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To evaluate the antimicrobial activities on leaf of distimake dissectus

R Nagaraju[1], P Kamalakar[2]
[1] Department of Botany, Plant Biochemistry and Physiology Laboratory, Osmania University, Hyderabad, Telangana, India
[2] Supervisor, Department of Botany, Plant Biochemistry and Physiology Laboratory, Osmania University, Hyderabad, Telangana, India

Abstract
Methanolic extracts inhibited zones exhibited antibacterial activity on various pathogenic bacteria (Table 15& Fig 20). Antibacterial activity test of methanolic extract on 4 bacterial strains (*Staphylococcus aureus*, *E. coli*, *Strepneumonia* and *Pseudomonas aeruginosa*) against ampicillin as a standard. The values of ampicillin and methanolic extract are all in microgrammes whilst the zones of inhibition are in millimetres.
For instance, with respect to *Staphylococcus aureus*; for both ampicillin and the methanolic extract, the zone of inhibition increased with increasing concentration as observed. Ampicillin: Zones 20 mm > 21 mm > 22 mm >23 mm Methanolic extract: Zones-10 mm,13mm,16mm,18mm For *E. coli* too, the zones of inhibition increased with increasing concentrations for both articles. Ampicillin showed 17 mm, 18 mm, 20 mm and 21 mm zones whereas methanolic extract exhibits 9 mm, 13 mm,16mm, and with hindrance of antibiotic zone-18mm.
Streptococcus pneumonia was sensitive only to ampicillin, the methanolic extract having no or very minimal zones of inhibition at lower concentration against this microorganism. For Pseudomonas aeruginosa all of which presented zones of 17 mm, 18 mm, 20 mm and 21 mm respectively while its methanolic extract showed zones of inhibition which were at 0 mm, 11 mm, 13 mm and 14mm respectively.

Keywords: Anti-microbial activities distimake dissectus convolvulaceae

Introduction
Inflammation, as an adaptive biological response to harmful stimuli, is a complex physiological process that extends beyond its conventional association with infections and tissue injuries (Medzhitov, 2010), Simo *et al.*, 2023). This response plays a crucial role in the body's defense against infections and injuries, promoting healing and tissue repair (Liu *et al*., 2018). It is a well-coordinated process involving various immune cells, cytokines, and chemical mediators (Lee *et al.*, 2020). One of the key components in the initiation and regulation of inflammation are transcription factors. These transcription factors act as molecular switches, turning on or off the genes responsible for producing pro-inflammatory or anti-inflammatory molecules. Through the coordinated action of transcription factors, inflammation can be tightly regulated to ensure an effective response without causing excessive tissue damage (Lee *et al.*, 2020; Xu *et al.*, 2021). Additionally, the resolution of inflammation is equally important. During the resolution phase, the immune system signals for anti-inflammatory molecules to be produced, which help to dampen the inflammatory response and restore tissue homeostasis. Inflammation is not only a localized response, but it can also have systemic effects. For instance, during an infection, the release of pro-inflammatory molecules can lead to fever, fatigue, and other systemic symptoms. Furthermore, chronic inflammation can contribute to the development and progression of various diseases, including cardiovascular disease, diabetes, cancer, and autoimmune disorders. Understanding the complex mechanisms of inflammation and its regulation is crucial for developing therapeutic strategies to target inflammation-related diseases (Medzhitov, 2008).

Materials and Methods
Antimicrobial activity and its MIC studies by using *D. dissectus* extract
Bacterial strains
The antibacterial potency of each plant extract was evaluated using four bacterial strains causing food poisoning diseases. The bacterial strains Gram positive of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400), and Gram negative of *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) used in the study were obtained from ATCC.

Inoculum preparation
The selected bacterial pathogens were inoculated into nutrient broth and incubated at 37°C for 24 hours and the suspensions were checked to provide approximately 10-5 CFU/ml.

Results
Determination of Anti-bacterial activity
In the present study, antibacterial activity of *D. dissectus* was determined against three standard pathogenic bacterial lab cultures such as Gram positive (-) *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400) and Gram negative (-) P*seudomonas aeruginosa* (ATCC27853), *E. coli* (ATCC25922) by using ampicillin as standard with different solvent extracts (hexane, and methanol).

