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APPLICATION OF EXTRACT OF PLANT TO CONTROL THE DRUG RESISTANT BACTERIA

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ABSTRACT

The three strains of bacteria that were used in the experiment were Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The purpose of the research was to evaluate the antibacterial activity of various medicinal plant extracts and their synergistic antibiotic and non-antibiotic medications. For the preparation of the alcoholic extract, a Soxhlet apparatus was used, and for the preparation of the aqueous extract, a water reflux apparatus was utilized. Both the disc diffusion technique and the well diffusion method were used in order to assess the antibacterial properties of the extracts; the inhibitory zones were measured in millimetres. The microdilution technique was used to determine the minimum inhibitory concentration (MIC) of plant extracts against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The disc diffusion technique was used in order to evaluate the synergistic impact that plants have when combined with the extraction of antibiotics and / or non-antibiotic medications. The findings of this investigation indicated that ethanolic extracts, when employed against E. coli, S. aureus, and P. aeruginosa, exhibited an antibacterial impact and a synergistic effect with the majority of antibiotics. These extracts performed much better than methanolic and aqueous extracts.

Keywords: Plant, Control, Drug, Paracetamol, Hcl

INTRODUCTION

Microorganisms have become more resistant to antibiotics over the course of the last three decades, despite the fact that the pharmaceutical industry has generated a number of novel medicines in that time period. In general, bacteria possess the genetic capability to both pass on and gain resistance to medications, which are exploited as therapeutic agents. Because of the large number of patients in hospitals who have compromised immune systems and because of the emergence of novel bacterial strains that are resistant to many antibiotics, this fact is reason for worry. As a direct consequence of this, the risk of contracting a new infection while in a hospital setting is significantly increased. Montelli and Levy (27) found that there was a significant frequency of resistant bacteria in clinical microbiology in Brazil during the years 1980 through 1990. This finding has also been confirmed in different medical facilities located all over the globe.

The emergence of bacterial resistance to the antibiotics that are already on the market has made it necessary to conduct research into the creation of new antibacterial drugs. Post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, and food poisoning are most often caused by Gram-positive bacteria like Staphylococcus aureus (Benayache et al., 2001). Gram-negative bacteria, such as Escherichia coli, are found in human intestines and may lead to infections of the lower urinary tract, coleocystitis, or septicemia (Benhassaini et al., 2003; Benjilali et al.,1986).

These antimicrobial compounds come from natural sources, and it is believed that their effects on the surrounding environment are minimal; hence, they are candidates for use as agents of biological control. However, certain medical herbs have not found widespread use for a variety of reasons, and as a result, they are frequently referred to as "forgotten plants." Taking into consideration the growing need for natural compounds that may be used in a variety of ways, such as food additives, as components of functional foods, as a means of avoiding plant diseases, and as nutraceuticals, amongst other potential uses, It is not unreasonable to reevaluate the 'lost plants' by determining their viability and advantages via the use of contemporary scientific research techniques (Abdel Rahman et al., 2011).

ANTIBIOTIC RESISTANCE

Methicillin-resistant Staphylococcus aureus (also known as MRSA) is a significant contributor to nosocomial infections and is one of the microorganisms that are resistant to antibiotics. Infections caused by MRSA are notoriously difficult to treat because MRSA

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

strains have developed resistance to the vast majority of medicines that are currently used in medical practise. Vancomycin and other glycopeptide-type medicines are the only antibacterial treatments that are effective against the vast majority of MRSA strains. On the other hand, the emergence of S. aureus strains resistant to vancomycin (VRSA) has been documented (Adwan and Mhanna, 2008). Because of its widespread distribution, ability to thrive in wet conditions, and resistance to a wide variety of medicines and antiseptics, Pseudomonas aeruginosa is also the causative agent of nosocomial infections. The rise of P. aeruginosa strains that are resistant to many classes of antimicrobial agents is a significant issue that has to be addressed.

