



Evaluation of Anti-Bacterial and Anti-fungal Activity of the *Alpinia purpurata* Flower Extract

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ABSTRACT

The aim of this study was to evaluate the antibacterial and antifungal properties of *Alpinia purpurata* flower extract. Phytochemical screening was also performed. The analysis revealed the presence of alkaloids, flavonoids, steroids, and phenolic compounds. With a view to assessing the fungitoxicity of *Alpinia purpurata* flower extract against the fungal pathogens, studies were conducted on the fungitoxicity of *Alpinia purpurata*. against fungal pathogens.

Keywords: *Alpinia purpurata*, antibacterial and antifungal properties, Phytochemical screening.

INTRODUCTION

Several species of the genus *Alpinia* have been reported to exhibit fungicidal, antioxidant and bactericidal properties^{1,2}. *Alpinia purpurata* (Vieill.) K. Schum (Family: Zingiberaceae) is locally known in the Philippines as “luyang pula” or red ginger, and is a native to the Pacific³. Studies on its chemical constituents revealed the presence of α -pinene, β -pinene⁴, 1,8-cineole, (*E*)-methoxycinnamate⁵, 6-shogaol, 8-gingerol, 6-gingerol, 10-gingerol, 10-shogaol and 4-shogaol. A US patent reported that its total anthocyanidin, shogaol and gingerol content shows promise in the treatment of inflammatory diseases such as arthritis^{6,7}.

Anthocyanins are flavonoid polyphenolic pigments commonly present in the stems, leaves, flowers, fruits, seeds, and other parts of plants⁸. Anthocyanins exist in plants in the form of glycosides that contain glucose, galactose, arabinose, rhamnose, and xylose⁹. Anthocyanins contain several phenolic hydroxyl groups in their structure, which help protect plants against oxidizable compounds. Owing to these special properties, they are known for scavenging free radicals and show anti-tumor, anti-cancer, and anti-inflammatory activities. They also help inhibit lipid peroxidation and platelet aggregation, prevent diabetes, support weight management, protect eyesight, and more. In vitro, anthocyanins can absorb oxygen free radicals and bind oxygen as well as hydroxyl radicals, enabling them to act as potent antioxidants that neutralize reactive oxygen species (ROS)¹⁰. By stimulating antioxidant enzymes like NAD(P)H oxidase, anthocyanins promote the activation of the Nrf2 signaling pathway¹¹. The ability of anthocyanins points to their potential role in preventing cardiovascular and neuronal diseases. Additionally, studies confirm their anti-inflammatory effects by blocking the NF- κ B pathway and stimulating the MAPK pathway¹². This study aims to assess the in vitro anti-bacterial and anti-fungal activity of anthocyanin used in traditional medicine. The extracts prepared were used to assess the anti-bacterial and anti-

fungal activity. This helps to identify which could be a therapeutic agent in treatment of disease.

MATERIALS AND METHODS

PLANT MATERIAL COLLECTION AND EXTRACTION

The *Alpinia purpurata* flower was collected from Peelamedu Coimbatore district, TamilNadu, India. It was authenticated from the botanical survey of India, Coimbatore. NO. BSI/SRC/5/23//2023/Tech – 556. Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. 20% of the extract was prepared in dissolving the different solvents of methanol, ethanol, and water (20g in 100ml). After dissolving, the flask was incubated in an orbital shaker for 24 hrs with 60 to 70 rpm at 400°C. After incubation the extract was filtered and used for further study.



Figure 1: *Alpinia purpurata*

Phytochemical Screening

Preliminary phytochemical tests were carried out with Methanol, Ethanol and Water solvent fraction using standard procedure described by Siddiqi and Ali Sofwara and Sazda et al^{13,14,15}.

