CONTENT

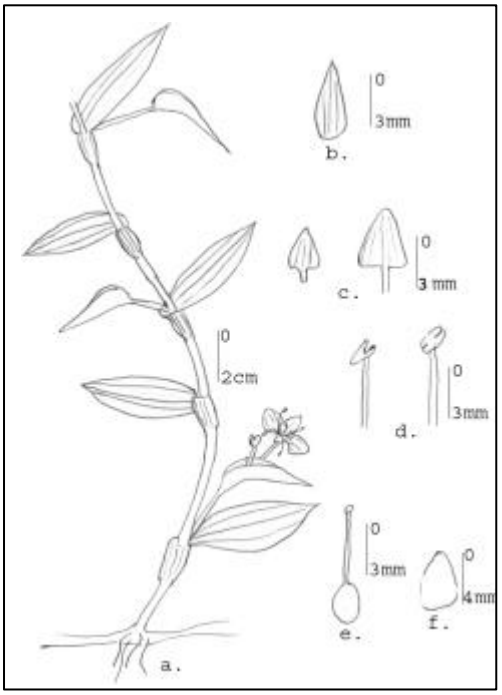
INTRODUCTION

➢ INTRODUCTION TO COMMELINA DIFFUSA (SPREADING DAYFLOWER)

Commelina diffusa, commonly referred to as the spreading dayflower, is a perennial herbaceous plant that belongs to the Commelinaceae family. It is native to tropical and subtropical regions, particularly in parts of the Americas but has also spread to regions in Asia and Africa. This plant is known for its striking blue flowers, which bloom in the morning and last until the afternoon, contributing to its nickname, "dayflower."

The plant exhibits a characteristic growth habit, spreading low to the ground with long, trailing stems that root at the nodes. Over time, Commelina diffusa can form dense mats or ground covers, making it an attractive option for ornamental gardens and a ground cover in certain landscaping designs. However, its fast-growing nature can also lead it to become invasive, especially in disturbed areas where it outcompetes native vegetation. This species has been observed to thrive in a variety of environments, from roadsides and grasslands to disturbed soil areas.

➢ PHYSICAL CHARACTERISTICS



Commelina diffusa is a herbaceous plant that typically grows between 30 cm to 60 cm in height, though its sprawling stems can extend much farther along the ground. Its stems are soft and often rooting at the nodes, helping the plant spread rapidly across the surface of the soil. The leaves are long, lance-shaped, and alternately arranged along the stems. They are typically dark green in color and provide a backdrop for the plant's distinctive blue flowers.

The flowers of Commelina diffusa are one of its most defining features. They are typically small, with a delicate blue or purple hue, and possess the classic three-petaled structure characteristic of the genus Commelina.

These flowers are usually about 2-3 cm in diameter and are often seen in clusters. The blooms have three petals, two of which are larger and blue or purple in color, while the third is smaller and white. A prominent yellow stamen in the center of the flower adds to the visual appeal. The flowers bloom during the morning and tend to fade by afternoon, which is why they are often referred to as "dayflowers."

➢ ECOLOGY AND HABITAT

The spreading dayflower is native to a wide range of tropical and subtropical regions, and it thrives in moist, well-drained soils. It is commonly found in disturbed or cultivated land, roadsides, grasslands, and forest edges, where the soil is rich and moisture is readily available. The plant is adaptable and can tolerate various environmental conditions, including partial shade and full sunlight. It is particularly abundant in areas where the soil is disturbed, making it both a resilient and opportunistic species.

The spreading dayflower prefers areas with moderate moisture and is often seen in wetlands or along riverbanks. However, it is not overly particular about soil type, as it can grow in sandy, loamy, or clay soils as long as the soil is well-drained. Its ability to grow in a wide variety of habitats has enabled it to spread across many different geographical regions.

➢ REPRODUCTIVE CHARACTERISTICS

Commelina diffusa reproduces primarily through both sexual and asexual means. It produces seeds from its flowers, but it also has the ability to spread quickly through vegetative growth. When the plant's stems come into contact with soil, they often root at the nodes, which leads to the rapid expansion of the plant. This asexual reproduction makes Commelina diffusa a strong competitor in areas with disturbed soils, as it can quickly take over spaces that would otherwise be populated by other vegetation.

In terms of reproduction, the plant’s flowers bloom in clusters, typically during the early morning, and remain open for a short period. This short bloom duration makes it a dayflower, and though it is not as showy as other flowering plants, it contributes an important ecological function by providing nectar to pollinators, including bees and butterflies. The flowers attract these pollinators in the morning before closing by the afternoon. After pollination, the flowers produce small, round fruits containing seeds that can be dispersed by wind or water, further aiding the plant’s spread.

➢ USES AND IMPORTANCE

Commelina diffusa is often used for ornamental purposes in gardens, particularly for ground cover. Its rapid growth and ability to quickly cover large areas make it an attractive option for gardeners looking to create low-maintenance, natural-looking ground cover. Its bright blue flowers can add color to the garden, and it is often planted in areas where a low-growing, spreading plant is needed.

In certain parts of the world, Commelina diffusa is considered to be a weed due to its invasive tendencies. In agricultural fields, it can quickly colonize spaces, potentially outcompeting more desirable crops. This aggressive spread can be problematic, particularly in areas with sensitive ecosystems or areas that are already facing issues with invasive plant species. Some farmers and land managers may find it difficult to control due to its rapid vegetative growth.

On the positive side, the plant has some medicinal and cultural significance in certain regions. In traditional medicine, parts of the plant, such as its leaves and roots, are believed to have various uses, including treatment for minor ailments such as skin irritations, fever, and even as a poultice for wounds. However, these uses are not widely researched, and caution is advised when considering medicinal applications.

➢ INVASIVENESS AND MANAGEMENT

While Commelina diffusa is not considered a major invasive species in most regions, it has shown the potential to become problematic in areas with disturbed soils. It can rapidly establish itself in disturbed habitats, such as roadsides, fields, and wastelands, and in some cases, outcompete native vegetation. Its ability to spread quickly through vegetative reproduction—when stems root at the nodes—enables it to cover large areas, making it difficult to eradicate once established.

