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Abrus precatorius is a Strong Competitor of ESBL Antibiotics

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Abstract:

Antibiotic resistance has been increased day by day. When the Bacterial infection occurs that time multidrug treatment is used while the drugs resistance bacteria get multiply and continue to spread as a result the person remain sick. Herbal medicines are in great demand for preliminary health care due to their wide medicinal values without any side effects. In present study ESBL producing pathogenic bacteria had shown sensitivity towards *Abrus precatorius* leaves extract.

Key words: *Pathogenic bacteria, Abrus precatorius, ESBL, Clinical samples, Antibiotic sensitivity.*

INTRODUCTION

Microorganism is an organism of microscopic size, which may exist in a single cell. The History of biology traces the study of the living world from ancient to modern times. The pathogenic bacteria is diseases causing bacteria which may leads to death of human being. The pathogenic bacteria like, *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae and Staphylococcus aureus* etc. are causing diseases that can affect your Skin, Lungs, Brain, Blood and Other parts of your Body. Hospitalized patients easily acquire these bacteria and eventually act as a reservoir. Antibiotic resistance of bacteria is commonly seen in daily medical practice with multidrug resistant Gram negative bacteria posing the greatest threat to human health (Baguma *et al.*, 2017).

Over the last 15 years numerous outbreak of infections with organisms producing Extended Spectrum of β-Lactamase have been observed worldwide. The advent of ESBL producers has posed a great threat to the use of many classes of antibiotics particularly cephalosporin. There are indication that poor outcome occurs when patients with serious infection due to ESBL producing organisms are treated with antibiotics to which the organism is resistant (Shukla *et al.*, 2004).

Introduction of the 3rd generation cephalosporin into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-Lactamase mediated bacterial resistance to antibiotics (Paterson & Bonomo, 2005). Antimicrobial resistance had long been regarded as an issue of human health alone, but recent years have witnessed a growing recognition of the imprudent use of antibiotics in multiple sectors (agriculture, food animals, aquaculture, and environment) as important drivers of resistance (Shivraman *et al.*, 2021).

Staphylococcus aureus is a gram-positive bacteria that cause a wide variety of clinical diseases. Infections caused by this pathogen are common both in community-acquired and hospital-acquired settings. The treatment remains challenging due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*). *S. aureus* does not normally cause infection on healthy skin, however, if it is allowed to enter the internal tissues or bloodstream, these bacteria may cause a variety of potentially serious infections. Transmission is typically from direct contact. However, some infections involve other transmission methods (Taylor *et al.*, 2022).

Escherichia coli is a gram-negative bacillus known to be a part of normal intestinal flora but can also be the cause of intestinal and extraintestinal illness in humans. Pathogenic *E. coli* strains each has distinctive virulence factors encoded on plasmids, transposons, and bacteriophages (Mueller *et al.*, 2023).

Pseudomonas aeruginosa is a Gram-negative aerobic rod-shaped bacterium that can be isolated from most environments, including soil, plants, and mammal tissue. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes several infections in humans. It has become an important cause of nosocomial infections and antibiotic resistance. This bacteria related to healthcare infections, including ventilator-associated pneumonia, intensive care unit infections, central line-related blood stream infections, surgical site infections, urinary tract infections, burn wound infections, keratitis, and otitis media. (Tuon *et al.*, 2022).

Proteus vulgaris is a Gram-negative, rod-shaped bacteria and can cause nosocomial infections. These include Urinary tract infections, Respiratory tract infections, Wound infections, Skin infections and Sepsis. The antibiotic resistance of *Proteus vulgaris* against a variety of antibiotic classes has considerably increased in recent years such as resistant to beta-lactams (Northage *et al.*, 2023).

Klebsiella pneumoniae is a gram-negative, encapsulated, non-motile bacterium found in the environment and has been associated with pneumonia in patient populations Today, *K. pneumoniae* pneumonia is considered the most common cause of hospital-acquired pneumonia in the United States, and the organism accounts for 3% to 8% of all nosocomial bacterial infections. Bacteria have a polysaccharide capsule made up of complex acidic polysaccharides and determine their pathogenicity. The capsule protects bacteria from phagocytosis and serum bactericidal proteins. (Ashurst *et al.*, 2023).

In the ayurvedic medicine leaf of *A. precatorius* has the potential as laxative, expectorant and aphrodisiac medicines. Almost all the parts of the *Abrus precatorius* has the anti-bacterial activity (Adelowotan, 2008). It was observed that the extract also resulted in increased levels of total serum protein, albumin, alanine amino transaminase, aspartate amino transferase, alkaline phosphatase and total bilirubin. (Bhakta *et al.*, 2020).

