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Isolation and identification of UTI bacteria and inhibition of their growth by some herbal extracts

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ABSTRACT

Urinary tract infections (UTIs) are caused by bacteria and are 10 times more common among women than men. More than 50% of women will have at least one UTI during their lifetime; for most of these infections, patients need to see a doctor and be treated with antibiotics[1]. About 30 - 40% of UTIs recur within 6 months after the initial episode. When UTIs do recur, it is often because the treatments used to suppress bacteria seem to work at first, but they do not produce a lasting cure[2]. UTIs can also recur if a woman is infected by different bacteria.

Signs of a UTI might include an urgency to urinate, pain when urinating, scalding urine, pelvic aching, lower/ middle back pain, fever and malaise[3]. UTIs have become increasingly 'antibiotic resistant', subsequently making herbal therapies critical. While upper UTIs (kidneys/ureters) are potentially serious, typically requiring medical attention, lower UTIs (bladder/urethra) are common and usually respond well to herbal therapies[4]. Treating a lower UTI early can help prevent it from advancing into an upper UTI[5]. During the present study 40 urine samples were collected from lab Meerut. Out of 40 urine samples, 30 (75%) wrer found positive for urinary tract infection. Out of the 30 infected samples 20 (66.7%) were female patients and 10(33.3%) samples were male patients. 58 bacterial strains were isolated from 30 urine samples. Different strains of E,coli, Klebsiella, Pseudomonas,Proteus, Staphylococcus, Enterobacter and Strepotococcus were isolated from the samples.

KEYWORDS

Urinary tract infection;
Colony forming units;
Biochemical tests.

INTRODUCTION

Urinary tract infections (UTI)

The 'urinary tract' consist of the various organs include the kidney, the ureters; the bladder and the urethra[6]. Urine cultures and pus cells counting diagnose infections. Our kidneys are chemical filters for our blood. About one-quarter of the blood pumped by the heart goes through kidneys[7]. The kidneys filter. This blood, and the filtrate is processed to separate out waste product and excess amounts of minerals, sugar, and other chemicals. The waste products and 'extras' make up the urine, which flows through 'ureters' (one per kidney) into the bladder, where it is held until you are ready

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to get rid of it. When you urinate, muscles in the bladder wall help push urine out of bladder, through the urethra, and out. When you are not urinate (which is most of time) a muscles called 'sphincter' squeezes the urethra shut to keep urine in, the sphincter relaxes when you urinate so that urine can flow[1,8].

Usually several things keep bacteria out of urine. These include the urethral sphincter: when the urethra is squeezed shut, bacteria can't climb up the urethra from the 'meauts' (the outside opening) into the bladder[9].

The length of urethra: it's a long way up to the bladder for a bacterium. -(Since a women's urethra is shorter than a man's, women are much more likely than men to get urinary tract infection).

Frequent washing: any bacteria that make it into the urethra are flushed out the next time you urinate, and since your bladder empties almost completely when you do urinate any bacteria that get that far will be flushed out too[11]. Furthermore, there are valve at the points where the ureters enters the bladder to prevent urine from 'refluxing' from the bladder to the kidneys, so even if the bladder and it's urine infected the bacteria shouldn't travels up to the kidney. The most common part of the urinary tract to get infection is bladder. Cystitis is more common in women than in man because the tube leading from the bladder to the outside (known as urethra) is shorter in women than in men[9,10]. Urinary tract infectio'n usually o'ccurs when-bacteria enter the opening of the. Urethra and multiply in the urinary tract. Men, women and children develop UTI[12]. Women are especially prone to UTIs for reason that is poorly understood. One woman in five develops a UTI during her lifetime. UTIs in men are not so common, but they can be very serious when they do occur[13].

