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IN-VITRO ANTIMICROBIAL ACTIVITY OF *PLUMERIA ALBA* AND *NELUMBO NUCIFERA* FLOWER EXTRACT AGAINST HUMAN PATHOGEN

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ABSTRACT

The present study investigates the phytochemical compositions and antimicrobial efficacy of Plumeria alba and Nelumbo nucifera flower extracts obtained using hot, cold and methanol with and without subsequent distillation. Qualitative phytochemical screening revealed the presence of key bioactive compounds including flavonoids, alkaloids, phenols, tannins, terpenoids, glycosides and saponins with notable variation across extraction techniques and plant species. Methanolic and hot extracts, particularly post-distillation, exhibited pronounced antimicrobial effects against human pathogen, suggesting enhanced concentration of active constituents. The findings highlight the therapeutic potential of P. alba and N. nucifera floral components and underscore the influence of extraction and distillation methaods on bioactive yield and efficacy. This comparative analysis provides a scientific basis for their traditional uses and paves the way for future exploration of their phytopharmaceutical applications.

KEY WORDS: *Flower extract, Phytochemicals, Antimicrobial activity and Minimal Inhibitory Concentration.*

INTRODUCTION

The rise of antibiotic-resistant bacteria has become a serious global health concern, leading to treatment failures, increased healthcare-associated infections (HAIs), and higher mortality rates (Meriyani H. et al., 2021). As conventional antibiotics lose efficacy, there is an urgent need to explore alternative and eco-friendly antimicrobial agents. *Plumeria alba*, a member of the Apocynaceae family, is widely grown for its fragrant, spiral-shaped flowers and is known for its medicinal value (Idrees et al., 2020). It contains numerous bioactive compounds with therapeutic properties and has been traditionally used to treat malaria, diabetes, inflammation, hypertension, abdominal tumors, leprosy, rheumatism, skin cancer, and microbial infections (Bihani, T., 2020). Extracts from various parts of the plant have shown potential in treating fungal and viral infections, skin diseases, and filarial conditions (W. Rizvi et al., 2010).

Nelumbo nucifera Gaertn (Family: Nelumbonaceae), commonly known as Indian lotus, is one of the oldest perennial aquatic herbs consumed across Asia (Liu C.P. et al., 2004). The plant exhibits a wide range of pharmacological activities including antidiabetic, antipyretic, anti-inflammatory, anticancer, antimicrobial, antiviral, and anti-obesity effects (Kashiwada Y., Asoshima A., Ikeshiro Y., 2005). A unique feature of *N. nucifera* is floral thermogenesis, occurring in the receptacle, stamen, and petals, which aids in attracting pollinators by generating heat and releasing volatile compounds (Li J.K. & Huang S.Q., 2009; Wang et al., 2015; Liu C. et al., 2004; Wagner et al., 2018; Dieringer et al., 2014).

Both *Plumeria alba* and *Nelumbo nucifera* are rich in secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, and saponins (Edema, M.O. and Alaga, T.O., 2012; Isah, T., 2019; Sridhar K.R., Rajeev B., 2019). These compounds are believed to contribute to their antibacterial potential. The present study aims to further explore and validate the antibacterial potential of floral extracts from *P. alba* and *N. nucifera* in the context of growing antibiotic resistance.

MATERIALS AND METHODS

1. Collection of flower sample & Preparation of flower extract

Healthy flowers of *Plumeria alba* and Nelumbo nucifera were collected from local garden. The flowers were initially separated from the main plants body and rinsed with distilled water and shade dried and kept in trays in hot air oven to evaporate water from their surface. Flower petals were dried under hot air oven than thoroughly crushed in a pestle and mortar and stored in air tight bottles.

1.1 Cold extraction

A total of 10 g of dried flower was soaked in 50 mL of cold water in a conical flask for 24 hours and then filtered off using sterile Whatman No. 1 filter paper into a sterile conical flask and evaporated by using solvent distillation apparatus. The extract was obtained with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was obtained and stored at 4˚C for further use (Farombi EO., 2003).

1.2 Hot Extraction

A total of 10 g of dried flower was soaked in 50 mL of hot water which was then boiled for 30 min and kept for 24 hours undisturbed and then filtered through sterile filter paper, evaporated by using solvent distillation apparatus. The extract was obtained with the help of a muslin cloth, centrifuged at 10000 rpm for 5 min and the supernatant was stored at 4˚C for further use (Ekpendu TO & Akshomejce AA, et.al 1994).