DRUG RESISTANCE IN BACTERIA: ALARMING NEED OF NEW ANTIBIOTICS

Infections caused by bacteria that are resistant to antibiotics are already commonplace around the world (Golkar et al. 2014). In February of 2017, the World Health Organization (WHO) issued its very first list of "priority infections" that are antibiotic-resistant and represent the greatest risk to human health. Carbapenem-resistant bacteria are at the top of the list for essential priorities. Acinetobacter baumannii, carbapenem-resistant Pseudomonas aeruginosa, and carbapenem-resistant and extended spectrum betalactamase (ESBL) generating Enterobacteriaceae are examples of bacteria that are resistant to these antibiotics.

The minimal inhibitory concentration (MIC) is the lowest concentration of an antibacterial agent that, after 18 to 24 hours of incubation, still allows observable development of a typical bacterial inoculum. It is common knowledge that plants contain a number of antibacterial chemicals, and these compounds are used in all forms of traditional medicine. In therapeutic settings, such as hospitals and animal hospitals, several unprocessed forms of the so-called "herbal medications" are in use. Researchers from a wide variety of scientific subfields are looking into plants to learn more about the antibacterial properties they possess.

SIGNIFICANCE

The synergistic impact of medicinal plant extracts in combination with antibiotics and non-antibiotic medications against S. aureus, E. coli, and P. aeruginosa will be the focus of this research, which I believe will be the first of its kind in Palestine. Because bacteria have become resistant to the antibiotics that are now on the market, it has become necessary to look for new antibacterial agents or a combination of medications that will be able to battle the newly resistant strains of dangerous bacteria. A synergistic impact of several plant

Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

extracts with antibiotic and non-antibiotic medications against certain antibiotic-resistant bacteria has been discovered in earlier research; hence, we will explore this potential in our study by employing traditional Palestinian plants.

OBJECTIVES

It was possible to accomplish the following particular goals:

1. The gathering and identification of several therapeutic herbs.
2. To extract the chosen medicinal plants by using a variety of solvents, including but not limited to methanol, ethanol, and water reflux.

REVIEW OF LITERATURE

Ibrahim Sani (2014) The development of antibiotic resistance in bacterial populations is becoming an increasingly serious obstacle in the treatment of a wide variety of illnesses. As a result, there is a pressing need to discover an alternative method that makes use of medicinal plants in order to circumvent antibiotic resistance and uncover a source of innovative drugs for the treatment of various bacterial infections. This study was conducted with the intention of determining whether or not it would be possible to use hexane extracts of Allium sativum bulbs, Calotropis procera leaves, Acacia nilotica pods, and Mitracarpus scaber whole parts to circumvent antibiotic resistance in a number of clinically isolated bacterial strains (Staphylococcus aureus, Klebsilla pneumoniae and Streptococcus pneumoniae).

Mustafa Oskay (2013) Traditional medicinal plants offer a diverse array of chemicals that have the potential to be effective in the treatment of a variety of infectious disorders. Therefore, the antibacterial properties of ethanolic extracts of 19 different plant species were investigated using the agar well diffusion technique against clinical isolates that were resistant to several drugs. Broad-spectrum antibacterial action was shown by extracts of Liquidambar orientalis, Vitis vinifera, Punica granatum, Cornus sanguinea, Euphorbia peplus, Ecballium elaterium, and Inula viscosa. The size of the inhibition zones ranged from 8 to 26 millimetres.

Nascimento Gislene G. F (2014) Both antibiotic-susceptible and antibiotic-resistant bacteria were used in this experiment to test the antimicrobial activity of plant extracts and phytochemicals. In addition, research was conducted to investigate whether or not the synergistic effects may occur when combined with antibiotics. Extracts from the following plants were utilised: Achillea millifolium (yarrow), Caryophyllus aromaticus (clove), Melissa offficinalis (lemon-balm), Ocimun basilucum (basil), Psidium guajava (guava), Punica granatum (pomegranate), Rosmarinus officinalis (rosemary), Salvia officinalis (sage), Syzygyum joabolanum (thyme). Additionally, the phytochemicals benzoic acid, cinnamic acid, eugenol, and farnesol were included into the process. The extracts of Caryophyllus aromaticus and Syzygyum joabolanum were shown to have the strongest antibacterial potentials.