In areas where Commelina diffusa is deemed invasive, management strategies include manual removal, frequent mowing, or the use of herbicides. However, control efforts need to be consistent, as the plant's spreading nature requires persistent effort to keep it in check.

➢ CONCLUSION

In summary, Commelina diffusa is a resilient, fast-growing perennial plant that can serve as both an ornamental ground cover and an invasive species in certain regions. Its ability to thrive in various environments, along with its striking blue flowers, makes it an interesting addition to gardens, though it requires careful management in areas where it might become too aggressive. While it has some medicinal applications in traditional practices, its spread and ecological impact make it an important plant for further study, particularly in terms of its management and role in local ecosystems. Its adaptability, rapid growth, and aesthetic appeal continue to make it a plant of interest in both horticultural and ecological discussions.

➢ Key words

Antibiotic resistance, Commelina Diffusa L., Ethanolic extract, Disk diffusion, Staphylococcus aureus, Escherichia coli

2.NEED AND SCOPE OF THE WORK

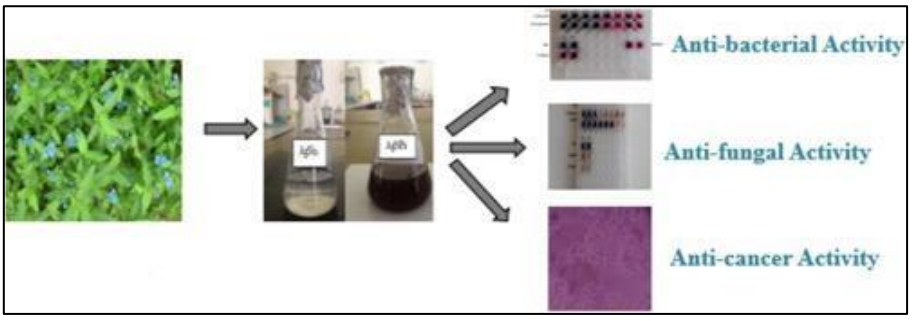
➢ AIM OF PRESENT WORK

The primary aim of this project is to investigate the antimicrobial properties of Commelina diffusa, a plant known for its medicinal uses, to determine its potential in combating various bacterial and fungal infections. The project seeks to isolate and analyze bioactive compounds present in the plant and evaluate their effectiveness against a range of pathogens.

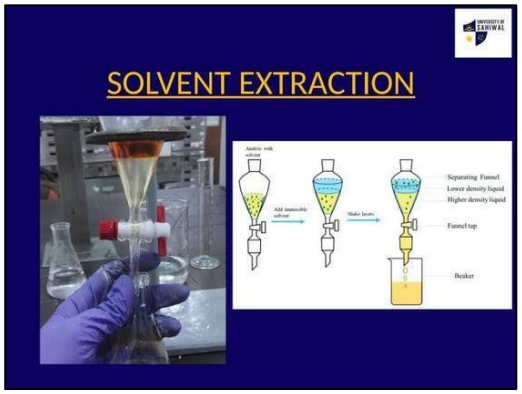
➢ SCOPE:

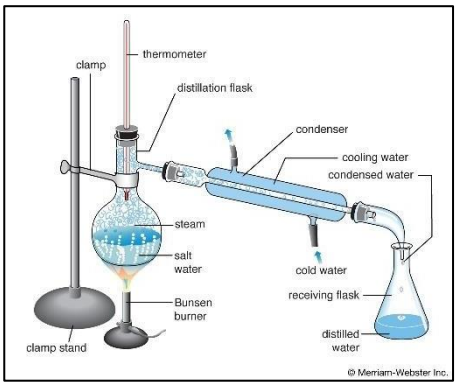
1. **Phytochemical Screening**: The project will focus on identifying and characterizing the bioactive compounds in Commelina diffusa, such as alkaloids, flavonoids, tannins, and saponins, which are known for their antimicrobial activity.

2. **Antimicrobial Activity Testing:** The antimicrobial effects of Commelina diffusa extracts will be tested on both Gram-positive and Gram-negative bacteria, as well as fungi. This will involve testing against common pathogens like Escherichia coli, Staphylococcus aureus, Candida albicans, and Aspergillus niger.

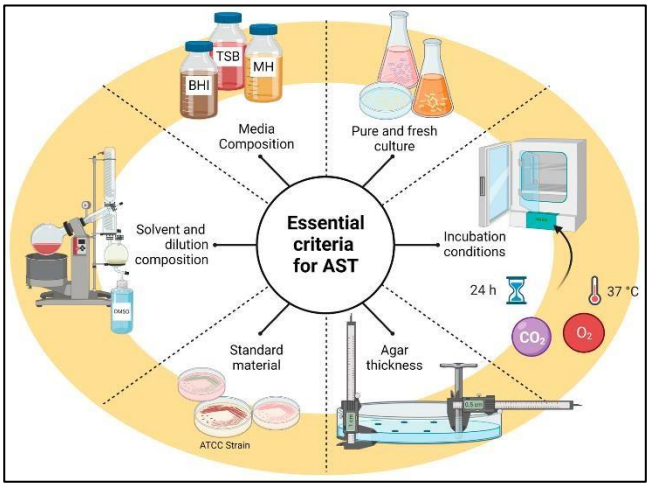


3. **Extraction Methods**: Different extraction techniques (e.g., solvent extraction, maceration, or distillation) will be employed to obtain the active compounds from the plant.





4. **Evaluation of Effectiveness :** The project will include experiments to assess the minimum inhibitory concentration (MIC) and zone of inhibition to determine the strength and spectrum of antimicrobial activity.
5. **Comparative Analysis:** The effectiveness of Commelina diffusa extracts will be



compared with conventional antibiotics and antifungal agents to understand its potential as an alternative or complementary treatment.