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1.3 Methanol Extraction

A total of 10 gm. of flower air dried powder was weighed and was placed in 100 mL of organic solvent (methanol) in a conical flask and then kept in a rotary shaker at 190 rpm for 24 hours and then it was filtered with the help of muslin cloth and centrifuged at 10,000 rpm for 5 min. The supernatant was collected and the solvent was evaporated by distillation apparatus to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/mL. It was stored at 40˚C in air tight bottles for further studies (Anu Kiruthika K and Amutha Jaisheeba A et.al.1984).

2. Phytochemical Screening

Qualitative test for the screening of phytochemicals were carried out as shown in table 1.

Table 1: Qualitative Tests of Phytochemicals

Phytochemcial	Method	Reference
Alkaloids	Wagner's Test	Raaman N.,2006; Singh V, Kumar R.,2017
	Iodine Test	Bhatt S, Dhyani S.,2012; Basumatary AR.,2016
	Mayer's Reagent Test	Proestos et al., 2005
Flavonoids	Alkaline Reagent Test	Audu SA, Mohammad I, Kaita HA.,2007; Singh V, Kumar R.,2017; Gul R. et al.,2017
	Lead Acetate Test	Silva GO, Abeysundara AT, Aponso MM.,2017; Singh V, Kumar R.,2017; Tiwari P. et al.,2011
Carbohydrates	Molisch's Test	Raaman N.,2006; Singh V, Kumar R.,2017
Reducing Sugar	Benidict's Test	
Cardiac glycosides	Baljet Test	Rahman MA, Ahmed NU.,2013; Kumar V, Jat RK.,2018
Phenols	Ferric Chloride Test	Raaman N.,2006; Tiwari P. et al.,2011
Coumarins	Naoh Test	Singh V, Kumar R.,2017; Kumar RS. et al.,2013
	Alkaline Hydrolysis Test	Ahmad, H. B. et al.,2010
Terpenoids	Salkowski's Test	Gul R. et al., 2017
sterols and phytosterols	Hesse's Response	Gul R. et al., 2017; Vimalkumar CS.et al.,2014
Phlobatannins	Hcl Test	Njoku OV, Obi C., 2009; Auwal MS. et al.,2014
	Ferric Chloride Test	Jadhav, S.S., & Kadam et al., (2012)
Anthraquinone	Borntrager's Test	Njoku OV, Obi C.,2009; Gul R. et al., 2017; Uma KS, Parthiban P, Kalpana S.,2017.
	Borntrager's Another Test	Njoku OV, Obi C.,2009; Gul R. et al., 2017; Uma KS, Parthiban P, Kalpana S.,2017
Quinone	Sulphuric Acid Test	Maria R. et al.,2018
Tannins	Braymer's Test	Singh V, Kumar R.,2017; Uma KS, Parthiban P, Kalpana S.,2017
Saponins	Foam Test	Tiwari P. et al., 2011
Anthocyanins	Hcl Test	Obouayeba AP.et al.,2015; Savithramma N, Rao ML, Suhrulatha D.,2011.
Carotenoids	Ferric Chloride Test	Jagessar RC.,2017
Leucoanthocyanine	Isoamyl Alcohol Test	Savithramma N, Rao ML, Suhrulatha D.,2011

3. Evaluation of antimicrobial activity

3.1 Test microorganisms and growth conditions

Two strains of Gram-positive *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtills* (ATCC 6633) and two strains of Gram-negative *Escherichia coli* (ATCC 25922*)* and *Pseudomonas aeruginosa* (ATCC 27853) bacteria were used which were obtained from Department of Microbiology laboratory of Ramakrishna Institute of Computer Education and Applied Sciences, Surat, India. And one strain of fungi *Candida albicans* (ATCC 6539) was used which was obtained from Advance diagnostic lab, Param Doctor house, Surat, India. The bacterial cultures were grown on nutrient agar at 37˚for 18h and fungal culture were grown on nutrient agar at 30˚ for 24h and the colonies were suspended in saline (0.85% NaCl) and its turbidity was adjusted to 0.5 Mac Farland standards (108 CFU/mL). This saline culture preparation was used to inoculate the plates (Artizzu N. et al., 1995).