1. Antibacterial activity against methanolic leaf extract of *D. dissectus*
Methanolic extracts inhibited zones exhibited antibacterial activity on various pathogenic bacteria (Table 15& Fig 20). Antibacterial activity test of methanolic extract on 4

bacterial strains (*Staphylococcus aureus*, *E. coli*, *Strepneumonia* and *Pseudomonas aeruginosa*) against ampicillin as a standard. The values of ampicillin and methanolic extract are all in microgrammes whilst the zones of inhibition are in millimetres.

For instance, with respect to *Staphylococcus aureus*; for both ampicillin and the methanolic extract, the zone of inhibition increased with increasing concentration as observed. Ampicillin: Zones 20 mm > 21 mm > 22 mm >23 mm Methanolic extract: Zones-10 mm,13mm,16mm,18mm For *E. coli* too, the zones of inhibition increased with increasing concentrations for both articles. Ampicillin showed 17 mm, 18 mm, 20 mm and 21 mm zones whereas methanolic extract exhibits 9 mm, 13 mm,16mm, and with hindrance of antibiotic zone-18mm.

Streptococcus pneumonia was sensitive only to ampicillin, the methanolic extract having no or very minimal zones of inhibition at lower concentration against this microorganism. For Pseudomonas aeruginosa all of which presented zones of 17 mm, 18 mm, 20 mm and 21 mm respectively while its methanolic extract showed zones of inhibition which were at 0 mm, 11 mm, 13 mm and 14mm respectively.

On various tested strains, the results will show different antibacterial effect of methanolic extract and most active against *Staphylococcus aureus* and *E. coli*. It also provides information about the anti-bacterial activity of crude plant extract with respect to that of a reference antibiotic ampicillin. No zones of inhibition (areas with no growth) at particular concentrations against specific bacterial strains may reflect to non-existent or minimal antibacterial activity at corresponding concentrations dose. Conclusions about broad spectrum antibiotic potential of the methanolic extract against these bacterial strains need to be drawn after conducting proper investigation and interpretation

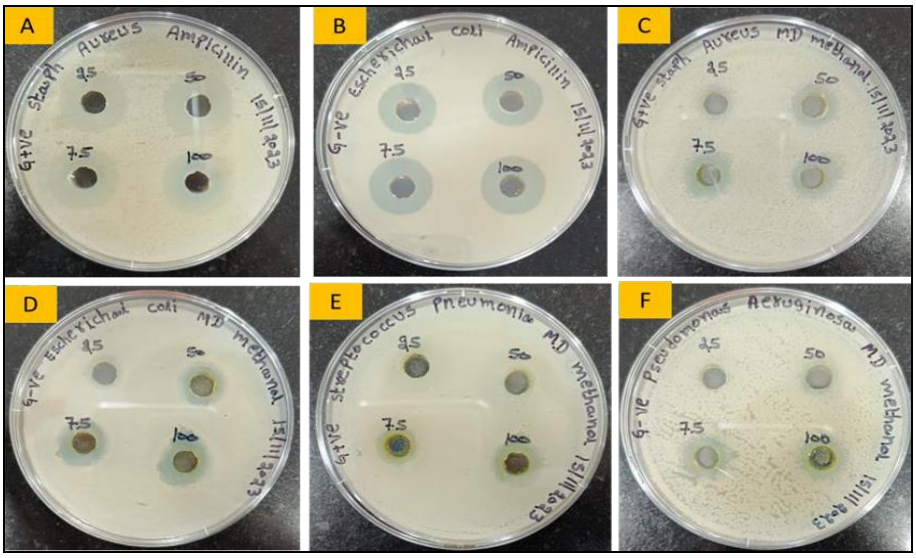

Fig 1. Anti-bacterial activity against various pathogenic bacteria. (A& B represents zone of inhibition with standard drug ampicillin whereas C, D, E & F are showing zone of inhibition with methanolic extract of *D. dissectus* with pathogenic bacterial stains)

Table 1: Anti-bacterial activity of the methanolic extract of D. dissectus leaves against pathogenic bacteria

