

Original Research Article

<https://doi.org/10.20546/ijcmas.2023.1204.029>

## Invitro Screening of Selected Medical Plants for their Anti-Bacterial Efficacy against few Clinical Isolates

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### ABSTRACT

#### Keywords

Phytochemicals,  
Antibiotics, MDRS

#### Article Info

##### Received:

08 March 2023

##### Accepted:

07 April 2023

##### Available Online:

10 April 2023

From ancient times, plants have been the primary source of several phytochemicals that have been crucial in maintaining human health. A variety of antibiotics including secondary metabolites that plants produce while under stress. Due to the advent of several multi-drug resistant strains, commonly available antibiotics lost their effectiveness, and it became the second biggest cause of death globally. It was critical to create brand-new, highly efficient antibacterial medications from plant sources that were affordable, had fewer side effects, and worked quickly to treat bacterial illnesses. This study examines the effectiveness of nine methanolic plant extracts against nine bacterial diseases that are frequently caused by bacteria.

### Introduction

India is one of the countries with the highest levels of biodiversity in the world, with 4 biodiversity hotspots and 47,000 plant species. Just 4635 of these are utilised commercially on a sizable basis as medicines (Punjabi *et al.*, 2014). Due to the alarming rise in the prevalence of new and re-emerging infectious illnesses, there is an ongoing and urgent need to develop new antimicrobial agents with varied chemical structures and unique modes of

action (Rojas *et al.*, 2003). Yet, leaves are the part of plants that are most frequently used in conventional medicine to treat a variety of ailments (Ugulu, 2011). In ayurveda medicine, natural medicinal herbs encourage self-healing, wellness, and durability (Atif Ali, 2012). Due to the existence of a wide range of bioactive secondary metabolites, including tannins, terpenoids, alkaloids, saponins, flavonoids, and phenolic compounds, which can have a clear physiological effect on the human body, medicinal plants serve as the primary sources of

anti-bacterial agents (Kumar Shakya and Arvind Kumar Shakya, 2016). In India, 70% of rural residents depend on utilising plant-based medications. The higher plant has a critical function in the treatment of numerous sorts of ailments in the Indian Ayurvedic medical system. Higher plants create a large number of varied chemical compounds with a range of biological actions, up to thousands (Rojas *et al.*, 2003). Being one of the safest treatments for the treatment of numerous bacterial illnesses, both industrialised and developing nations rely on plant-derived medications. Due to their capacity to thrive in a variety of settings, various bacteria include *Staphylococcus*, *Bacillus*, *Tuberculosis*, *Pneumonia*, *Salmonella*, *E.coli*, and *Pseudomonas* species mostly caused various forms of bacterial illnesses (Uddin *et al.*, 2021).

In most cases, antibiotic resistance is an adaptation to antibacterial drugs that occurs naturally. When bacteria develop resistance to an antibiotic, they can either horizontally or vertically transfer this trait to their offspring. These days, new resistant bacterial strains that are somewhat more deadly than the original strain have emerged as a result of the indiscriminate and illogical use of antibiotics (Chandra *et al.*, 2017; Anand *et al.*, 2021)

Over the world, medicinal plants have been used to cure a variety of illnesses caused by bacteria. Many chemical compounds having pharmacological uses can be found in medicinal plants (Al-Ansari *et al.*, 2019). Methicillin Resistance *Staphylococcus aureus* is just one of the bacterial strains that have emerged as a result of the majority of commonly used modern allopathic antibiotic drugs. This alarming clinical situation makes it difficult to treat infections that have serious negative effects on the human body.

