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An evaluation of antimicrobial activity in medicinal plants, *Cassia fistula* Linn., *Delonix regia* (Hook.) Raf. & *Albizia lebbeck* (L.) Benth.

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ABSTRACT

The global burden of infectious illnesses and drug abuse necessitates the rapid discovery of novel medications derived from medicinal plants. The study's goal was to analyze the antibacterial activity of ethno-medicinal plants. In the research region, medicinal plants *Cassia fistula* Linn. (Amaltas), *Delonix regia* (Hook.) Raf. (Gulmohar), and *Albizia lebbeck* (L.) Benth. (Siris) have been utilized to cure a range of ailments. According to this investigation, hexane and acetone extracts of all three plants had the highest MIC value against *Sclerotinia sclerotiorum*. All three plants' ethanolic extracts have a considerable MIC against *Aspergillus niger*. Ethanolic extracts of *Delonix regia* (Hook.) Raf. (Gulmohar) and *Albizia lebbeck* (L.) Benth. (Siris) also have a large MIC against *Pseudomonas syringae*; however, acetone extracts of *Cassia fistula* Linn. (Amaltas) have an effective MIC against *Pseudomonas syringae*. Results against *Erwinia carotovora* are encouraging for the ethanolic extract of *Delonix regia* (Hook.) Raf. (Gulmohar), the acetone extract of *Albizia lebbeck* (L.) Benth. (Siris), and the ethanolic and acetone extracts of *Cassia fistula* Linn. (Amaltas). The outcome presented suggests prospective sources for the medicinal plants under investigation as antimicrobial agents; hence, more *in vitro* and *in vivo* antimicrobial activity investigations are advised.

Key Words - Antimicrobial activity in medicinal plants, *Cassia fistula* Linn. (Amaltas), *Delonix regia* (Hook.) Raf. (Gulmohar), *Albizia lebbeck (*L.) Benth. (Siris).

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INTRODUCTION

Medicinal plants have significant commitments in the social insurance arrangement of neighborhood networks as the primary wellspring of medication for most of the local populace. Plants have dietary benefits and, according to the nearby individuals, have therapeutic and custom or enchanted qualities (Abbink J., 1995) . The ethno-restorative mending frame works change across societies. In India, there is a decent social variety with different examples of utilizing greenery. As indicated by the World Health Organization (WHO), more than 3.5 billion individuals in the creating scene depend on medicinal plants as part of their human services (Balick & Cox. 1996) . Most by far of individuals (70-80%) in Africa counsel Traditional Medical Practitioners (TMPs) for their social insurance (Cunningham, 1993) . Traditional medication has been brought into the center for meeting the objectives of a more extensive inclusion of essential medicinal services conveyance in Africa and all nations of the world. It is the primary decision human services treatment for, at any rate, 80% of Africans who experience the ill effects of high fever and other regular afflictions. Consequently, restorative plants are generally utilized to treat various human and domesticated animals' sicknesses in different parts of the world (Sofowora, *et al.* 2013). Ethnobotany, the biggest sub-discipline

of ethnobiology, is commonly characterized as the "study of individuals' collaboration with plants". This circumscription incorporates the investigation of plants that have restorative applications. While the essential targets of current ethnobotany are neither to grow new pharmaceuticals nor to find new bioactive compound moieties, explaining the pharma- cological exercises of a specific plant is a piece of some ethnobotanists' examination (McClatchey *et al.* 2009). The bioactive constituents or plant might be utilized for the treatment of different ailments, and these future utilized as another definition for the novel medications disclosure in pharmaceutical ventures.

Antimicrobial resistance (AMR) has emerged as one of the most serious public health issues of the twenty-first century, threatening the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi that are no longer susceptible to common antibiotics. The issue of AMR is especially pressing in light of bacterial drug resistance. Bacteria that cause common or serious illnesses have evolved resistance to each new antibiotic that enters the market over several decades, to variable degrees. In light of this fact, immediate action is required to prevent a looming global healthcare disaster.