Types of UTI

Urinary tract infections usually^ develop first in the lower urinary tract (urethra, bladder) and, if not treated progress to the upper urinary tract (ureters, kidneys)[14]. Bladder infection (cystitis) is by far the most common UTI. Infection of the urethra is called urethritis[15]: Kidney infection (pylonephritis) requires urgent treatment and can lead to reduced kidney function and possibly even death in untreated, severe cases[16].

Incidence and prevalence

UTIs occur in as many as 5% of girls and 1 to 2% of boys. The incidence of UTIjn infantsranges from approximately 0.1 to 1.0% in all newborn infants to as high as 10% in low birth weight infants[17]. Infection of the urinary tract before age one occurs more frequentlyin boys than girls. After age one both Bacteriuria and UTI are' more common in girls[1]. Some risk factors for urinary tract infection in,younger children include poor hygiene, and the use of soap or bubble bath that irritate, the urethra. Different types of bacteria such as *E. coli, Klebsiella, Enterobacter,* and *Proteus* may cause UTIs[18].

Causes of UTI

Normal urine is sterile. It contains fluids, salt, and waste products, but free of bacteria, viruses, and fungi. An infection occurs when microorganisms, usually bacteria from the digestive tract, cling to the opening of urethra and begin to multiply[19]. Most of the bacteria. that cause urinary tract infections are derived from the patient's normal flora. Often urinary tract infections are caused by coliforms bacteria such as *E. coli,* found in the commensal flora of the bowel[20].

Sexual intercourse triggers UTI in some women, for unknown reasons. The introduction of a urinary catheter greatly increases the risk of the patient developing an infection. Neurological problems that affect bladder emptying, including spina bifida and multiple sclerosis, are associated with an increased risk of urinary tract infections[21]. Recently, researchers found that women whose partners use a condom with spermicidal foam also tend to have growth *ofE. coli* in the vagina[22].

Risk factors

Bladder outlet obstructions *(e.g.* kidney stones, BPH)[23].

- Conditions that cause incompletes bladder emptying *(e.g.* spinal cord injury).
- Congenital (present at birth) abnormalities of the urinary tract *(e.g.* vasocoureteral Regulax).
- Suppressed immune system.
- Being uncircumcised.
- Abnormalities of urinary tract function *(e.g.*, indwelling catheter, neurological bladder, vesicoureteric refluxes, anatomical abnormalities).
- Incomplete bladder emptying *(e.g.*, obstruction in men with prostatic enlargement, Chronic indwelling catheter).
- Previous urinary tract surgery.
- Anal intercourse, (www. Urologychannel.com\uti\

indexhtml 14 may 2004).

Agents that causes UTI

The causative agents of urinary tract infections in hospitalized infection show a different distribution from those that occur in the community.

Hospital patients

(i) *Escherichia coli:* 40%
(ii) Coagulase- negative *Staphylococci:* 3%
(iii) Other Gram- negative bacteria: 25%
(iv) Other Gram-positive bacteria: 16%
(v) *Candida albicans: 5%*
(vi) *Proteus mirabilis:* 11%

Community acquired urinary tract infections

(i) *$FEscherichia coli*; 80%
(ii) Coagulase- negative *Staphylococci:* 7%
(iii) Other Gram- negative bacteria: 4%
(iv) Other Gram- positive bacteria: 3%
(y) *Proteus mirabilis:* 6%

Gram-negative bacteria other than *Escherichia coli* causing urinary tract infections, particularly in hospitalized patients, commonly include *Klebsiella* spp., *Enterobacter* spp.[24] *Serratia* spp. and *Pseudomonas aeruginosa*.

***IM.* site of UTI**

(a) Urethra

Urethritis is the infection inflammation of the urethra. Bacteria present in stool, which more often than not are found on the skin near the meatus cause urethritis. Many sexuallytransmitted diseases initially appear to be urethritis *(e.g.* Chlamydia, Gonorrhoea)[25].

(b) Urinary bladder

Cystitis is an infection of the urinary bladder. This is the most common form of UTI. It can be aggravated further if the' bladder does not empty completely when an individual urinates.

