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Phytochemical and Antibacterial Efficacy of Medicinal Plant Extracts against *Xanthomonas oryzae* pv. *Oryzae*

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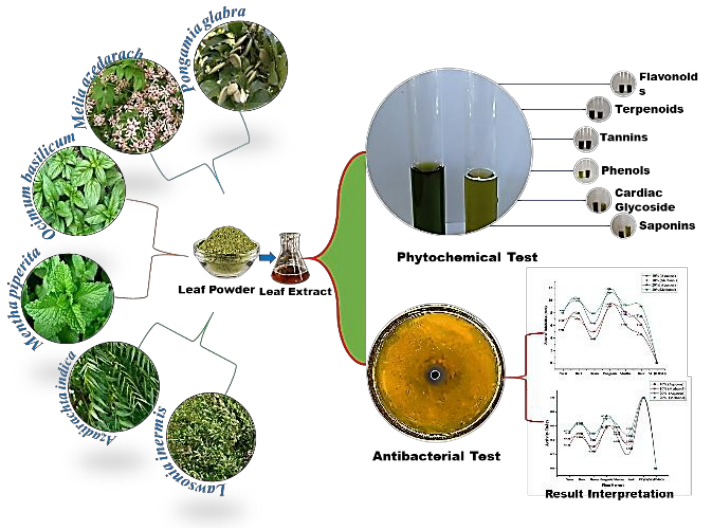
ABSTRACT

Rice is one of the most extensively cultivated and economically significant crops worldwide. However, bacterial blight, is caused by Xanthomonas oryzae pv. oryzae, poses a significant threat, leading to considerable yield losses. The rising challenge of antibiotic resistance among bacterial pathogens in plant, including Xanthomonas oryzae pv. oryzae, underscores the need to explore sustainable alternatives to synthetic chemicals. This study investigates the phytochemical composition and antibacterial activity of leaf extracts from six medicinal plants: Azadirachta indica, Melia azedarach, Pongamia glabra, Lawsonia inermis, Mentha piperita, and Ocimum basilicum. Phytochemical analysis revealed the presence of bioactive compounds such as flavonoids, terpenoids, tannins, cardiac glycosides, phenols, and saponins in varying combinations. The antibacterial activity was evaluated using the well-diffusion method at 10 per cent and 20 per cent concentrations. Among the aqueous extracts, Pongamia glabra demonstrated the highest antibacterial potential, with a zone of inhibition (ZOI) of 11.83 ± 0.10 mm at 20 per cent concentration, corresponding to a relative percent inhibition (RPI) of 73.96 per cent and an activity index (AI) of 0.74. Melia azedarach followed, exhibiting a ZOI of 10.25 ± 0.22 mm (RPI = 64.06%, AI = 0.64). Similar trends were observed with methanolic extracts, where Pongamia glabra and Melia azedarach showed the strongest activity. The positive control (Streptocycline, 100 ppm) produced a ZOI of 16.00 ± 0.45 mm, validating the experimental setup, while the negative control Dimethyl salphoxide (DMSO) showed no inhibitory effect. The results highlight the potential of locally available medicinal plants, particularly Pongamia glabra and Melia azedarach, as eco-friendly anti-microbial agents against Xanthomonas oryzae pv. oryzae.

Keywords: *Antibiotic resistance, Medicinal plants, Antibacterial activity, Xanthomonas oryzae pv. oryzae, well diffusion, Phytochemicals, Rice*



Graphical Abstract

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Introduction

Rice (*Oryza sativa* L.*)* is an important staple food crop, sustaining over 3.5 billion people globally (Khush, 2005). In India, rice occupies a significant position as the second major cereal crop after wheat, cultivated on approximately 45.06 million hectares, yielding 122.57 million tonnes with a productivity of 27.13 q/ha (GOI, 2022). However, rice production is highly affected by various diseases, among which bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, poses a significant threat, particularly in Asia (Swings *et al.*, 1990). This destructive disease is responsible for substantial crop losses, reducing yield by up to 81 per cent under favourable conditions (Srinivasan and Gnanamanickam, 2005), with economic losses impacting nearly 50 per cent of the affected plant parts (Sharma *et al.*, 2017). The escalating issue of antibiotic resistance has emerged as one of the most pressing global health challenges, threatening the efficacy of antimicrobial agents that have historically revolutionized medicine and agriculture. The overuse and misuse of antibiotics in human health, veterinary medicine, and agriculture have accelerated the emergence of multidrug-resistant pathogens. This includes *Xanthomonas oryzae* pv. *oryzae,* a devastating rice pathogen responsible for bacterial blight, causes significant yield losses and economic repercussions in rice-producing regions worldwide (Sibanda and Okoh, 2007; Levy and Marshal, 2004). In agricultural practices, antibiotics have been used at subtherapeutic levels to promote growth and manage infections. However, the continuous use of antibiotics has been directly linked to the development of acquired resistance, which

significantly limits the efficacy of antibiotics (Ulka and Karadge, 2010; Bandow *et al.,* 2003).