Many studies have recently looked at the potential use of certain plant extracts as efficient natural preservatives (Clarke *et al.* 2017; Fernández-López, *et al.* 2005; Suppakul *et al.* 2016). In the past, many medicinal plant components, including as the root, stem, flower, fruit, and twigs, were widely employed to cure a variety of human ailments[8]. Numerous phyto chemicals found in medicinal plants, including flavonoids, alkaloids, tannins, and terpenoids, have antibacterial and antioxidant activities (Talib & Mahasneh. 2010). Numerous studies have been conducted on certain plant species' antibacterial properties. For instance, a variety of Gram-positive and Gram-negative bacteria are resistant to the antibacterial effects of the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs (Alzoreky & Nakahara, 2003). Furthermore, it has been claimed that extracts from Chinese chives and cassia can successfully slow the growth of *Escherichia coli* and other germs when meat, juices, and milk are being stored (Mau *et al.* 2001). The first step in making the best use of these extracts as natural antimicrobial agents is comprehending the mechanism of antimicrobial activity of medicinal plant extracts. Thus, this research is focused to identify the potency of hexane, acetone, and ethanol extract of three well-known ayurvedic plants, namely; *Cassia fistula* Linn. (Amaltas), *Delonix regia* (Hook.) Raf. (Gulmohar), *Albizia lebbeck* (L.) Benth. (Siris) against pathogenic microbes. For evaluating the significant antimicrobial effect, we have selected two pathogenic bacteria, namely; *Erwinia carotovora* and *Pseudomonas syringae*, and the two fungal isolates; *Aspergillus niger* and *Sclerotinia sclerotiorum*. The research work includes a dedicated wet lab study to identify the above stated activity by performing an antimicrobial assay and minimum inhibitory concentration (MIC) estimation.

MATERIALS & METHODS

Preparation and Selection of plant extract

In this work, four plants were selected based on their traditional usage in folk medicine. The plants were obtained from the topographical area of Siwan, Bihar. Three different plants have been opted: *Cassia fistula* Linn. (Amaltas), *Delonix regia* (Hook.) Raf. (Gulmohar), and *Albizia lebbeck* (L.) Benth. (Siris) to prepare plant extract. Hexane, ethanol, and acetone were used to prepare the plant extract mentioned above. The soxhlet extraction method was followed for extract preparation, whose detailed procedure is mentioned in our previous study. The total number of extracts obtained is nine extracts, that is, three solvent extracts for three different plants.

Preparation of inoculum:

Through the use of the Agar well diffusion technique, the activity was assessed. The Muller Hinton Agar (MHA) Media for the bacterial isolates was made according to the standard formulation

provided by Himedia, and the Sabouraud Dextrose Agar (SDA) Media for the fungal isolates was made according to the standard formulation. Following sterilization, the medium was put into sterile glass petridishes while utilizing aseptic procedures (Toshiba, India). Using the spread plate technique, the medium was inoculated (100 µl of the culture broth) with the appropriate bacterial isolates (*Erwinia carotovora* and *Pseudomonas syringae* on the MHA media and *Aspergillus niger* and *Sclerotinia sclerotiorum* on the SDA media) once the plates had adequately solidified.

Antimicrobial activity:

The plant extract samples for all nine extracts were made with a DMSO (dimethyl sulfoxide) solvent concentration of 1 mg/ml. Using sterile microtips, wells were created in the media plates, and 20 ml of each plant extract sample that will be examined were then added to each well. After allowing the samples to fully saturate the medium in the wells, the plates were paraffin-sealed and incubated at 37°C for 24 hours (for bacteria) or 27°C for 48 hours (for other organisms i.e., Fungus). The plates featured two wells; one was used as a positive control and contained antibiotics Ciprofloxacin (500 ppm concentration for bacteria) and Luliconazole (500 ppm concentration for fungi). The other well served as a negative control and contained DMSO.

Determination of the Minimum Inhibitory concentration (MIC):

The pathogen was treated with a range of concentrations of the plant extract in order to calculate the MIC value of each extract against each chosen pathogen. Each extract's concentration ranged from 500 µg to 31.25 µg. As was already indicated, nutrient broth for bacteria and potato dextrose broth for fungi were also employed in the test. As a result, 1 ml of each of these media was divided among 5 tubes and labeled with the respective extract concentrations of 500 µg, 250 µg, 125 µg, 62.5 µg, and 31.25 µg. These tubes were then filled with the appropriate bacterial and fungal pathogen cultures once this was completed for all of the plant extracts. Once the culture was added, the tubes were sealed and incubated at 37°C (for bacteria) or 27°C (for fungi) for the specified amount of time (fungus). In addition to the sample tubes, (+) C culture media for each pathogen were created, along with (-) C uninoculated culture media. The MBC/MFC value was then calculated for all the tubes that had no turbidity or apparent growth, and a 100 µl aliquot from each of these tubes was inoculated on the appropriate medium using the spread plate technique, which is nutrient agar media for bacteria isolates and Potato Dextrose Agar Media for fungal isolates.