(c) Ureter

Uriteritis is an infection of the ureters. This can occur if the bacteria enter the ureter from above or if the valve between ureter and the urinary bladd.er malfunction, allowing the urine to reflux (spill over) from the bladder into the ureters.

(d) Kidney

Pylonephritis is an infection of the kidney itself. This happens when an untreated systematic infection infects the kidney. In rare case the reflux (spillage) into the ureter from the bladder is so bad that the infected urine refluxes all the way up to the kidney.

Starting of UTI

The urinary tract infections can start from up to down (descending) or down to up (ascending). 95% of the UTI are, of ascending type and approximately 5% are of descending type *e.* the bacteria enter the kidneys from the blood stream and infect the renal parenchyma and travel down to urethra[26].

In the ascending infection the bacteria enter the urethra and travel upward to kidneys. The descending type of infection is more often seen in the newborns with a generalized infection (sepsis). Since in neonates the kidney filters are still immature, if there are many bacteria in1 the blood stream some are likely to get through the filters of the kidney into the urine subsequently infected the ureters, bladder and the urethra. In older children and adult, infections generally start from below, where organism colonies the periuretheral area before infection, and then move up.

In children using diapers, stool (largely bacteria) can sit for sometime right at the bottom of penile foreskin of males or the opening of the vagina in females. The bacteria; may then enter the urethra causing infection[27].

Older girls\women may become prone to UTI is through wiping back to front[28]. This pulls stool into the vaginal area, causing colonization and subsequent urinary tract infection[29].

Sexually active teenage and adult women are prone to UTI since due to the friction, during intercourse; the bacteria present at the male or female meatus are pushed deeper into the vagina[30,31].

Complications from a UTI

While uncomplicated UTIs by themselves cause enough misery like fever, chills and pain, if left untreated a UTI can become more serious than a set Of uncomfortable symptoms[32].

An untreated UTI can lead to the following complications:

(i) Acute or chronic infection of the kidneys (pylonephritis)
(ii) The bacteria that infect the kidneys enter the blood stream causing life-threatening infections like sep-

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ticemia or septic shock[33].

(iii) Chronic kidney disease may lead to the kidneys becoming permanently damaged thereby scarring the pressure regulation, tissues. Dysfunction of the blood pressure mechanism can lead to strokes, heart disease and various irreversible organ pathologies.

(iv) Due to the impairment of the filtration abilities of the kidney, fluid is retained into the body. Fluid retention leads to the toxic waste accumulation within the body. This may disable the patient permanently and make him depend on dialysis or kidney transplants, (v) Pregnant mothers who have UTI are at increased risk of delivering low birth weight or premature babies[34].

(vi) Much of the renal damage from urinary tract infections occurs in young children of the preschool age group. If the treatment is not initiated immediately it would lead to life long kidney complication[35].

MATERIALS AND METHODS

Site of investigation

The samples were collected from Kapil Seth pathology lab, situated at Begum Bride road, Meerut.

Sterilization of glassware

All the glassware were washed thoroughly and were sterilized by autoclaving at 15 pa for 15 minutes.the glasswares were then heated at 16^{O}c and dried in oven.

Preparation of media

During the present study the following media were used

1. Nutrient broth
2. Nutrient agar medium
3. Mc Conkey agar medium
4. Eosin methylene blue agar

Quantitave analysis of urine sample

Pour plate dilution method was used for quantitative estimation of bacteriuria In this method original urine sample was serially diluted (ten fold) in 0.9% saline in sterile screw capped tubes to get dilution 10^{-1} to 10^{-4}, Each of these dilution was poured on the surface of nutrient agar plates for isolation of colonies.

Bacteriruria per ml of urine= no of colonies x dilution factor

Isolation of bacteria from urine samples

One loopfull of urine sample was streaked on the surface of nutrient agar plates and Macconkey agar plates and incubated at 37^{0}c for 24 hours.