Drug-resistant pathogenic strains will emerge as a result of the misuse and abuse of antibiotics. Recently, a lot of focus has been placed on extracts and physiologically active chemicals obtained from plant species used in herbal therapy due to side effects and the resistance that harmful bacteria

acquire against antibiotics (Essawi and Srour, 2000). *Escherichia coli* is a member of the Enterobacteriaceae family. It is a gram-negative, facultatively anaerobic, rod-shaped, motile, non-sporulating bacteria that mostly causes bloodstream infections (BSI), urinary tract infections (UTI), and intestinal infection (Desmarchelier, 2016; Yu *et al.*, 2021). In the majority of biotechnology and microbiology labs, *E. coli* was regarded as a model organism. Normal *Bacillus subtilis* is a gram-positive, rod-shaped, aerobic, non-pathogenic soil bacterium that primarily affects some animals' gastrointestinal tracts (GITs) (Kimelman and Shemesh, 2019; Su *et al.*, 2020). In the majority of biotechnology and microbiology labs, *E. coli* was regarded as a model organism. Normal *Bacillus subtilis* is a gram-positive, rod-shaped, aerobic, non-pathogenic soil bacterium that primarily affects some animals' gastrointestinal tracts (GITs) (Bar, 2021).

A relatively common pathogenic microbe in humans, *Staphylococcus aureus* may cause a number of infectious illnesses, including bacteremia, minor skin and soft tissue infections, infective endocarditis, osteomyelitis, and deadly pneumonia (Guo *et al.*, 2020). It is a cluster-forming, grape-like stricture made up of gram-positive, non-motile cocci bacteria. Due to the production of "staphyloxanthin" in all medium, *S. aureus* often forms colonies that are golden and yellow in hue. *S. aureus* occasionally creates pink colonies as a result of the hydrolysis of the chromogenic substrate for -glucosidase, and it occasionally develops unique green colonies as a result of the synthesis of -glucosidase (Perry *et al.*, 2004; Perry and Freydière, 2007; Balaji *et al.*, 2022).

Antibiotics and upholding a sanitary atmosphere can be used to treat staph infections. Yet, the rise of bacterial strains that are multi-drug resistant makes therapy difficult. Penicillin-binding protein 2a or 2' (PBP2a or PBP2') (*mecA*), which was integrated into the chromosomal element (SCC*mec*) of methicillin-sensitive cells, was created by them as a result of carrying the *mecA* gene on their

chromosome (Guo *et al.*, 2020). Even in the presence of numerous antibiotics, the PBP continues to produce peptidoglycan. The emergence of methicillin-resistant *S. aureus* strains has brought about a serious problem in both healthcare settings and community settings (Ahmad-Mansour *et al.*, 2021).

For the treatment of MRSA infections, there are currently no effective antibiotic medications on the market. The goal of the current study was to screen nine plants that are widely accessible in India's north-eastern areas for high-efficiency plants by infecting them with three different bacterial strains (*Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*).

## Materials and Methods

### Collection of plant material

In April and May 2022, a total of 9 plants' healthy, disease-free leaves and stems were harvested. The lab received the freshly gathered specimens and used the Plant-Net programme and the herbarium kept in the Department of Botany at the University of Calcutta to identify them. The identified plant specimens were cleaned under running water from the faucet to get rid of any dust that had settled on the plant's components. To produce an air-dried product, plant pieces were once again cleaned with distilled water and kept at room temperature in the clean shade house. In order to prevent the growth of microorganisms (fungi or bacteria), the air-dried was periodically checked on and the position of the plant portion was rotated. After three to four weeks, plant portions were divided into small pieces and ground using an electric grinder, generating 300 g of powdered samples that were kept at room temperature in clean, labeled sealed polyethylene bags.

### Preparation of Plant Extract

Plant extract preparation required the collection of pulverised plant material. A glass column containing

200g of the powdered material was filled with petroleum benzene, a non-polar solvent. The chemicals from the samples were released into the solvent after 3–4 days. After that, a 0.22 m pore-size filtering device was used to filter the solvent. The filtrate was then put through a rotating vacuum evaporator to create a thick slurry.

The slurry was allowed to dry naturally before being weighed. The final concentration of extracts, 20 mg/ml at room temperature, was obtained by dissolving the slurry in 50% dimethyl sulfoxide (DMSO) solvent. Following serial dilution processes, plant extracts were once more diluted from the final concentration and made at 10 mg/ml, 5 mg/ml, and 2.5 mg/ml, respectively.