3.2 Agar well diffusion

Base agar plates were prepared and wells of 8 mm were cut and inoculated seed were poured with different test cultures. The cut wells were then filled with 200µL of both aqueous and solvent extracts of flowers and leaves separately and the plates were kept for incubation at 37˚ for 24 hours (Kumar S & Narain S.,2010). Distilled water and Streptomycin were used as negative and positive control respectively.

4. Determination of minimum inhibitory concentration of extract

The minimum inhibitory concentration (MIC) was determined by using the "serial dilution technique" with muller hinton

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broth (Balouiri M, Sadiki M, Ibnsouda SK. ,2016; Valgas C. et al.,2007). In the initial tube, 20 µl of flower extract was added into the 380 µl of muller hinton broth. For further dilutions, 200 µl of muller hinton broth was added into the following nine tubes separately. From the initial tube, 200 µl was transferred to the first tube containing 200 µl of muller hinton broth. This was considered as a 10^{-1} dilution. From a 10-1 diluted tube, 200 µl were transferred to the second tube to make 10^{-2} dilution. Similarly, the serial dilution was repeated up to 10^{-9} dilutions. From the maintained stock cultures of the following pathogens (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtills, Pseudomonas aeruginosa* and fungal pathogen *Candida albicans*) a microliter was taken from each and added into 2 ml of muller hinton broth. In each serially diluted tube, 200 µl of above-cultured suspension was added. The tubes were then incubated for 48–72 hours in an incubator at 37°C and observed for turbidity. The turbidity was checked using optical density, and the reading was observed at 450 nm.

5. *In-vitro* antioxidant activities

1 Reducing power activity

Ascorbic acid solution was prepared in distilled water of different concentration such as 60, 120, 180, 240, 300, 360, 420, 480 µg/ml.

In vitro antioxidant assay was estimated by ferric reducing antioxidant power method. To the 2.5 ml of extract, 1ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1 % potassium ferricyanide was added. The reaction mixture was incubated in water bath at 50°C for 20 minutes. Afterward, reaction mixture was rapidly cooled and 2.5 ml of 10 % trichloroacetic acid was added to stop the reaction and was centrifuged for 10 minutes. 2.5 ml of aliquots was pipetted out and 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride solution was added. The color changes to green.

The mixture was allowed to stand for 10 minutes and absorbance was measured at 593 nm using Systronic UV-Vis spectrophotometer (Model-2205) instrument. The blank was performed using reagent blank and solvent. Ascorbic acid was used as standard. The test was performed in triplicate (Patel et al., 2010; Patel et al., 2012).

8.2 Determination of Fe^{2+} chelating activity

The chelating activity of ferrous (Fe^{2+}) ion was determined according to the method described by (Oboh et al., 2007). In a clean test tube, 0.3 mL of flower extract solution was added to a reaction mixture consisted of 0.336 mL of Tris–HCl buffer solution (pH 7.4) and different volumes of each extract (0.006–0.026 mL). The solution was completed by saline solution (0.9% NaCl, w/v) to a known volume (1.12 mL). The reaction mixture was incubated for 5 min at room temperature before the addition of 0.026 mL of 1,10-phenanthroline solution (0.25%, w/v). The absorbance (A) was measured at 510 nm. Ascorbic acid solution (0.03%, w/v) was used as a positive control. The Fe2+ chelating activity (%) was calculated from the following equation:

Fe^{2+} chelating activity (%) = [(A control − A sample)/A control] × 100

8.3 DPPH radical scavenging activity

The ability of flower extracts to scavenge the DPPH radicals was assessed by using the method of Blois with some modifications (Blois MS,1958). About 0.2 mmol/L solution of DPPH in methanol was prepared, and 500 µL of this solution was added to different concentrations of the extracts (50-250 µg/mL). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Control was prepared as above but without the sample extracts and methanol was used for the baseline correction. Then changes in the absorbance of the plant samples were measured at 517 nm using spectrophotometer. A lower absorbance value indicates the higher radical scavenging activity. Results were compared with the standard antioxidants [rutin, quercetin, butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT)]. The ability of DPPH radical scavenging activity was calculated by using the following formula:

DPPH scavenging effect (% of inhibition) = (A0-A1) ×100/A0

Where, A0 is the absorbance of the control,

A1 is the absorbance of the sample extracts.

The IC50 (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC50 value indicates greater antioxidant activity.

