homogenized, and approximately 100 µl was dispensed into each sterile well of 1.5 cm diameter on microscopic slides. Plugs measuring 1 mm of fungal mycelium, excised from the periphery of an actively growing colony, were inoculated

at the center of the agar wells and incubated in a humidified environment at 25ºC. Control cultures received a corresponding volume of DMSO.

Table 5: Antimicrobial Activity

Organisms	100 µL	150µL	200µL	Standard
C.albicans	-	-	11mm	20mm
S.aureus	-	-	13mm	21mm
S.epidermidis	15mm	18mm	22mm	24mm
MRSA		18mm	21mm	23mm

Table 6: Antibacterial sensitivity for sample

S.NO	Agar	Name of organisms	Zone of inhibition
1	Nutrient agar	*Staphylococcus aureus*	6mm
2	Nutrient agar	*Staphylococcus epidermidis*	12mm
3	Sabouraud agar	*Candida albicans*	16mm
4	Nutrient of SDA	Control +ve	38mm

Table 7: Antibacterial activity represented by zone of inhibition

Test organism	Standard disc (gentomycin)	Extract (oil)	Extract (Et + DMSO)
Psuedomonas aurigona	2cm	-	1.8cm

Table 8: Invitro Antibacterial and Antifungal Activity of WT ET and Oil

S.No	Extract	Organism	Zone Of Inhibition (Mm)	Average
1	**WTET**	*S.aureus*	**20/24**	**22 mm**
2		*S.aureus MDR*	**20/25**	**23m**
3		*C.albicans*	**-**	**-**
4		*Acitinobacter bowmanii*	**-**	**-**
5				
6	**WT OIL**	*S.aureus*	**-**	**-**
7		*S.aureus MDR*	**-**	**-**
8		*C.albicans*	**-**	**-**
9		*Acitinobacter bowmanii*	**-**	**-**
10				
11	**STD AB**	*S.aureus*	**15/18**	**17**
12		*S.aureus MDR*	**18**	**18**
13		*C.albicans*	**11**	**11**
14		*Acitinobacter bowmanii*	**-**	**-**

RESULT & DISCUSSION

Ethanolic extract of WTET has shown anti-inflammatory and antioxidant properties. *WTET* extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At 100 mg/ml WTET, the inhibition percentage is 80.5% (Table 1), and the IC_{50} value was found to be 86.1861 mg/ml compared to the standard ascorbic acid IC_{50} value of 33.7334 mg/ml (Table 2). As a result, even a high concentration of antioxidant activity is significant compared to the standard ascorbic acid vitamin C. The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract WTET nor its separated constituents exhibit any appreciable anti-inflammatory efficacy. When compared to standard diclofenac sodium, the aqueous extracts of WTET exhibit moderate anti-inflammatory efficacy. At 300 mg/ml, WTET had a 47.6% (Table 3) IC_{50} value of 326.2789 mg/ml compared to the standard diclofenac sodium IC_{50} value of 121.29 mg/ml (Table 4). While we were carrying out different

concentrations in a dose-dependent way and comparing them with the standard, many of them showed the antioxidant and anti-inflammatory properties of WTET. Overall investigation results, such as WTET, had significant antioxidant and moderate anti-inflammatory activity. WTET and WTET OIL were investigated for invitro antimicrobial activity. At 250 µL, the antimicrobial activity of C. albicans is 11 mm, and S. aureus is 13 mm compared to the standard. At 100 µL, 200 µL, and 250 µL of S. epidermidis, the diameters are 15 mm, 18 mm, and 21 mm, and the standard is 24 mm, as shown in Table 5. Antifungal activity of methanolic extract of WTET and WTET oil is shown in Table 8. WTET is active against Staphylococcus aureus; the zone of inhibition is 22 mm, and methicillin-resistant Staphylococcus aureus has a zone of inhibition of 23 mm. As a result, WTET has good antifungal activity compared to the standard. Antibacterial activity of WTET and WTET oil is shown in tables 6 & 7. The zone of inhibition of WTET against Staphylococcus aureus is 6 mm, Staphylococcus epidermidis is 12 mm, Candida albicans is 16 mm, and Pseudomonas is 18 mm. As a result, WTET is more active against the Pseudomonas bacteria.

