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Study of the antimicrobial Properties of *Nyctanthes arbor-tristis* and *Sphagneticola trilobata* extracts against multidrug-resistant bacteria

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ABSTRACT

Global health is seriously threatened by the rise of multidrug-resistant (MDR) bacteria, which makes the hunt for new natural antimicrobial medicines imperative. The purpose of this study was to assess the potential of the antimicrobial compounds present in the extracts of Night-blooming flower (Nyctanthes arbor-tristis) and Trailing Daisy (Sphagneticola trilobata) that are effective against a variety of multidrug-resistant bacterial strains. Following the extraction of plant materials using solvent-based techniques, using water, DMSO, chloroform and methanol, the disc diffusion method was used to test for antibacterial activity. Multidrug-resistant strains of Staphylococcus aureus, were used to assess the antibacterial effectiveness of the extracts. Significant inhibitory zones were observed in the extracts, suggesting the presence of strong antimicrobial agents. Broad-spectrum action was demonstrated by the antimicrobial compounds; against S. aureus. To evaluate the potency of the active fractions, the minimum inhibitory concentration (MIC) was calculated.

These results highlight the potential of Night-Blooming Flower and Trailing Daisy as important sources of bioactive chemicals with antibacterial qualities, providing a substitute for traditional antibiotics in the treatment of diseases triggered by microorganisms that are resistant to several drugs. The development of plant-based antimicrobial therapies requires more research on their pharmacokinetics and toxicity.

Keywords: *Multidrug Resistant, Antimicrobial, Night Blooming Flower, Trailing Daisy, Diseases, Antibiotics, Microorganisms.*

INTRODUCTION

The rise in resistance of harmful bacteria to conventional therapy has made alternative treatments necessary. Thus, the use of multiple antibacterial agents may lead to increased toxicity problems, which is not desirable, especially in the treatment of immunocompromised patients. Resistance of bacteria to different medications causes conflict between "humans and bacteria". Therefore, the continuous search for new methods to combat harmful microorganisms is essential [1]. This study investigated the antibacterial activity of methanolic extracts derived from Lippia graveolens, *Sphagneticola trilobata*, and *Gliricidia sepium* plants against four pathogenic bacterial species: Pseudomonas aeruginosa, *Escherimonia Klebsiella*, and *Escherimonia Klebsi, Staphylococcus*. Among the tested extracts, *Pseudomonas aeruginosa* proved to be the most sensitive to the inhibitory effect of the agent, followed by *E. coli* and *S. aureus*. The results showed that *Sphagneticola trilobata* exhibited the strongest activity. Analysis of antagonistic and synergistic effects involving the combination of extracts from different parts of the plant (in a 1:1 ratio) [2-3]. These findings are promising, given that these bacteria are currently the subject of extensive research aimed at elucidating the mechanisms underlying their high levels of resistance to various antibiotics.

The emergence of multidrug-resistant (MDR) microorganisms is a major public health challenge today [4]. The Infectious Diseases Society of America (IDSA) has identified antimicrobial resistance as one of the greatest threats to human health worldwide. There are a number of problems that result in the serious threat of emergence of the MDR bacteria. First, patients infected with multidrug-resistant bacteria generally have worse outcomes than patients infected with more sensitive organisms. Rising rates of antibiotic resistance thus affect all parts of today's medicine and threaten the success of many accomplishments, such as cancer therapy, transplantation, and surgery. Second, these infections have huge additional costs. In the US, the disease caused by resistant microbes, rather than vulnerable species, costs an estimated $21-$34 billion per year. Third, the frequency of specific MDR microorganisms is strongly associated with the use of broad-spectrum antibiotics for empirical and definitive treatment [5]. This increased use leads to a vicious loop, which further increases the frequencies of MDR bacteria.

Trailing Daisy (*Sphagneticola trilobata*)

Nowadays, increasing interest in herbal remedies has highlighted the therapeutic potential of plant metabolites.

One such valuable plant is *Sphagneticola trilobata* (L.) Pruski (Asteraceae), formerly known as *Wedelia trilobata*. Commonly called creeping daisy, creeping wedelia, rabbit's paw, and trailing daisy, it is an herbaceous plant native to tropical regions and widely distributed across Central and South America, Australia, the Pacific Islands, Southeast Asia, and India [6-7]. *S. trilobata* is a highly invasive and resilient plant characterized by rapid growth, an extensive root system, efficient nutrient uptake, and strong antioxidant defenses that help it tolerate environmental stresses [8-12]. It also exhibits antifungal activity against several soil-borne pathogens, including *Rhizoctonia solani, Sclerotinia sclerotiorum, Phytophthora capsici*, and *Fusarium oxysporum* [13-15].