6. **Safety and Toxicity Evaluation:** In addition to antimicrobial testing, the project will also investigate the safety profile of Commelina diffusa extracts through preliminary toxicity assessments to ensure that the plant's medicinal use is safe.

7. **Applications and Future Potential**: The results will be analyzed to suggest practical applications of Commelina diffusa in developing natural antimicrobial agents, especially in the context of growing antibiotic resistance. The project may lay the groundwork for further research into its commercial and therapeutic potential.

This project is particularly significant in the current context of antimicrobial resistance, offering the possibility of discovering plant-based, sustainable alternatives to synthetic antibiotics.

3.MATERIAL AND METHOD

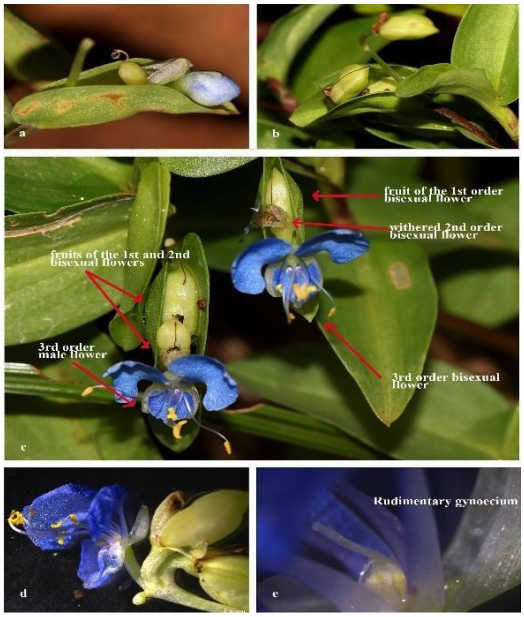
To study the antimicrobial properties of Commelina diffusa (a plant in the Commelinaceae family), you would typically follow a systematic approach. Here's a general outline of the materials and methods for such an investigation:

MATERIALS:

- **Plant Material:**

- Fresh or dried Commelina diffusa leaves, stems, or roots (depending on the focus of your study).

- Aerial parts can also be used if you are looking at broader antimicrobial effects.

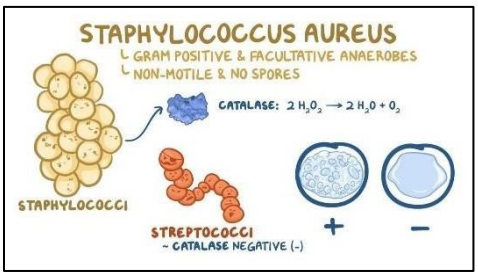


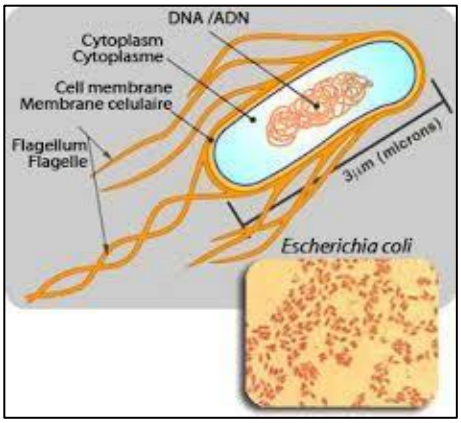
- **SOLVENTS FOR EXTRACTION:**

- Distilled water, ethanol (95%), methanol, acetone, or chloroform (depending on the extraction method).

- Ethyl acetate or hexane may also be considered based on the solvent's ability to extract specific compounds.

- **Microorganisms**:





- Bacterial Strains: Common test bacteria include Escherichia coli (Gram-negative), Staphylococcus aureus (Gram-positive), Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, etc.

- Fungal Strains: Candida albicans, Aspergillus niger, Trichophyton rubrum, etc.

- Nutrient Agar (NA): Used for bacterial growth.
- 5. Potato Dextrose Agar (PDA): Used for fungal growth.
- 6. Sterile Petri dishes for culturing the microorganisms.
- 7. Sterile test tubes or conical flasks for preparing plant extracts.
- 8. Incubator (for optimal temperature conditions during incubation of microorganisms).
- 9. Control Solutions:

- Positive control: A known antibiotic (e.g., amoxicillin, tetracycline) for bacteria and antifungal agents

for fungi.

- Negative control: Solvent used for extraction (e.g., ethanol, methanol).

METHODS:

o COLLECTION AND PREPARATION OF PLANT MATERIAL:

- Collect Commelina diffusa from a natural habitat.

- Clean thoroughly to remove dirt and contaminants.

- Dry the plant material (leaves, stems, etc.) in a shaded, well-ventilated area or use an oven set at

low temperature.

- Once dried, grind the plant material into a fine powder using a mortar and pestle or mechanical

grinder.

-The parts of plant are harvested in the near wadibhokar, Dhule Maharashtra 424002. In Octomber. A voucher are deposited to Dhule Charitable Society's Annasaheb Ramesh Ajmera College of Pharmacy, Nagaon, Dhule.

o EXTRACTION OF BIOACTIVE COMPOUNDS:

- Solvent Extraction:

- Weigh the dried powdered plant material (e.g., 100g).

- Add the powdered plant material to a beaker containing a solvent (e.g., ethanol or methanol) in a

1:3 ratio (plant:solvent).

- Leave the mixture for 48 hours, shaking occasionally (cold maceration method).

- Filter the solution through a fine mesh or Whatman filter paper to remove solid particles.

- Concentrate the filtrate using a rotary evaporator or evaporate under reduced pressure.