Identification of bacteria

Identification of isolated bacteria was done with the hewlp of colony morphology, microscopic examination and biochemical characterization.

Various biochemical test permormed were

- Catalase test
- Oxidase test
- Urease test
- IMViC test
- Indole test,Methyl red test, voges Proskauer test,Citrate utilization test.
- Carbohydrate fermentation test
- H_2S production test

Effect of herbal extracts on growth of isolated bacteria

Following herbal extracts were selected to observe the effect of the herbal extract on the growth of UTI bacteria.

1. Garlic (Alliium Sativum)
2. Khadir extract (Acrcia catechn)
3. Nee, Bark extract (Azardiea indica)
4. Black Pepper extract (Piper nigrum)
5. Neergurnde (Vitex nigundio)
6. Neem Leaf extract (Azardica indica)
7. Lemon (Citus orentifolium)
8. Neem (Azardiea indica)

Preparation of herbal extract

10gm of the herb sample was taken, fresh weight or dry weight, it is grinded and mixed with 100ml of sterilized water. Then it is centrifuged at 10000 rpm for 10 min and preserved at 4^{0}C in sterilized vials.

Agar well diffusion method

To observe the effect of herbal extract on UTI bacteria agar well diffusion method was used.

Nutrient agar plates were prepared and the liquid culture of isolated bacteria was spreded on the surface of the agar plate. Wells were cut with the help of cork borer (6mm) in the nutrient agar plates and the wells wre filled with 50 µl of crude and dilutions of the herbal extract. The control was filled with sterilized

distilled water and then these plates were incubated at 37^0 C for 24h. These zone of inhibition was observed around the well were measured in mm with the help of scale.

RESULTS AND DISCUSSION

During the present study 40 urine samples were collected from Kapil Seth pathology lab. Meerut. Out of these 40 urine samples, 30 (75%) were-found to be positive for urinary tract infection. Out of these 30 infected samples 20 (66.7%) samples were of female patients and 10 (33.3%) samples were of male patients (TABLE 1). Sexvise break-up revealed that females are more prone to urinary tract infections as compared to male: Our study is similar to Obi *et.al,* (1996) who also confirmed that urinary tract infections are common in females as compared to male.

TABLE 1 : Morphological characteristics of bacteria isolated from urine sample.

S.No	Bacterial isolate	Colony colour	Colony shape	Elevation	Size (mn
1.	£co/f CCSUB93	White	Circular	Convex	1.0
2.	*Pseudomonas aeruginosa* CCSUB 94	White	Circular	Convex	1.0
3.	*Proteus vulgaris* CCSUB 95	White	Circular	Convex	1.0
4.	*Klebsiella pneumoniae* CCSUB 96	White	Circular	Convex	1.0
5.	*Staphylococcus aureusCC$>\JB97*	White	Circular	Convex	1.0
6.	*Staphylococcus* spp. CCSUB 98	White	Irregular	Convex	3.0
7.	*Enterobacter-aeruginosa* CCSUB 99	White	Irregular	Raised	3.0
8.	*Streptococcus* spp. CCSUB 100	Fluorescent	Irregular	Flat	2.0

TABLE 2 : Microscopic characterization of bacteria isolated from urine samples.

S. No.	Bacterial isolate	Gram's staining	Motility
1.	*Kcoli* CCSUB93	-ve, rod	Yes
2.	*Pseudomonas aeruginosaCCS\JB94*	-ve,rod	Yes
3.	*Proteus vulgaris* CCSUB 95	-ve, rod	Yes
4.	*Klebsiella pneumoniae* CCSUB 96	-ve, rod	Yes
5.	*Staphylococcus aureus* CCSUB 97	-ve, rod	Yes
6.	*Staphylococcus* spp. CCSUB 98	-ve, rod	Yes
7.	*Enterobacter aeruginosa* CCSUB 99	-ve, rod	No
8.	*Streptococcus* spp. CCSUB 100	-ve, rod'	No

TABLE 3 :IMViC test for the identification of bacteria isolated from urine samples.