The plant has a long history of traditional medicinal use for conditions such as colds, bronchitis, wounds, infections, kidney problems, diabetes, and menstrual disorders [16-18]. Phytochemical studies have identified various metabolites, including terpenes, phenolic compounds, sterols, and fatty acids, which contribute to its reported antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, wound-healing, and antidiabetic activities.

Night-Blooming Flower (*Nyctanthes arbor-tristis*)

Nyctanthes arbor-tristis L., commonly known as night-blooming jasmine or parijat, is a shrub or small tree native to South Asia and widely distributed from Pakistan and Nepal through India to Thailand. It has a long history of use in traditional medicine, with different plant parts employed for various ailments [19]. The leaves are traditionally used for fever, rheumatism, liver and biliary disorders, loss of appetite, piles, and intestinal worms. Flowers are used for menstrual disorders, digestive problems, skin diseases, and as a sedative. Seeds are traditionally employed for alopecia and as an anthelmintic, while the bark is used for bronchitis and snakebites. Other traditional applications include treatment of cough, hiccups, dysentery, diabetes, ulcers, scabies, and rheumatism [20-23]. The plant is also reported to possess anthelmintic, antibacterial, antiallergic, antihistaminic, anti-inflammatory, expectorant, antidiabetic, and immunomodulatory properties, supporting its importance in traditional herbal medicine. *Nyctanthes arbor-tristis* L., commonly known as Parijata, Harsingar, Night Jasmine, or Tree of Sorrow, is an important medicinal plant of the family Oleaceae. It is widely distributed throughout India, the sub-Himalayan region, Bangladesh, Pakistan, and parts of Southeast Asia. The plant is a shrub or small tree reaching up to 10 m, with fragrant white flowers having orange-red corolla tubes that open at dusk and close at dawn The plant has long been used in Ayurvedic, Siddha, and Unani medicine for treating various ailments. Different plant parts, including flowers, bark, leaves, and seeds, have traditionally been used for fever, malaria, rheumatism, sciatica, cough, digestive disorders, intestinal worms, piles, skin diseases, snakebites, and hair loss [24]. Flowers are also used in religious ceremonies, garlands, and as a natural dye for silk and cotton. The orange pigment nyctanthin, chemically related to α-crocetin, contributes to its traditional use as a dye. Despite extensive traditional applications, further scientific research is needed to bridge gaps in the phytochemical characterization, pharmacological validation, and toxicological evaluation of *N. arbor-tristis*. Such studies could provide stronger scientific evidence for its traditional medicinal claims and help identify its therapeutic potential.

Phytochemical Composition and Biological Activities

N. arbor-tristis contains a wide range of bioactive phytochemicals. Leaves contain D-mannitol, β-sitosterol, flavonoid glycosides such as astragalin and nicotiflorin, oleanolic acid, nyctanthic acid, tannins, ascorbic acid, carotene, lupeol, friedelin, iridoid glycosides, and phenolic compounds. Flowers contain essential oils, nyctanthin, tannins, carotenoids, glycosides, and crocetin derivatives. Seeds contain arbortristosides A and B, fatty acids, nyctanthic acid, triterpenoids, and polysaccharides, while the bark and stem contain glycosides, alkaloids, and β-sitosterol. Flower essential oil includes compounds such as α-pinene, p-cymene, phenylacetaldehyde, and anisaldehyde [25-26].

Phytochemical studies suggest that *N. arbor-tristis* possesses significant antioxidant and antimicrobial potential. Flower extracts have demonstrated free-radical-scavenging activity, while leaf and flower extracts have shown antibacterial effects against pathogenic and multidrug-resistant (MDR) bacteria, including *Staphylococcus aureus*. Solvent extracts prepared using water, DMSO, chloroform, and methanol have produced notable inhibition zones, indicating the presence of potentially active antimicrobial constituents. *Sphagneticola trilobata* is another medicinally important plant containing phenolics, sterols, fatty acids, and terpenes. It has traditionally been used for ulcers, fever, wounds, kidney problems, hepatitis, and digestive disorders, and has been reported to exhibit antioxidant, antibacterial, anti-inflammatory, antifungal, antimalarial, hepatoprotective, antidiabetic, wound-healing, and anticancer activities.