### Test cultures

Three bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, were used in an in vitro antibacterial experiment. Using a sterile loop, all of the bacterial strains were brought back to life from the glycerol stock (stored at -80°C). The stock culture was kept alive by incubating cultures at 37°C for 16–24 hours on sterile Luria agar slants. The finished culture was kept in storage for later use at 4°C.

### Preparation of Inoculums

Using stringent aseptic procedures, pathogenic (*Staphylococcus aureus*) and non-pathogenic (*E. coli* and *Bacillus subtilis*) bacterial strains were sub-cultured on the sterile Luria broth growth medium. In a shaker incubator, the cultures were incubated at 37°C for 18 to 24 hours. Cultures were removed from the incubation period after which a disc diffusion experiment was carried out.

### Preparation of paper disc

Whatman No 1 filter paper was used for the paper plate preparation. The size of a 5mm paper plate was prepared by using punching machines and later autoclaved it.

## Screening of extracts for anti-bacterial activity

Nine plant extracts were tested for antibacterial activity using the Kirby- Bauer disc diffusion methods. Under strictly aseptic circumstances, 5 ml of the tested bacterial culture was combined with 100 ml of sterile Luria agar media. The media eventually harden and become cemented. To infect the test organism, previously autoclaved paper discs were dipped into various plant extract concentrations and then put on solid agar plates using sterile forceps (if the paper disc contain more extracts, touched in a sterile tissue paper to remove excess extract). As reference standards, the approved antibiotics Ampicillin (100 mg/ml) and Kanamycin (50 mg/ml) were employed, while the solvent DMSO (50% conc.) served as the adverse control. After that, plates were incubated inverted for 12–16 hours at 37°C. Antibacterial activity was detected following the incubation period as a distinct zone of growth inhibition (ZOI) surrounding the infected disc. A clear ruler with a millimetre (mm) scale was used to measure the inhibition's diameter, including the disc, and the findings were recorded. The greatest value was used in all trials, which were carried out in duplicate.

## Results and Discussion

This study assessed the value of medications produced from plants in treating various human illnesses brought on by bacterial pathogens. The Kirby Bauer disc diffusion test was used to assess the antibacterial activity against often causing bacterial illnesses of a total of nine methanolic plant extracts at four different doses. Without a distinct zone of inhibition surrounding the disc, the absence of antibacterial action is indicated, and a distinct zone denotes the bactericidal activity of the test plant extracts.

*Catharanthus roseus* extracts (20 mg/ml conc.) revealed the lowest inhibitory activities (7 mm) against non-pathogenic *E. coli* bacterial infections, whereas *Andrographis paniculata* extracts at 20 mg/ml demonstrated the largest inhibition zone (18

mm). Only *Acacia nilotica* extracts, however, demonstrated the greatest inhibition at the remaining values of 10 mg/ml, 5 mg/ml, and 2.5 mg/ml. Notably, none of the plant extracts from *Carica papaya*, *Psidium guajava*, or *Moringa oleifera* exhibited any inhibitory action at any of the doses. Also, several combinations of extracts from *Ocimum tenuiflorum*, *Andrographis paniculata*, *Acacia nilotica*, *Catharanthus roseus*, *Murraya koenigii*, and *Tinospora cordifolia* showed strong antibacterial action against *E. coli* infections.

DMSO solvent was employed as a negative control while Ampicillin and Kanamycin were utilised as reference standards to evaluate the activity of plant extracts. As a result, we may presume that the inhibition is caused by plant extracts since they include bioactive substances.

At a concentration of 20 mg/ml conc, *Carica papaya* extracts have the maximum inhibitory activity (20 mm) against non-pathogenic *Bacillus subtilis* infections. The strongest antibacterial activity, however, is found in *Ocimum tenuiflorum*, which has concentrations of 10 mg/ml conc. (13mm), 5 mg/ml conc. (11mm), and 2.5 mg/ml conc. (8mm), respectively.