S. No	Bacterial isolate	Indole	MR	VP	Citrate utilization
1.	*E.coli* CCSUB93	+ve.	-ve	-ve	-ve
2.	*Pseudomonas aeruginosa CCSUB* 94	+ve	-ve	-ve	-ve
3.	*Proteus vulgaris* CCSUB 95	+ve	-ve	-ve	-ve
4.	*Klebsiella pneumoniae* CCSUB 96	+ve	-ve	-ve	-ve
5.	*Staphylococcus 'aureus* CCSUB 97	+ve	-ve	-ve	-ve
6.	*Staphylococcus* spp. CCSUB 98	+ve	+ve	-ve	-ve
7.	*Enterobacter aeruginosa* CCSUB 99	-ve	-ve	+ve	+ve
8.	*Streptococcus* spp. CCSUB 100	-ve	-ve	-ve	+ve

TABLE 4 : Sugar fermentation tests for the identification of bacteria isolated from urine samples.

S.No.	Bacterial isolate	Lactose	Dextrose	Sucrose
1.	*E. coli* CCSUB93	AG	AG	A
2.	*Pseudomonas aeruginos*CCSU94	AG	AG	A
3.	*Proteus vulgaris* CCSUB 95	AG	AG	A
4.	*Klebsiella Pneumoniae* CCSUB 96	AG	AG	A
5.	*Staphylococcus aureus* CCSUB 97	AG	AG	A
6.	*Staphylococcus* spp. CCSUB 98		AG	AG
7.	*Enterobacter aeruginosa* CCSUB 99	AG	AG	AG
8.	*Streptococcus* spp. CCSUB 100	-	-	-

AG = Acid gas, A= Acid

TABLE 5 : Biochemical tests for the identification of bacteriaisolated from urine samples.

S.No.	Bacterial isolate	Catalase	Oxidase	Urease	H2S
1.	*E. coli* CCSUB93	+ve	-ve	-ye	-ve
2.	*Pseudomonas aeruginosa* CCSUB 94	+ve.	-ve	-ve	-ve
3.	*Proteus vulgaris* CCSUB 95	+ve	-ve	-ve	-ve
4.	*Klebsiella pneumoniae* CCSUB 96_	+ve	-ve	-ve	-ve
5.	*Staphylococcus aureus* CCSUB 97	+ve	-ve	-ve	-ve
6.	*Staphylococcus* spp. CCSUB 98	+ve	-ve	+ve	+ve
7.	*Enterobacter aeruginosa* CCSUB 99	+ve	-ve	+ve	-ve
8.	*Streptococcus* spp. 'CCSUB 100	+ve	-ve	-ve	-ve

We isolated 58 bacterial strains from these 30 urine samples. Different strains of*E.coli, Klebsiella, Pseudomonas, Proteus, Staphylococcus,*

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Enterobacter and *Streptococcus* were isolated from these urine samples.

During our study *E.coli* has been found the predominant organism isolated from urine sample 80% of the samples were positive for *E.coli.* No significant change has occurred in this picture over the last so many decades. The researchers who assessed the microbiological pattern of the urinary isolates in the 70s and 80s found that *E.coli* the most prominent isolate in acute UTI with an isolation frequency of more than 70% every year, in chronic UTI, *E.coli* also the most frequent species with isolation rates of 17-37%[35].

Besides *E.coli, Klebsiella pneumoniae* (30%), *Pseudomonas aeruginosa* (23.33%), *Proteus vulgaris* (13.33%), *Staphylococcus aureus* (23.33%), *Staphylococcus* sp. (10%), *Enterobacter aerogenus* (10%) and *Streptococcus* sp. (6.66%) were isolated from these urine samples.

Microscopic and bioche These isolated bacterial strains were identified on the basis of colony rhorphoIogy,mical characterization test.