Sr. No	Bacterial Strain	Ampicillin				Methanolic extract			
		Concentration (µg)/ Zone of Inhibition (mm)							
		25	50	75	100	25	50	75	100
1	*Staphylococcus aureus*	20	21	22	23	10	13	16	18
2	*E coli*	17	18	20	21	9	13	16	18
3	*Streptococcus pneumonia*	20	21	22	23	0	0	11	13
4	*Pseudomonas aeruginosa*	17	18	20	21	0	11	13	14

Antibacterial activity of hexane leaf extract of *D. dissectus*

The zone of inhibition was tested for the antibacterial activity against various pathogenic bacteria by using hexane extract listed in Table 16. Effect of Hexane Extract on Antibacterial Susceptibility Test in Comparison with Ampicillin - traditional antibiotic with this investigation the action of two different agents in the inhibition of four bacterial strains being *Staphylococcus aureus*, *E. coli*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa* was evalued. MIC values of ampicillin used were denoted in microgram (µg) present by prefixed symbols and diameters of the zone if inhibition which matches with respect mg/ml was calculated.

Ampicillin showed a concentration dependent increase in sizes of zones of inhibition ranged from 20 mm to 23 mm against S. On the other hand, hexane extract showed no inhibitory images below 25 µg and very low inhibition (from 9 mm up to 10 mm) with higher concentrations. The up-trend in the zones of inhibition was observed with increasing concentration in *E. coli*, maximum 21 mm by ampicillin and 17 mm at the lowest concentration. On the other hand, none of the concentrations of the hexane extract showed inhibitory effect with all values =0 mm.

With *Streptococcus pneumonia* ampicillin showed increase in zones of inhibition with a range of 20 mm to 23 mm with increasing concentrations. On the other hand, although hexane extract showed poor inhibition at less than 75 µg, mild to moderate inhibitions (10–12 mm) were shown at higher concentrations of mechanisms of action. In the case of *Pseudomonas aeruginosa*, at maximum concentration ampicillin has shown an increase in zone of inhibition increasing from 17 mm to 21 mm. In contrast, the hexane extracts showed no inhibition for concentrations below 75 µg and partial inhibition at the highest ccc (10 mm to 14 mm).

In short, the data indicate that moving from lower to higher concentrations of ampicillin inhibits the growth of four bacterial strains. Although, hexane extract showed very less or no inhibition activities against *Staphylococcus aureus*, *E. coli* and *Streptococcus pneumonia* in low concentration as compare to other two extracts. *P. aeruginosa* was only slightly inhibited by the hexane extract at higher concentrations.

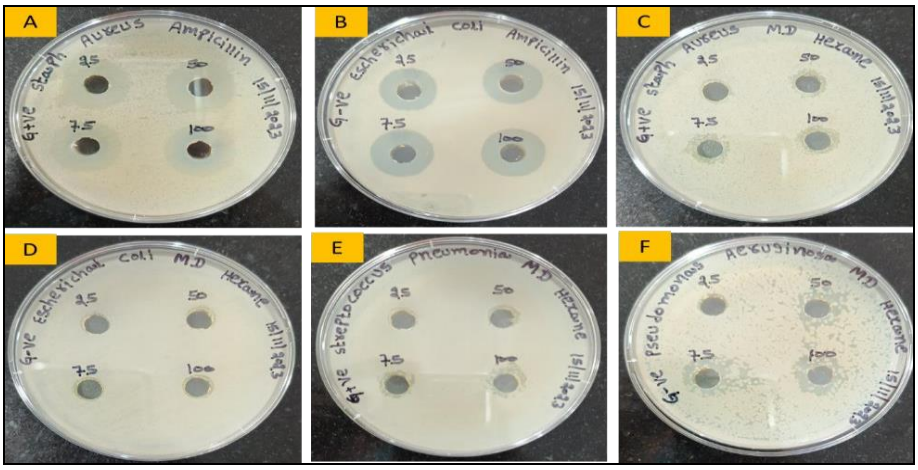

Fig: 2. Anti-bacterial activity against various pathogenic bacteria. (A& B represents zone of inhibition with standard drug ampicillin whereas C, D, E & F are showing zone of inhibition with Hexane leaf extract of *D. dissectus* with pathogenic bacterial stains.