Antibacterial Assays

DISC Assay:

Screening plant extracts for their antibacterial activity was conducted using the disc assay described by Bauer et al. (1966). Extracts of acetone or BM were evaporated in vacuum at 30 jC using a rotary evaporator (Eyela, Tokyo, Japan). Residues were dissolved in sterile DMSO at 10 mg ml⁻¹. Solutions of phenolic compounds (controls) commonly found in plants were also prepared (10 mg ml⁻¹ DMSO) and those were quercetin (Sigma, St. Louis, MO, USA), naringenin (Extrasynthese, Genay, France) and chlorogenic acid (ICN Biomedicals, Aurora, OH, USA). Two hundred microliters of prepared culture were spread on surfaces of Mueller –Hinton agar. Sterile filter paper discs (10 mm in diameter, Toyo Roshi Kaisha, Japan) were aseptically put on agar surfaces and immediately impregnated with 80 Al (800 Ag) of prepared extracts or phenolic compounds. Similarly, 80 Al of DMSO was used as a control. Antibiotic discs (6 mm in diameter) of vancomycin (30 Ag) and amoxicillin (25 Ag) were also used as positive controls. Spread plates were then kept at ambient temperature for 30 min to allow diffusion of extracts prior to incubation at 36 jC for 21 h. Inhibition zones (including the diameter of disc) were measured, and values < 12 mm were considered as nonactive extracts against bacteria¹⁶.

Antimicrobial Assay

DISC Assay:

Antifungal activity was tested against *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus flavus*. The well diffusion method was used to evaluate the antimicrobial activity of the *Alpinia purpurata* flower extract. The antimicrobial activity of the extract was investigated against three fungi (*A.niger* and *A.flavus*, *A.terreus*) 15ml of nutrient Agar and malt agar (fungi) were prepared poured onto the surface of sterile petriplate as a basal layer. After solidification 80 µl of the indicator strains (10⁸ cfu) were swabbed uniformly on the surface of the agar plate and allowed to dry for 5 minutes. Five wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with 30 µl of sample. Standard reference antibiotics were used as the positive controls (Antibiotic) and Dimethyl Sulfoxide (DMSO) serve as the negative control. It was then incubated at 37°C for 18-24 hours for the bacteria and 30°C for 3 to 5 days for the fungi. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm¹⁷.

RESULT AND DISCUSSION

Phytochemical Screening

Qualitative preliminary phytochemical analysis the results confirm the presence of alkaloids, saponin, flavonoids, steroids, terpenoids, phenols, quinines, proteins in the hydro-ethanolic and aqueous extract. The methanolic extracts showed the presence of steroids, terpenoids, phenols, saponins, flavonoids, quinones. Among the various extracts, the hydro-ethanolic extract contains higher concentration of phytochemical constituents (Table 1).

Table 1: Qualitative phytochemicals analysis of *Alpinia purpurata* flower extract in different solvent systems

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	++	++	++
Flavonoids	+++	+++	+++
Terpenoids	+	+	+
Phenol	+++	++	++
Saponins	+++	++	++
Quinones	+	+	+
Proteins	++	+	-
Steroids	++	+	-