Smruti Mahapatra (2021) Ayurvedic medicine comes from India and is considered a traditional kind of treatment due to its long history and well-established basis. Herbal treatments have an important part to play in both the prevention and treatment of illnesses that affect humans. Plants have been utilised as a kind of traditional medicine for millennia. Bioactive phytochemicals are a secondary metabolite that are created by plants and medicinal plants are an abundant source of these phytochemicals. Studies conducted by scientists in the last several decades have provided compelling evidence that these phytochemicals play a vital part in the prevention of infectious diseases. In recent decades, there has been a resurgence of interest in the use of medicinal plants as a source of possible treatments due to the rediscovery of these plants.

RESEARCH METHODOLOGY

Nerium oleander, Artemisia herba alba, Withania somnifera, Lantana camara, Ficus sycomorus, Allium sativum, and Eucalyptus camaldulensis are all plants that can be found growing in Palestine. These plants provided the plant materials that were employed in this research project. These plants were obtained from a variety of locations around the Gaza strip In order to continue with the experiments, pathogenic strains of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli were obtained from the microbiology department of Al-Shifa hospital. These pathogenic strains were kept on Brain Heart Infusion (BHI) agar medium (HiMedia) at a temperature of 4 degrees Celsius. It was necessary to use many different types of media in order to conduct this research, including Brain Heart Infusion broth, Nutrient agar (biolife), and Mueller-Hinton agar (HiMedia). In the process of extraction, both ethanol and methanol were also used. These recording medium, as well as the solvent, were acquired from a Gaza-based firm.

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

DATA ANALYSIS AND RESULT

EVALUATION OF ANTIBIOTICS ACTIVITY

Against Escherichia coli

The efficiency of a variety of antibiotics against E. coli was evaluated using the disc plate technique, which is described in section 3.2.5. (Table 4.1 and Figure 4.1). When tested against E. coli, chloramphenicol exhibited the greatest degree of inhibition (24 mm). Tetracyclines, ofloxacin, ampicillin, cefazolin, nalidixic acid, and co-trimoxazole were all ineffective against the resistant strain.

Against Staphylococcus aureus

In accordance with the findings shown in Table 4.1, the antibiotics Gentamicin, Chloramphenicol, and Tetracyclines exhibited the greatest inhibitory zone (21 mm), which was followed by Ofloxacin, Amikacin, and Neomycin (20 mm). Ceftazidime, Ampicillin, Penicillin G, and Cefazolin had no impact against S. aureus, although they did have an effect against other bacteria.

Against Pseudomonas aeruginosa

As can be seen in Table 4.1, the medicines that exhibited the highest level of action against P. aeruginosa were amikacin, ceftazidime, and gentamicin. The other antibiotics had no impact.

Table 1 Evaluation of antibiotics activity against S. aureus, E. coli and P.aeruginosa

Microorganism / Antibiotics	Staphylococcusaureus	Escherichia coli	Pseudomonas aeruginosa
	Inhibition zone (mm)		
Vancomycin	15mm	*	*
Cefotaxime	11mm	8 mm	0mm

Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

Ofloxacin	20mm	0 mm	0mm
Ceftriaxone	12mm	9 mm	0mm
Ceftazidime	0 mm	11 mm	9 mm
Tetracyclines	21mm	0 mm	*
Amikacin	20mm	10 mm	17 mm
Chloramphenicol	21mm	24 mm	*
Gentamicin	21mm	7 mm	8mm
Ampicillin	0 mm	0 mm	*
Erythromycin	17 mm	*	*
Rifampicin	19 mm	*	*
Neomycin	20 mm	14 mm	0mm
Co-trimoxazole	10 mm	0 mm	*
Pencillin G	0 mm	*	*
Cefazolin	0 mm	0 mm	*
Ceflexin	10 mm	7 mm	0 mm
Nalidixic acid	*	0 mm	*

*= Have not been tested.mm= millimeter.

VA: Vancomycin; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime TE: Tetracycline; AK: Amikacin; C: Chloramphenicol, E: Erythromycin;AMP: Ampicillin; N: Neomycin; CN: Ceflexin.

Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

EVALUATION OF PLANT EXTRACTS BIO ACTIVITY

Against Escherichia coli

The findings shown in Table 4.2 demonstrated that the disc diffusion approach was superior to the well diffusion method when it came to determining the antibacterial activity of plant extracts when tested against E. coli. Extracted by methanol for eight hours, the leaves of Artemisia herba-alba and the bark of Ficus sycomorus demonstrated the greatest effectiveness against E. coli, with a zone of inhibition measuring 9 millimetres. As can be shown in Table 4.2, the antibacterial properties of the plant species Allium sativum, Ficus sycomorus, and Lantana camara were not detected at a concentration of 200 mg/ml after being extracted with methanol for 8 hours.

After being extracted by ethanol for 8 hours, Ficus sycomorus (Leaves and Bark), Eucalyptus camaldulensis, and Withania somnifera showed the highest activity against E. coli with a zone of inhibition equal to 8 millimetres, followed by Artemisia herba-alba and Nerium oleander, which showed a zone of inhibition equal to 7 millimetres. When tested against E. coli, the antibacterial properties of Lantana camara and Allium sativum (after being extracted by ethanol for 8 hours) were absent.

Except for Artemisia herba-alba, which demonstrated a modest level of antimicrobial activity with a zone of inhibition equal to 6 millimetres, none of the plant extracts that were extracted by water for two hours showed any antimicrobial activity against Escherichia coli (as shown in Table 4.2).

Table. 2 Antimicrobial Activity of Plant extracts on Escherichia coli by welldiffusion method and disc diffusion method

A.A.A* Plant extract	Well diffusionmethod				Disc diffusion method			
	M	E	W	Control°	M	E	W	Control°
Nerium oleander	-	-	-	-	7	7	-	-

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

Artemisia herba-alba		-	-	-	-	9	7	6	-
Withaniasomnifera		-	-	-	-	7	8	-	-
Lantanacamara		-	-	-	-	-	-	-	-
Ficus sycomorus	L	-	-	-	-	-	8	-	-
	B	-	-	-	-	9	8	-	-
Alliumsativum		-	-	-	-	-	-	-	-
Eucalyptus camaldulensis		-	-	-	-	7	8	-	-

Against Staphylococcus aureus

Well Diffusion Method

The findings of the effects of the methanolic, ethanolic, and aqueous extracts of the plants using 20 l from the extracts (200 mg/ml crude extract) are shown in table format. These extracts were used to assess their efficacy against S. aureus (4.3). It has been shown that the methanolic and ethanolic extracts of Artemisia herba-alba had the greatest impact against S. aureus, with a zone of inhibition that is respectively 19 mm and 20 mm in size.

It was shown that aqueous extracts of Ficus sycomorus had the greatest antibacterial activity against S. aureus. In such case, the zone of inhibition found on the Ficus sycomorus leaves was 14 millimetres wide and proved to be more efficient than the zone of inhibition found on the bark (12 mm). As can be shown in Table 4.3 and Figure 4.10, the effectiveness of Lantana camara against S. aureus was much lower than that of Nerium oleander, Withania somnifera, and Allium sativum. Lantana camara had no impact whatsoever against S. aureus.

Disc Diffusion Method

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

The methanol and ethanol extracts of the Ficus sycomorus bark exhibited the greatest activity against S. aureus (with a 15 mm zone of inhibition), followed by the Lantana camara extracts (with a 14 mm zone of inhibition) (by methanol extract). Leaves of the Eucalyptus camaldulensis tree that were extracted with ethanol and exhibited a zone of inhibition (13 mm). Extraction with methanol and ethanol revealed very little biological activity in Nerium oleander, Artemisia herba-alba, Allium sativum, and Withania somnifera.

The aqueous extracts of Ficus sycomorus (leaves and bark) taken for two hours demonstrated the maximum efficacy against S. aureus, with a zone of inhibition of 15 and 12 millimetres, respectively. The Lantana camara and Eucalyptus camaldulensis plants did not demonstrate significant antibacterial action against S. aureus (with 8 mm inhibition zone). As can be shown in Table 4.1 and Figure 4.10, Nerium oleander, Artemisia herb-alba, Withania somnifera, and Allium sativum did not demonstrate any antibacterial action when tested against S. aureus.