RESULTS & DISCUSSION

Antimicrobial activity analysis

The antimicrobial activity of all the nine extracts was evaluated against the plant pathogenic microbes, the bacterial species include *Erwinia carotovora* and *Pseudomonas syringae*, and the fungal species include *Aspergillus niger* and *Sclerotinia sclerotiorum*. All the agar well diffusion plates with different microbes and plant extract are shown in Figures 1, 2, 3, and 4. In figures, P1, P2 and P3 are the plant extract code for *Delonix regia* (Hook.) Raf., *Cassia fistula Linn. and Albizia lebbeck* (L.) Benth. Respectively, (+) C *luliconazole* antifungal agent and (-) C DMSO. In Table 1 zone of inhibition is tabulated for all extracts and microbes.

Fig. 1- Antifungal activity of all the plant extracts against the fungal pathogen *Sclerotinia sclerotiorum*

Fig. 2- Antifungal activity of all the plant extracts against the fungal pathogen *Aspergillus niger*

Fig. 3- Antibacterial activity of all the plant extracts against the bacterial pathogen *Erwinia carotovora*

Fig.4- Antibacterial activity of all the plant extracts against the bacterial pathogen *Pseudomonas syringae*

Table 1: Zone of Inhibition (mm) obtained for all the nine plant extract against the bacterial and fungal pathogens

Solvent Name	Sample loaded	Zone of Inhibition in mm			
		A. niger	*S. sclerotiorum*	*E. carotovora*	*P. syringae*
n- hexane	P1	13	15	12	11
	P2	10	15	11	11
	P3	12	15	11	11
	+C	17	21	25	25
	- C	Nil	Nil	Nil	Nil
Acetone	P1	12	11	11	12
	P2	13	16	12	13
	P3	12	15	13	11
	+C	17	22	26	25
	-C	Nil	Nil	Nil	Nil
Ethanol	P1	13	16	13	14
	P2	14	15	12	11
	P3	13	18	11	13
	+C	17	21	25	25
	-C	Nil	Nil	Nil	Nil

P1, P2, P3 are the plant extract code for *Delonix regia* (Hook.) Raf*., Cassia fistula* Linn. and *Albizia lebbeck* (L.) Benth. Respectively; (+) C Ciprofloxacin antibitioc (bacteria)/luliconazole antifungal agent (fungus) and (-) C DMSO

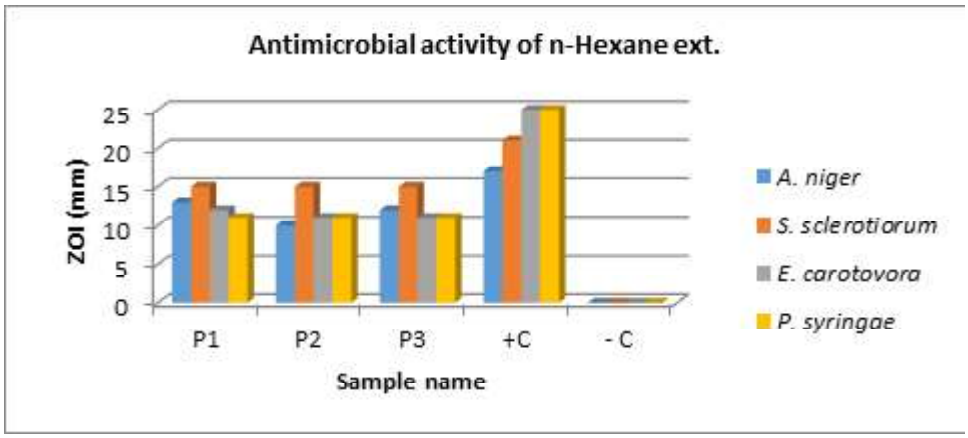

Fig. 5- Graphical representation of the antimicrobial potential of n-Hexane extract of all three plant against bacterial and fungal plant pathogen