The increasing prevalence of multidrug-resistant bacteria has created an urgent need for new antimicrobial agents [27-28]. Therefore, investigating the bioactive compounds and antimicrobial properties of *N. arbor-tristis* and *S. trilobata* may help identify promising natural sources for the development of alternative antimicrobial agents.

Methicillin-resistant *Staphylococcus aureus*

Between 2010 and 2018, the percentage of methicillin-resistant S. aureus isolates dropped from 31.30% to 14.30%, which is not statistically significant (p =.25). On a total of 28 cultures, methicillin-resistant *S. aureus* was found. Individuals of Methicillin-resistant Staphylococcus aureus showed high resistance to erythromycin (89.29%), clindamycin (35.7%), levofloxacin (50%), and ciprofloxacin (80%). They were also highly susceptible to all other antimicrobials that were tested. The MRSA samples that were tested did not show resistance to vancomycin, daptomycin, or linezolid.

Material and Methods

I. Sample collection

Samples (Leaves) were collected from Public Gardens (Bagh-e-aam) located in Nampally, Hyderabad. The collected samples were Night Blooming Flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*). These leaves sample were made into powder with the help of mortar and pestle separately and stored in Ziplock bags to avoid any moisture.

II. Preparation of Plant Extract

Chloroform Extraction

Chloroform extraction of the material was carried out by suspending 0.25 grams of the powders of collected samples in 1.5 ml of Chloroform and sealed with parafilm, so as not to evaporate.

They were transferred into 2 ml of separate sterile eppendorfs and kept in the refrigerator for further method.

DMSO Extraction
DMSO (Dimethyl sulfoxide) extraction of the material was carried out by suspending 0.25 grams of the powders of collected samples in 1.5ml of DMSO. They were transferred into 2 ml of sterile eppendorfs and kept in the refrigerator for further method.

Water extraction
Water extraction of the material was carried out by suspending 0.25 grams of the powders of collected samples in 1.5 ml of Water. They were transferred into 2 ml of sterile Eppendorfs tubes and kept in the refrigerator for further method.

Methanol extraction
Methanol extraction of the material was carried out by suspending 0.25 grams of the powders of collected samples in 1.5 ml of methanol. They were transferred into 2 ml of sterile eppendorfs and kept in the refrigerator for further method.

Multidrug resistant bacteria against antibiotics
For regular testing in a clinical laboratory when many isolates are evaluated for susceptibility to various plant extracts, the disk-diffusion approach (Kirby-Bauer) is more appropriate. A paper disc impregnated with a predetermined concentration of a plant extract is placed on the surface of an agar plate that has been uniformly infected with the test organism. The organism's growth and the plant extract's diffusion start at the same time, creating a circular zone of inhibition where the amount of the extract surpasses inhibitory concentrations. The amount of medication in the disc and the microorganism's susceptibility determine the inhibition zone's diameter.

Testing of Antimicrobial activity in disk diffusion method
For regular testing in a clinical laboratory when many isolates are evaluated for susceptibility to various plant extracts, the disk-diffusion approach (Kirby-Bauer) is more appropriate. A paper disc impregnated with a predetermined concentration of a plant extract is placed on the surface of an agar plate that has been uniformly infected with the test organism. The organism's growth and the plant extract's diffusion start at the same time, creating a circular zone of inhibition where the amount of the extract surpasses inhibitory concentrations. The amount of medication in the disc and the microorganism's susceptibility determine the inhibition zone's diameter. Since zone size is also influenced by inoculum size, medium composition, incubation temperature, excess moisture, and agar thickness, this test needs to be strictly standardized. The disc diffusion method was used to assess the produced extracts' antibacterial activity. The antibacterial activity of the inoculated extracts was then assessed using a zone reader to look for inhibition zones (measured in millimetres). After being impregnated with 20 µg/m sample extracts (20 µg/disc), the discs (6 mm in diameter) were put on infected agar.

Preparation of nutrient agar-
Agar media is made by combining nutritional medium with commercially available agar powder; if nutrient medium is unavailable, water, peptone, beef extract, and agar can be created separately. 1gm of agar was weighed, mixed with nutrient medium in 100ml of water, and placed into petri plates, which were autoclaved at 121°C for 15lbs.