The alarming rise in antibiotic-resistant strains has driven research toward sustainable alternatives, with medicinal plants emerging as a viable solution. Natural products derived from higher plants have demonstrated significant antimicrobial potential due to their unique bioactive compounds and diverse mechanisms of action. Unlike synthetic drugs, plant-derived medicines are often safer, more cost-effective, and eco-friendly (Nita *et al.,* 2002; Ateb and ErdoUrul, 2002). For centuries, traditional medicinal plants have been employed in various cultures to combat bacterial, fungal, and viral infections (Rizwan *et al.,* 2012; Bandow *et al.,* 2003). For instance, *Pongamia glabra*, commonly known as Karanja, has gained attention for its antioxidant, anti-inflammatory, and antibacterial, antifungal properties (Bensky *et al.,* 2004; Dhami *et al.,* 2018).

Similarly, leaf extracts of plants *viz*; Neem (*Azadirachta Indica*), Drek (*Melia azedarach*), Henna (*Lawsonia inermis*), Pongamia (*Pongamia glabra*), Mentha (*Mentha piperita*), Basil (*Ocimum basilicum*), have exhibited notable antibacterial activity against plant pathogens like *Xanthomonas oryzae* pv. *oryzae.* These plants are rich in bioactive compounds such as polyphenols, terpenes, and phytosterols disrupt bacterial membranes and inhibit critical metabolic pathways, making them highly effective against resistant pathogens (Lillehoj *et al.,* 2018; Hua *et al.,* 2018). Furthermore, aqueous extraction methods have been recognized for their affordability and reduced toxicity, ensuring safer applications in both medicinal and agricultural contexts (Fang *et al*., 2019).

In the context of plant pathology, *Xanthomonas oryzae* pv. *oryzae* poses a severe challenge due to its ability to rapidly spread under favorable conditions and significantly reduce rice productivity. Existing control measures particularly chemical treatment, have faced limitations due to environmental concerns, pathogen resistance, and residual toxicity in crops. Consequently, exploring the safer, cost-effective, and eco-friendly plant-based antimicrobials approach for the management of disease are of major concern. The antibacterial potential of medicinal plants offers a new frontier for developing alternative management practice to manage bacterial diseases in crops (Fern, 2020).

This study aims to investigate the phytochemical composition and antibacterial properties of six medicinal plant extracts against *Xanthomonas oryzae* pv. *oryzae.* These plants, including *Azadirachta Indica, Melia azedarach, Lawsonia inermis, Mentha piperita, Ocimum basilicum,* are not only well-documented in traditional medicine but are also hardy perennials that can be cultivated with minimal maintenance across diverse climatic regions such as Asia, Europe, and the United States (Anonymous, 2020). By establishing their efficacy against phytopathogen, this research seeks to identify promising plant-based alternatives that align with sustainable agricultural practices and address the growing issue of antibiotic resistance.

Materials and Methods

Isolation and purification of Pathogen

The *bacterium* was isolated from samples collected from the experimental research farm of the Division of Plant Pathology, Sher-e-Kashmir University of Agriculture Science and Technology, Jammu, India, and small leaf bits, 1-2 cm in size, were cut from the junction point between infected and healthy leaf areas using a sterilized scalpel.

These bits were surface sterilized in a sodium hypochlorite (4% v/v) solution for 30 second, then rinsed three times in double-distilled water (DDW) for 40 seconds. Sterilized pieces were subsequently dipped in sterilized double-distilled water in centrifuge tube for 20 minutes to ooze out of bacteria. This bacterial suspension was streaked onto the surface of Nutrient Agar (NA) medium and incubated for 5-7 days at 28 ± 2°C. Lemon-yellow colonies (LYC) with a shiny, circular appearance were selected and further purified on fresh Nutrient agar (NA) plates by serial dilution method (Ahsan *et al*., 2021). The isolated bacterium was identified and confirm using the Pathogenicity test, Gram staining and Biochemical tests *viz;* H_2S production test, Nitrate reduction test and Starch hydrolysis (Aneja, 1996; Goszczynska, 2000). The purified and identified culture of *Xanthomonas oryzae pv. oryzae* was used for evaluation of the antimicrobial activity of Plant extracts.

Plant materials

Plant's leaves were collected. Leaves are separated and washed out in tap water, dried in the shade and finely powdered, stored in a ziplock plastic cover.