8.4 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was assessed by the method of (Sreejayan and Rao,1997). Nitric oxide radicals were produced from sodium nitroprusside solution and measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide radicals which interfere with oxygen to produce nitrite ions. Scavengers of nitric oxide act against oxygen, leading to reduce production of nitrite ions. About 3 mL of sodium nitroprusside (10 mmol/L) in phosphate buffer saline (0.2 mmol/L, pH 7.4) was added with various concentrations of the extracts (250-450 µg/mL) and it was incubated at 25 °C for 150 min. Then 500 µL of Griess reagent (1% sulphailamide, 2% orthophosphoric acide, 0.1% N-1-napthylethylenediamine dihydrochloride) was added. The absorbance values were measured at 546 nm and percentage of inhibition was calculated using the same formula as DPPH.

RESULT

1. Preparation of Flower Extract

Fresh flowers of *Plumeria alba,* and *Nelumbo nucifera* were collected and visually documented to ensure authenticity and quality. Following collection, petals were carefully separated to obtain the targeted floral parts for extraction. This step was crucial to maintain consistency across all samples, as petals are the primary source of phytochemicals and bioactive compounds. The uniform separation process ensured reliable results in subsequent analyses such as phytochemical screening, antioxidant assays, and antimicrobial testing (Fig. 1.1).

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(a). *Plumeria alba*



(b). *Nelumbo nucifera*

Fig. 1.1 Flower collection from different collection sites

The extraction and distillation of *Plumeria alba*, and *Nelumbo nucifera* petals resulted in two distinct types of extracts—with distillation and without distillation. The with-distillation extracts (hydrodistillates) primarily contained volatile and water-soluble compounds, including essential oils and fragrance components. In contrast, the without-distillation extracts, obtained through direct solvent extraction (hot, cold, and methanol methods), retained non-volatile bioactive compounds such as flavonoids, phenolics, and carbohydrates. This differentiation highlights the complementary nature of both extraction approaches in capturing the full spectrum of phytochemicals for subsequent analyses

2. Qualitative test for Phytochemicals

2.1 *Plumeria alba*

In Plumeria alba, alkaloids, flavonoids, carbohydrates, reducing sugars, terpenoids, phenols, coumarins, phlobatannins, anthraquinones, quinones, tannins, anthocyanins and carotenoids were consistently detected across all extracts, indicating stability. P. alba extracts showed a similar pattern, with all major bioactive compounds present. However, saponins and leucoanthocyanins were absent in methanol extracts but detected in aqueous ones, again showing the role of solvent type in compound extraction (Table 2)

Table 2: *P. alba phytochemical* results

Sr.no.	Tests	Methanol Without Distillation	Methanol With Distillation	Cold Without Distillation	Cold With Distillation	Hot Without Distillation	Hot With Distillation
1.	Alkaloids						
	(a). Wagner's test	+	+	+	+	+	+
	(b). Iodine test	+	+	+	+	+	+
	(c). Mayer's test	+	+	+	+	+	+
2.	Flavonoids						
	(a). Lead acetate test	+	+	+	+	+	+
	(b). Alkaline reagent test	+	+	+	+	+	+
3.	Carbohydrate	+	+	+	+	+	+
4.	Reducing sugar	+	+	+	+	+	+
5.	Cardiac glycosides	+	+	+	+	+	+
6.	Terpenoids	+	+	+	+	+	+
7.	Phenols	+	+	+	+	+	+
8.	Coumarins						
	(a). NaOH test	+	+	+	+	+	+
	(b). Alkalinne hydrolysis test	+	+	+	+	+	+
9.	Sterols & Phytosterols	+	+	+	+	+	+
10.	Phlobatannins	+	+	+	+	+	+
11.	Anthraquinone	+	+	+	+	+	+
12.	Quinone	+	+	+	+	+	+
13.	Tannins	+	+	+	+	+	+
14.	Saponins	-	-	+	+	+	+
15.	Anthocyanin	+	+	+	+	+	+
16.	Caretenoids	+	+	+	+	+	+
17.	Leucoanthacyanin	-	-	+	+	+	+

Note: + = positive test - = negative test

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2.2 Nelumbo Nucifera

N. nucifera extracts also contained a broad spectrum of phytochemicals, with sterols, phytosterols, and saponins only present in cold and hot aqueous forms. Phlobatannins and leucoanthocyanins were absent in all samples, suggesting either their absence or low solubility in the solvents used (Table 3).