Using a standard, prepare a suspension of the bacterial culture to be tested with an appropriate turbidity.1 to 2 drops of gram-positive and gram-negative bacteria were distributed on separate sterile nutrient agar plates. A sterile spreader was used for spreading. Using sterilised forceps, discs of the following plant extracts were placed on the plate: Ajwain, Terminalia, Henna, and Cumin. Plates were incubated within 15 minutes of adding the discs. The plates should be incubated as soon as the discs are placed because the test is standardized under conditions where plant extract diffusion and bacterial growth begin. A table of antibacterial susceptibilities is used to determine whether the strain is resistant, intermediate, or susceptible to the antibiotics tested.

Determination of minimum inhibitory concentration (MIC)-
The researchers determined the least inhibitory doses by conducting antibacterial activity tests on the isolates (MIC). The above-mentioned strain subcultures were chosen for their antibacterial properties.MIC is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after 24-hour incubation. This will lower the opportunity for microbial resistance to specific antimicrobial agents. Antibacterial properties of each plant extract were found by testing it at various doses. Activate is a test used to detect the antibacterial activity of a substance. Aliquots of antibiotic A were added to wells 2 cm away and incubated for 24–96 hours at 30 0 C, depending on the strain. Water was employed to maintain control. The percentage of inhibition (I percent) was determined. Observation of the diameter of the zone of inhibition as a parameter of the growth.

RESULTS

I. Sample Collection
Samples were collected from Public Gardens (Bagh-e-aam) located in Nampally, Hyderabad. The collected samples were Night Blooming Flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*).

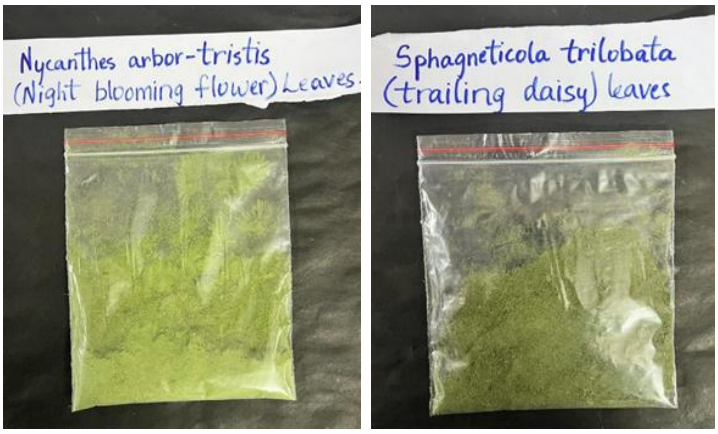


Fig 1 and 2: showing Night blooming flower (Nyctanthes arbor-tristis) and Trailing Daisy (Sphagneticola trilobata) in powdered form

These collected samples were cleaned and air dried. These were grinded into fine powder as shown in fig 1 and 2 and mixed with different solvents (DMSO, Chloroform, methanol, water) and were stored for further work.

II. Multidrug resistant bacteria against antibiotics

Bacteria were incubated for a standardized period of 24 hours to allow for the development of observable zones of inhibition around plant extract discs. The zones of inhibition, indicative of the effectiveness of each plant extract against the respective isolates, were measured and recorded. The interpretation of these results is essential in guiding healthcare practitioners towards the selection of appropriate medicine for the treatment.