Preparation of Aqueous and Ethanol-Based Leaf Extracts

To evaluate the antibacterial activity of six medicinal plant leaf extracts Neem (*Azadirachta indica*), Drek (*Melia azedarach*), Pongamia (*Pongamia glabra*), Henna (*Lawsonia inermis*), Mentha (*Mentha piperita*), and Basil (*Ocimum basilicum*) against the target pathogen, fresh leaves were collected from the vicinity of Sher-e-Kashmir University of Agricultural Science and Technology, Jammu, 180009, Jammu and Kashmir, India. The collected leaves were thoroughly washed with tap water, and rinsed with double distilled water (DDW) to eliminate any surface contaminants. The leaves were air-dried at room temperature and ground into a fine powder with the help of mortar and pestle for extraction. This leaves powder (20 g) was mixed in 40 ml of double distilled water (1:2 w/v) and then heated at 60°C for 30 minutes in a water bath, followed by filtration through muslin cloth and then through Whatman no.1 filter paper. Extracts was then be concentrated using a rotary evaporator (BIOBASE RE 5000), and the concentrated extracts were dissolved separately by adding 5ml of organic solvent dimethyl sulfoxide (DMSO) having 10 per cent concentration for further study.

For methanol extraction twenty-grams Dried and powdered leaves of all above mentioned medicinal plants were dissolved in 90 ml (1:9) of ethanol (80%) separately in round bottom flask and incubate at 37°C at 150 rpm for 24 h in a shaking incubator. Liquid extract were obtained and separated by filtration with Whatman no.1 (Hi-Media) filter and then concentrated using a rotary evaporator. The dried extracts were dissolved separately by adding 5ml of organic solvent dimethyl sulfoxide **(**DMSO**)** having 10 per cent concentration for antibacterial property evaluation**.**

Determination of antibacterial activity *in-vitro*

Medicinal plant extracts were assessed about the antibacterial activity against the pathogen (*Xanthomonas oryzae* pv. *oryzae*) using the well diffusion method (Jayaraj *et al.,* 2019) against. Bacterial culture of *Xoo* in Nutrient broth (Himedia M002) were incubated over 24 h at 28 ± 2 ◦C for bacterial growth.

The bacterial suspension was adjusted by adding sterilized distilled water to an optical density (OD) ± 0.1 equivalent to bacterial count 1×10^8 Cells per ml (Singh *et al.,* 2011). Nutrient agar plates were prepared by pouring 20 ml of nutrient agar medium into each sterile petri dishes and allowing them to solidify. Hundred microliter (100 µl) bacterial suspension was evenly spread over the agar surface by L- spreader. Using, the wells of 5 mm diameter were prepared by sterile cork borer in the nutrient agar plate. Each well was filled with 80 µl of each plant extract solution at two different concentrations i.e., 10 and 20 per cent. The inoculated plates were stored in a refrigerator at 5°C for 1 hour to facilitate the diffusion of the extracts into the medium. Ten per cent of DMSO solution was used as negative control whereas streptocycline sulphate at 100 ppm was used as a positive control. The plates were incubated at 28±1°C for five days. The experimentation was conducted in four replications. After five days of incubation, the zones of inhibition were measured in terms of diameter (mm) and observation recorded as effectiveness of the plant extracts against the pathogen. Activity Index (AI) and Relative percent inhibition (RPI) was calculated by following the formula given by Singariya *et al*., 2011; Paluri *et al.,* 2012

$$\text{Activity Index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

$$\text{Relative Per Cent Inhibition (RPI)} = \frac{100\,(X - Y)}{(Z - Y)}$$

Where

X = Zone of inhibition of test sample
Y = Zone of inhibition of Solvent
Z = Zone of inhibition of Standard drug

Determination of Phytochemical Analysis

Methanolic plant extracts were evaluated for bioactive compounds of alkaloids, tannins, Saponins, cardiac glycosides, steroids, phenols and flavonoids according to standard protocols for detecting the presence of different phytochemicals in the plant extracts (Harborne JB, 1998).

Flavonoids Test (Alkaline Reagent Test)- Flavonoid's test was conducted by taking 1 ml of methanolic plant extract in the test tube and added 5-6 drops of a 5% aqueous ferric chloride ($FeCl_3$) solution in it. Colour change represented the presence of Flavonoid compounds.

Terpenoids Test (Salkowski's Test)- Terpenoids test was conducted by taking 1 ml of methanolic plant extract in the test tube and added 0.5 ml of chloroform along with 3-5 drops of concentrated sulphuric acid (H_2SO_4) in it. Colour change represented the presence of Terpenoids compounds.