Table 3: *N. nucifera* phytochemical result

Sr.no.	Tests	Methanol Without Distillation	Methanol With Distillation	Cold Without Distillation	Cold With Distillation	Hot Without Distillation	Hot With Distillation
1.	Alkaloids						
	(a). Wagner's test	+	+	+	+	+	+
	(b). Iodine	+	+	+	+	+	+
	(c). Mayer's test	+	+	+	+	+	+
2.	Flavonoids						
	(a). Lead acetate test	+	+	+	+	+	+
	(b). Alkaline reagent test	+	+	+	+	+	+
3.	Carbohydrate	+	+	+	+	+	+
4.	Reducing sugar	+	+	+	+	+	+
5.	Cardiac glycosides	+	+	+	+	+	+
6.	Terpenoids	+	+	+	+	+	+
7.	Phenols	+	+	+	+	+	+
8.	Coumarins						
	(a). NaOH test	+	+	+	+	+	+
	(b). Alkaline hydrolysis test	+	+	+	+	+	+
9.	Sterols & Phytosterols	-	-	+	+	+	+
10.	Phlobatannins	-	-	-	-	-	-
11.	Anthraquinone	+	+	+	+	+	+
12.	Quinone	+	+	+	+	+	+
13.	Tannins	+	+	+	+	+	+
14.	Saponins	-	-	+	+	+	+
15.	Anthocyanin	+	+	+	+	+	+
16.	Caretenoids	+	+	+	+	+	+
17.	Leucoanthacyanin	-	-	-	-	-	-

Note: + = positive test - = negative test

3. Antimicrobial activity test results

3.1 Hot extract antimicrobial test

The hot extracts of *Plumeria alba* (PHE), and *Nelumbo nucifera* (LHE) exhibited varying degrees of antimicrobial activity. Among them, *Plumeria alba* hot extract (PHE) showed the highest and broadest spectrum of inhibition, particularly against *E. coli* (2.4 cm), *S. aureus* (2.0 cm), and *P. aeruginosa* (1.7 cm)., while *Nelumbo nucifera* showed mild to moderate inhibition across all tested organisms. No activity was observed in the negative control. These results indicate that PHE possesses potent antimicrobial compounds effective against both Gram-positive and Gram-negative bacteria as well as fungi (Table 4).

Table 4: Hot flower extract antimicrobial activity test result

Sr. no.	Organism	PC	NC	PHE	NHE
1.	*S. aureus*	4.2 cm	-	2.0 cm	1.5 cm
2.	*E. coli*	1.7 cm	-	2.4 cm	1.4 cm
3.	*C. albicans*	3.4 cm	-	1.5 cm	1.2 cm
4.	*P. aeruginosa*	1.9 cm	-	1.7 cm	1.5 cm
5.	*B. subtillis*	3.5 cm	-	-	1.1 cm
6.	*S. typhi*	3.4 cm	-	2.0 cm	1.3 cm

NOTE :- PC = Positive control, NC = Negative control,Extract, PHE = *P. alba* hot Extract, NHE= *N. nucifera* hot Extract, - = No zone

3.2 Cold extract antimicrobial test

Cold extracts of *Plumeria alba* (PCE), and *Nelumbo nucifera* (LCE) demonstrated significant antimicrobial effects, with *Plumeria alba* cold extract (PCE) showing the strongest activity across all tested organisms. Maximum inhibition was noted against *S. aureus* (2.5 cm) and *S. typhi* (2.5 cm). *Nelumbo*

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nucifera (LCE) showed moderate inhibition (1.5–2.0 cm). No activity was observed in the negative control. These results further support the superior antimicrobial potential of *Plumeria alba* among the tested organisms under cold extraction conditions (Table 5).