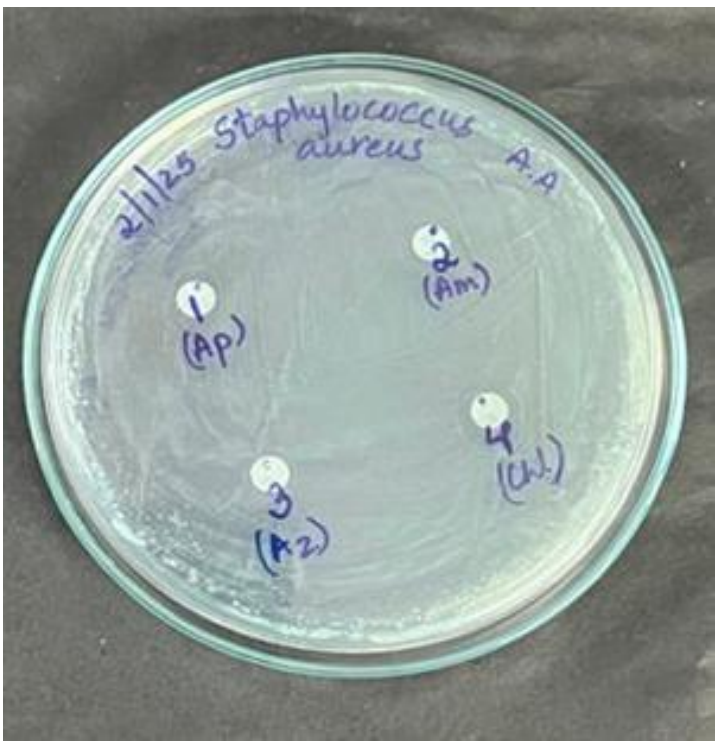


Fig 3: showing antibiotics against drug resistant bacteria Staphylococcus aureus

Antibiotic sensitivity test was done for *Staphylococcus aureus* using four different antibiotics where 1 is ampicillin; 2 is amoxicillin; 3 is azithromycin; 4 is clindamycin, inhibition zones were not observed in all the four antibiotics shows resistant to multiple drugs as shown in fig 3.

III. Antimicrobial activity of medicinal plant extracts

Following inoculation, the bacteria were incubated for a standardized period of 24 hours to allow for the development of observable zones of inhibition around plant extract discs. The results of the antimicrobial activity test were systematically documented and are summarized in the table. The zones of inhibition, indicative of the effectiveness of each plant extract against the respective isolates, were measured and recorded. The interpretation of these results is essential in guiding healthcare practitioners towards the selection of appropriate medicine for the treatment.

Antimicrobial activity of Trailing Daisy (*Sphagneticola trilobata*)

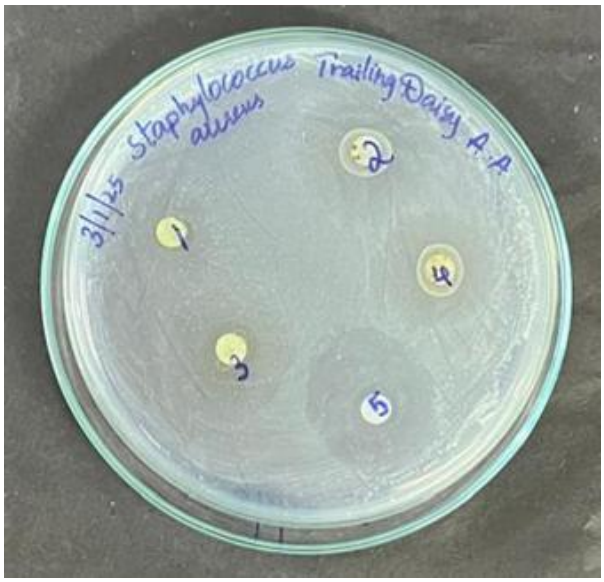


Fig 4: showing antimicrobial activity of Trailing Daisy (Sphagneticola trilobata) against Staphylococcus aureus

Antibacterial activity test was conducted against *Staphylococcus aureus* with Trailing Daisy (*Sphagneticola trilobata*) treating with different solvents where 1 is Antibiotic; 2 is Water+ Trailing Daisy; 3 is Methanol + Trailing Daisy; 4 is Chloroform + Trailing Daisy; 5 is DMSO + Trailing Daisy. Here, DMSO + Trailing Daisy is showing more antimicrobial activity. Formation of zone as shown in fig 4 indicates more activity towards DMSO + Trailing Daisy.