Except for *Andrographis paniculata* extracts (only 20mg/ml and 10mg/ml conc. exhibited inhibition), all six of the studied plant extracts, *Ocimum tenuiflorum*, *Andrographis paniculata*; *Carica papaya*, *Tinospora cordifolia*, *Moringa oleifera*, and *Psidium guajava* showed inhibitory zone in all concentrations.

Only the extracts from *Acacia nilotica* shown bactericidal action against the pathogenic *Staphylococcus aureus* infections that were evaluated at doses of 20 mg/ml, 10 mg/ml, 5 mg/ml, and 2.5 mg/ml. *Murraya koenigii* and *Catharanthus roseus* have also demonstrated some inhibitory effect, but only at concentrations of 20 mg/ml of the extracts. Notably, no plant, with the exception of *Acacia nilotica*, shown antibacterial activity in any concentration of the extracts.

Table.1

Name of the plant	Family	Local Name
1. <i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulshi
2. <i>Andrographis paniculata</i> (Burm.f.) Nees	Acanthaceae	Chirota
3. <i>Carica papaya</i> L.	Caricaceae	Amita
4. <i>Catharanthus roseus</i> (L.) G. Don	Apocynaceae	Nayan tora
5. <i>Acacia nilotica</i> (L.) P.J.H.Hurter and Mobb.	Fabaceae	Torua Kadam or Kher
6. <i>Murraya koenigii</i> (L.) Sprengel	Rutaceae	Noro-xinkho
7. <i>Psidium guajava</i> L.	Myrtaceae	Modhuri- Aam
8. <i>Tinospora cordifolia</i> (Thunb.) Miers	Menispermaceae	Giloy
9. <i>Moringa oleifera</i> Lam.	Moringaceae	Sajina

Fig.1 Pictorial diagram of antibacterial assay of nine plants against *E. coli* infections.