CONCLUSION

Although the spectrum of pathological bacteria isolated from the urine of patients across the globe remained largely unchanged over the past few decades there have been drastic changes in the resistance pattern and sensitivity in most countries. Drug resistance in the isolates of urinary tract infection is common in past few decades[23].

Many herbals have been used through the centuries in the urinary tract infections. During this study effect of different herbal extract such as garlic extract, khadir extract, neem bark extract, black pepper extract, lemon extract, neem leaf extract, neergurnde extract, was observed against these isolates and zone of inhibition was measured in mm.10"1 and 10"2 dilutions of these herbal extracts inhibited different bacteria that were isolated from UTI. The effects of different extracts were also observed at crude preparation of herbals. The garlic extract inhibited the growth of most of the isolated UTI bacteria and lemon extract and black pepper was next to garlic extract in showing inhibitory effects. Khadir and neem bark extract was less inhibitory against the growth of UTI bacteria.

The growth *of Escherichia colt* CCSUB 93 was inhibited by garlic extract, lemon extract and black pepper extract and whereas less effect was shown by neem bark extract and neem leaf extracts.(TABLE 8 Figure 1)

TABLE 6 : Effect of different herbal extract on the growth of *Escherichia coli* CCSUB 93.

Name of the Herbal extract	Zone of inhibition (mm)		
	Crude (undiluted)	10"1 dilution	10^{-2} dilution
Garlic	17	-	~
Khadir	3	-	-
Neem Bark	-	-	-
Black Pepper	16	-	-
Lemon	10	9	-
Neergurnde	2	1	-
Neem Leaf	-	-	-

TABLE 7 : Effect of different herbal extract on the growth of *Pseudomqnas aeruginosa* CCSUB 94.

Name of the herbal extract	Zone of inhibition (mm)		
	Crude (undiluted)	10"1 dilution	10"^ dilution
Garlic	12	-	-
Khadir	2	-	.
Neem Bark	4	-	-
Black Pepper	16	5	-
Lemon	19	2	-
Neergurnde	10	-	-
Neem Leaf	3	1	-

TABLE 8 : Effect of different herbal extract on the growth *of Proteus vulgaris* CCSUB 95.

Name of the herbal extract	Zone of inhibition (mm)		
	Crude (undiluted)	10"1 dilution	102 dilution
Garlic	17	-	-
Khadir	2	-	-
Neem Bark	5	-	-
Black Pepper	12	-	-
Lemon	18	4	-
Neergurnde	6	1	-
Neem Leaf	-	-	-

The growth of *Pseudomonas aeruginosa* CCSUB 94 was inhibited by lemon black pepper, garlic and neergunde extract (TABLE 9 Figure 2).

The growth of *Proteus vulgaris* CGSUB 95 was inhibited by the lemon, garlic black pepper and neergunde extract while no effect were shown by neem leaf extract Figure 3.

The growth of *Klebsiella pneumoniae* CCSUB 96 was inhibited by lemon neergunde khadir and black

pepper extract while no effects was shown extra The growth of *Staphylococcus aureus* CCSUB 97 was inhibited by garlic, lemon black pepper, neergunde extract and neem bark extract while no effect was shown by khadir extract Figure 4.

TABLE 9 : Effect of different herbal extract on the growth of *Klebsiella pneumoniae* CCSUB96.

Name of the herbal extract	Zone of inhibition (mm)		
	Crude (undiluted)	10"1 dilution	10"2 dilution
Garlic	7	-	-
Khadir	10	-	-
Neem Bark	-	-	-
Black Pepper	8	-	-
Lemon	14	4	-
Neergurnde	12	6	2
Neem Leaf	2	-	-

The growth of *Enterobacter aerogenus* CCSUB 9.9 was inhibited by lemon, garlic, neem leaf, neem bark and khadir extracts respectively, while no effect was shown by black pepper.