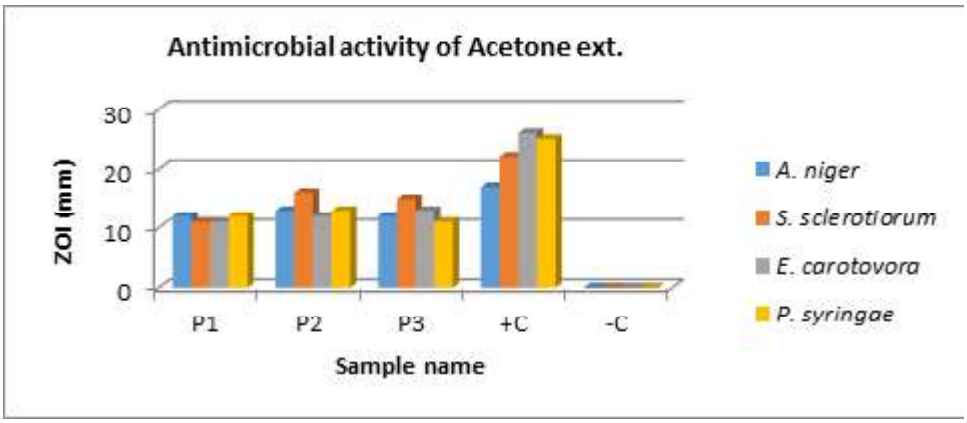

Fig. 6- Graphical representation of the antimicrobial potential of Acetone extract of all three plant against bacterial and fungal plant pathogen

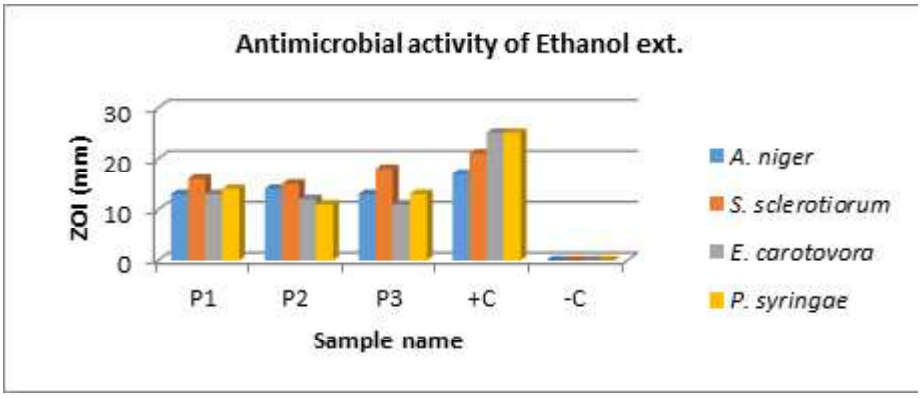

Fig. 7- Graphical representation of the antimicrobial potential of Ethanol extract of all three plant against bacterial and fungal plant pathogen

Analysis of the Minimum Inhibitory concentration (MIC)

The broth dilution method determined the minimum inhibitory concentration (MIC) value for all nine extracts against all the pathogens. This method used nutrient broth for the bacterial isolates and potato dextrose broth for the fungal isolates. MIC and MBC values of all plant extract against selected four microbes are tabulated in Tables 2, 3, 4, and 5. From the tabulated results, we can easily interpret that ethanolic extract of *Delonix regia* has prominent MIC and MBC values against *Erwinia carotovora, Pseudomonas syringae*, *Sclerotinia sclerotiorum*, and *Aspergillus niger*. In the case of *Cassia fistula,* acetone and ethanolic both show the same MIC and MBC against *Erwinia carotovora*; however, its acetone extracts show low MIC and MBC against Pseudomonas syringae, and ethanolic extract show better results against *Aspergillus niger.*

Moreover, acetone extract of *Albizia lebbeck* proved to be more potent against *Erwinia carotovora,* whereas its ethanolic extract showed significant MIC and MFC against *Pseudomonas syringae* and *Aspergillus niger.* Furthermore, *Cassia fistula* acetone extract shows good MIC and MFC against *Sclerotinia sclerotiorum*. Nevertheless, both hexane and acetone of *Albizia lebbeck* show the same and better MIC and MFC values against *Sclerotinia sclerotiorum*.

Table 2: MIC and MBC value of different solvent extracts of each three plant against the bacterial pathogen *Erwinia carotovora*

Plant Name	Solvent name	Concentration of extract (µg)					MIC value	MBC value
		500µg	250µg	125µg	62.5µg	31.25µg		
Delonix regia	n-Hexane	NT	NT	NT	WT	MT	125µg	250µg
	Acetone	NT	WT	MT	HT	HT	500µg	500µg
	Ethanol	NT	NT	NT	WT	MT	125µg	125µg
Cassia fistula	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	NT	NT	WT	MT	125µg	250µg
	Ethanol	NT	NT	NT	WT	MT	125µg	250µg
Albizia lebbeck	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	NT	NT	WT	MT	125µg	125µg
	Ethanol	NT	WT	MT	HT	HT	500µg	500µg

HT= High Turbidity; MT= Moderate Turbidity; WT= Weak Turbidity; NT= No Turbidity (No growth)

