



---

**ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT OF *CAESALPINIA SAPPAN* AGAINST *PSEUDOMONAS AERUGINOSA***

**Vikraman K S**

Graduate, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, Tamilnadu, India.

**Dr. P. Sankar Ganesh**

Assistant Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600 077, Tamilnadu, India.

**Dr. A.S. Smiline Girija**

Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai -600 077, Tamilnadu, India.

**Dr. J. Vijayashree Priyadharshini**

Associate Professor, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai- 600 077, Tamilnadu, India.

**Corresponding Author**

**Dr. P. Sankar Ganesh**

**Abstract:**

**Background:** According to the World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents an extended history of human interactions with the environment. *Caesalpinia sappan* Linn. a little thorny tree, 6-9 m high is found in India, is a well-established alternative medicine to treat various ailments. *Pseudomonas* infections are diseases caused by a gram-negative bacterium from the *Pseudomonas* and is considered as a potent nosocomial pathogen with multi-drug resistant properties.

**Methods:** *C.sappan* (10g) was collected and mixed with 100 ml of methanol and kept in a shaker and the extract was filtered using Whatman No:1 filter paper and crude extract was used for further study. MHA agar plate was used and *C.sappan* was loaded in each well of different concentrations. After 24 hours of incubation the zone of inhibition was measured using a standard scale.

**Results:** The result showed that 10 µL, 20 µL, 30 µL and 40 µL concentrations of methanol extract of *Caesalpinia sappan* has predominantly inhibited *Pseudomonas aeruginosa* in a concentration dependent manner.

**Conclusion:** The present study showed that low concentration of methanol extract of *C.sappan* inhibited the growth of *Pseudomonas aeruginosa*. The methanol extract of *C. sappan* is of potential value as natural antimicrobial inhibitors and could be used safely for treatment of Gram-negative bacterial infections and also can be used safely in the food industry.

**Keywords:** *Caesalpinia sappan*, *Pseudomonas aeruginosa*, MHA agar, Environment.

**Running title:** *Antimicrobial activity of methanol extract of C. sappan*

### **Introduction:**

According to the World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents an extended history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Ammara, 2012). Several screening studies have been carried out in several parts of the countries. There are several reports on the antimicrobial activity of various herbal extracts in several regions of the world due to the side effects and therefore the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Lokhande, Chandrashekhar J.Chandekar and Priya L. Lokhande Chandrashekhar J.Chandekar and Priya L. Lokhande and ijrbat, 2014).

*Caesalpinia sappan* Linn. a little thorny tree, 6-9 m high is found in India, Peru, Malaya etc. it's getting used traditionally for a big number of ailments and reported to possess a large sort of medicinal properties. Its anticonvulsant, anti-inflammatory, anti-proliferative, antimicrobial, anticoagulant, antiviral, immuno-stimulant and antioxidant activities have been reported (Rossolini, Valentini and Satta, 1989). In Ayurveda, the heartwood is used to treat Pitta, burning sensation, and to cure many other systemic ailments. The *Caesalpinia sappan* Linn. is mostly used in Kerala, India for its blood purifying, anti-diabetic properties and this plant is the main ingredient in many traditional Ayurveda medicines (Tupe *et al.*, 2010).

*Pseudomonas* infections are diseases caused by a Gram-negative bacterium. The bacteria are found widely within the environment, like in soil, water, and plants(Banso *et al.*, 2007). They typically don't cause infections in healthy people. More severe infections occur in people that are already hospitalized with another illness or condition, or people having a weak immune system. *Pseudomonas* are fairly common pathogens involved in infections acquired during a hospital setting (Verma *et al.*, 2010). *Pseudomonas* infections are difficult to treat due to their propensity for multi-drug resistant property. Our team has extensive knowledge and research experience that has translate into high quality publications(Krishnamurthy *et al.*, 2009; Abdul Wahab *et al.*, 2017; Eapen, Baig and Avinash, 2017; Ravindiran and Praveenkumar, 2018;

Subramaniam and Muthukrishnan, 2019; Anita *et al.*, 2020; Kumar *et al.*, 2020; Rajasekaran *et al.*, 2020; Arumugam, George and Jayaseelan, 2021; Dhanraj and Rajeshkumar, 2021)

This study is thus aimed to evaluate the antimicrobial effect of the methanol extract of *Caesalpinia sappan* against *P.aeruginosa*.