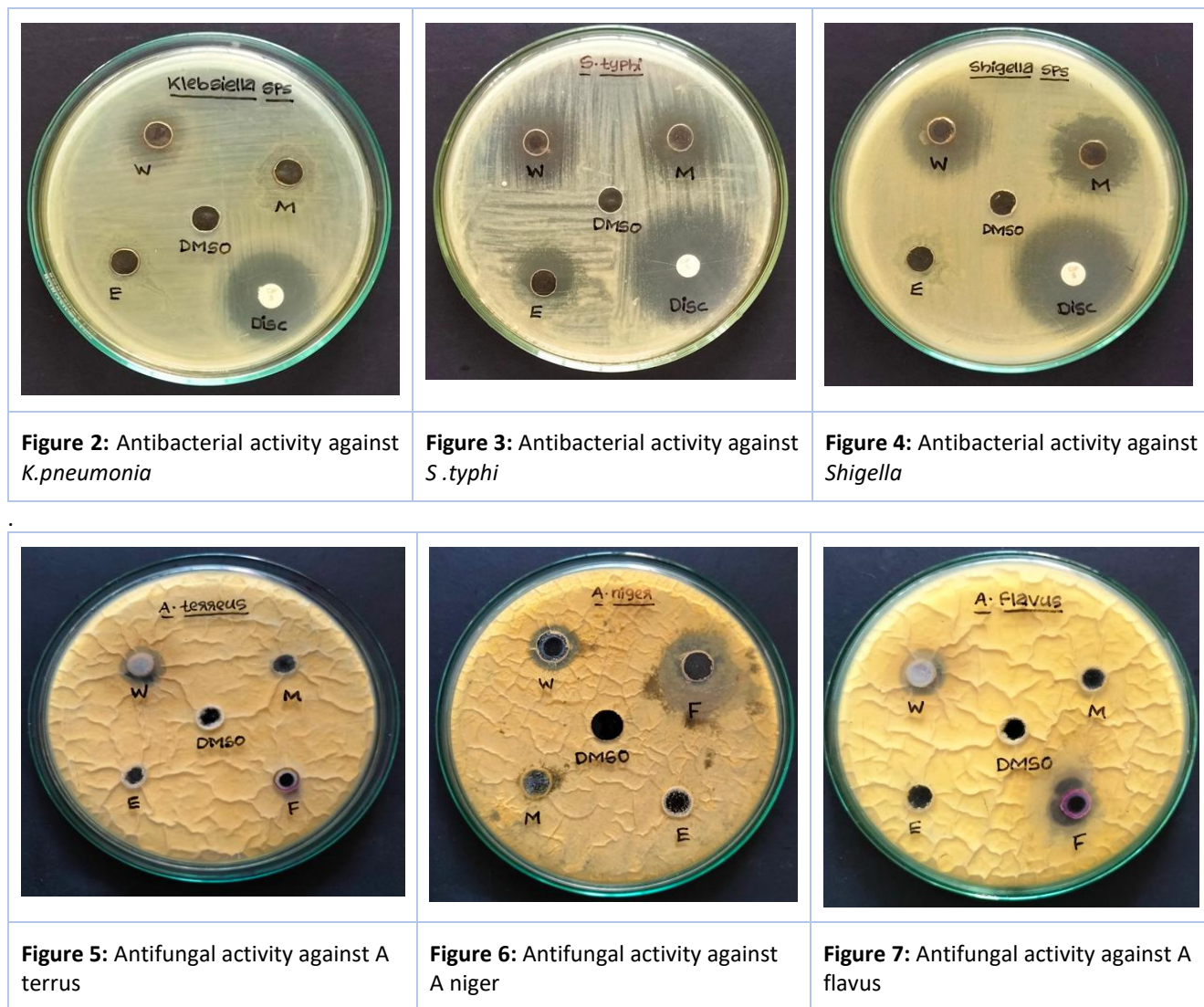
Antibacterial Activity

The antibacterial potential of the extracts was evaluated against three bacterial strains: *Klebsiella pneumoniae*, *Salmonella typhi*, and *Shigella* sp., using the agar well diffusion method. The results are presented in Table X. Among the tested extracts, the water extract (W) exhibited moderate antibacterial activity against all tested organisms, showing zones of inhibition of 12 mm, 18 mm, and 20 mm for *K. pneumoniae*, *S. typhi*, and *Shigella* sp., respectively. The methanol extract (M) displayed comparable activity against *S. typhi* (18 mm) and *Shigella* sp. (19 mm), but no inhibition was observed against *K. pneumoniae*. The ethanol extract (E), however, showed no antibacterial effect against any of the tested bacteria. The standard antibiotic disc (Disc) demonstrated the highest antibacterial activity, producing inhibition zones ranging from 20 mm to 28 mm across all bacterial strains, confirming the sensitivity of the test organisms. The DMSO control showed no zone of inhibition, indicating that the solvent had no intrinsic antibacterial effect. Overall, these findings suggest that the aqueous and methanolic extracts possess notable antibacterial properties, particularly against *S. typhi* and *Shigella* sp., whereas the ethanolic extract was inactive.