Antimicrobial activity of Night blooming flower (*Nyctanthes arbor-tristis*)

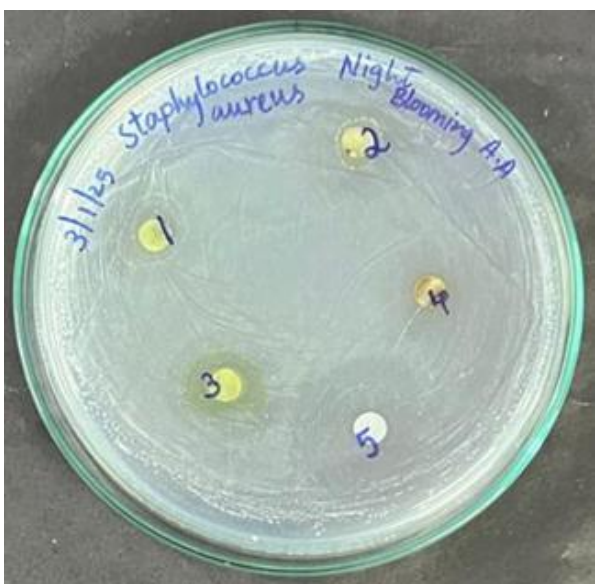


Fig 5: showing antimicrobial activity of Night blooming flower (Nyctanthes arbor-tristis) against Staphylococcus aureus

Antibacterial activity test was conducted against *Staphylococcus aureus* with Night blooming flower (*Nyctanthes arbor-tristis*) treating with different solvents where 1 is Methanol + Night blooming flower; 2 is Antibiotic; 3 is DMSO + Night blooming flower; 4 is Water + Night blooming flower; 5 is Chloroform+ Night blooming flower. Here, Chloroform + Night blooming flower is showing more antimicrobial activity. Formation of zone as shown in fig 5 indicates more activity towards Chloroform + Night blooming flower.

Table 1: Antimicrobial activity of Night blooming flower (Nyctanthes arbor-tristis) and Trailing Daisy (Sphagneticola trilobata) with different solvents against multidrug resistant bacteria

Staphylococcus	DMSO	Chloroform	Methanol	Water	Antibiotic
Trailing Daisy	1.6±1	1.0±1	1.4±1	1.3±1	0.8 ±1
Night blooming flower	1.0 ±1	1.5 ±1	1.0 ±1	1.1 ±1	0.9±1

IV. Minimum Inhibitory concentration of Plant Extracts

In the assessment of antimicrobial efficacy, DMSO + Trailing Daisy and Chloroform + Night blooming flower demonstrated significant inhibitory effects against multi drug resistant bacteria (*Staphylococcus aureus*), with discernible zones of inhibition. This observation underscores the potential of Night blooming flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*) with solvents DMSO and chloroform as a promising antibacterial agent, exhibiting notable activity multidrug resistant bacterial species.

Minimum inhibitory concentration of Trailing Daisy (*Sphagneticola trilobata*)

Fig 6: Minimum inhibitory concentration of Trailing Daisy + DMSO against Staphylococcus aureus

Minimum inhibitory concentration was done for Trailing Daisy + DMSO against *Staphylococcus aureus* using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml; 5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.5 ±1 cm and 0.625ug/ml was showing low activity which is 0.6 ±1 cm among five concentrations for Trailing Daisy + DMSO against *Staphylococcus aureus* as shown in fig 6.

Minimum inhibitory concentration of Night blooming flower (*Nyctanthes arbor-tristis*)

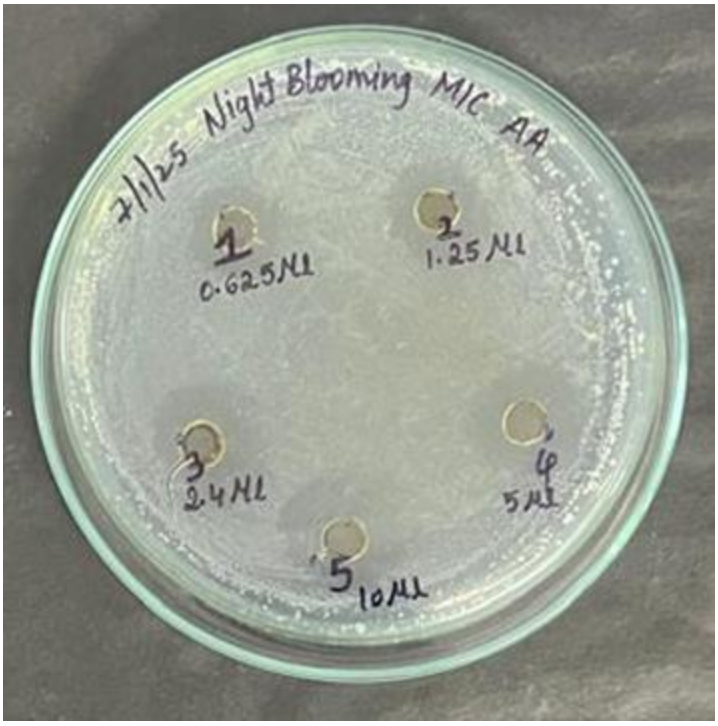
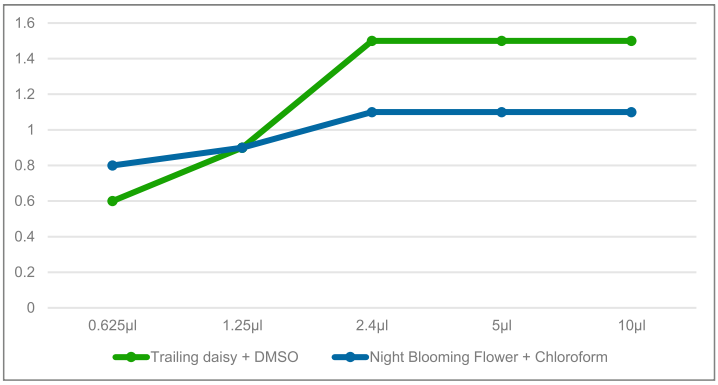