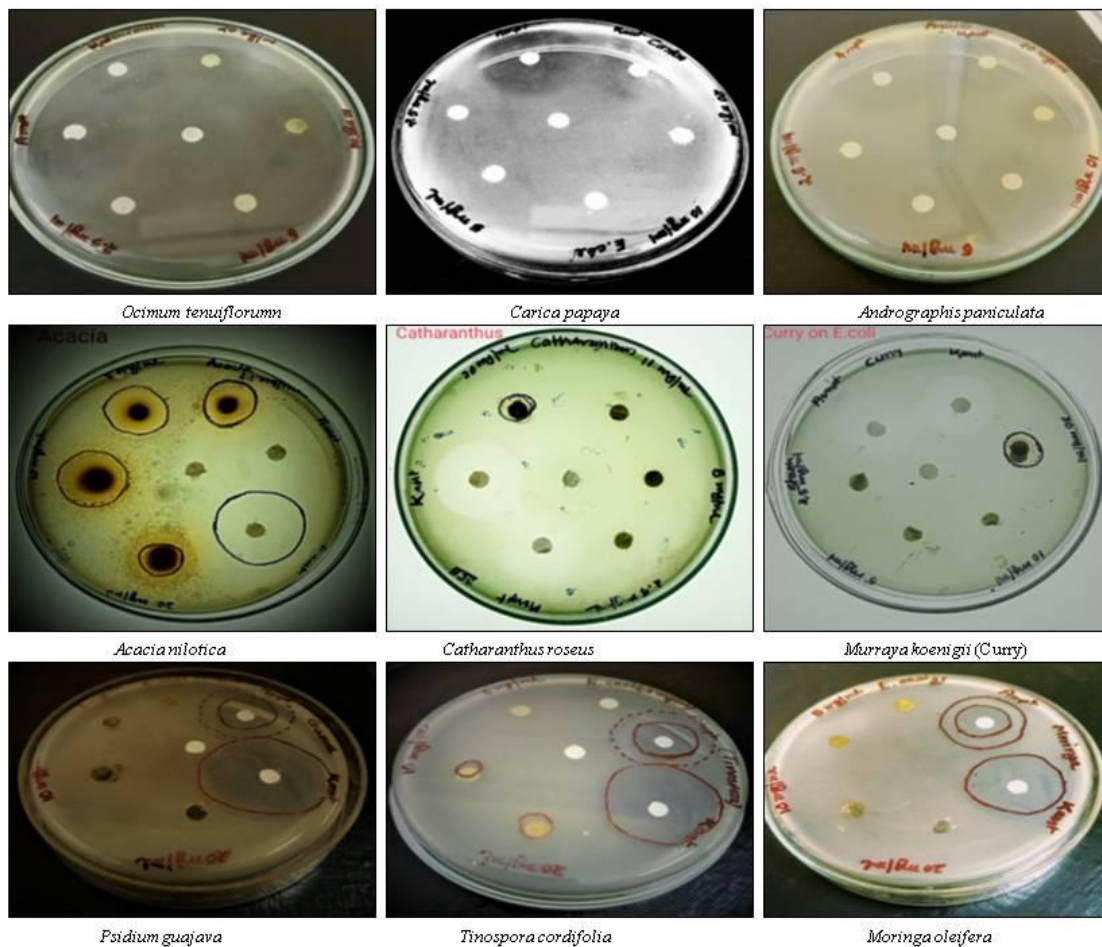


Fig.2 Graphical representation of the results of inhibition against *E. coli* infections.

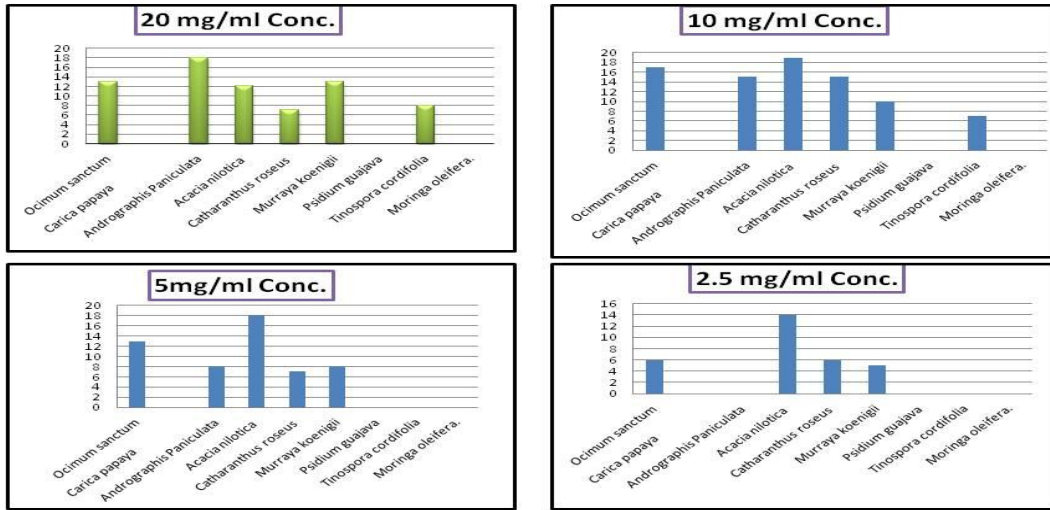


Fig.3 Pictorial diagram of the antibacterial assay against *Bacillus subtilis* infections.