## **Materials and Methods:**

### ***Isolation and identification of P.aeruginosa***

The plaque sample was collected from the patient and the plaque was transferred into the potato dextrose broth. The tube was incubated at 28-30°C for 48 hours. After 48 hours a loop full culture was streaked on the SDA. The plate was incubated at 28°C for 48 hours. A clean glass slide was taken and, on the center, two drops of Bromophenol Cotton blue were added. Then a loop full culture was mixed with Bromophenol Cotton blue stain. A clean-over slip was placed on the strain without air bubbles. Then the slide was observed under the microscope (40x,100x).

### ***Collection of plant bark***

*C. sappan* bark was purchased from a local market in Chennai, Tamil Nadu. The collected plant was washed thoroughly with running tap water to remove the surface microflora and other adherents.

### ***Preparation of methanol extract***

The collected plant leaves were washed thrice with sterile distilled water and leaves were dried under shade. The dried leaves were ground to a coarse powder by the mechanical grinder. 10 g of *C. sappan* powder was mixed with 100 ml of methanol and was kept in a shaker for 40 hours with agitation at 150 rpm. After 48 hours all the extracts were filtered with a Whatman no.1 filter paper. Then the methanol was evaporated and the crude extract was used for further study.

### ***Antimicrobial activity of ethanol extract of C. sappan***

The test culture was swabbed on Muller Hinton Agar (MHA) plates and wells were created using a well cutter. The methanol extract of *C. sappan* was loaded into the corresponding well with varying concentrations. Control and sterility control also maintained. The plate was incubated for 48 h at 37°C. After the 48 hours of the incubation period, the minimum inhibitory concentration was observed.

## **Results and Discussion**

The result showed that 10 µL, 20 µL, 30 µL and 40 µL concentrations of methanol extract of *Caesalpinia sappan* has inhibited *Pseudomonas aeruginosa* at different concentration levels. Antibacterial activity of plant extract is attributed to the presence of bioactive compounds in the extracts of plants tested (Sayekti, Subiwahyudi and Prasetyo, 2016). The presence of these bioactive compounds in crude extracts shows their antibacterial activity against disease-causing microorganisms offer protection to plants themselves against pathogenic microbial infections (Verma *et al.*, 2010). The antibacterial activity of *C. sappan* (bark) were assayed by the agar disc diffusion and broth dilution method against six bacterial and two fungal strains. Methanol extracts from the this plants were found to have highest antibacterial

activity, whereas the aqueous extracts were less effective in inhibiting bacterial growth. Among the six bacterial strains tested for antibacterial activity, *S.aureus* was most susceptible with inhibition zones ranging from 9-20 mm and *K.pneumoniae* was least susceptible to the plant extracts. Further, the methanol extracts of *C.sappan* were found to be more effective than *M.pudica*.

**Table 1:** Minimum inhibitory concentrations of *C. sappan* against *P. aeruginosa* : At the lowest concentration, the extract (0.375mg/ml) inhibited the bacterial growth of *P. aeruginosa*

Methanol Extract	Clinical Isolate	Concentration	Observation
<i>C.sappan</i>	<i>P.aeruginosa</i>	10mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	5mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	2.5mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	1.25mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.75mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.375mg/ml	Growth inhibition
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.187mg/ml	Growth
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.093mg/ml	Growth
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.046mg/ml	Growth
<i>C.sappan</i>	<i>P.aeruginosa</i>	0.023mg/ml	Growth

### Conclusion

The present study concluded that low concentration of methanol extract of *C.sappan* inhibited the growth of *Pseudomonas aeruginosa*. The methanol extract of *C. sappan* is of potential value as natural antimicrobial inhibitors and could be used safely for treatment of Gram-negative bacterial infections and also can be used safely in the food industry.

**Conflict of Interest:** The authors declare that there is no conflict of interest.

**Acknowledgements:** The authors thank Dr. Deepak Nalaswamy, director, Saveetha Dental College and Hospitals, Saveetha institute of Medical and Technical sciences (SIMATS), for providing facilities and ideas to carry out this work.

**Funding Sources:** The authors sincerely acknowledge the financial support from Saveetha institute of Medical and Technical sciences (SIMATS), Chennai, Tamilnadu, India.