Saponins Test (Foam Test)- Presence of Saponins was detected by taking 1 ml of methanolic plant extract in the test tube and added 5 ml of distilled water in it and shaked well for 5 min. Appearance of foam in the test tube represented the presence of Saponins compounds.

Tannins Test (Braymer's Test)- The presence of Tannins was detected by taking 1 ml of methanolic plant extract in the test tube and treating it with 1 ml of 10% alcoholic ferric chloride ($FeCl_3$) solution. Colour change represented the presence of Tannins.

Phenols Test (Ferric Chloride Test)- Presence of Phenols was detected by taking 1 ml of methanolic plant extract in the test tube and adding 5-6 drops of 5% aqueous ferric chloride solution in it. Colour change represented the presence of Phenols.

Cardiac Glycosides Test (Keller Kelliani's Test)- Taken 1 ml of methanolic plant extract in the test tube and treated it with 1 ml of glacial acetic acid and 2-3 drops of 5% ferric chloride ($FeCl_3$) solution. Adding 0.5 ml dilute HCl to this mixture, color change indicated the presence of Cardiac Glycosides.

Statistical analysis

The Antimicrobial and phytochemical analyses were conducted in four replications, with results expressed as the mean of the replications ± standard deviation. Statistical analysis was performed using RStudio software (version 4.1.1). Line graphs were created using OriginPro software (version 2024b), and the text was grammatically revised and proofread with the assistance of AI tools.

Acknowledgment

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Results

Antibacterial Activity

The antibacterial activity of various medicinal plant extracts against *Xanthomonas oryzae* pv. *oryzae* was evaluated using the well diffusion method at two different concentrations i.e., 10 percent and 20 per cent. The result of the experimentation is presented in Tables 1 and 2. A graphical representation of the same is presented in (Fig, 1, 2, and 3), revealing significant variation in the antibacterial potential of the extracts, which was listed (Plate 1 and 2).

Table 1: Antibacterial Activity (Zone of inhibition) of different Aqueous based extract of six medicinal plants against Xanthomonas oryzae pv. Oryzae

Aqueous Extract	Zone of Inhibition (mm) (mean ± SD)					
	10%	AI	RPI	20%	AI	RPI
Neem (*Azadirachta Indica*)	5.28 ± 0.13	0.33	32.99	8.00 ± 0.02	0.50	50.00
Drek (*Melia azedarach*)	7.92 ± 0.11	0.49	49.48	10.25 ± 0.22	0.64	64.06
Henna (*Lawsonia inermis*)	3.82 ± 0.04	0.24	23.87	6.33 ± 0.40	0.40	39.58
Pongamia (*Pongamia glabra*)	9.42 ± 0.06	0.59	58.85	11.83 ± 0.10	0.74	73.96
Mentha (*Mentha piperita*),	6.11 ± 0.01	0.38	38.19	7.75 ± 0.33	0.48	48.44
Basil (*Ocimum basilicum*)	4.51 ± 0.11	0.28	28.21	7.42 ±0.02	0.46	46.35
Positive Control (Streptocycline, 100 ppm)	16.00 ± 0.45	1.00			1.00	
Negative Control (DMSO)	-	-	-	-	-	-
CD (0.05%)	**0.14**			**0.28**		
SeM ±	**0.05**			**0.095**		

Zone of inhibition (mm) (mean ± SD), (-) No zone of inhibition

Aqueous Extraction

In the case of 10 percent concentration Pongamia (*Pongamia glabra*) showed the maximum antibacterial activity, with the inhibition of 9.42 ± 0.06 mm (RPI = 58.85%, AI = 0.59) followed by Drek *(Melia azedarach*), which exhibited inhibition of 7.92 ± 0.11 mm (RPI = 49.48%, AI = 0.49). Mentha (*Mentha piperita*) produced a zone of inhibition of 6.11 ± 0.01 mm (RPI = 38.19%, AI = 0.38). Henna (*Lawsonia inermis*) showed the least activity with a lowest inhibition zone of 3.82 ± 0.04 mm (RPI = 23.87%, AI = 0.24).

At 20 per cent dose the same trend was observed. Pongamia (*Pongamia glabra*) gave maximum inhibition of 11.83 ± 0.10 mm with RPI = 73.96 per cent and AI = 0.74, which was followed by Drek *(Melia azedarach*) with an inhibition zone of 10.25 ± 0.22 mm (RPI = 64.06%, AI = 0.64). Mentha (*Mentha piperita*) inhibited the pathogen with inhibition zone of 7.75 ± 0.33 mm (RPI = 48.44%, AI = 0.48). Minimum inhibition was showed by Henna (*Lawsonia inermis*) with an inhibition zone of 6.33 ± 0.40 mm (RPI = 39.58%, AI = 0.40).