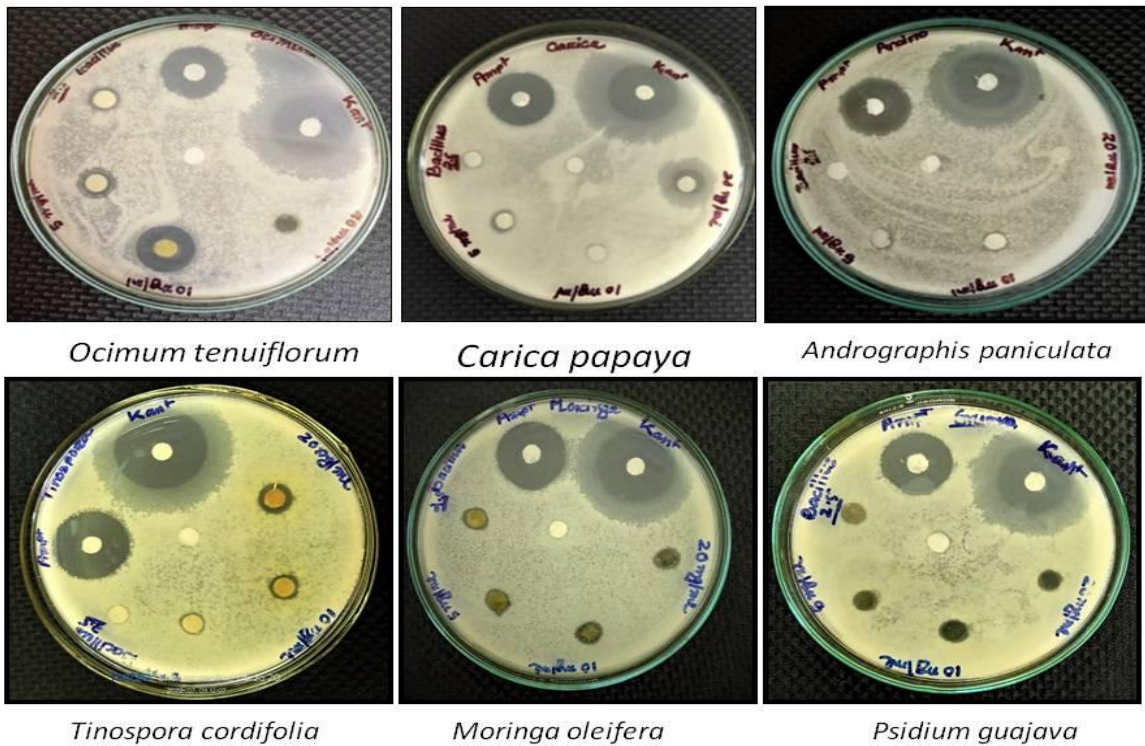


Fig.4 Graphical representation of inhibition against *Bacillus subtilis* bacterial infections.