Table 2: Anti-bacterial activity of Hexane leaf extract of *D. dissectus* against pathogenic

Sr. No	Bacterial Strain	Ampicillin				Hexane leaf extract			
		Concentration (µg)/ Zone of Inhibition (mm)							
		25	50	75	100	25	50	75	100
1	*Staphylococcus aureus*	20	21	22	23	0	9	10	10
2	*E coli*	17	18	20	21	0	0	0	0
3	*Streptococcus pneumonia*	20	21	22	23	0	0	10	12
4	*Pseudomonas aeruginosa*	17	18	20	21	0	10	12	14

Minimum inhibitory concentration (MIC) Studies on *D. dissectus*

The methanolic extract of the plant had significant antibacterial potential with minimum inhibitory concentration done with Ampicillin as a standard antimicrobial for the bacterial species (*E-coli* and *Staphylococcus aureus*).

All the samples and standards were incubated with different concentration of test compounds into nutrient broth by keeping at 370 C temperature, for an overnight culture. Results of the MIC determinations are given in Table **17** and **Fig.**

Fig 3: A. MIC activity of Ampicillin on *S. aureus*, B: MIC activity of Ampicillin on *E. coli*, C: MIC activity of methanolic leaf extract of *D. dissectus* on *S. aureus*, D: MIC activity of methanolic extract of plant on *E. coli*

Discussion

The global prevalence of contagious epidemics stemming from microbial agents has become a significant contributor to both morbidity and mortality on a worldwide scale (Martínez, 2012). While antimicrobials play an indispensable role in the battle against infectious diseases, the escalating and alarming trend of antimicrobial resistance poses a formidable threat to human health. Microbes can exhibit intrinsic resistance to antimicrobials, or they may acquire resistance through genetic mutations. Of particular concern is the ability of antimicrobial-resistant bacteria to transfer resistant gene (rgenes) via horizontal gene transfer (HTG) mechanisms to nearby bacteria, resulting in previously susceptible bacteria becoming resilient even in the face of high concentrations of antimicrobials (Martínez, 2012).

The emergence of multi-drug resistant (MDR) bacteria, colloquially termed "super bugs," introduces an additional layer of complexity to this issue. Pathogens such as *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, Pseudomonas *aeruginosa*, and Enterobacter *sp* collectively known as the "ESKAPE" group, are particularly prone to developing MDR (Gómez-Núñez *et al*., 2020). This not only extends the duration of illness and recovery periods but also poses a significant challenge for individuals who are immunocompromised or undergoing hospitalization, thereby exacerbating the financial burden on both individuals and healthcare systems (Gómez-Núñez *et al*., 2020) Spp.

In essence, the surge in antibiotic-resistant pathogens underscores the critical need for the implementation of effective infection control measures and the ongoing development of innovative antibiotics. It is imperative to address this escalating problem to minimize its far-reaching impact on public health and to alleviate the strain placed on healthcare systems globally (Gómez-Núñez *et al.*, 2020; Martínez, 2012). Urgent attention and concerted efforts are necessary to curb the rise of antimicrobial resistance and safeguard the effectiveness of existing treatments while exploring novel approaches to combat infectious diseases.

Present study revealed the potential of antibacterial activity from leaves methanolic and hexane extract using cup plate method against four pathogenic bacteria with zone of inhibition used as a measure of the antibacterial activity. Specifically, by *Staphylococcus aureus*, *Escherichia coli* (E. coli), *Streptococcus pneumonia*, and *Pseudomonas aeruginosa* with ampicillin as a control.

The antibacterial potential of the methanolic extract was evaluated against four pathogenic bacteria, *Staphylococcus aureus, Escherichia coli* (E. coli), *Streptococcus pneumonia* and *Pseudomonas aeruginosa* using ampicillin as standard for comparison. the methanolic extract (zone of inhibition being larger at higher concentrations) and ampicillin exhibited similar increasing patterns in zones of inhibition concerning *Staphylococcus aureus* and E. coli. *Staphylococcus aureus* had an inhibition zone ranging from 20 mm-23 mm, while methanolic extract showed a zone of 10 mm-18 mm.