Table 2: Antibacterial activity against *K.pneumoniae*, *S. typhi* and *Shigella*

Name of the organism	Zone of inhibition (in mm)				
	W	M	E	Disc	DMSO
<i>K.pneumoniae</i>	12	Nil	Nil	20	Nil
<i>S. typhi</i>	18	18	Nil	28	Nil
<i>Shigella</i>	20	19	Nil	27	Nil





Antifungal Activity

The antifungal efficacy of the extracts was assessed against *Aspergillus niger*, *A. flavus*, and *A. terreus* using the agar well diffusion method. The results are summarized in Table X. Among the tested extracts, only the aqueous extract (W) exhibited detectable antifungal activity, while the methanolic (M) and ethanolic (E) extracts showed no inhibition against any of the fungal strains tested. The aqueous extract demonstrated zones of inhibition of 12 mm, 14 mm, and 13 mm against *A. niger*, *A. flavus*, and *A. terreus*, respectively. the aqueous extract exhibited inhibition zones that were comparable to or greater than those of the standard antifungal drug, fluconazole, which produced inhibition zones of 10 mm, 8 mm, and 17 mm for *A. niger*, *A. flavus*, and *A. terreus*, respectively. This suggests that the aqueous extract contains water-soluble antifungal compounds capable of inhibiting fungal growth, particularly against *A. flavus* and *A. niger*. The DMSO control showed no activity against any of the test organisms, confirming that the observed antifungal effects were due to the bioactive constituents of the plant extract and not the solvent. The results indicate that the aqueous extract possesses moderate to strong antifungal activity, while the absence of

inhibition in the methanolic and ethanolic extracts suggests that the active antifungal constituents are likely polar and water-soluble in nature.

Table 3: Antifungal activity against *A .niger*, *A .flavus* and *A .terreus*

Name of the organism	Zone of inhibition (in mm)				
	W	M	E	F- Fluconazol	DMSO
<i>A .niger</i>	12	Nil	Nil	10	Nil
<i>A .flavus</i>	14	Nil	Nil	8	Nil
<i>A .terreus</i>	13	Nil	Nil	17	Nil

CONCLUSION

The present study highlights the phytochemical richness and antimicrobial potential of aqueous, methanolic, and hydro-ethanolic extracts of the investigated plant. Preliminary qualitative phytochemical analysis confirmed the presence of various bioactive compounds, including alkaloids, saponins, flavonoids, steroids, terpenoids, phenols, quinones, and proteins. Among the different extracts, the water extract exhibited the highest concentration of phytochemicals,



The antibacterial evaluation revealed that both aqueous and methanolic extracts possessed notable inhibitory effects against *Klebsiella pneumoniae*, *Salmonella typhi*, and *Shigella* sp. The aqueous extract showed moderate activity against all tested bacteria, while the methanolic extract exhibited selective inhibition against *S. typhi* and *Shigella*. The absence of antibacterial activity in the ethanolic extract indicates that the key antibacterial constituents are predominantly polar and better extracted in water or mixed solvent systems.

Antifungal studies demonstrated that only the aqueous extract exhibited measurable inhibition against *Aspergillus niger*, *A. flavus*, and *A. terreus*, with inhibition zones comparable to or greater than those of the standard antifungal agent fluconazole. The methanolic and ethanolic extracts showed no antifungal activity, supporting the hypothesis that the active antifungal compounds are water-soluble and polar in nature.

Overall, the study establishes a strong correlation between solvent polarity, phytochemical content, and antimicrobial efficacy. The aqueous extracts, being rich in polar phytochemicals, demonstrated promising antibacterial and antifungal activities. These findings suggest that the *Alpinia purpurata* flower extract under study could serve as a valuable source of natural antimicrobial agents. Further research focusing on the isolation, purification, and characterization of the active compounds is recommended to elucidate their mechanisms and potential therapeutic applications.

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