- **ANTIMICROBIAL TESTING:**

- Agar Well Diffusion Method (for bacterial and fungal assays):
  - Prepare nutrient agar or potato dextrose agar plates.
  - Inoculate the plates with the bacterial or fungal cultures.
  - Using a sterile cork borer, create wells in the agar plates.
  - Fill the wells with a specific amount of plant extract (e.g., 50 µL, 100 µL, 200 µL).
  - Incubate the plates at 37°C for bacterial cultures and 25°C for fungal cultures for 24–48 hours.
  - Measure the zone of inhibition (clear zone around the well) to assess antimicrobial activity.
- Minimum Inhibitory Concentration (MIC) Test (if applicable):
  - Prepare a series of concentrations of the plant extract.
  - Inoculate these with bacterial or fungal cultures.
  - The lowest concentration of the extract that shows no visible growth after incubation is noted as

the MIC.

- Disk Diffusion Method (alternative for bacterial assays):
  - Soak sterile paper discs in different concentrations of the plant extract.
  - Place the discs on the inoculated agar plate.
  - Incubate and measure the zone of inhibition around each disc.

- **4. ANALYSIS:**

- Compare the zones of inhibition of the plant extract with the control (antibiotic/antifungal) and the

negative control.

- Statistical analysis (e.g., ANOVA) can be conducted to determine the significance of the

antimicrobial effect.

- **5. PHYTOCHEMICAL SCREENING (OPTIONAL):**

- Perform preliminary phytochemical analysis (e.g., using tests for alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds) to correlate the antimicrobial activity with the chemical compounds present in Commelina diffusa.

- **6. DATA INTERPRETATION:**

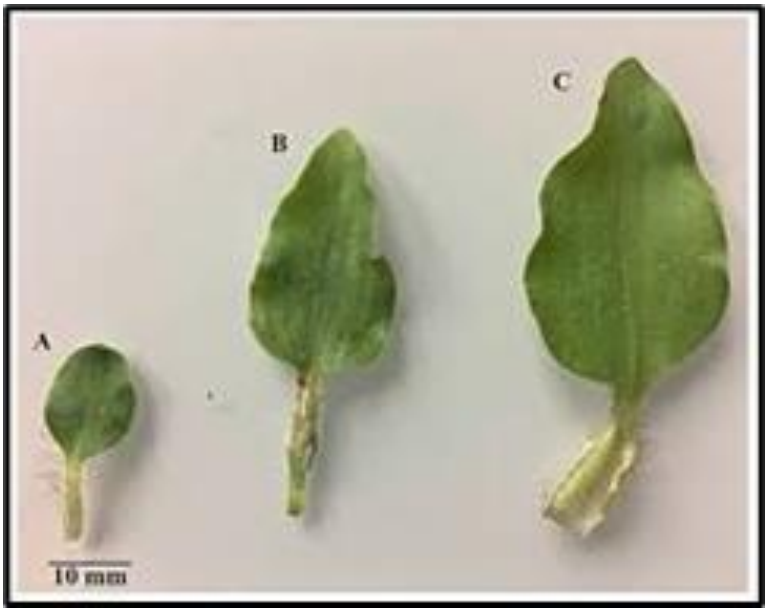
- Evaluate the antimicrobial activity based on the size of the inhibition zones.

- Determine if the extract is more effective than the positive control or comparable to the antibiotics

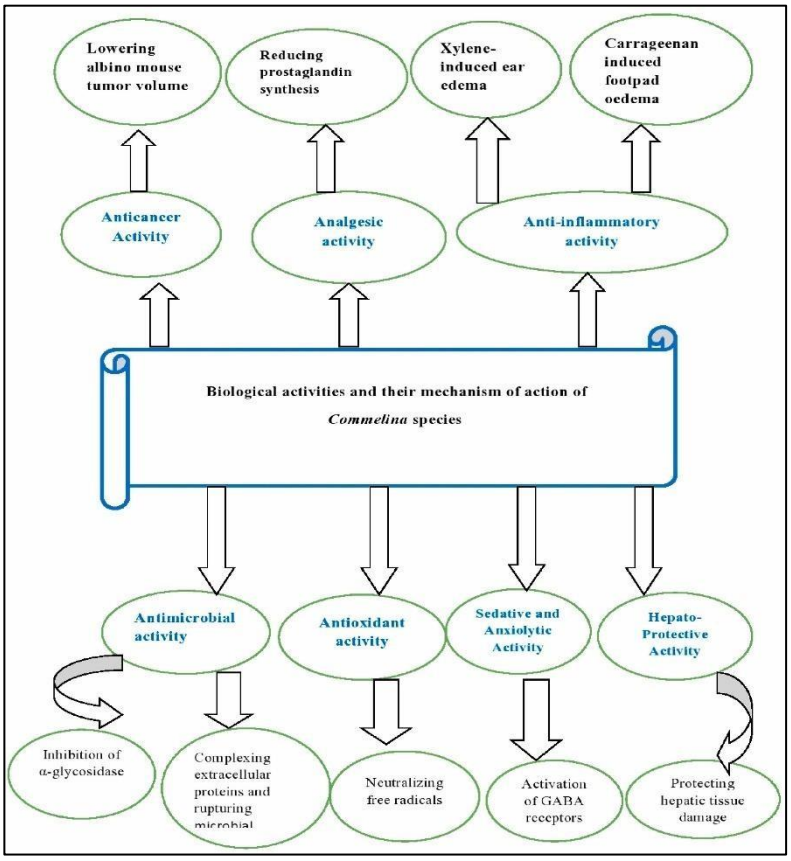
tested.

This approach can be modified based on specific research needs (e.g., testing on specific microbes or

extraction methods).



➢ PLAN OF WORK



- **ANALGESIC ACTIVITY:**

Ethanol extract of Commelina Diffusa root showed, analgesic activity by performing athetoid movement, hot-plate and tail-flick tests in Swiss Albino mice in a dose dependent method . Aerial part extracts of Commelina Diffusa shows analgesic activities against Nalbuphine as standard drug . Commelina Diffusa is containing sterol, alkaloids, caffine, anthocynins and carotenoids which might be responsible for analgesic activities .