MATERIAL AND METHODS

The present work "*Abrus precatorius* is a competitor of ESBL drugs" was conducted prospectively in the P. G. Department of Microbiology, Ghulam Nabi Azad College of Arts, Commerce and Science, Barshitakli Dist. Akola from September 2022 to April 2023. In assessment to isolate and identify the ESBL producing pathogenic bacteria from Clinical Samples and comparative study with their susceptibility and resistance pattern with various antibiotics and leaf extract of *Abrus precatorius* herbal plant, present work was under taken.

Collection of sample:-

A Total 25 clinical samples were collected from different laboratories, blood in EDTA bulb, pus sample in syringe or cotton swab and urine and sputum samples air tie container and transferred immediately to laboratory for further processing.

Isolation and Identification of Pathogenic Bacteria.

- **Propagation of Samples:** Samples were transferred in 0.5 ml of Nutrient Broth for enrichment and incubated at 37^{o}C for 24 hours. Culture medium supports the growth of bacteria (Kaye *et al*, 2003).
- **Plating of Enriched Sample:** All the samples after enrichment were compared with the control tube. Prior incubation loopful of each enriched culture was then inoculated on the plates of Nutrient media (Hi-media, Mumbai) agar used for the growth of several bacteria. All the samples were inoculated by striking method. After inoculation all the plates were kept for incubation aerobically at 37 ^{o}C for 24 hours.
- **Cultural characterization (Plating on Selective Media):** Colonies of relevant pathogens, with different morphological characters and biochemical characters were selected to analyze their cultural properties and inoculated on respective selective media viz. Blood agar, Nutrient agar, Mannitol salt agar, EMB agar (Eosin Methylene Blue), MacConkey agar. All the plates were incubated at 37°C for 24 hours. All culture media were procured from Hi-media, Mumbai.
- **Maintenance and preservation of culture strains:** Organisms grown in appropriate media for 18 hrs were preserved in a nutrient agar slant at 4°C in a refrigerator and this culture was used for routine laboratory works.

Morphological Characterization:

As per the standard literature (Bergey's manual of Systematic bacteriology, 2nd edition, 1984), next day all the typical colonies on Nutrient media agar were screened for colony characteristics and examined microscopically for Gram character using Gram's staining method as well as motility testing using hanging drop method.

Biochemical characterization:

All the pathogenic bacteria were then subjected for conventional biochemical analysis as per the following table (Bergey's manual of Systematic bacteriology, 2nd edition, 1984; Dubey and Maheswari, 2006).

Antimicrobial susceptibility testing:

After isolation and identification, the pathogenic bacteria were subjected for antibiogram. The antimicrobial susceptibility testing was done by the agar Disk Diffusion Method as described by NCCLS 2002, and Kirby Bauer disk diffusion method, now known as the Clinical and Laboratory Standards Institute (CLSI) (Bauer *et al*., 1966; *CLSI*, 2015; Jain and Kamble, 2017).

Antibiotics used: Ampicillin (Amp 10mcg), Gentamicin (Gen 10mcg), Penicillin (P 10mcg). Cephalosporine antibiotics as, Cefixime (CFM 05mcg), Cefotaxime (CTX 30mcg). Ciprofloxacin (CIP 5 mcg) and Tetracyclin (Te 30 mcg)

Alternate Remedies to Combat with Drug Resistant pathogenic bacteria from ESBL producing bacteria:

Herbal Remedies

Many hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infections. Plants are prospective source of antimicrobial agents in different countries. Traditionally, crude plant extracts are used as herbal medicine for the treatment of ESBL causing diseases. Plants are rich in a variety of phytochemicals which have been found in vitro to have antimicrobial properties. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses (Khan *et al*., 2013).

Acording to the study of Solanki *et al*., (2012) *Abrus precatorius* is a woody twinning plant with characteristic toxic red seeds with black mark at the base. Leaves resemble tamarind leaves having 20-40 leaflets. It is native to India, at altitudes up to 1200 m on the outer Himalayas but now found in all tropical countries.