### References:

1. Abdul Wahab, P.U. *et al.* (2017) 'Risk Factors for Post-operative Infection Following Single Piece Osteotomy', *Journal of maxillofacial and oral surgery*, 16(3), pp. 328–332. doi:10.1007/s12663-016-0983-6.
2. Ammara, A. (2012) 'Antimicrobial Evaluation of Some Dental remedial Plant Extracts from Pakistan', *Medicinal Plant Research* [Preprint]. doi:10.5376/mpr.2012.02.0003.
3. Anita, R. *et al.* (2020) 'The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients', *American journal of cancer research*, 10(8), pp. 2546–2554. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/32905518>.
4. Arumugam, P., George, R. and Jayaseelan, V.P. (2021) 'Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma', *Archives of oral biology*, 122, p. 105030. doi:10.1016/j.archoralbio.2020.105030.
5. Banso *et al.* (2007) 'Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*', *African Journal of Biotechnology*, pp. 1785–1787. doi:10.5897/ajb2007.000-2262.
6. Dhanraj, G. and Rajeshkumar, S. (2021) 'Anticariogenic Effect of Selenium Nanoparticles Synthesized Using *Brassica oleracea*', *Journal of nanomaterials*, 2021. doi:10.1155/2021/8115585.
7. Eapen, B.V., Baig, M.F. and Avinash, S. (2017) 'An Assessment of the Incidence of Prolonged Postoperative Bleeding After Dental Extraction Among Patients on Uninterrupted Low Dose Aspirin Therapy and to Evaluate the Need to Stop Such Medication Prior to Dental Extractions', *Journal of maxillofacial and oral surgery*, 16(1), pp. 48–52. doi:10.1007/s12663-016-0912-8.
8. Krishnamurthy, A. *et al.* (2009) 'Glandular odontogenic cyst: report of two cases and review of literature', *Head and neck pathology*, 3(2), pp. 153–158. doi:10.1007/s12105-009-0117-2.
9. Kumar, S.P. *et al.* (2020) 'Targeting NM23-H1-mediated Inhibition of Tumour Metastasis in Viral Hepatitis with Bioactive Compounds from *Ganoderma lucidum*: A Computational Study', *Indian Journal of Pharmaceutical Sciences*. doi:10.36468/pharmaceutical-sciences.650.
10. Lokhande, C.J.C.A.P.L.C.J.C.A.P.L., Chandrashekhar J.Chandekar and Priya L. Lokhande Chandrashekhar J.Chandekar and Priya L. Lokhande and *ijrbat* (2014) 'Efficacy of Solvent Extracts of Some Medicinal Plants for Potential Antibacterial Activity', *International Journal of Researches in Biosciences and Agriculture*

- Technology* [Preprint]. doi:10.29369/ijrbat.2014.03.iii.0002.
11. Rajasekaran, S. *et al.* (2020) 'Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends', *Fuel*, 277, p. 118166. doi:10.1016/j.fuel.2020.118166.
  12. Ravindiran, M. and Praveenkumar, C. (2018) 'Status review and the future prospects of CZTS based solar cell – A novel approach on the device structure and material modeling for CZTS based photovoltaic device', *Renewable and Sustainable Energy Reviews*, 94, pp. 317–329. doi:10.1016/j.rser.2018.06.008.
  13. Rossolini, G.M., Valentini, S. and Satta, G. (1989) 'Evaluation of in vitro antimicrobial activity of lomefloxacin against staphylococci, enterococci, Enterobacteriaceae, and Pseudomonas aeruginosa', *Diagnostic Microbiology and Infectious Disease*, pp. 57–64. doi:10.1016/0732-8893(89)90068-0.
  14. Sayekti, S.F., Subiwahyudi, A. and Prasetyo, E.A. (2016) 'Perbedaan Efektivitas Daya Antibakteri Ekstrak Daun Mimba (*Azadirachta Indica* A. Juss) Dibanding NaOCl 2,5% Terhadap Enterococcus Faecalis (The Difference of Antibacterial Effectiveness of Neem Leaf Extract (*Azadirachta Indica* A. Juss) Than NaOCl 2,5% Against Enterococcus Faecalis)', *Conservative Dentistry Journal*, p. 71. doi:10.20473/cdj.v6i2.2016.71-76.
  15. Subramaniam, N. and Muthukrishnan, A. (2019) 'Oral mucositis and microbial colonization in oral cancer patients undergoing radiotherapy and chemotherapy: A prospective analysis in a tertiary care dental hospital', *Journal of Investigative and Clinical Dentistry*. doi:10.1111/jicd.12454.
  16. Tupe, P. *et al.* (2010) 'Hepatoprotective effect of Averrhoa bilimbi Linn. methanol extract on carbon tetrachloride induced liver damage in albino rats', *Planta Medica*. doi:10.1055/s-0030-1264297.
  17. Verma, R. *et al.* (2010) 'Antibacterial activity of plants used in Indian herbal medicine', *International Journal of Green Pharmacy*, p. 22. doi:10.4103/0973-8258.62161.