Table 2: Antibacterial Activity (Zone of inhibition) of different methanol-based extract of six medicinal plants.

Aqueous Extract	*Zone of Inhibition (mm) (mean ± SD)*					
	10%	AI	RPI	20%	AI	RPI
Neem (*Azadirachta Indica*)	6.75± 0.14	0.42	42.19	8.4 2 ± 0.08	0.53	52.60
Drek (*Melia azedarach*)	7.01± 0.09	0.44	43.84	9.90 ± 0.15	0.62	61.88
Henna (*Lawsonia inermis*)	5.00± 0.06	0.31	31.25	7.92 ± 0.14	0.49	49.48
Pongamia (*Pongamia glabra*)	9.06 ±0.08	0.57	56.59	11.25± 0.12	0.70	70.31
Mentha (*Mentha piperita*),	8.17± 0.23	0.51	51.04	9.27 ± 0.19	0.58	57.97
Basil (*Ocimum basilicum*)	6.08± 0.05	0.38	38.02	9.00 ± 0.06	0.56	56.25
Positive Control (Streptocycline, 100 ppm)	16.00 ± 0.45	1.0	-	-	1.0	-
Negative Control (DMSO)	-	-	-	-	-	-
CD (0.05%)	**0.19**			**0.20**		
SeM ±	**0.066**			**0.070**		

Zone of inhibition (mm) (mean ± SD), (-) No zone of inhibition

Methanol-based Extracts

The antibacterial potential of methanol-based extracts was evaluated and the data shows that at 10 per cent concentration, Pongamia extract again exhibited the strongest antibacterial efficacy, with a zone of inhibition of 9.06 ±0.08 mm (RPI = 56.59%, AI = 0.57) followed by Mentha, which showed inhibition of 8.17± 0.23 mm (RPI = 51.04%, AI = 0.51%). Drek was observed as third best treatment inhibition zone of 7.01± 0.09 mm (RPI = 43.84%, AI = 0.44). Henna was found as least effective plant extract with a zone of 5.00± 0.06 mm (RPI = 31.25%, AI = 0.31).

In case of 20 percent concentration *Pongamia glabra* exhibited maximum zone of 11.25± 0.12 mm with AI of 0.70 and RPI - 70.31 per cent followed by Drek (*Melia azedarach*) showed inhibition zone of 9.90 ± 0.15 mm with AI - 0.62 and RPI - 61.88 per cent. Mentha was observed as third best treatment having the inhibition of 9.27 ± 0.19 mm (RPI = 57.97%, AI = 0.58%). Henna was found as least effective plant extract with a zone of 7.92 ± 0.14 mm (RPI = 49.48%, AI = 0.49).

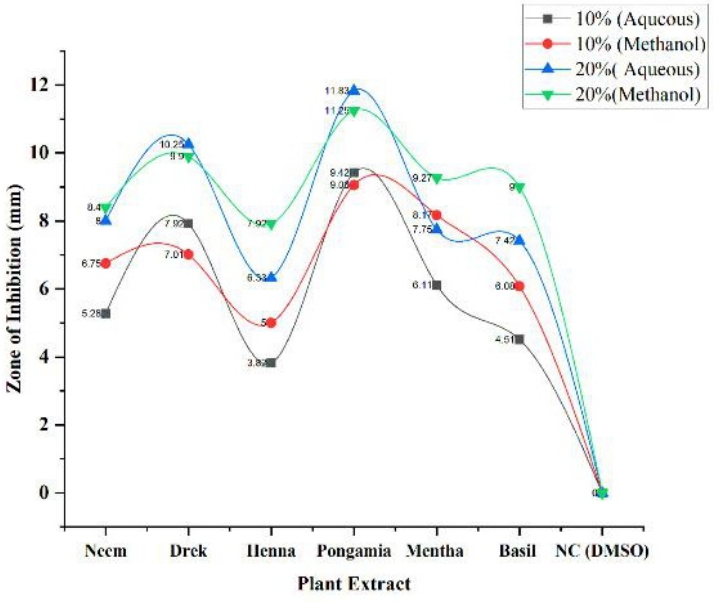

Fig. 1: Comparative analysis of Zone of Inhibition of Six methanolic and Aqueous medicinal plants extract of methanolic and Aqueous medicinal plant extracts.
NC - (Negative control)

