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Frangipani: A Common Plant with Antimicrobial Potential against Common Bacterial Pathogens

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ABSTRACT

A lot of microflorae especially bacteria present throughout the human body help in protecting the individual against foreign particles, but with the rapid increase in antibiotic resistance, it has become a major threat to public health around the world. The risk of synthetic drugs becoming resistant against bacterial illnesses makes it vital to identify alternative treatments that are safe, accessible and has greater efficacy than synthetic drugs. *Plumeria* also known as Frangipani is a flowering plant belonging to the family *Apocynaceace*. The bioactive compounds of Frangipani are used to heal a variety of infections, the petals are edible and utilized in several meals, cosmetics, and spiritual rites. In this study, we have studied the potential of Frangipani to be used as an alternative traditional medicine as an antibacterial. It has antimicrobial capabilities that are almost equivalent to wide spectrum synthetic antibiotics against gastrointestinal pathogens. The prime goal of this study was to assess the antibacterial activity of the flower and leaf extracts of the frangipani plant against pathogenic *E. coli, Staphylococcus spp., Streptococcus spp., and Pseudomonas spp*. We have extracted 5gm/ml of the plant samples in two different solvents namely methanol and hexane. The maximum zone of inhibition was observed in methanolic leaf extract against gram negative *Pseudomonas* (64.28%) and gram-positive *Streptococcus* (91.15%), respectively.

INTRODUCTION

Inadequate disease surveillance, incorrect antibiotic prescriptions or lenient doses of antibiotics have resulted in the development and spread of Antibiotic Resistant Microorganisms, especially Bacteria. This has now become a major public health Issue all over the world. Various Multi-drug resistant pathogens like *Escherichia coli* or *Acinetobacter baumannii*, or methicillin resistant *Staphylococcus aureus* have been identified as nosocomial diseases. According to a study, in Egypt a 2 year research project of nosocomial infections in a number of university hospitals displayed that almost 90% of the gram negative and staphylococcal strains were MDR strains and the potential of exposure to infectious diseases at hospitals is significant (Hamam, et al. 2021). Unfortunately, the existing therapeutics approaches for the disease caused by pathogens are limited (Deep, et al. 2004, Venmugil and Kumar 2018). Problems like these suggest that Antibiotic resistance is a great challenge in the healthcare sector, and most importantly in developing countries (Al Bshabshe, et al. 2020). As a result, discovery of effective antimicrobial agents for antibiotic resistant strains is the need of the hour.

So, researchers are now focusing on developing novel natural or synthetic drugs and effective methods to fight against these drug-resistant bacteria. For example, Halicin, a promising wide spectrum antimicrobial agent, was discovered via repurposing varieties of FDA-approved drugs (Stokes, et al. 2020), darobactin obtained from *Bacillus* was found effective against most Gram-negative pathogens (Hart, et al. 2019) and piperine isolated from black pepper is an alkaloid effective against Tuberculosis (Hegeto, et al. 2019).

Similarly, the plants from genus *Plumeria* (frangipani) which belongs to *Apocynaceae* family, are cultivated worldwide for medicinal, food and cosmetic purposes. Frangipani plants are well known for its visual attractiveness and olfactory senses. The essential oils from the flowers are used for perfumery and aromatherapy purposes (Shaida, et al. 2008). The different parts of frangipani plant possess antibacterial activities that are equivalent to a wide spectrum of synthetic antibiotics against the most common gastrointestinal pathogen, namely Escherichia coli, this bacterium is usually resistant to synthetic drugs. This plant has the potential to be used as the source of natural anti-toxin drugs as well as a new antibacterial component (Sura , Dwivedi and Dubey 2021).

Written records of the usage of traditional remedies, such as Ayurveda, Chinese, Tibetan, Siddha and Unani traditional medicine, dates back more than 5,000 years ago and addresses

disease resulting from an imbalance between the mind, body, and environment (Schneeman, et al. 2005). In Ayurveda or traditional Indian medicine, *Plumeria spp*. have played an extremely important role in antibacterial, wound healing, natural remedy for diarrhoea, cure for itching, asthma, tumours, blood disorders and bronchitis (Nadkarni 1976).

According to the World Health Organization, 2003 about 80% of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, primarily plant based, to sustain their basic health care facilities (Goyal and Mehta 2008).

In our present study, we have focused on the species *Plumeria obtusa* (flower and leaf parts), which emerges as a small shrub or plant with broadly spreading thick fleshy branches typically coated with knob like thick ends with a height of 0.9-6.1 metres (Shinde, Patil and Bairagi 2014). The flower of this plant has a characteristic feature containing five petals fused at the base like a small funnel-shaped tube. The flower is white in colour with a small brilliant yellow at centre. The leaves are present in clusters or groups near the branches or at the tips. They are obtuse at the end and slightly oblong in shape. In colour, it is dark greenish and leathery in texture which appears shiny on the surface. *Plumeria obtusa* is most commonly found in the Bahamas and the Greater Antilles in Central America and it is widely cultivated in tropical regions of Africa and Asia (Shinde, Patil and Bairagi 2014).