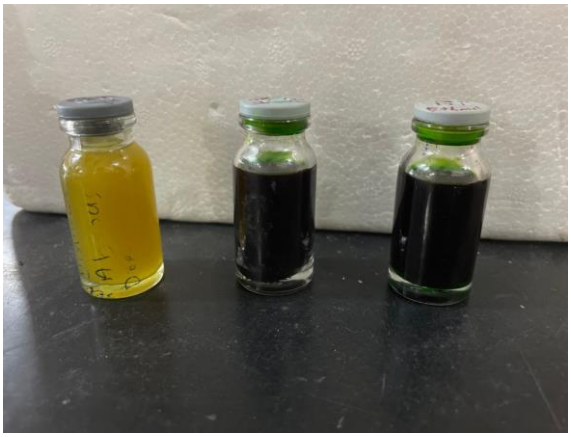
Leaves Constituents of Abrus precatorius:

Several compounds like abrine, trigonelline, abruslactone A, hemiphloin, abrusoside A, abrusoside B, abrusoside D, arabinose, galactose, xylose, choline, hypaphorine, precatorine, glycyrrhizin, montany alcohol, inositol, D monomethyl ether, pinitol are identified in the leaves of *A. precatorious*.

a. Extraction of Plant Material

25g of *Abrus precatorius* leaves were weighed on weighing machine and transferred into a conical flask, 125ml of hot distilled water (100^{o} C) measured using a glass cylinder poured into the conical flask and kept it for 24 hours. The mixture was filtered out with a Whatman No 1 filter paper. The filtrate was then poured into 6 glass petri dishes and allowed to dry in a laboratory dry oven at 45 degree celcius to 70 ^{o}C, to concentrate the extract.

The methanolic and Methanolic extraction of *Abrus precatorius* was done by using the Soxhlet apparatus (Redfern *et al.*, 2014). In-vitro antibacterial activities of *Abrus Pricatorius* herbal leaves extracts were tested against ESBL producing bacteria isolated from different clinical samples by using well diffusion method.



b. Sensitivity testing:

Sensitivity test medium (Hi-media M-296) was used to test the antibacterial activity. Turbidity of the inoculums was matched with McFarland 0.5 turbidity standards. The antimicrobial activity was studied by agar well diffusion method (Perez *et al*., 1990). Wells were cut out in the solid medium with flamed 6mm cork borer equidistantly from each other and in the centre. Extracts of different plant parts (50 micro liter of each) were transferred to the wells. The plates were incubated at 37 ^{o}C for 18-24 hours. Zone of inhibition, if any, were recorded in mm.

RESULTS AND DISCUSSION

Resistance against the ESBL antibiotics is increasing worldwide. It is associated with bacterial infections. Severe bacterial infection can take much time to cure due to the resistant of ESBL antibiotic. Consumption of antibiotic in abundant amount can be causes the other health issue like, habitual to intake drugs, renal diseases, and hypertension and so on. Herbal drugs is most preferable in now a days due to its less side effect.

Sources of clinical sample and distribution of demographic variables

Collection of clinical sample:

A total 25 samples were collected during period of September 2022 to February 2023 from clinical laboratory with different age group and gender. Out of 25 samples 18 samples were found to be positive for bacterial infection and total 19 isolates were isolated.

Demographic study

ESBL producing bacterial infection is very common in hospital admitted patient. The various antibiotic is used against the bacterial infection which get severe day by day. The study aimed to determine the prevalence of ESBL producing bacterial infection by considering different demographic variables like age and gender.

Table 1: Demographic study

No. of sample	Type of sample	Area	Location	Age in years	Gender
1	Blood	Urban	Lab	40	Female
2	Blood	Urban	Lab	34	Male
3	Blood	Urban	Lab	45	Female
4	Blood	Urban	Lab	52	Female
5	Pus	Urban	Hospital	27	Female
6	Pus	Urban	Hospital	26	Male
7	Pus	Urban	Hospital	28	Male
8	Pus	Urban	Domestic	23	Female
9	Blood	Rural	Lab	36	Male
10	Blood	Rural	Lab	45	Male
11	Blood	Rural	Lab	53	Male
12	Blood	Rural	Lab	25	Female
13	Urine	Rural	Lab	35	Female
14	Urine	Rural	Lab	28	Female
15	Urine	Rural	Hospital	35	Male
16	Urine	Urban	Domestic	26	Female
17	Sputum	Urban	Hospital	56	Female
18	Sputum	Urban	Hospital	46	Male

19	Sputum	Urban	Hospital	25	Male
20	Pus	Rural	Domestic	23	Male
21	Urine	Rural	Lab	26	Female
22	Blood	Urban	Hospital	42	Male
23	Blood	Urban	Hospital	29	Female
24	Blood	Urban	Hospital	80	Male
25	Blood	Urban	Hospital	45	Male

Collection of samples

Isolation and identification of pathogenic bacteria from clinical sample by phenotypic methods.