Fig 7: Minimum inhibitory concentration of Chloroform + Night blooming flower against Staphylococcus aureus

Minimum inhibitory concentration was done for Chloroform + Night blooming flower against *Staphylococcus aureus* using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml; 5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.1 ±1 cm and 0.625ug/ml and 1.25ug/ml was showing low activity which is 0.8 ±1 cm and 0.89±1 cm among five concentrations for Chloroform + Night blooming flower against staphylococcus aureus as shown in fig 7.

Table 2: Minimum inhibitory concentration of Night blooming flower (Nyctanthes arbor-tristis) and Trailing Daisy (Sphagneticola trilobata) against multidrug resistant bacteria

S.no.	Concentration	Trailing Daisy + DMSO	Night blooming flower + Chloroform
1.	0.625µl	0.6cm	0.8cm
2.	1.25µl	0.9cm	0.9cm
3.	2.4µl	1.5cm	1.1cm
4.	5µl	1.5cm	1.1cm
5.	10µl	1.5cm	1.1cm



Graph 1: Minimum inhibitory concentration of Night blooming flower (Nyctanthes arbor-tristis) and Trailing Daisy (Sphagneticola trilobata) against multidrug resistant bacteria

All the DMSO + Trailing Daisy and Chloroform + Night blooming flower were showing highest value at concentration 10ug/ml and low value at concentration 0.625ug/ml as shown in table 2. These findings highlight the variability in antimicrobial properties among different plant extracts and underscore the importance of comprehensive screening to identify potent antimicrobial agents with broad-spectrum activity. Further investigations into the underlying mechanisms of action and potential therapeutic applications of these plant extracts are warranted to harness their full potential in combating bacterial infections.

Discussion

It is well known that one of the most important problems in public health today is the multidrug-resistant (MDR) microorganisms. The Infectious Diseases Society of America (IDSA) has called antimicrobial resistance "one of the greatest threats to human health worldwide." There are a number of problems that result in the serious threat of emergence of the MDR bacteria. First, patients infected with multidrug-resistant bacteria generally have worse outcomes than patients infected with more sensitive organisms. Rising rates of antibiotic resistance thus affect all parts of today's medicine and threaten the success of many accomplishments, such as cancer therapy, transplantation and surgery. Second, these infections have huge additional costs. In the US, the disease caused by resistant microbes, rather than vulnerable species, costs an estimated $21-$34 billion per year [29-33]. Third, the frequency of specific MDR microorganisms is strongly associated with the use of broad-spectrum antibiotics for empirical and definitive treatment. This increased use leads to a vicious loop, which further increases the frequencies of MDR bacteria.

In the assessment of antimicrobial efficacy, DMSO + Trailing Daisy and Chloroform + Night blooming flower demonstrated significant inhibitory effects against multi drug resistant bacteria (*Staphylococcus aureus*), with discernible zones of inhibition. To evaluate the potency of the active fractions, the minimum inhibitory concentration (MIC) was calculated. DMSO + Trailing Daisy and Chloroform + Night blooming flower showed the highest value at concentration 10ug/ml and low value at concentration 0.625ug/ml. This observation underscores the potential of Night blooming flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*) with solvents DMSO and chloroform as a promising antibacterial agent, exhibiting notable activity against multidrug resistant bacterial species [34-41]. These findings highlight the variability in antimicrobial properties among different plant extracts and underscore the importance of comprehensive screening to identify potent antimicrobial agents with broad-spectrum activity [42-48]. Further investigations into the underlying mechanisms of action and potential therapeutic applications of these plant extracts are warranted to harness their full potential in combating bacterial infections.