Table 3 Antimicrobial Activity of Plant extracts on Staphylococcus aureus by well diffusion method and disc diffusion method

A.A.A* Plant extract		**Well diffusionmethod**				**Disc diffusion method**			
		M	**E**	**W**	**Contral ^{o}**	**M**	**E**	**W**	**Control ^{o}**
Nerium oleander		**-**	**-**	**-**	**-**	**7**	**6**	**-**	**-**
Artemisia herba-alba		**19**	**20**	**-**	**-**	**8**	**9**	**-**	**-**
Withaniasomnifera		**-**	**-**	**-**	**-**	**7**	**8**	**-**	**-**
Lantanacamara		**9**	**11**	**7**	**-**	**14**	**10**	**8**	**-**
Ficus sycomorus	L	**14**	**15**	**-**		**11**	**12**	**-**	

Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

	B	**13**	**14**	**-**		**15**	**15**	**-**	
Allium sativum		**-**	**-**	**-**	**-**	**7**	**7**	**-**	**-**
Eucalyptus camaldulensis		**13**	**14**	**7**	**-**	**11**	**13**	**8**	**-**

Against Pseudomonas aeruginosa

Well Diffusion Method

Only two of the eight plants that were harvested showed any antibacterial activity against the strain of P. aeruginosa that was tested. These findings were disclosed by the antibacterial activity test. P. aeruginosa was found to be susceptible to the activity of methanol and ethanol extracts of Ficus sycomorus bark, which had a zone of inhibition measuring 12 and 11mm, respectively, and ethanol and methanol extracts of Eucalyptus camaldulensis leaves, which had a zone of inhibition measuring 11 and 10mm, respectively.

On the other hand, the only aquatic extract of Eucalyptus camaldulensis that exhibited any efficacy against P. aeruginosa was this one.

Disc diffusion method

As can be seen in Table 4.4, the methanolic extracts of Ficus sycomorus bark and Eucalyptus camaldulensis had the biggest zone of inhibition against P. aeruginosa, measuring in at 10 millimetres in diameter. This was shown to be the case for both of these substances.

The ethanolic extract of Ficus sycomorus bark exhibited the maximum efficacy against P. aeruginosa (10mm), followed by Artemisia herba-alba and Eucalyptus camaldulensis, all of which had a zone of inhibition of 8mm for each of them. In contrast, the aqueous extracts of Eucalyptus camaldulensis and Artemisia herba-alba showed only a moderate level of antibacterial activity against P. aeruginosa, with zones of inhibition of 8 and 7 mm, respectively. Although the extracts of Nerium oleander, Withania somnifera, Lantana camara, and Allium sativum did not demonstrate any antibacterial action against P.

Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

aeruginosa, using either the well diffusion technique or the disc diffusion method, respectively.

Table 4 Antimicrobial Activity of Plant extracts on Pseudomonas aeruginosa byWell Diffusion Method and Disc Diffusion Method

A.A.A* Plant extract		Well diffusionmethod				Disc diffusion method			
		M	E	W	Control ⁰	M	E	W	Control ⁰
Nerium oleander		-	-	-	-	-	-	-	-
Artemisia herba-alba		-	-	-	-	-	8	7	-
Withaniasomnifera		-	-	-	-	-	-	-	-
Lantanacamara		-	-	-	-	-	-	-	-
Ficus sycomorus	L	-	-	-	-	-	7	-	-
	B	12	11	-	-	10	10	-	-
Alliumsativum		-	-	-	-	-	-	-	-
Eucalyptus camaldulensis		11	10	10	-	10	8	8	-

MINIMUM INHIBITORY CONCENTRATION OF PLANT EXTRACTS ALONE USINGMICRODILUTION METHOD

The results of the minimum inhibitory concentration (MIC) test revealed that all of the plant extracts that were put to the test had antibacterial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, with MIC values ranging from 0.19 to 100 mg/ml. As can be seen in Table 4.5, the examined extracts demonstrated varying degrees of antibacterial activity based on the species that were evaluated.