Morphological and cultural characteristic of bacterial isolates

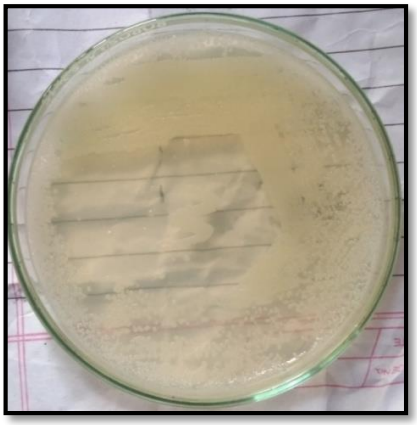
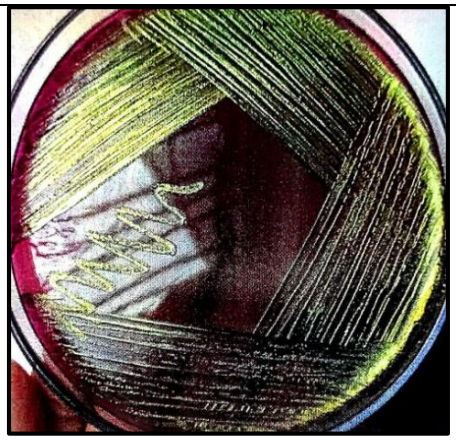
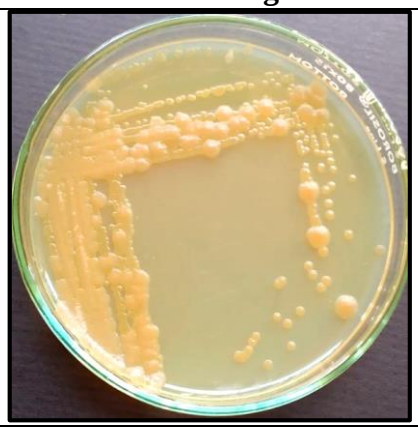
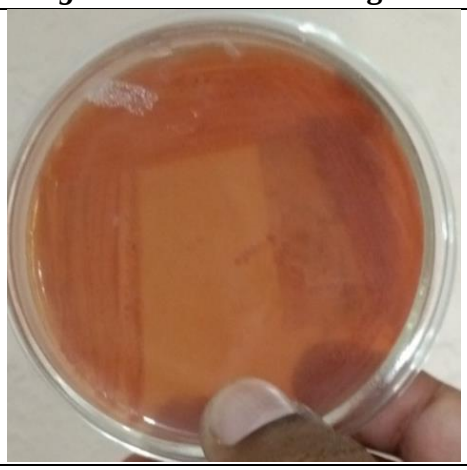
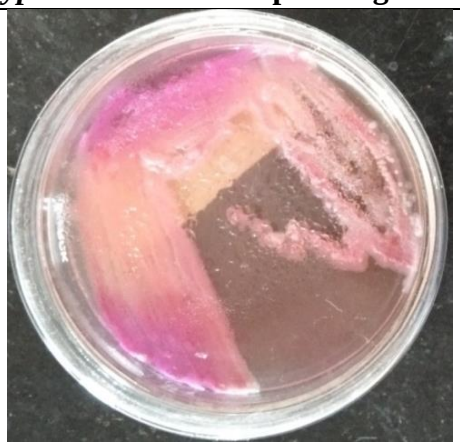
Table 2: Morphological Characteristics of bacterial isolates

Sr. No.	**Character**	**Bacteria coded as**					
		A	**B**	**C**	**D**	**E**	**F**
1	Size	1-4mm	1-3mm	1-3mm	1-3mm	2-3mm	2-3mm
2	Shape	Circular	Circular	Circular	Circular	Circular	Circular
3	Margin	Regular	Regular	Irregular	Entire	Entire	Irregular
4	Elevation	Convex	Convex	Flat	Convex	Convex	Convex
5	Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Sticky
6	Opacity	Opaque	Tranlucent-Opaque	Opaque	Tranlucent	Tranlucent	Tranlucent
7	Gram's character	Gm+ve cocci	Gm-ve rod	Gm-ve rod	Gm-ve rod	Gm-ve rod	Gm-ve rod
8	Motility	Non motile	Motile	Motile	Motile	Motile	Non motile

Table 3: Cultural characteristics

Sr. No	Media	A	B	C	D	E	F
1	Nutrient agar	Golden yellow	Off white	Greenish	Pale yellow	Mudy white	White mucoid
2	MacConkey agar	Yellow	Pink	-	Colourless	-	Pink mucoid
3	EMB agar	-	Green Metallic Sheen	-	-	-	-
4	MSA agar	Yellow	-	-	-	-	-
5	Cetrimide agar	-	-	Yellow Greenish	-	-	-
6	BSA agar	-	-	-	-	Black coloured	-