Fig. Ethanol extract of Commelina Diffusa

- **ANTI-INFLAMMATORY & WOUND HEALING ACTIVITY:**

Ethanol extract of Commelina Diffusa root shows anti inflammatory activity by using different models which is due to the presence of resins, balsams, flavonoids and tannins . Commelina Diffusa whole plant alcoholic extracts also have anti-phlogistic and substance P inhibition activity . It is also reported that Commelina Diffusa might be a potent and safe drug for acute and long term use inflammations . Aqueous and alcoholic root extract of Commelina Diffusa showed significant wound-healing activities . Methanol extract of dried leaf of Commelina Diffusa showed anti-asthmatic activities by inhibit 15-lipoxygenase enzyme. Plant with Commelinaceae family shows antipyretic and diuretic activities . anti-inflammatory,

- **ANTI-MICROBIAL ACTIVITIES:**

Ethanolic Leaf extract of Commelina Diffusa showed potential inhibitiory activities against Streptococcus lactis (Gram +ve) and Enterobacter aerogenes(Gram –ve) bacteria by performing Agar well diffusion method . Phytoconstituents obtained from plant active against plant and human pathogenic bacteria . In Bangladesh, Antimicrobial activities were found on different extracts of plant by disc diffusion method . Last few years, multiple drug resistance in both plant and human pathogenic bacteria have been developed due to the random use of commercial antimicrobial drugs which is commonly used in the treatment of infectious diseases . Antimicrobial activities of this plant have paying attention for upcoming research.

- **ANTI-OXIDANT & RADICAL SCAVENGING ACTIVITIES**:

Acetone and Methanol extract from aerial parts of Commelina Diffusa shows antioxidant activities which work as anti-aging, anti-cancer, cardio-vascular and neurological agent, anti-diabetes and other autoimmune diseases. Methanolic leaf extracts of Commelina Diffusa shows high amount of antioxidants, which contains phenols, can help to neutralize free radical so used in pharmaceutical industries . Phytoconstituents like phenolics, flavonoids, protocatechuic acid, vanillic acid, ferulic acid, apigenin, and kaempferol present in plant which shows antioxidant and radial scavenging activities .

- **DIURETIC PROPERTIES:**

Commelina Diffusa contain flavonoids, tannin, alkaloids, saponins which are secondary metabolites and they show pharmacological activities like diuretic effect . Methanolic extract of whole plant of Commelina Diffusa L. shows diuretic effect in albino wistar rats . Larvicidal Activity: Generally, fever like dengue, chikungunya and Zika infections spread by A. aegypti mosquito is foremost health problem worldwide. Petroleum ether extracts of Commelina Diffusa showed larvicidal activities due to the presence of phytoconstituents like phenol, Flavonoids and resins .

- **ANTIDIARRHEAL AND ANTHELMINTIC ACTIVITY:**

Methanol extract of Commelina Diffusa prevent diarrheal conditions and shows anthelmintic properties which took maximum 22.17 min for the paralysis of the parasite and almost one hour for complete death of the parasite .

- **HEPATOPROTECTIVE ACTIVITY:**

Alcoholic and aqueous extracts of this plant showed to have great hepatoprotective activity against paracetamol induced hepatic tissue damage from which alcoholic extract shows more effective than aqueous extract. Many therapeutic plants are used to treat degenerative fibrotic hepatic diseases . Fertility-Inducing Property: Leaf extract of Commelina Diffusa is use to treat female infertility. Furthermore study showed that plant extract became potential applicant to treat male infertility by prevents testicular toxicity induced by environmental toxic substances .

- **THROMBOLYTIC & CYTOTOXIC ACTIVITY:**

Toxicity of plant materials is a main worry to scientists and medical practitioners, researchers and therefore Brine Shrimp Lethality test was conducted to know cytotixicity which provide important primary data to help select plant extracts with potential antineoplastic properties for future research. Methanol extract of Commelina Diffusa showed to have a significant thrombolytic activity using streptokinase enzymes as standard .

- **ANTI-CANCER ACTIVITY:**

Commelina Diffusa contain secondary metabolites like flavonoids, alkaloids, steroids, triterpenes, lactones, courmarins, resins, phenols, carbohydrates and tannins which have anticancer activities. activity Methanol extract of Commelina Diffusa shows anti cancer by suppressing malignancy cell development and reducing tumor size in Swiss albino mice and normalize haemoglobin level and additional increase the life of mice . Semi-pure extracts of Commelina Diffusa can lead to the development of an effective anti-neoplastic drug . The crude methanolic extract of Commelina Diffusa contains bioactive phytoconstituents which are useful to suppress tumor growth using tumor protein p53 and Bax and Bcl- 2. Additionally, this anti-cancer activity is a result of deregulated expression of apoptosis-responsive genes .

- **SEDATIVE AND ANXIOLYTIC PROPERTIES :**

From last decade, new phytopharmacotherapy from medicinal plants for psychiatric disorder were developed . Chloroform and petroleum ether extract of Commelina Diffusa have significant therapeutic efficiency to treat anxiety along with other related neuropsychiatric disorders . Aerial part of Commelina Diffusa contains alkaloid and flavonoids which shows strong sedative and anxiolytic activities . Commelina Diffusa is used to treat excited mental disorder such as psychosis, insanity and epilepsy.

- **ANTIDEPRESSANT ACTIVITY:**

World Health Organization showing that depression is the fourth leading disease of the worldwide, best by lower respiratory infections, perinatal conditions and HIV/AIDS . Around, two third of depressed patients get suicide thoughts and 10-15% of them try suicide . Methanolic extract of leaf of Commelina Diffusa shows antidepressant activity by decrease the duration of calmness in animal models, forced swimming and tail suspension tests .

- **ANTI-VIRAL ACTIVITY:**

Five different extracts of Commelina Diffusa accordingly methanol, ethanol, chloroform, n hexane and benzene were shows the inhibitory effects against dengue virus. Therefore, Commelina Diffusa have good anti-viral properties by prophylactic treatment. Viral disease like dengue that represents a major health, economic and social problem in tropical and subtropical areas of the world.