Against Escherichia coli

Table 4.5 provides a summary of the minimum inhibitory concentrations (MIC) values for each plant extract that was tested against E. coli. The minimum inhibitory concentration (MIC) of the methanol extract of the leaves of N. oleander and F. sycomorus ranged from 6.25 to 12.5 mg/ml. While W. somnifera and L. camara had a concentration of 25 mg/ml, A. sativum and F. sycomorus (Bark) ranged from 12.5-25 mg/ml, and A. herba-alba had a concentration of 25 mg/ml. The minimal inhibitory concentration (MIC) for E. camaldulensis against E. coli was determined to be 3.125 mg/ml.

The minimum inhibitory concentration (MIC) for ethanolic extracts of N. oleander, F. sycomorus (leaves and Bark), and W. somnifera was 12.5 mg/ml; the MIC value for L. camara and A. sativum was 25 mg/ml; the MIC value for A. herba-alba ranged from 6.25-12.5 mg/ml; and the MIC value for E. camaldulensis As indicated in Table 4.5, the MIC findings for aquatic extracts of N. oleander, A. herba-alba, W. somnifera, and F. sycomorus were 25 mg/ml; for L. camara and A. sativum, it was 12.5 and 50 mg/ml respectively; and for E. camaldulensis, it ranged from 12.5-6.25 mg/ml.

Table 5 Minimal inhibitory concentrations (MIC) of the plants extracts against E. coli.

MIC (mg/ml)								
Plant / Solvent	**N. oleander**	**A. herba-alba**	**W. somnifera**	**L. camara**	**F. sycomorus (leaves)**	**F. sycomorus (Bark)**	**A. sativum**	**E. camaldule-nsis**
Methanol	**12.5-6.25**	**12.5**	**25**	**25**	**12.5-6.25**	**25-12.5**	**25-12.5**	**3.125**
Ethanol	**12.5**	**12.5-6.25**	**12.5**	**25**	**12.5**	**12.5**	**25**	**6.25**
Water	**25**	**25**	**25**	**12.5**	**25**	**25**	**50**	**12.5-6.25**

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

Against Staphylococcus aureus

Table 4.6 displayed the results of the minimal inhibitory concentration (MIC) tests conducted on plant extracts against S. aureus. The minimum inhibitory concentration (MIC) of the methanol extracts against S. aureus was 12.5 mg/ml for each of N. oleander, A. herba-alba, W. somnifera, and L. camara, while it was 6.25 mg/ml and 50 mg/ml for F. sycomorus (Bark) and A. sativum, respectively. While this was going on, the F. sycomorus (leaves) concentration ranged from 3.125 to 6.25 mg/ml, while the E. camaldulensis concentration ranged from 12.5 to 6.25 mg/ml.

The minimum inhibitory concentration (MIC) of the ethanol extract of S. aureus was determined to be 25, 6.25, 12. 5, 25, 50, and 6.25 mg/ml for N. oleander, A. herba-alba, L. camara, F. sycomorus (leaves), A. sativum, and E. camaldulensis, respectively. On the other hand, it ranged from 6.25 to 12.5 mg/ml for W. somnifera and F. sycomorus (Bark).

CONCLUSION

Based on the results of the antibacterial test that was conducted for this research, it was discovered that S. aureus was more vulnerable to the plant extracts that were used than E. coli and P. aeruginosa. The minimum inhibitory concentration (MIC) of each plant extract was determined with respect to E. coli, S. aureus, and P. areuginosa. The minimum inhibitory concentration (MIC) of methanolic extract of E. camaldulensis for E. coli was determined to be 3.125 mg/ml. In addition, the methanol and aqueous extract of F. sycomorus (leaves) had an antibacterial activity of between 6.25 and 3.125 mg/ml against S. aureus. In addition, the ethanol extract of E. camaldulensis has a potency of 6.25 mg/ml against P. areuginosa. E. camaldulensis (methanol extract), leaf extract of F. sycomorus (methanol and aquatic extract), and E. camaldulensis (ethanol extract) had very potent activity against E. coli, S.aureus, and P. areuginosa, suggesting that only very small amounts of the extracts are needed to inhibit the growth of the bacteria. In comparison to methanolic and aqueous extracts, ethanolic plant extracts shown superior antibacterial and synergistic efficacy with antibiotics.

 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*

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 Priya Ranjan Sharma *, *University Department of Botany: B.R.A. Bihar University, Muzaffarpur. India.*