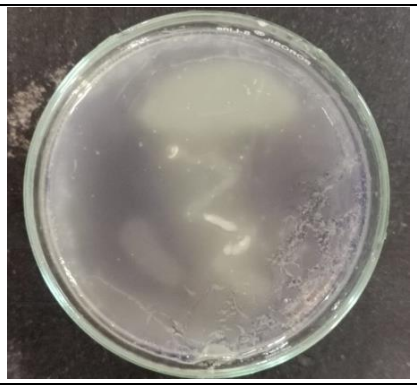
Isolation and Identification of Isolates

	
***S. aureus* on Nutrient Agar**	***E. coli* on EMB agar**
	
***P. aureginosa* on Cetrimide Agar**	***S. typhi* on Bismuth sulphate agar**
	
***P. vulgaris* on MacConkey Agar**	***K. pneumoniae* on MacConkey Agar**

Biochemical characteristics of bacterial isolates

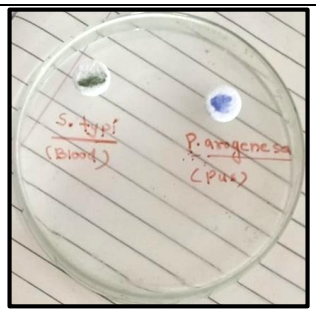
Table 4: Sugar fermentation

Test		A	B	C	D	E	F
Sugar							
Glucose	Acid	+ve	+ve	+ve	+ve	+ve	+ve
	Gas	-ve	+ve	-ve	+ve	+ve	+ve
Lactose	Acid	+ve	+ve	-ve	-ve	-ve	+ve
	Gas	-ve	+ve	-ve	-ve	-ve	+ve
Mannitol	Acid	+ve	+ve	+ve	-ve	+ve	+ve
	Gas	-ve	+ve	+ve	-ve	+ve	+ve
IMViC test							
Indole		-ve	+ve	-ve	+ve	-ve	-ve
MR		+ve	+ve	-ve	+ve	+ve	-ve
VP		+ve	-ve	-ve	-ve	-ve	+ve
Citrate		+ve	-ve	+ve	-ve	-ve	+ve
Enzyme test							
Coagulase		+ve	-ve	-ve	-ve	-ve	-ve
Catalase		+ve	+ve	+ve	+ve	+ve	+ve
Oxidase		-ve	-ve	+ve	-ve	-ve	-ve
Urease		-ve	-ve	-ve	+ve	-ve	+ve
Amylase		-ve	+ve	-ve	-ve	-ve	-ve
Hemolysis		γ	γ	γ	α	γ	β