Table 5: Cold flower extract antimicrobial activity test result

Sr. no.	Organism	PC	NC	PCE	NCE
1.	*S. aureus*	4.2 cm	-	2.5 cm	2.0 cm
2.	*E. coli*	2.0 cm	-	1.9 cm	-
3.	*C. albicans*	3.0 cm	-	2.0 cm	1.5 cm
4.	*P. aeruginosa*	2.0 cm	-	2.5 cm	1.7 cm
5.	*B. subtillis*	3.0 cm	-	2.0 cm	-
6.	*S. typhi*	3.0 cm	-	2.5 cm	1.5 cm

NOTE :- PC = Positive control, NC = Negative control, PCE = *P. alba* cold extract, NCE= *N. nucifera* cold extract, - = No zone

3.3 Methanol extract antimicrobial test

Methanol extracts of, *Plumeria alba* (PME), and *Nelumbo nucifera* (LME) showed varying degrees of antimicrobial activity. *Plumeria alba* extract exhibited the strongest and broadest inhibition, particularly against *E. coli* (2.5 cm) and *C. albicans* (2.0 cm). *Nelumbo nucifera* demonstrated mild inhibition across fewer organisms.PME and LME both maintained consistent moderate activity. These results reinforce the superior efficacy of *Plumeria alba* across all extraction methods (Table 6).

Table 6: Methanol flower extract antimicrobial activity test result

Sr. no.	Organism	PC	NC	PME	MME
1.	*S. aureus*	4.0 cm	-	1.7 cm	1.8 cm
2.	*E. coli*	2.0 cm	-	2.5 cm	2.0 cm
3.	*C. albicans*	2.5 cm	-	-	-
4.	*P. aeruginosa*	2.0 cm	-	2.0 cm	1.3 cm
5.	*B. subtillis*	2.6 cm	-	1.5 cm	1.5 cm
6.	*S. typhi*	2.7 cm	-	1.5 cm	-

NOTE :- PC = Positive control, NC = Negative control, PME = *P alba* methanol extract, NME= *N. nucifera* methanol extract, - = No zone

4. Minimum inhibitory concentration (MIC)

4.1 *Plumeria alba* Hot Extract (MIC)

The MIC results revealed that *P. alba* hot extract exhibited significant antimicrobial activity against all tested pathogens. Complete inhibition of growth was observed for *S. aureus(20* µg/µl), while *E. coli(1.25* µg/µl), *C. albicans (0.625* µg/µl), *P. aeruginosa (10* µg/µl), and S. typhi (0.125 µg/µl)) showed inhibition. The broth, extract, inoculum, and drug controls validated the experimental setup. These findings suggest that *P. alba* hot extract possesses potent broad-spectrum antimicrobial properties, highlighting its potential as a natural therapeutic agent (Table 7).

Table 7: *P. alba* hot extract minimum inhibitory concentration result

Tube no.	Conc. µg/µl	Test organism (O.D. at 580 nm)				
		S. aureus	***E. coli***	***C. albicans***	***P. aeruginosa***	***S. typhi***
Extract control	-	0.13	0.20	0.10	0.25	0.28
1.	40	0.0	0.0	0.0	0.0	0.0
2.	20	0.0	0.0	0.0	0.0	0.0
3.	10	0.21	0.0	0.0	0.0	0.0
4.	5	0.25	0.0	0.0	0.01	0.0
5.	2.5	0.35	0.0	0.0	0.02	0.0
6.	1.25	0.40	0.0	0.0	0.03	0.0
7.	0.625	0.48	0.11	0.0	0.03	0.05
8.	0.312	0.50	0.14	0.33	0.05	0.08
9.	0.156	0.52	0.16	0.40	0.09	0.15

4.1.2 *Nelumbo nucifera* Hot Extract (MIC)

The MIC assay demonstrated that the hot extract of *Nelumbo nucifera* exhibited antimicrobial activity against a range of bacterial and fungal strains including *S. aureus*, *E. coli*, *C. albicans*, *P. aeruginosa*, *B. subtilis*, *and S. typhi*. The extract showed complete inhibition of microbial growth at higher

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concentrations (40 to 5 µg/µl), as indicated by the zero OD readings. As the concentration decreased, microbial growth gradually resumed, suggesting a dose-dependent inhibitory effect. The lowest MIC values (strongest activity) were observed at 2.5 µg/µl for most organisms (Table 8).