METHODOLOGY

Collection and Authentication of the Sample:

Plumeria obtusa leaf and flower was collected from the university campus. Fresh leaves and flowers were washed properly with distilled water and ethanol for sterilization and kept for drying. The dried specimen was powdered and stored in airtight containers.

Extraction of Plant Material and Preparation of Crude Sample:

For baseline antimicrobial analysis, crude sample was prepared by weighing 5 g of the dried powder of leaf and flower as described previously (Singh et al., 2016). The powder sample was homogenized using methanol and hexane, and different sets were prepared. The homogenized samples, with respective solvents, were kept overnight in airtight bottles. An excess amount of methanol and hexane was dried off. After 24 hours, 2ml of DMSO was added into each of the samples and the supernatant (crude sample) was obtained by centrifugation at 10,000 rpm for 5 minutes at RT, stored in Eppendorf tubes at 4ºC.

Selection of Microorganisms:

For the antimicrobial assay to carry out, two gram-negative bacterial strains – *E. coli*, *Pseudomonas spp.* and two gram-positive bacterial strains – *Staphylococcus spp.*, *Streptococcus spp.* are collected from lab of Amity Institute of Biotechnology, Lucknow campus.

Antimicrobial Assay:

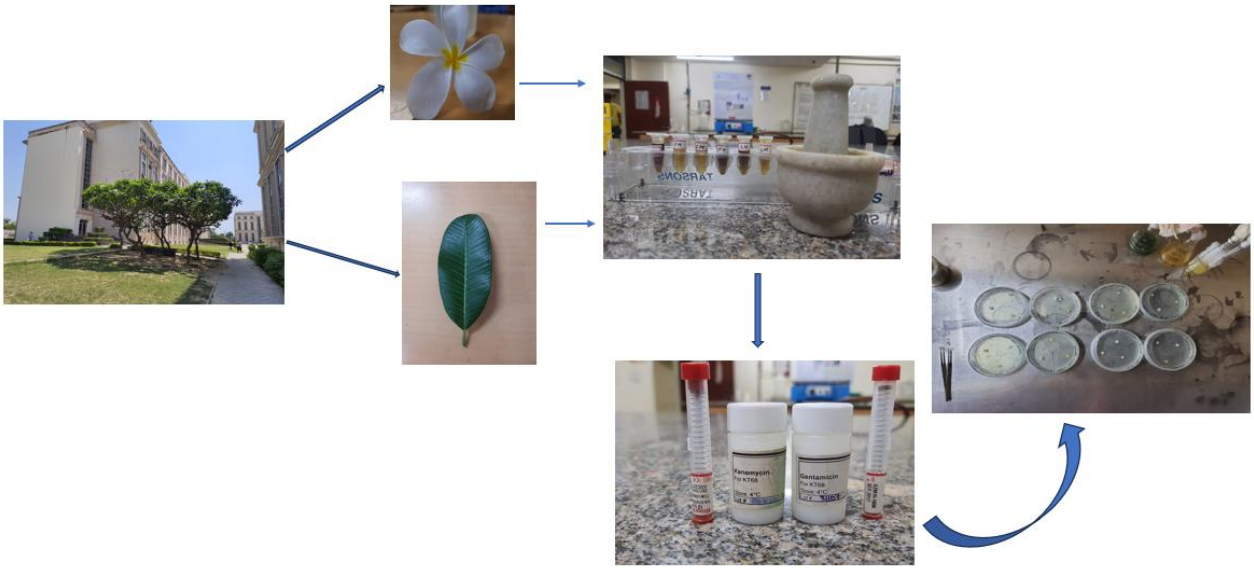
The disc diffusion method was used in this experiment to test the antimicrobial activity of leaf and flower extract of *Plumeria obtusa* against four bacterial strains (2 Gram positive and 2 Gram negative). Dried and sterilized filter paper discs was impregnated with considerable concentration of test or crude sample using a micropipette. Residual solvents were allowed to evaporate and placed on Nutrient Agar medium plates, uniformly seeded with test microorganisms.

Same solvents were used as negative control. For positive control Kanamycin (10µg/ml) was used for *E.coli*, Azithromycin (10µg/ml) was used for *Streptococcus spp.* , Amoxicillin (10µg/ml) was used for *Staphylococcus spp.* , and Gentamycin (10µg/ml) was used for *Pseudomonas spp.* The inoculated plates were then incubated at 37°Celsius for 24 hours for bacterial strain. The test materials containing our antimicrobial activity displayed a zone of inhibition.

Antimicrobial activity

Antimicrobial activity was determined using disc diffusion method (Ahaotu, et al. 2020). For testing, 5g of sample powder (flower and leaf of *Plumeria obtusa*) was weighed, using a weighing balance. Then 5 ml of methanol and hexane were used to homogenize the test sample powder and then 2 ml of DMSO was added into all the powdered samples and centrifuged at 10,000 rpm for 5 minutes at RT to obtain the supernatant as test sample. Different set of vials were prepared homogenized with three different solvents. Both gram positive viz., *Streptococcus spp.*, *Staphylococcus spp.* and gram negative viz., *E.coli*, *Pseudomonas spp.*, were used as test microorganism. In this experiment, sterile filter paper disc was prepared and impregnated with known potency of test samples using a micropipette and then dried to evaporate the excess. The discs are then carefully placed on petri plates containing nutrient agar medium lawned with specific test microorganisms. An antibiotic disc of positive control was also placed that proved to be specific for positive and negative

bacterial strains. The plates are then incubated at 37°C to allow maximum diffusion of the test sample material in the gel and for 18-24 hours to allow the growth of test microorganism. After the incubation period, the antimicrobial activity of the test samples was determined by calculation the diameter of zone of inhibition in millimetre.