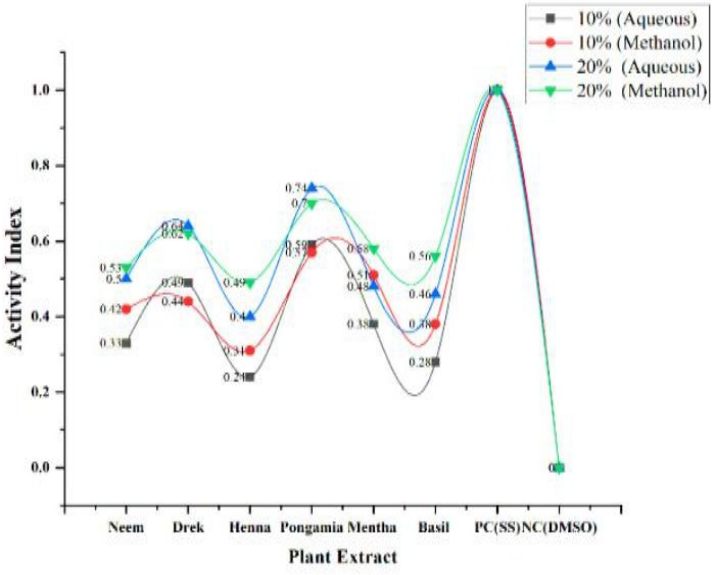

Fig. 2: Comparative analysis of Activity Index of Six methanolic and Aqueous medicinal plant extract
NC - (Negative control), PC - (Positive Control)

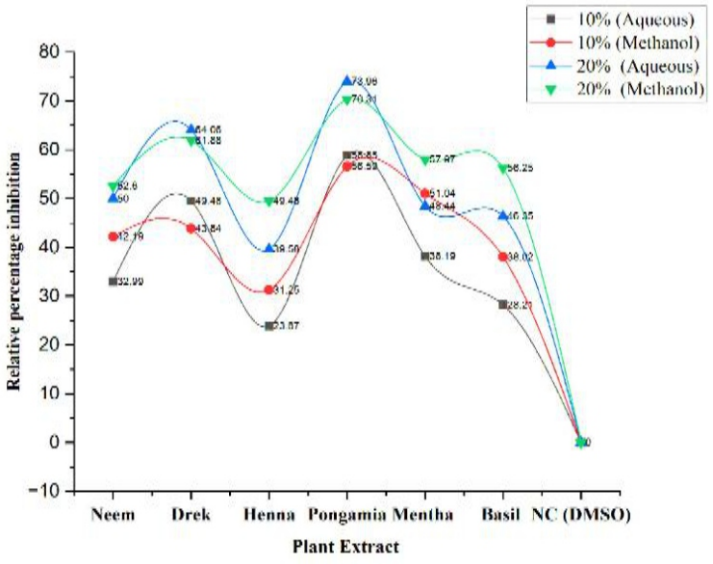

Fig. 3: Comparative analysis of Relative percentage of inhibition of Six methanolic and Aqueous medicinal plants extract
NC - (Negative control)

Qualitative phytochemical Screening

The phytochemicals analyzed in the leaf of six methanolic plant extracts *viz;* Neem (*Azadirachta indica*), Drek (*Melia azedarach*), Pongamia (*Pongamia glabra)*, Heena (*Lawsonia inermis)*, Mentha (*Mentha piperita)*, and Basil (*Ocimum basilicum*) examined the presence, absence of bioactive compounds and results was summarized in (Table 3).

The extracts of *Azadirachta indica* and *Melia azedarach* tested positive for all six tested phytochemicals i.e, Flavonoids, Terpenoids, Tannins, Cardiac Glycosides, Phenols, and Saponins. The extracts of *Pongamia glabra* showed the presence of Flavonoids, Terpenoids, Tannins, Cardiac Glycosides, and Phenols, but were found negative for Saponins. The extracts of *Lawsonia inermis* contained five phytochemicals i.e., Flavonoids, Terpenoids, Tannins, Cardiac Glycosides, and Phenols, while Saponins was not present. The extracts of *Mentha piperita* were positive for Flavonoids, Terpenoids, Tannins, Phenols, and Saponins, but Cardiac Glycosides and Phenols were absent. Similarly, the extracts of *Ocimum basilicum* contained all the tested phytochemicals.

Discussion

The antibacterial activity of the medicinal plant extracts, as evaluated through the well diffusion method, demonstrated a direct relation between the presence of bioactive phytochemicals and their effectiveness against *Xanthomonas oryzae* pv. *oryzae*. Phytochemical screening revealed that all the tested plant extracts inhibited the pathogen. To plants extracts i.e., *Pongamia glabra* and *Melia azedrach* was highly effective in inhibiting the pathogen and was observed very near to positive control in both extraction method.