- **TOXICITY:**

Hydroalcoholic extract of leaves of Commelina Diffusa was evaluated for acute and sub-acute toxicity in female Wistar rats. This plant extract found Median lethal dose (LD50) was safe. Cytotoxic determination of Commelina Diffusa was carried out by performing the Brine Shrimp lethality test which help to evaluate plant's anti-cancer properties and can further help to develop new anticancer compounds . Methanol extract of this plant demonstrated a significant cytotoxic properties against the brine shrimp at a concentration of 278.68 µg/ml. Even high dose 2000/kg of Hydroethanolic extracts of leaves of Commelina Diffusa did not showed any toxic reactions thus LD50 of hydroethanolic extracts of Commelina Diffusa must be greater than 2000 mg/kg .

- **HEAVY METAL PHYTOREMADIATION:**

Commelina Diffusa are good applicant for phytostabilisation of lead, Cadmium, Copper, Zinc and Manganese in rural drainage ecosystem. Commelina Diffusa is use for heavy metal appropriation from urban/rural stream sediments, with good accumulation in roots which lead good phytostabilization. In Commelina Diffusa heavy metal sedimentation found to the shoot

and stem which is noticeable for phytoremediation of Copper of urban watercourse ecosystem and wastewaters. Antiproliferative/Anti-Lymphoma: Acetone extract of Commelina Diffusa shows anti-proliferative properties against Wil-2NS lymphoma cells .

- **POTENTIAL FEED FOR RUMINANTS:**

Commelina Diffusa as feed for ruminants, effects of plant maturity on composition, rumen degradability, digestibility and Nitrogen balance. Results showed advancing maturity affected the chemical composition, but not rumen degradability. Inclusion of this plant in Sorghum almum diet improved intake, digestibility and Nitrogen intake, suggesting its potential as food supplement .

- **GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES:**

Silver nanoparticles were effectively prepared using leaf extracts of Commelina Diffusa. To develop safe, cost-effective and environmental-friendly technologies for nanoparticals synthesis, Green synthesis play major role. The bioreduction of aqueous Silver ions by the leaf extracts of Commelina Diffusa has been established. This bioreduction of the Silver ions leads to the formation of Silver nanoparticals of comparatively well defined dimensions .

- **NAPHROPROTECTIVE ACTIVITY:**

Study showed the protective and curative effect of Commelina Diffusa against Quinalphos induced oxidative stress in kidney tissue which Results in Nephroprotection from cell damage caused by Quinalphos.

EXPERIMENTS

- **Antibacterial activity test**

To test the antimicrobial activity of Commelina diffusa, an experiment can be conducted using the following steps. This experiment typically involves preparing extracts from the plant and testing them against various microbial strains (e.g., bacteria or fungi) using well-established methods like agar well diffusion or disk diffusion.

Materials Needed:

1. Plant material (fresh or dried Commelina diffusa leaves, stems, or roots)

2. Solvents (e.g., methanol, ethanol, water, or chloroform) for extracting the antimicrobial compounds

3. Microbial cultures (e.g., Escherichia coli, Staphylococcus aureus, Candida albicans)

4. Agar plates (e.g., Nutrient Agar for bacteria, Sabouraud Dextrose Agar for fungi)

5. Sterile paper discs or wells for applying the extracts

6. Inoculum of the microorganisms

7. Incubator (for growing the microorganisms)

8. Sterile pipettes and pipette tips

9. Autoclave (for sterilizing materials)

Procedure:

1. PREPARATION OF EXTRACT FROM COMMELINA DIFFUSA (PLANT MATERIAL)

A. COLLECTION AND DRYING (IF NECESSARY):

- If using fresh plant material, collect the leaves of Commelina diffusa. Rinse them thoroughly to remove any dirt or contaminants, then air-dry them at room temperature. This ensures you get a concentrated extract when you prepare it.

B. EXTRACTION PROCESS:

- Solvent Extraction : The most common solvents for extracting bioactive compounds are ethanol, methanol, or distilled water. Ethanol is preferred as it can dissolve both polar and non-polar compounds.

- METHOD :

1. Weigh about 10–20 g of the dried leaves (or 100–150 g of fresh leaves).

2. Grind the plant material into a fine powder using a mortar and pestle or a mechanical grinder.

3. Soak the powdered leaves in 100–200 mL of solvent (e.g., ethanol or methanol). Use a 1:10 ratio of plant material to solvent.

4. Allow the mixture to macerate for 24–48 hours at room temperature or heat in a water bath (if needed) to enhance extraction.

5. After the extraction time, filter the mixture using a fine sieve or filter paper to separate the liquid extract from the plant residue.

6. Evaporate the solvent under reduced pressure (if necessary) to concentrate the extract or use it directly in the assay.

C. STORAGE :

- Store the extract in an airtight container, away from light and heat, to prevent degradation of bioactive compounds.

2. PREPARATION OF AGAR PLATES

A. MEDIA PREPARATION:

- Use nutrient agar or Mueller-Hinton agar. These media support the growth of a wide range of bacterial species.

- Mueller-Hinton Agar (for bacterial testing) : Mix the appropriate amount of Mueller-Hinton powder with distilled water, autoclave it to sterilize, and pour it into sterile petri dishes.

B. BACTERIAL CULTURES :

- Obtain pure cultures of the test microorganisms (e.g., E. coli, Staphylococcus aureus, Bacillus subtilis). These can be obtained from microbiological supply companies or from a laboratory culture collection.

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C. INOCULATION :

- Bacterial Inoculum Preparation : Grow the bacterial culture overnight in a sterile broth medium (e.g., nutrient broth) at 37°C. Then, dilute the culture to obtain a standardized inoculum (usually 0.5 McFarland standard, equivalent to ~10^8 CFU/mL).

- Inoculation Method : Using a sterile cotton swab, evenly spread the bacterial suspension over the surface of the agar plate. Ensure the plate is covered uniformly for even growth.