RESULTS AND DISCUSSION

An example of a prokaryotic organism without a nuclear envelope is bacteria (Sinaga and Jaya 2022). The two forms of bacteria with differing cell wall structures are gram-positive bacteria and gram-negative bacteria (Sinaga and Jaya 2022). Antibiotics are chemical substances created by fungi or bacteria that can kill or inhibit the growth of dangerous microorganisms by suppressing bacterial cell wall formation, causing the cell wall to become brittle and causing cell lysis while having a low toxicity for humans (Sinaga and Jaya 2022). *Plumeria obtusa* (Frangipani) has been cultivated around the world and utilized for thousands of years for food, essential oil applications, and traditional medicine (Idrees, et al. 2020). *Plumeria spp.* is known to contain active compounds of alkaloids and saponins that possess antimicrobial assay (Pasaribu, et al. 2020). Several pharmacologically and medicinally significant compounds, including plumeride, isoplumeride, fluvoplumericin, irriod glycoside, and other diverse minor secondary metabolites, are found in various species of Plumeria (Shinde, Patil and Bairagi 2014). Research on the pharmacological effects of extracts taken from different plant sections using various in vitro and in vivo models has revealed that the chemicals are effective against a variety of bacteria (Trivedi et al., 2013; Tripathi et al., 2016; Tripathi & Singh, 2020).

In this experimental study, *Plumeria obtusa* extract was evaluated for antimicrobial activity using disc diffusion method against *E.coli*, *Pseudomonas spp.*, *Streptococcus spp.*, *Staphylococcus spp.* It was observed that the zones of inhibition in methanolic leaf extract and hexane leaf extract were **12.6mm** and **10mm** against gram-negative *Pseudomonas*, respectively**.** Similarly, the methanolic flower extract (**9.6mm**) and hexane flower extract (**9.0mm**) displayed maximum inhibitory zone against *Pseudomonas* which is resistant to most antimicrobial agents**.** Furthermore, the methanolic leaf and hexane leaf extracts were effective against *Streptococcus spp.* since the zone of inhibition was **10.3mm** and **8.6mm**, respectively. *Streptococcus* is sensitive to the methanolic flower extract (**11.3mm**) and hexane flower extract (**9.0mm**). *E.coli* was sensitive to only hexane leaf extract (**9mm**), but it was resistant to all other extracts. Lastly, *Staphylococcu*s was resistant to both the methanolic and hexane extracts of leaf and flowers (Table 1).

Table 1 Antimicrobial assay test of methanolic and hexane extracts of leaf and flowers of *Plumeria obtusa*

Test Organisms	**Zone of Inhibition (in mm)**				
	Leaf (hexane)	**Leaf (methanol)**	**Flower (hexane)**	**Flower (methanol)**	**Controls**
E.coli	9	-	-	-	21 (Kanamycin)
Staphylococcus	-	-	-	-	- (Amoxicillin)
Pseudomonas	10	12.6	9	9.6	19.4 (Gentamycin)
Streptococcus	8.6	10.3	9	11.3	15.3 (Azithromycin)

CONCLUSION

The results of the current investigation suggest that several extracts have potent pharmacological effects. So, there is a lot of potential for *Plumeria obtusa* to isolate different phytochemical constituents and assess their pharmacological screening to obtain higher therapeutic value (Devprakash, et al. 2012).

According to classification based on strength, our results show that *Staphylococcus* is resistant whereas *Pseudomonas spp* is highly sensitive to the extracts of Frangipani leaf and flowers.

The plant is a versatile plant with a broad spectrum of medicinal activity that has been extensively explored for its pharmacological properties and is regarded as a universal cure-all in Ayurvedic medicine. The creation of contemporary pharmaceuticals from Plumeria species should be highlighted as the worldwide scenario is currently shifting towards the usage of non-toxic plant products (Tripathi & Singh, 2015). Its therapeutic usage should be supported by clinical trials. It is also vital to realize that its extracts may be useful not just in isolation but may really have a modifying effect when given in combination with others (Shinde, Patil and Bairagi 2014). Research on future molecular medicine on *Plumeria alba* and *Plumeria obtusa* is also under progress (Annggoro, Istyastono and Hariono 2020). Potential for treatment of Antimicrobial Resistance by *Plumeria obtusa* is also now into focus for future studies (Eloutify, et al. 2023). New uses and applications of frangipani by-products are continually added. It is necessary to conduct more research on how to gather and prepare frangipani leaves and flowers as efficiently as possible, especially in underdeveloped nations (Idrees, et al. 2020).

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