Zones given by ampicillin varied from 17 mm to 21 mm and methanolic extract gave zones ranging from 9 mm to 18 mm for *E. coli* as well. Nevertheless, *Streptococcus pneumonia* was susceptible only to ampicillin (zones of inhibition), while the methanolic extract showed less or no zones. Ampicillin demonstrated zones of 17-21 mm against *Pseudomonas aeruginosa*, while that of methanolic extract lied at 0-14mm. The hexane extract showed 0.00 mm inhibitory effect against *Staphylococcus aureus* and *E. coli* at all concentration, respectively. Hexane extract did not inhibit the growth of Streptococcus pneumonia at concentrations less than 75 µg and displayed moderate inhibition (10 mm–12 mm) at higher concentrations. In the same way for *Pseudomonas aeruginosa*, whereas there was no inhibition with hexane extract in concentrations 75 µg and below, but mild – moderate inhibition (10 −14 mm) were seen only at high concentration. A study which was comparing the Minimum inhibitory concentration values and the antibacterial activity *in vitro* against some solated bacterial strains such as *Staphylococcus aureus* and *Escherichia* Biradar et al. When using Ampicillin (5 to 200 µg/ml), *Staphylococcus aureus* exhibited inhibition zones declining from 0.34 mm to 0.1 mm, while the range for Escherichia coli comprised of values between 0.29 and 0.11 mm on the other hand, in methanolic extract inhibitory zones were observed from 0.41 mm upto 0.12 mm for E-coli and between 0.33mm to 0.20 mm for S-aureu.

Both ampicillin and the methanolic extract showed antibacterial activity against the 2 strains, but at different rates according to the concentration of the compounds.

Jawad Ahmad *et al*. (2023) investigated the antibacterial properties of *Distimake dissectus* extracts, revealing that aqueous leaf extracts effectively targeted gram- negative *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus*. Additionally, their ethanolic extract displayed antimicrobial activity against *Bacillus thuringiensis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida*, *Candida albicans*, and *Aspergillus niger*. Furthermore, the diethyl ether extract of *Distimake dissectus* leaves exhibited significant antibacterial and antibiofilm activities against the biofilm of the phenotypic variant of *Pseudomonas aeruginosa* ATCC27853.

In contrast, the current study investigated the anti-bacterial and antifungal activity of methanolic and hexane extracts from plant leaves. The methanolic and hexane extracts in our study showed limited or no activity against *Pseudomonas aeruginosa* strain below 75 µg, which contrasts with the findings of Jawad Ahmad *et al*. (2023) regarding aqueous, ethanolic, and diethyl ether plant extracts. However, our study demonstrated antifungal activity against the *Candida albicans* strain. In addition, the methanolic extract showed antifungal activity with higher concentrations being more efficacious. When the Candida albicans strain did not respond to the lowest concentration, as happened with hexane extract, it became antifungal at lower concentrations, suggesting a change of effect according to the concentration.

Comparatively, in a study by S. Parkavi *et al.* (2020), the methanolic extract of *Merremia emarginata* leaves demonstrated superior antibacterial efficacy compared to the aqueous extract. This methanolic extract displayed effectiveness against a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* B, *Acinetobacter baumannii*, and *Enterococcus faecalis*. Whereas the current study has not included the *Klebsiella pneumoniae, Proteus mirabilis, Salmonella paratyphi* B, *Acinetobacter baumannii*, and *Enterococcus faecalis* in the study. However, the anti-microbial activity agrees with our study. Also, it is

important to note that study by S. Parkavi *et al.* (2020) have not included the *candida albicans* in the antimicrobial study. Study by Abdel Karim M. *et al.* (2021) [1] investigated the antimicrobial properties of oil extracted from *Distimake dissectus* seeds in contrast with current study where plant leaves were used for demonstrating antimicrobial activity. Their findings revealed significant efficacy against *Bacillus subtilis*, with an inhibition zone of 15 mm observed at a concentration of 100 mg/mL However, the oil did not exhibit notable activity against other tested microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.

Conclusion

The study also identifies a number of promising avenues for future research, such as determining the mode or modes of action in connection to important bioactive compounds, locating the most effective screening and extraction techniques, and conducting clinical trials that may validate pharmacological effects observed at preclinical stages. All of these are examples of these promising avenues. In this study, the emphasis placed on the bioactive character of a considerable number of the components found in *D. dissectus* makes a significant contribution to the growth of the phytochemical and pharmacological profile of the flowering plant. It has been stated that the results are significant for the advancement of these plants as possible sources of natural medicine.

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