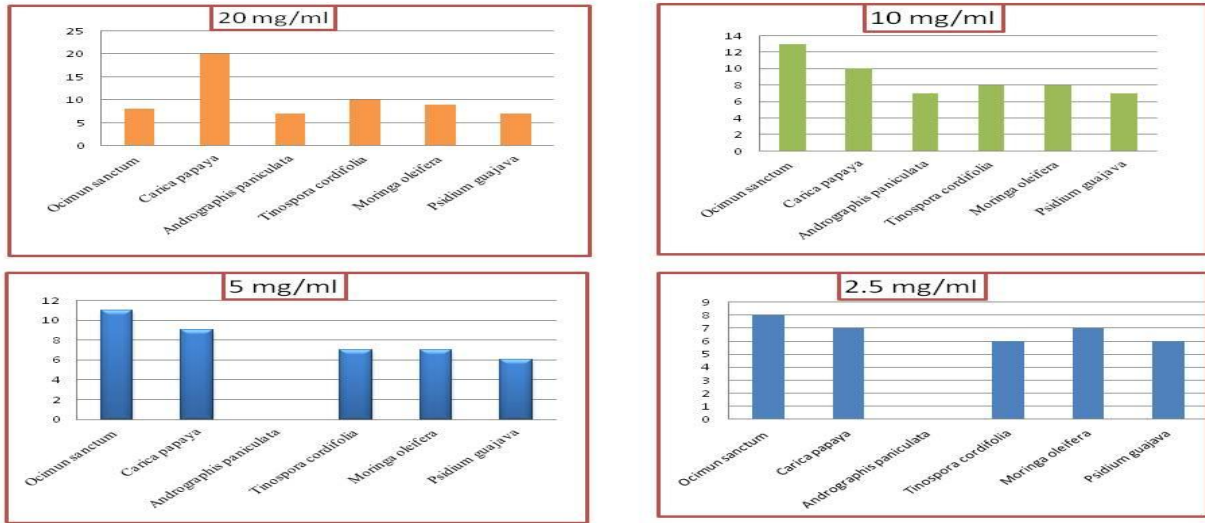
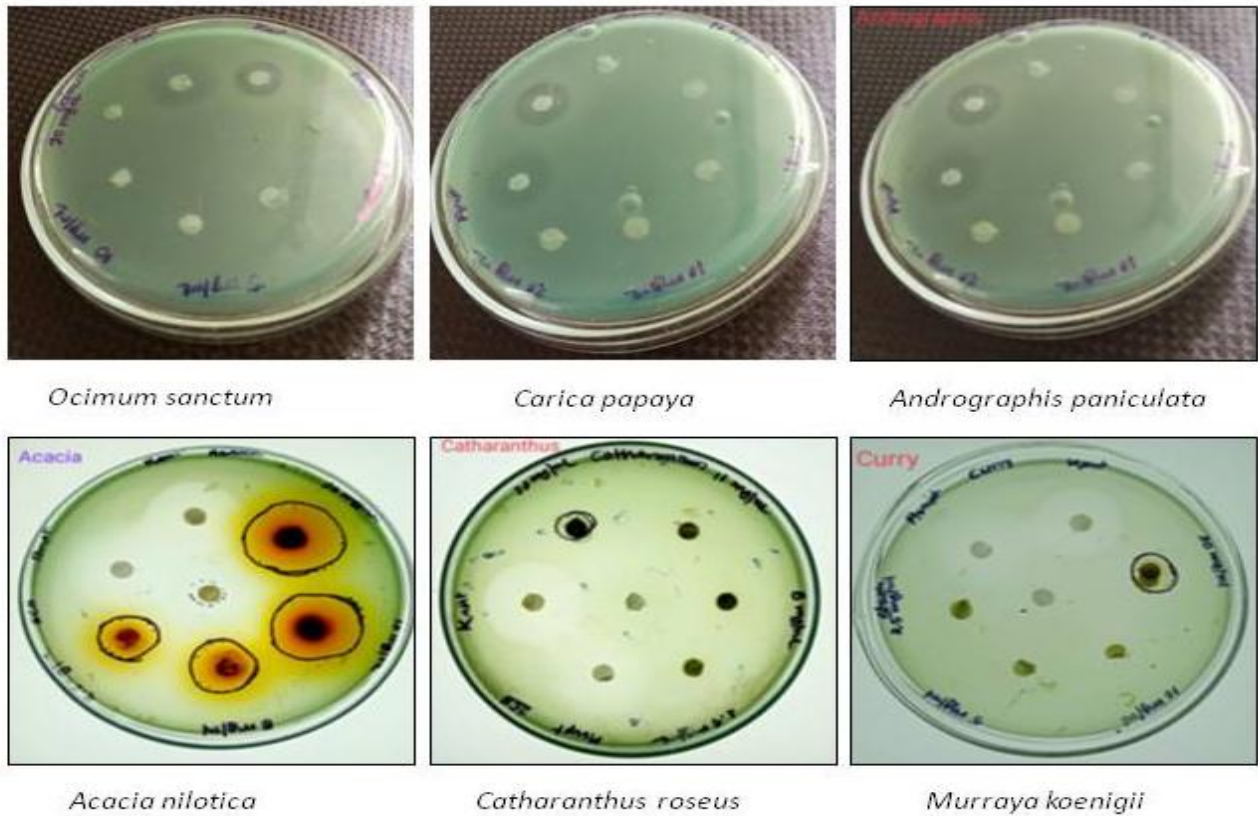
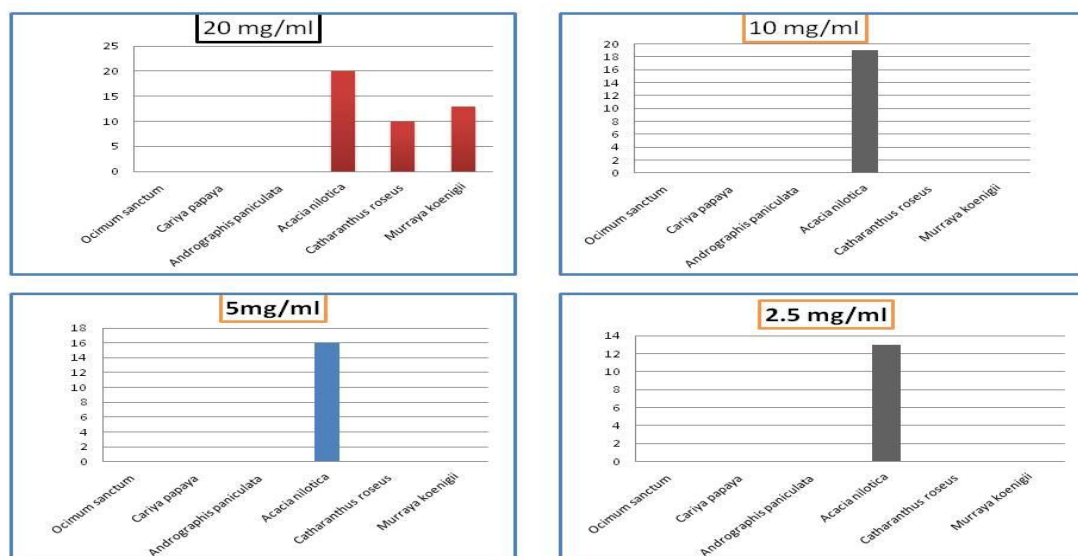