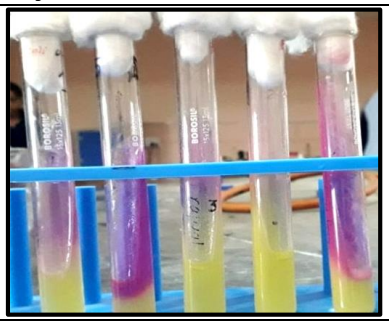


Amylase Test

β-hemolysin



Oxidase Test

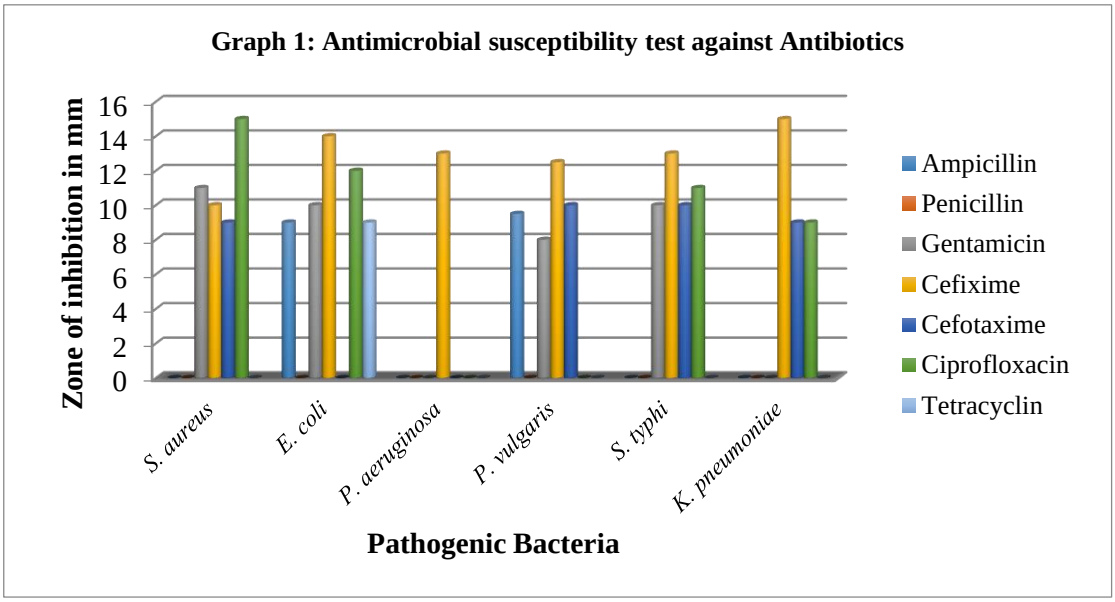


Urease Test

Table 5:- Frequency Distribution of Isolated Organisms

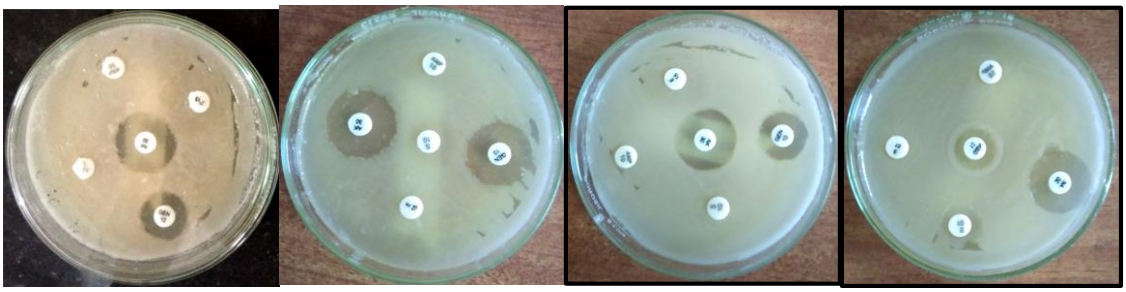
Sr. No.	Isolated organism	No of isolates	% of isolates	code
1	*Staphylococcus aureus*	1	4 %	A
2	*Escherichia coli*	11	44 %	B
3	*Pseudomonas aeruginosa*	2	8 %	C
4	*Protus vulgaris*	1	4 %	D
5	*Salmonella tpyhi*	2	8 %	E
6	*Klebsiella pneumoniae*	2	8 %	F

Antimicrobial susceptibility testing:

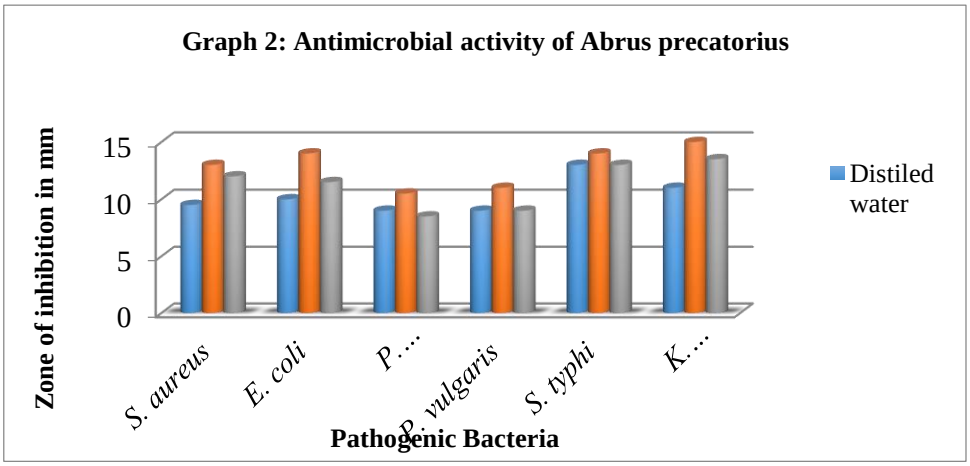


In present study *Staphylococcus aureus* isolates were highly resistant to Ampicillin, Penicillin and Tetracyclin, intermediately sensitive towards Gentamycin, Cifixime and Cifotaxime and highly sensitive to Ciprofloxacin. *Escherichia coli* isolates were highly resistant towards Penicillin and Cefotaxime, intermediately sensitive towards Ampicillin, Gentamycin, Ciprofloxacin and Tetracyclin while Cifixime were sensitive. *Pseudomonas aeruginosa* isolates were highly resistant towards Ampicillin, Penicillin, Gentamycin, Cefotaxime, Ciprofloxacin and Tetracyclin were sensitive towards Cifixime.