Mentha was observed third best treatments in comparison to others in case of water extraction method whereas stand on second position at 10 per cent concentration of methanol based extraction method the change of effectiveness may be due to the increased availability of antibiotic photochemical compounds, which are soluble in organic solvent, qualitative analysis of methanol based extract showed that *Pongamia glabra* and *Melia azedrach*, which were effective at higher concentration that is 20 percent were positive for five photochemical, *Pongamia glabra* extract was not having the Saponins. Show it may be concluded that Saponins are not having the antimicrobial efficacy against the *Xanthomonas oryzae* pv. *oryzae* (Kagale *et al.,* 2004; Soltani and Aliabadi, 2013). The phytochemical profile of *Melia azedarach*, similar to (Farook *et al*., 2019), rich in flavonoids, terpenoids, and tannins, likely underpins its inhibitory effect on Pathogen.

The activity index (AI) and relative percent inhibition (RPI) calculations further highlighted the effectiveness of *Pongamia glabra* and *Melia azedarach*. At 20% concentration, the RPI values for these extracts reached highest in aqueous and methanol-based extraction, respectively, relative to the positive control (Streptocycline, 100 ppm).

Moderate activity was observed in both extracts of plants i.e., *Azadirachta indica* and *Mentha piperita* inhibitory effect against the pathogen and These findings are supported by (Kagale *et al.,* 2004; Soltani and Aliabadi, 2013). Which contain a varied range of secondary metabolites but at lower concentrations or with variations in bioavailability of key antimicrobial compounds like saponins and cardiac glycosides might explain their comparatively reduced effectiveness, as observed in another study by (Zouine *et al.,* 2024 and Solanki *et al*., 2017)

The reduced efficacy of *Ocimum basilicum* and *Lawsonia inermis*, exhibited comparatively lower antibacterial activity further underscores the importance of specific phytochemicals, such as phenols and flavonoids, in imparting antimicrobial activity. The absence or lower levels of such compounds might account for their comparatively lower inhibition of the pathogen findings similar to those (Vengadesh Kumar and Balabaskar, 2013). The absence of saponins and tannins in their phytochemical profiles may contribute to the reduced efficacy observed.

The results of this study highlight the potential of plant-derived extracts as natural alternatives for managing bacterial plant pathogens. The activity index (AI) and relative percent inhibition (RPI) further reinforce the promise of *Pongamia glabra* and *Melia azedarach* as effective bio-control agents. These findings suggest that secondary metabolites such as flavonoids, terpenoids, and phenols are critical contributors to antibacterial activity and provide a foundation for further exploration of plant-based antimicrobial formulations. Underscoring the relevance of bioactive compounds in plant disease management. Future research should focus on optimizing extraction techniques, identifying active components, and evaluating field efficacy for these promising natural antimicrobial agents.

Conclusion

The study highlights the significant antibacterial potential of medicinal plant extracts against *Xanthomonas oryzae* pv. *oryzae*, establishing a clear link between the presence of bioactive phytochemicals and their effectiveness. Among the tested extracts, *Pongamia glabra* and *Melia azedarach* exhibited the highest antimicrobial activity, attributed to key secondary metabolites such as flavonoids, terpenoids, phenols, and tannins. The findings suggest that saponins may not play a crucial role in antibacterial efficacy, as evidenced by the strong performance of *Pongamia glabra* extracts lacking this compound. The variation in effectiveness between different extraction methods underscores the importance of solvent choice in maximizing the availability of bioactive compounds. Methanol-based extracts consistently demonstrated higher antibacterial activity, likely due to the enhanced solubility of key phytochemicals in organic solvents.

These results demonstrate the potential of *Pongamia glabra*, *Melia azedarach*, and other medicinal plants as eco-friendly bio-control agents. Their use could reduce dependence on synthetic chemicals in plant disease management, supporting sustainable agricultural practices. Further research should focus on refining extraction techniques, isolating active components, and assessing field-level efficacy to facilitate their integration into plant protection strategies. These findings contribute to the development of eco-friendly and cost-effective strategies for sustainable disease management in crops.