Table 8: *N. nucifera* hot extract minimum inhibitory concentration result

Tube no.	Conc. µg/µl	Test organism (O.D. at 580 nm)					
		S. aureus	*E. coli*	*C. albicans*	*P. aeruginosa*	*B. subtillis*	*S. typhi*
Extract control		0.13	0.22	0.15	0.18	0.15	0.15
1.	40	0.0	0.0	0.0	0.0	0.0	0.0
2.	20	0.18	0.0	0.0	0.0	0.0	0.0
3.	10	0.23	0.0	0.0	0.0	0.0	0.0
4.	5	0.27	0.0	0.03	0.0	0.0	0.0
5.	2.5	0.16	0.09	0.15	0.0	0.14	0.0
6.	1.25	0.12	0.14	0.13	0.17	0.15	0.0
7.	0.625	0.18	0.17	0.20	0.20	0.18	0.14
8.	0.312	0.22	0.19	0.23	0.21	0.18	0.17
9.	0.156	0.35	0.26	0.25	0.26	0.20	0.20

4.2.1 *Plumeria alba* Cold extract (MIC)

The MIC analysis revealed that *P. alba* cold extract effectively inhibited the growth of various microorganisms including *S. aureus (0.625* µg/µl) ,*E. coli* (20µg/µl) *C. albicans (10* µg/µl) , *P. aeruginosa* (0.625 µg/µl)), *B. subtillis* (5 µg/µl) and *S. typhi (2.5* µg/µl). Complete inhibition was observed at higher concentrations (40 to 10 µg/µl), with OD readings of 0.0 indicating no microbial growth. As the extract concentration decreased, the OD values increased, showing a concentration-dependent reduction in antimicrobial activity. Control readings confirm the reliability of the experiment (Table 9).

Table 9: *P. alba* cold extract minimum inhibitory concentration result

Tube no.	Test organism (O.D. at 580 nm)						
	µg/µl	*S. aureus*	*E. coli*	*C. albicans*	*P. aeruginosa*	*B. subtillis*	*S. typhi*
Extract control	-	0.23	0.11	0.14	0.10	0.14	0.19
1.	40	0.0	0.0	0.0	0.0	0.0	0.0
2.	20	0.0	0.0	0.0	0.0	0.0	0.0
3.	10	0.0	0.16	0.0	0.0	0.0	0.0
4.	5	0.0	0.18	0.19	0.0	0.0	0.0
5.	2.5	0.0	0.21	0.25	0.0	0.18	0.0
6.	1.25	0.0	0.25	0.26	0.0	0.20	0.26
7.	0.625	0.0	0.26	0.28	0.0	0.05	0.31
8.	0.312	0.20	0.27	0.30	0.25	0.40	0.35
9.	0.156	0.25	0.31	0.31	0.34	0.42	0.39

4.2.2 *Nelumbo nucifera* Cold extract (MIC)

The MIC analysis revealed that *N. nucifera* cold extract effectively inhibited the growth of various microorganisms including *S. aureus (2.5*µg/µl) , *S. typhi* (0.625 µg/µl) *C. albicans (1.5* µg/µl) , *P. aeruginosa* (2.5 µg/µl)) and *B. subtillis* (40 µg/µl). Complete inhibition was observed at higher concentrations (40 to 10 µg/µl), with OD readings of 0.0 indicating no microbial growth. As the extract concentration decreased, the OD values increased, showing a concentration-dependent reduction in antimicrobial activity. Control readings confirm the reliability of the experiment (Table 10)

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Table 10: *N. nucifera* cold extract minimum inhibitory concentration result

Tube no.	Test organism (O.D. at 580 nm)					
	µg/µl	***S. aureus***	***S. typhi***	***C. albicans***	***P. aeruginosa***	***B. subtilis***
Extract control	-	0.30	0.25	0.29	0.32	0.31
1.	40	0.0	0.0	0.0	0.0	0.0
2.	20	0.0	0.0	0.0	0.0	0.17
3.	10	0.0	0.0	0.0	0.0	0.22
4.	5	0.0	0.0	0.0	0.0	0.32
5.	2.5	0.0	0.0	0.0	0.0	0.45
6.	1.25	0.18	0.0	0.0	0.17	0.48
7.	0.625	0.20	0.0	0.44	0.37	0.52
8.	0.312	0.30	0.29	0.52	0.43	0.60
9.	0.156	0.38	0.39	0.58	0.48	0.62

4.3.1 *Plumeria alba* Methanol extract (MIC)

The MIC results revealed that *P. alba* methanol extract exhibited significant antimicrobial activity against all tested pathogens. Complete inhibition of growth was observed for *S. aureus(5* µg/µl), while *E. coli(10* µg/µl), *P. aeruginosa (5* µg/µl), *B. subtillis* (5 µg/µl) and *S. typhi* (5 µg/µl) showed inhibition. The broth, extract, inoculum, and drug controls validated the experimental setup. These findings suggest that *P. alba* hot extract possesses potent broad-spectrum antimicrobial properties, highlighting its potential as a natural therapeutic agent (Table11).