A prior study indicated that the methanolic extract exhibited significant activity in a dose-dependent manner, comparable to the reference standard diclofenac sodium. Phytochemical analyses revealed that alkaloids and phenols are the primary constituents of the extract, suggesting that these components may contribute to its antioxidant and anti-inflammatory properties (Sawale, J.A., et al 2014). Additionally, Ramalakshmi, S., et al conducted antibacterial studies on the ethanolic flower extract at various concentrations, finding that a concentration of 250 mg/mL demonstrated substantial inhibitory effects against all tested pathogens when compared to standard antibiotics such as streptomycin and penicillin. The cytotoxicity of the flower extract was assessed using the brine shrimp lethality bioassay, yielding an LC50 value of 3.544 µg/mL. Furthermore, Ponnusamy, K., et al 2015 reported that the indole compound indirubin derived from Wrightia tinctoria exhibited antifungal properties, indicating its potential utility in treating dermatophytosis. The Wrightia tinctoria extract (WTET) possesses a robust phytochemical profile with therapeutic applications, as its polyherbal formulation contains numerous bioactive compounds that effectively neutralize free radicals and inhibit inflammation

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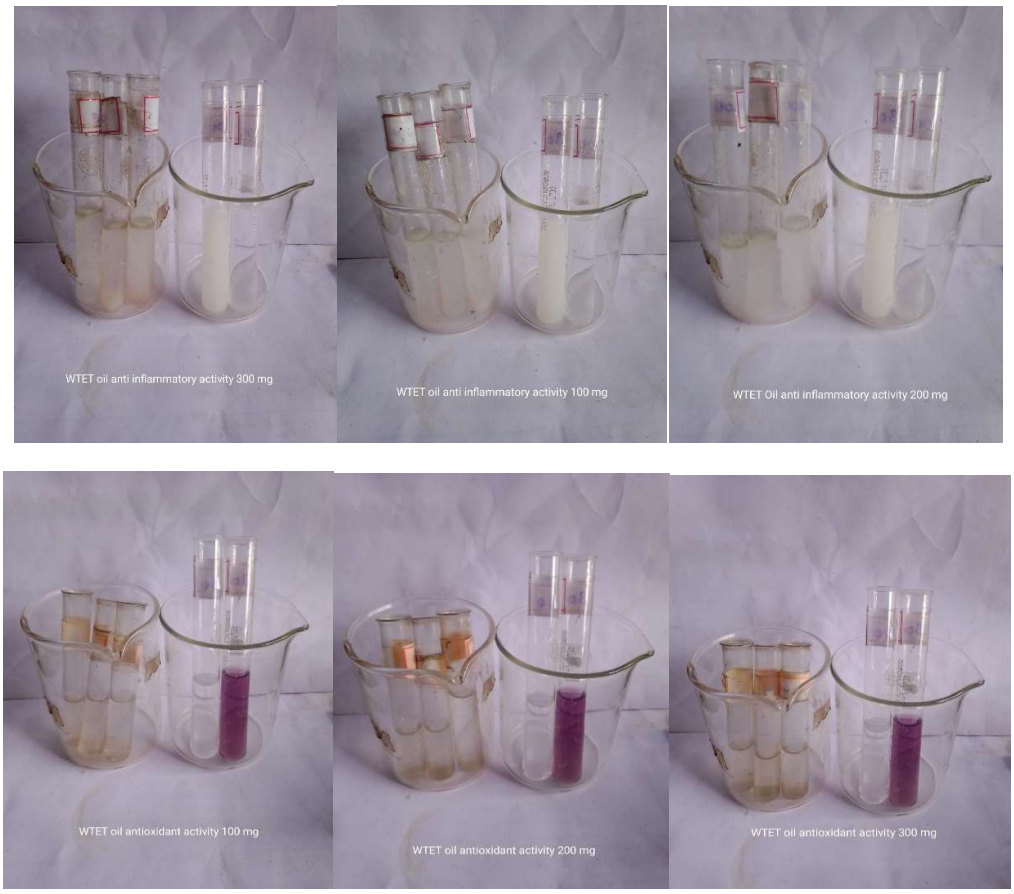