Table 3: MIC and MBC value of different solvent extracts of each three plant against the bacterial pathogen *Pseudomonas syringae*

Plant Name	Solvent name	Concentration of extract (µg)					MIC value	MBC value
		500µg	250µg	125µg	62.5µg	31.25µg		
Delonix regia	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	NT	NT	WT	MT	125µg	250µg
	Ethanol	NT	NT	NT	WT	MT	125µg	125µg
Cassia fistula	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	NT	NT	WT	MT	125µg	125µg
	Ethanol	NT	WT	MT	HT	HT	500µg	500µg
Albizia lebbeck	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	WT	MT	HT	HT	500µg	500µg
	Ethanol	NT	NT	NT	WT	MT	125µg	250µg

HT= High Turbidity; MT= Moderate Turbidity; WT= Weak Turbidity; NT= No Turbidity (No growth)

Table 4: MIC and MBC value of different solvent extracts of each three plant against the fungal pathogen *Aspergillus niger*

Plant Name	Solvent name	Concentration of extract (µg)					MIC value	MFC value
		500µg	250µg	125µg	62.5µg	31.25µg		
Delonix regia	n-Hexane	NT	NT	NT	WT	MT	125µg	125µg
	Acetone	NT	NT	WT	MT	HT	250µg	500µg
	Ethanol	NT	NT	NT	WT	MT	125µg	125µg
Cassia fistula	n-Hexane	NT	WT	MT	HT	HT	500µg	500µg
	Acetone	NT	NT	NT	WT	MT	125µg	250µg
	Ethanol	NT	NT	NT	WT	MT	125µg	125µg
Albizia lebbeck	n-Hexane	NT	NT	MT	HT	HT	250µg	500µg
	Acetone	NT	NT	MT	HT	HT	250µg	500µg
	Ethanol	NT	NT	NT	WT	MT	125µg	125µg

HT= High Turbidity; MT= Moderate Turbidity; WT= Weak Turbidity; NT= No Turbidity (No growth)

Table 5: MIC and MBC value of different solvent extracts of each three plant against the fungal pathogen *Sclerotinia sclerotiorum*

Plant Name	Solvent name	Concentration of extract (µg)					MIC value	MFC value
		500µg	250µg	125µg	62.5µg	31.25µg		
Delonix regia	n-Hexane	NT	NT	NT	NT	WT	62.5µg	125µg
	Acetone	NT	WT	MT	HT	HT	500µg	500µg
	Ethanol	NT	NT	NT	NT	WT	62.5µg	62.5µg
Cassia fistula	n-Hexane	NT	NT	NT	NT	WT	62.5µg	125µg
	Acetone	NT	NT	NT	NT	WT	62.5µg	62.5µg
	Ethanol	NT	NT	NT	NT	WT	62.5µg	125µg
Albizia lebbeck	n-Hexane	NT	NT	NT	NT	WT	62.5µg	62.5µg
	Acetone	NT	NT	NT	NT	WT	62.5µg	62.5µg
	Ethanol	NT	NT	NT	NT	NT	31.25µg	31.25µg

HT= High Turbidity; MT= Moderate Turbidity; WT= Weak Turbidity; NT= No Turbidity (No growth)

CONCLUSION

Medicinal plants *Cassia fistula* Linn. (Amaltas), *Delonix regia* (Hook.) Raf. (Gulmohar), *Albizia lebbeck* (L.) Benth. (Siris) have been used to treat a variety of diseases in the study area. This study indicated that hexane and acetone extract of all three plants shows the best MIC value against *Sclerotinia sclerotiorum.* Ethanolic extract of all three plants shows significant MIC against *Aspergillus niger.* Also, ethanolic extract of *Delonix regia* (Hook.) Raf. (Gulmohar), *Albizia lebbeck* (L.) Benth. (Siris) shows significant MIC against *Pseudomonas syringae*; however, acetone extracts of *Cassia fistula* Linn. (Amaltas) show effective MIC against *Pseudomonas syringae.* Ethanolic extract of *Delonix regia* (Hook.) Raf. (Gulmohar), Acetone extract of *Albizia lebbeck* (L.) Benth. (Siris), and both acetone and ethanolic extract of *Cassia fistula* Linn. (Amaltas) show promising results against *Erwinia carotovora*. The result here indicates the potential sources of the studied medicinal plants as antimicrobial agents; thus, further, *in vitro* and *in vivo* antimicrobial activity studies are recommended. Isolation of active compounds is also recommended.

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