One of the most critical dangers to the healthcare sector today is the increasing prevalence of antibiotic-resistant bacteria. Deadly pathogenic multi drug resistance (MDR) bacteria are increasing day by day and are highly serious threat to human health [49-53]. In the past, these kinds of antibiotic resistant bacterial strains were unusual and were only seen in nosocomial-acquired infections, but lately they have become increasingly frequent.

This problem is more common in Gram positive and Gram-negative bacterial species, such as *A. baumannii, E. coli, P. aeruginosa* and *K. pneumoniae* (Gram negative) and *S. aureus*, *S. pneumoniae, E. faecium and E. faecalis* (Gram positive). It is seen that this antibiotic resistance in these bacterial species was related to the acquisition of plasmids through the transfer of resistance genes. Some bacterial species have particular method to escape from the detrimental effects of antibiotics including efflux pumps, decreased permeability of LPS layer, secretion of degrading enzymes and modification of targets [54-61]. Some of the variables responsible for increasing this antibiotic resistance may include widespread development, overexploitation of antibiotics, overuse of broad-spectrum medications and lack of target orientated antimicrobial treatments.

The purpose of this work is to assess the potential of the antimicrobial compounds present in the extracts of Night blooming flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*) that are effective against a variety of MDR bacterial strains. Following the extraction of plant materials by solvent-based techniques, using water, DMSO, chloroform and methanol, the disc diffusion method was used to test for antibacterial activity [62-67]. Multidrug-resistant strains of Staphylococcus *aureus*, were used to assess the extracts of antibacterial effectiveness. Significant inhibitory zones were shown by the extracts, suggesting the presence of strong antimicrobial agents.

Conclusion

Medicinal plant antimicrobial activity is a new hope to combat the dangerous threats posed by increasing evidence of antimicrobial resistance. Therefore, there is an urgent need to identify and isolate new bioactive compounds from medicinal plants, which have yet to be adequately explored. The large diversity of these compounds has proved to have therapeutic potentials as antimicrobials and as antimicrobial resistance modifiers. Further research is warranted to elucidate the mechanisms underlying the antimicrobial activity of the plant extracts. These findings pave the way for the development of natural, sustainable, and effective preservation strategies that uphold food quality, safety, and sustainability.

In the assessment of antimicrobial efficacy, DMSO + Trailing Daisy and Chloroform + Night blooming flower demonstrated significant inhibitory effects against multi drug resistant bacteria (*Staphylococcus aureus*), with discernible zones of inhibition. To evaluate the potency of the active fractions, the minimum inhibitory concentration (MIC) was calculated. DMSO + Trailing Daisy and Chloroform + Night blooming flower showed the highest value at concentration 10ug/ml and low value at concentration 0.625ug/ml. This observation underscores the potential of Night blooming flower (*Nyctanthes arbor-tristis*) and Trailing Daisy (*Sphagneticola trilobata*) with solvents DMSO and chloroform as a promising antibacterial agent, exhibiting notable activity multidrug resistant bacterial species.

Future prospective:

More research is needed to understand the processes underlying the antibacterial action of plant extracts. These data illustrate the heterogeneity in antimicrobial activities across plant extracts, emphasizing the significance of thorough screening to discover powerful antimicrobial agents with broad-spectrum activity.

Further research into the underlying mechanisms of action and potential therapeutic applications of these plant extracts is required to fully realise their potential in treating bacterial infections. The prospective utilization of novel bioactive chemicals remains challenging. It is critical to emphasize that rigorous in vitro and in vivo testing are required to ensure the selection of active and harmless antibacterial plant-derived chemicals. It is also difficult to harness the potential synergistic or antagonistic effects of substances within and amongst medicinal plant extracts. Studies into the mechanisms of action, interactions with antibiotics or other medicinal plants or substances, as well as the extracts' pharmacokinetic and pharmacodynamic profiles, should receive top consideration.

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