Fig.5 Pictorial results of the zone of inhibition by infecting with *Staphylococcus aureus* Strains.



**Fig.6** Graphical representation of results against pathogenic staph infections.



Finding a phytomedicine will be highly important for treating numerous viral disorders (Al-Ansari *et al.*, 2019) Due to their accessibility and lack of significant adverse effects, traditional medicines (i.e., plants) have emerged as a godsend in the medical sciences (Chandra *et al.*, 2017).

Since ancient civilizations, the plant has played a significant part in a number of remedies for bacterial illnesses, although not being aware of the precise mechanism of inhibition. The development of multi-drug resistant strains (MDRS) makes it difficult to treat many bacterial illnesses over the world.

It is imperative to use herbal products for the bio-control of illnesses as a cutting-edge replacement for antibacterial therapies that will result in nontoxic and more environmentally friendly management of virulent diseases (Atef *et al.*, 2019). Although it is not critical for us to find new herbal medications with fewer side effects, lower costs, etc., finding new potential medications is a highly challenging and time-consuming procedure.

Since they contain a variety of primary and secondary metabolites, including phenolics, polyphenols, tannins, quercetin, flavones, flavonols, alkaloids, terpenoids, lectins, polypeptides, and

complex combinations, plants are effective at killing bacterial infections (Doddanna *et al.*, 2013) According to my observations, extracts from *Acacia nilotica*, *Ocimum tenuiflorum*, *Carica papaya*, and *Andrographis paniculata* were the plants with the most antibacterial activity, and all of these species were widely accessible in Northeast India.

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<https://doi.org/10.22207/JPAM.16.2.35>

#### How to cite this article:

Himangsu Sharmah, M. Mathiyazhaghan, Binay Chaubey, K. Meenakshi Sundaram and Lavanya prathap. 2023. *In vitro* Screening of Selected Medical Plants for their Anti-Bacterial Efficacy Against Few Clinical Isolates. *Int.J.Curr.Microbiol.App.Sci*. 12(04): 255-264. doi: <https://doi.org/10.20546/ijcmas.2023.1204.029>