Protus vulgaris isolated were intermediate sensitive towards Ampicillin and Cefotaxime were sensitive towards Cifixime and highly resistant towards Penicillin, Gentamycin, Ciprofloxacin and Tetracyclin. *Salmonella tpyhi* were isolated sensitive towards Cifixime and highly resistant towards Ampicillin, Penicillin, and Tetracyclin were intermediate sensitive towards Gentamycin, Cefotaxime and Ciprofloxacin. *Klebsiella pneumoniae* were isolated highly resistant towards Ampicillin, Penicillin, Gentamycin and Tetracyclin isolated were sensitive towards Cifixime and intermediate sensitive towards Cefotaxime and Ciprofloxacin.



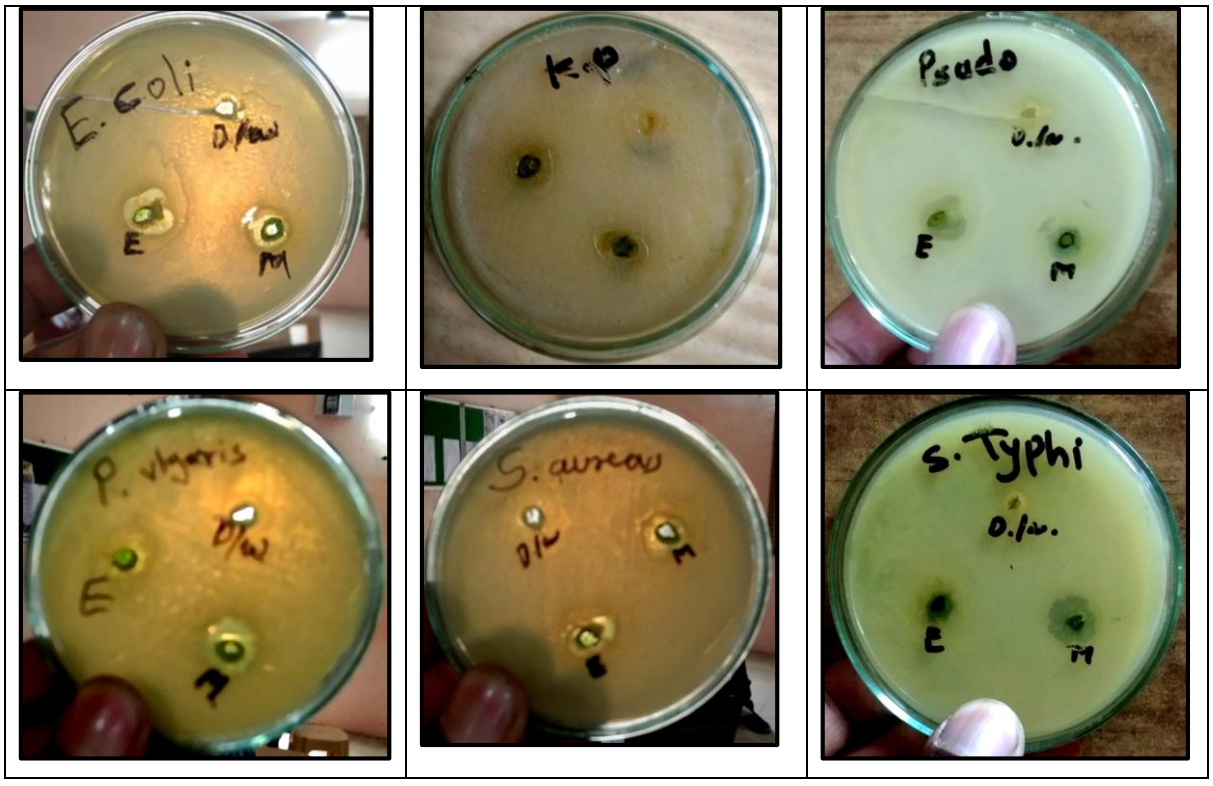
Antimicrobial activity of herbal extracts:



Our country is known as a hub of medicinal plant. Plants are prospective source of antimicrobial agents in different countries. Herbal plant extracts are used as herbal medicine for the treatment of ESBL Producing bacterial infection. The *Abrus precatorius* plant extracts were used against pathogenic bacteria.