3. APPLICATION OF PLANT EXTRACT

a. WELL OR DISC DIFFUSION :

- For Disc Diffusion: Soak sterile filter paper discs (6 mm diameter) in different concentrations of Commelina diffusa extract. Place the discs on the surface of the inoculated agar plates.

- For Well Diffusion : Use a sterile cork borer or a sterile pipette tip to create small wells (5–8 mm in diameter) in the agar. Add different volumes of the extract (e.g., 50 µL, 100 µL, 200 µL) into these wells.

B. CONCENTRATIONS :

- You can prepare a series of concentrations of the extract (e.g., 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL) to determine the dose-response relationship of antimicrobial activity.

C. CONTROLS :

- Positive Control : Include discs or wells containing a standard antibiotic (e.g., ciprofloxacin, tetracycline) to compare the effectiveness of the plant extract.

- Negative Control : Include discs or wells with the solvent only (ethanol, methanol, or water) to ensure that any observed activity is due to the plant extract and not the solvent.

4. INCUBATION

- After applying the extract and controls, incubate the plates upside down at 37°C for 24–48 hours.

MICROORGANISM	TYPE OF ORGANISM	INCUBATION TEMPERATURE	ICUBATION TIME	NOTES
Escherichia coli	Bacteria (gram-negative)	37 °c	24-48 hrs	Optimal growth temperature for E. coli is 37°c
Staphylococcus aureus	Bacteria (Gram-Positive)	37 °c	24-48 hrs	Common pathogen; grows well at 37°c
Pseudomonas aeruginosa	Bacteria (Gram-negative)	37 °c	24-48 hrs	Similar to E.coli, optimal growth at 37°c
Candida albicans	Fungi (Yeast)	25-30°c	24-48 hrs	Yeast grows best at slightly lower temperature than bacteria.
Aspergillus niger	Fungi (Mold)	25-30°c	48-72 hrs	Mold requires slightly longer incubation times usually 48-72 hours

- Ensure that the incubation conditions (temperature, time) are optimized for the growth of the test organisms.

5. OBSERVATION AND MEASUREMENT OF ZONE OF INHIBITION

A. OBSERVING GROWTH:

- After incubation, examine the plates for any inhibition zones around the discs or wells.

- The absence of bacterial growth in the area surrounding the well or disc indicates antimicrobial activity.

B. MEASURING ZONE OF INHIBITION :

- Measure the diameter of the clear zone (in mm) using a ruler or caliper. This zone represents the area where bacteria could not grow due to the antimicrobial activity of the plant extract.

6. DATA ANALYSIS

A. ZONE OF INHIBITION :

- Record the zone diameter for each concentration of the plant extract. A larger zone of inhibition suggests stronger antimicrobial activity.

- Compare the zones of inhibition of the plant extract with those of the positive control (antibiotic).

Diameter of Inhibition Zone (MM)	Interpretatioin	Activity
0-5 mm	No Inhibition	No antimicrobial activity
6-10mm	Very weak inhibition	Weak antimicrobial activity
11-15mm	Moderate Inhibition	Moderate antimicrobial activity
16-20mm	Strong inhibition	Strong antimicrobial Acivitiy
>20 mm	Very strong inhibition	Very strong antimicrobial acitivity

B. INTERPRETATION OF RESULTS :

- No Zone : No antimicrobial activity.
- Small Zone (< 10 mm) : Low antimicrobial activity.
- Moderate Zone (10–15 mm) : Moderate antimicrobial activity.
- Large Zone (> 15 mm) : Strong antimicrobial activity.

RESULT : Diameter – 6 mm (very weak inhibition)

C. MIC (MINIMUM INHIBITORY CONCENTRATION) TEST :

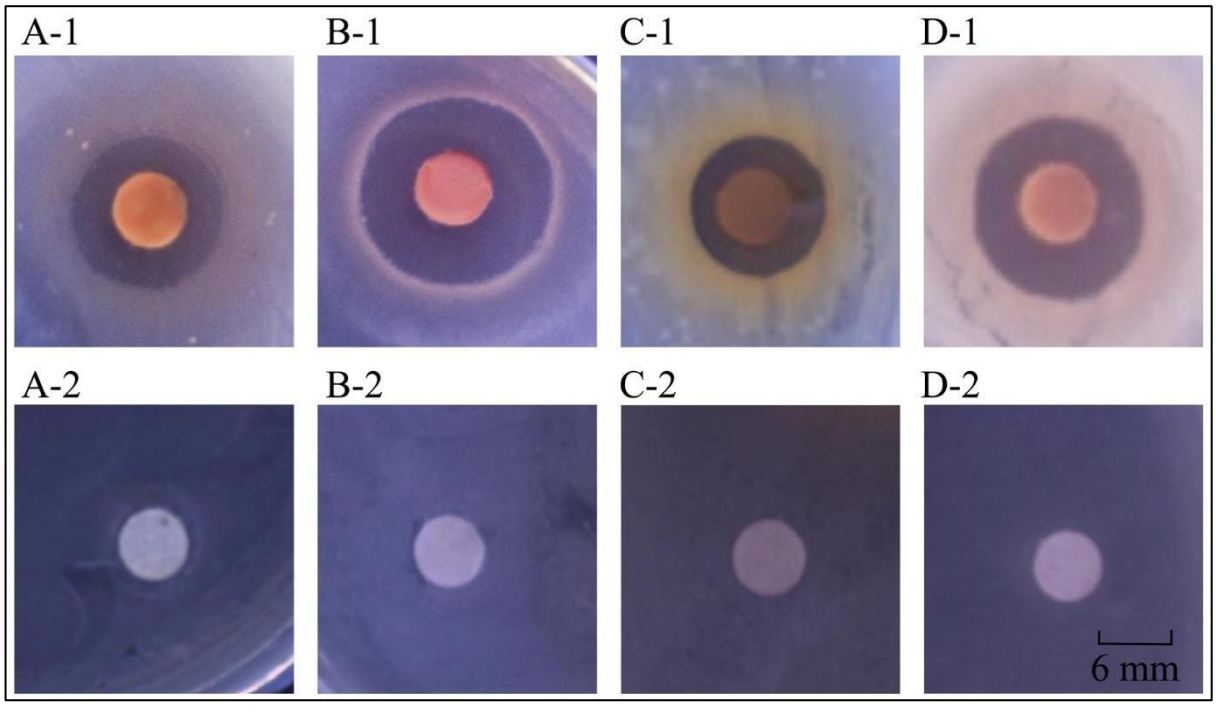
- If you want to further quantify the effectiveness of the extract, you can perform an MIC test. This involves preparing serial dilutions of the plant extract and testing them to determine

- the lowest concentration that prevents visible bacterial growth.