Fig 1: WTET OIL antioxidant and antinflammatory

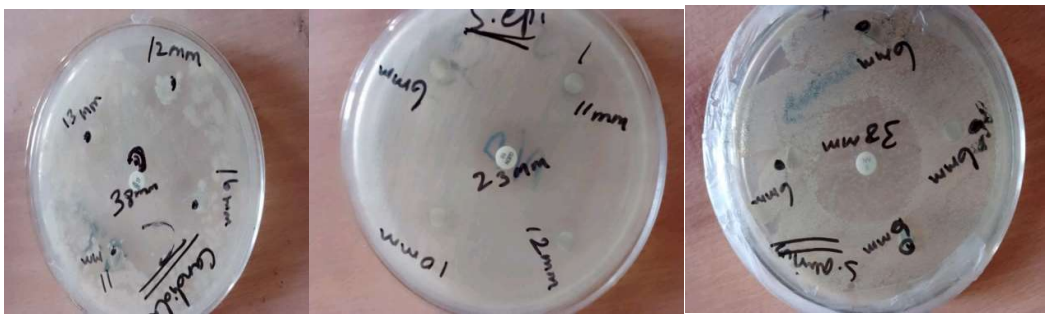

Fig 2: Antibacterial activity

Fig 3: Antimicrobial activity

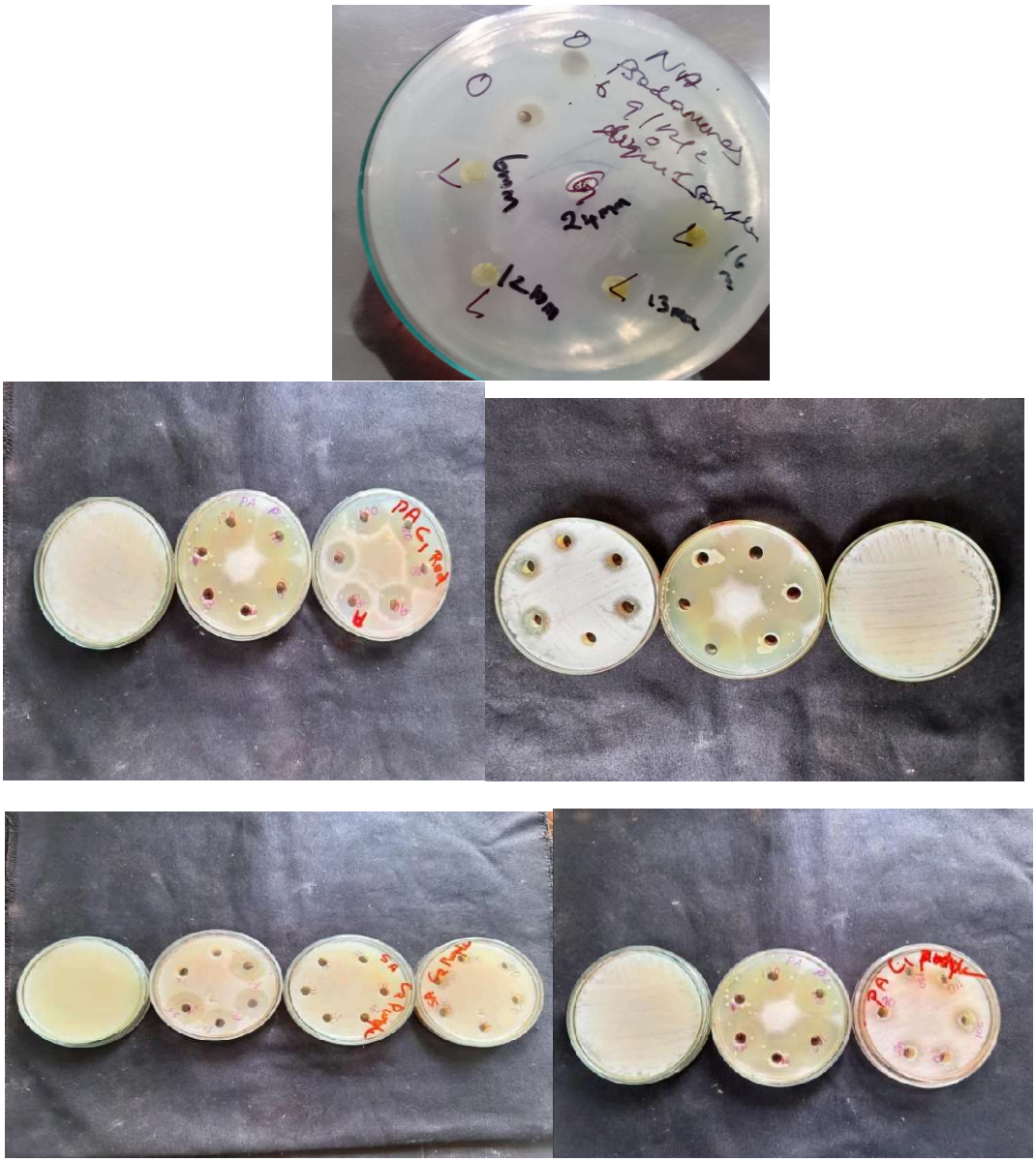

Fig 4: Antibacterial activity represented by zone of inhibition

CONCLUSION

The ethanolic extract of *Wrightia tinctoria* oil exhibits substantial pharmacological activity, including significant antioxidant, moderate anti-inflammatory, and promising antimicrobial properties. The extract demonstrated high efficacy in DPPH assays, with inhibition percentages comparable to ascorbic acid. Its moderate anti-inflammatory activity and effective antimicrobial action against pathogenic organisms further establish its potential as a natural therapeutic agent.These findings support the traditional medicinal uses of *Wrightia tinctoria* and provide a foundation for further studies to isolate bioactive compounds and elucidate their mechanisms of action. Future research could explore its applications in treating oxidative stress-related disorders, inflammatory diseases, and microbial infections, paving the way for its development into a standardized natural therapeutic.

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