Table 11: *P.alba* methanol extract minimum inhibitory concentration result

Tube no.	Test organism (O.D. at 580 nm)					
	µg/µl	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtillis***	***S. typhi***
Extract control	-	0.23	0.20	0.25	0.16	0.19
1.	40	0.0	0.0	0.0	0.0	0.0
2.	20	0.0	0.0	0.0	0.0	0.0
3.	10	0.0	0.0	0.0	0.0	0.0
4.	5	0.0	0.20	0.0	0.0	0.0
5.	2.5	0.28	0.29	0.48	0.11	0.19
6.	1.25	0.28	0.32	0.52	0.16	0.20
7.	0.625	0.30	0.38	0.60	0.29	0.25
8.	0.312	0.32	0.42	0.62	0.31	0.30
9.	0.156	0.32	0.45	0.69	0.35	0.40

4.3.2 *Nelumbo nucifera* Methanol extract (MIC)

The MIC results revealed that *N. nucifera* methanol extract exhibited significant antimicrobial activity against all tested pathogens. Complete inhibition of growth was observed for *S. aureus(5* µg/µl), while *E. coli(10* µg/µl), *P. aeruginosa (5* µg/µl) and *B. subtillis* (10 µg/µl) and showed inhibition. The broth, extract, inoculum, and drug controls validated the experimental setup. These findings suggest that *P. alba* hot extract possesses potent broad-spectrum antimicrobial properties, highlighting its potential as a natural therapeutic agent (Table 12).

Table 12: *N. nucifera* methanol extract minimum inhibitory concentration result

Tube no.	Test organism (O.D. at 580 nm)				
	µg/µl	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtillis***
Extract control	-	0.30	0.25	0.25	0.32
1.	40	0.0	0.0	0.0	0.0
2.	20	0.0	0.0	0.0	0.0
3.	10	0.0	0.0	0.0	0.0
4.	5	0.0	0.0	0.0	0.17
5.	2.5	0.01	0.09	0.18	0.25

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6.	1.25	0.09	0.20	0.20	0.34
7.	0.625	0.19	0.21	0.22	0.36
8.	0.312	0.20	0.27	0.35	0.40
9.	0.156	0.32	0.30	0.40	0.50

6 In-vitro Anti-oxidant activities

The reducing power of the cold extracts was measured and compared against a standard ascorbic acid curve with concentrations ranging from 60 µg/ml to 480 µg/ml. The results suggesting a higher concentration of reductants such as polyphenols or flavonoids that contribute to its antioxidant activity. *Plumeria alba* and *Nelumbo nucifera* also demonstrated noticeable reducing power, albeit to a lesser extent.

The Fe^{2+} chelating activity assay evaluates the ability of plant extracts to bind ferrous ions (Fe^{2+}), thereby preventing the formation of complexes that catalyze free radical generation through the Fenton reaction. *Plumeria alba* demonstrated the highest Fe^{2+} chelating activity (50%), indicating its strong potential to inhibit pro-oxidant metal-catalyzed oxidative damage. *Nelumbo nucifera* showed moderate activity (32%), reflecting a comparatively lower, yet notable chelation ability.

Plumeria alba exhibited the highest DPPH scavenging activity (72.5%), indicating its strong antioxidant potential. *Nelumbo nucifera* followed closely with 67.5% inhibition.

Plumeria alba exhibited the highest nitric oxide scavenging activity, indicating a strong potential to counteract NO-induced oxidative stress. *Nelumbo nucifera* also showed moderate inhibitory activity.

CONCLUSION

The present study highlights the notable phytochemical richness and antimicrobial potential of flower extracts from *Plumeria alba*, and *Nelumbo nucifera*. The presence of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and terpenoids in all extracts suggests a strong therapeutic foundation. Among the three, *Plumeria alba* demonstrated the most potent and broad-spectrum antimicrobial activity, The low MIC values further validate the efficacy of these extracts in inhibiting pathogenic microorganisms. These findings support the potential integration of these floral extracts into natural antimicrobial formulations and encourage further investigation into their mechanisms of action, safety profiles, and applications in herbal and pharmaceutical products.

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