7. CONCLUSION

- Based on the size of the inhibition zones, you can conclude whether Commelina diffusa has antimicrobial activity and the relative potency of its extract.

- If the extract shows significant inhibition, it could be a promising candidate for further study and development as a natural antimicrobial agent.



Discussion

Commelina diffusa is a plant widely used in traditional medicine across various cultures, primarily for its healing and therapeutic properties. Recent scientific studies have provided substantial evidence supporting its antimicrobial activity, which contributes to its medicinal value. The plant's extracts, including those derived from its leaves, stems, and roots, exhibit inhibitory effects against a range of pathogenic microorganisms, including both bacteria and fungi. These findings suggest that *Commelina diffusa* has significant potential in modern pharmacology, particularly in combating infections caused by resistant microorganisms.

Antimicrobial Activity

Several studies have reported that *Commelina diffusa* exhibits broad-spectrum antimicrobial activity, with its extracts showing effectiveness against common bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Additionally, the plant has shown antifungal properties, particularly against fungi such as *Candida albicans* and *Aspergillus niger*. The antimicrobial effects are believed to stem from various bioactive compounds, including flavonoids, alkaloids, and saponins, which possess the ability to interfere with microbial cell walls, membranes, or enzymatic activities, disrupting pathogen growth and replication.

One important aspect of *Commelina diffusa's* antimicrobial properties is its potential to target antibiotic-resistant strains. With the rise of multidrug-resistant bacteria, there is an urgent need to discover and develop new antimicrobial agents. *Commelina diffusa* may provide an alternative solution, offering an accessible, natural source of antimicrobial compounds that can be used either independently or in conjunction with conventional antibiotics to treat resistant infections.

Medicinal Uses

The antimicrobial properties of *Commelina diffusa* provide the foundation for its medicinal applications. Traditionally, this plant has been used for the treatment of various ailments, such as wounds, infections, and gastrointestinal disturbances. Modern studies have supported many of these uses, particularly in the treatment of external infections, wounds, and skin conditions. *Commelina diffusa* extracts can be incorporated into topical formulations like creams, ointments, or poultices to treat skin infections, ulcers, and fungal conditions, offering a natural remedy for these common issues.

Beyond external uses, *Commelina diffusa* has been investigated for its internal applications, including gastrointestinal infections. The plant's antimicrobial activity extends to pathogens responsible for digestive disorders, and it may be useful in treating conditions like diarrhea, dysentery, or infections caused by *Helicobacter pylori*. Additionally, its potential as a treatment for respiratory infections suggests it could be beneficial in managing conditions like bronchitis or pneumonia.

Practical Considerations

Despite its promising antimicrobial effects, the practical use of *Commelina diffusa* in modern medicine faces several challenges. First, while laboratory studies have demonstrated its potential, there is a need for further clinical trials to establish the appropriate dosage, safety,

and efficacy of the plant for human use. Standardized extracts and formulations need to be developed to ensure consistency and potency. Furthermore, the identification of the active compounds responsible for the antimicrobial effects will be crucial in maximizing the plant's therapeutic potential.

In addition, the safety profile of *Commelina diffusa* must be thoroughly evaluated. While traditional uses suggest it is safe, adverse effects or interactions with other medications need to be assessed through rigorous testing.

CONCLUSION

Conclusion on the Antimicrobial Properties and Medicinal Uses of *Commelina diffusa*

Commelina diffusa, a plant commonly used in traditional medicine, has garnered attention for its notable antimicrobial properties. Research has shown that extracts from *Commelina diffusa* possess activity against a wide spectrum of microorganisms, including various bacterial and fungal strains. This antimicrobial effect is attributed to the bioactive compounds present in the plant, which have demonstrated the ability to inhibit the growth of harmful pathogens. These findings suggest that *Commelina diffusa* holds potential as a natural source for developing antimicrobial treatments.

In practical terms, the medicinal applications of *Commelina diffusa* are diverse. The plant's antimicrobial effects make it suitable for treating infections, particularly skin-related conditions, such as wounds, ulcers, and fungal infections. Its natural antimicrobial action could be utilized in topical creams or ointments to prevent infection and promote healing. Additionally, the plant's medicinal potential could extend to internal uses, such as for treating gastrointestinal and respiratory infections, offering a promising alternative to synthetic antibiotics.

The plant’s antimicrobial properties also indicate a possible role in managing infections caused by antibiotic-resistant microorganisms. As the global challenge of antimicrobial resistance continues to grow, the use of *Commelina diffusa* could serve as a valuable component in the search for new, natural antimicrobial agents.

However, while the antimicrobial effects of *Commelina diffusa* are promising, further research is needed to fully understand the plant's mechanism of action, the active compounds involved, and the most effective methods of preparation and dosage. Additionally, clinical trials are necessary to assess the safety, efficacy, and potential side effects of the plant in human use.

In conclusion, *Commelina diffusa* shows considerable promise as a natural antimicrobial agent with practical medicinal uses, especially in the treatment of infections and wound care. Continued research into its bioactive components and therapeutic potential will help determine its viability as a reliable and safe treatment in modern healthcare.

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