Table 3: Phytochemical analysis of methanolic plant extract

S. no.	Phytochemicals	Test name	*Azadirachta indica*	*Melia azedarach*	*Pongamia glabra*	*Lawsonia inermis*	*Mentha piperita*	*Ocimum basilicum*
1	Flavonoids	Alkaline reagent test	+	+	+	+	+	+
2	Terpenoids	Salkowskis test	+	+	+	+	+	+
3	Tannins	Braymre's test	+	+	+	-	+	+
4	Cardiac Glycoside	Keller Kelliani test	+	+	+	+	-	+
5	Phenols	Ferric chloride test	+	+	+	+	-	+
6	Saponins	Foam test	+	+	-	+	+	+

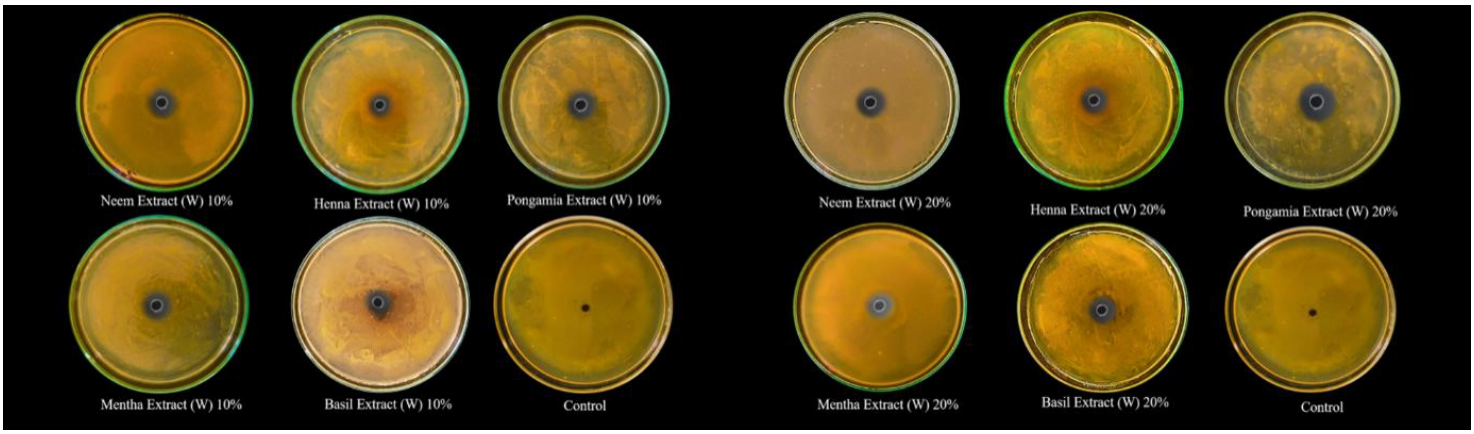

Plate 1: Zone of Inhibition (mm) of Six medicinal plants Aqueous extracts

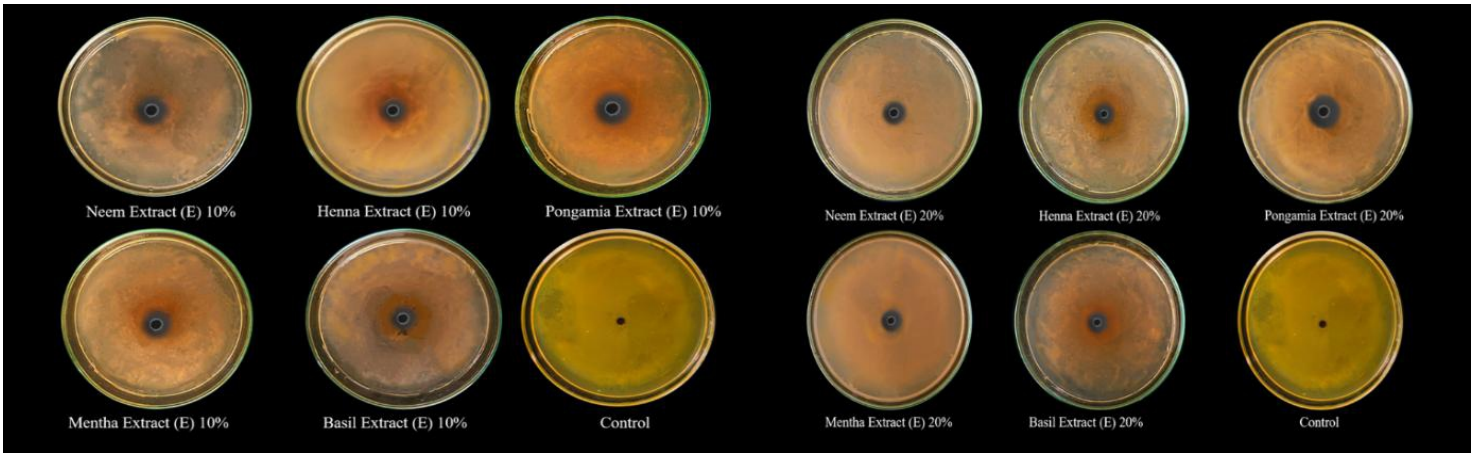

Plate 2: Zone of Inhibition (mm) of Six medicinal plants methanolic extracts

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