In present study, all the isolates were found to be sensitive toward the extract of *Abrus precatorius*. Consider the zone of inhibition with more than 8mm because the Ethanol and Methanol both are shown the 8mm zone against the all the isolates. So we consider as the affective on all the bacteria with more than 8mm zone.

Antimicrobial Activity against *Abrus precatorius* Extract



DISCUSSION

The 3rd generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against B-Lactamase mediated bacterial resistance to antibiotics. (Paterson & Bonomo *et. al*, 2005). Common antibiotics are used against various types of Pathogenic bacterial infection. Especially antibiotics group like cephalosporins, penicillin, B-lactam and Aminoglycoside this is used against the ESBL producing bacterial infection. More resistance are shown in my study then we did the comparative study between ESBL antibiotics and herbal plant. Herbal plant is shown the more sensitivity against the ESBL producing bacteria. Antimicrobial resistance had long been regarded as an issue of human health alone, but recent years have witnessed a growing recognition of the imprudent use of antibiotics in multiple sectors (agriculture, food animals, aquaculture, and environment) as important drivers of resistance (Shivraman *et al.*, 2021).

The treatment remains challenging due to the emergence of multi-drug resistant. According to the Taylor *et al.,* 2022 study showed the strains such as Methicillin-Resistant *Staphylococcus aureus*. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes several infections in humans by the Tuon *et., al* 2022. The antibiotic resistance of *Proteus vulgaris* against a variety of antibiotic classes has considerably increased in recent years shows in the Olympus *et al.,* (2023). *Salmonella typhi* resistant to all MDR strains. Multiple resistance has been encoded by plasmids of the H1 incompatibility group shows in the study of Rowe *et al* (2023). The alternate pathway of complement activation is more active in *Klebsiella pneumoniae* infection shows by the Ashurst *et al.,* (2023). All the problems has only solution that is shows the effect of *Abrus precatorius* is against the pathogenic bacteria is very effective than ESBL antibiotics and other antibiotic. The *Abrus precatorius* is sensitve and intermediate sensitive on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi* and *Klebsiella pneumoniae.*

In our study *Staphylococcus aureus* were resistant to Ampicillin, Penicillin and Tetracyclin but with *Abrus precatorius* it gives sensitive. As other pathogenic bacteria like, *Escherichia coli* resistant to Penicillin and Cefotaxime, as compare to *Abrus precatorius* it gives sensitive. *Pseudomonas aeruginosa* resistant to Ampicillin, Penicillin, Gentamycin, Cefotaxime, Ciprofloxacin and Tetracyclin, this is the most resistant pathogenic bacteria but with *Abrus precatorius* it also shows sensitive result. Proteus vulgaris shows resistant towards Penicillin, Gentamycin, Ciprofloxacin and Tetracyclin. On other hand with *Abrus precatorius* it gives effective on it. *Klebsiella pneumoniae* were resistant to Ampicillin, Penicillin, Gentamycin and Tetracyclin. Instead of this antibiotic *Abrus precatorius* is also effective so it is an alternative antibiotic of ESBL producing pathogenic bacteria.

It is regrettable that resistance to ciprofloxacin has now emerged in MDR *S. typhi* (Rowe *et al.,* 2023) and in our study we found resistance against Ampicillin, Penicillin, and Tetracyclin. In our study, antimicrobial activity of *Abrus precatorius* by distilled water, ethanol, and methanol was effective against ESBL producing clinical isolates of bacteria.

CONCLUSION

Antibiotic resistance is a serious problem throughout the world wide. Extended Spectrum of Beta-Lactamase enzyme producing pathogenic bacteria which causing diseases that leads to affect the mortality. This bacterial infection is a wild spread disease to cure the infection antibiotic is not effect fully on the infection so the resistance against the bacterial infection goes to increasing. The relatively high prevalence reported in this study was clearly indicated lack of treatment against the ESBL causing bacterial infection.

The Herbal drugs are effective as antibiotic, so the herbal drugs is a competitor of ESBL antibiotics. India is the hub of herbal plant, there is a less amount of side effect on herbal drugs as compare to antibiotic.

In our study the resistance against the ESBL producing bacteria is increasing by the antibiotics as cephalosporin group antibiotic, penicillin, which is used as the treatment on pathogenic bacterial infection. The Antimicrobial activity of *Abrus precatorius* is shows the sensitive or effective on the ESBL producing pathogenic bacterial infection. Instead of using Antibiotics the leaves Herbal drug (Extract of *Abrus precatorius*) is effective or less harmful for human health.

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