The growth of *Streptococcus* sp. CCSUB 100 was inhibited by lemon, garlic, black pepper, neem leaf and khadif extracts respectively, while no effect was shown by neergurnde extract.

During the present study it was shown that the crude herbal extracts were more effective as compared to dilution, agains isolated bacteria strains.

Lemon garlic and black pepper extracts have been shown to have antibacterial activity against almost all isolated urinary tract infectious bacteria.

Lemon, garlic and black pepper prevents the. bacteria from adhering to interior of the urinary tract and make the urine more acidic. So that these bacteria are unable to survive, garlic extract also purifies the urine and prevents urinary tract disease.

Many doctors recommended 5,000mg or more of vitamin C per day for an acute UTI, as well as long term supplementation for individuals who are prone to recurrent UTI. Although no controlled studies have demonstrated the effectiveness of vitamin C for this purpose, this vitamin has been shown to inhibit the growth of *E.coli*, the most common bacterial cause of UTIs. In addition ingestion of 4,000 mg or more of vitamin C per day results in a slight increases in the acid ness of the urine, creating an infrequently environment for certain bacteria[3].

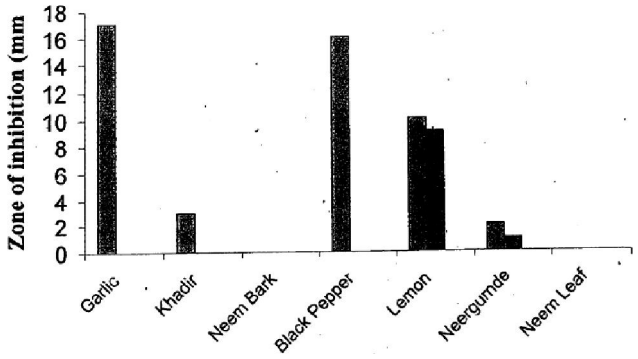

Figure 1 : Effect of different extract on *E.coli* CCSUB93.

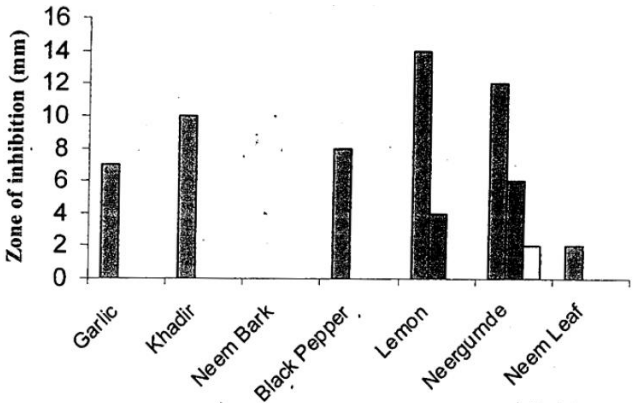

Figure 2 : Effect of different herbal extracts on *Pseudomomas aerogenosa* CCSUB94.

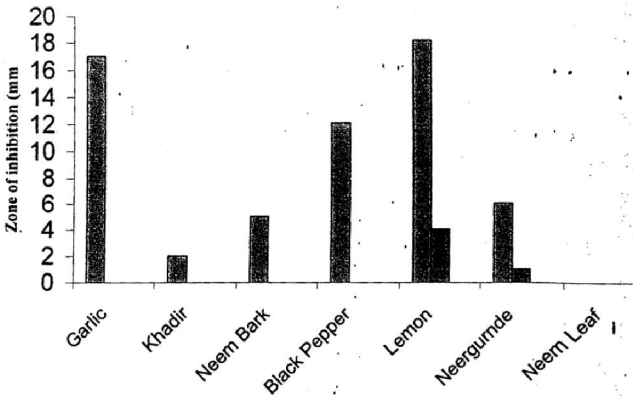

Figure 3 : Effect of different extract on Proteus vulgaris CCSUB95.

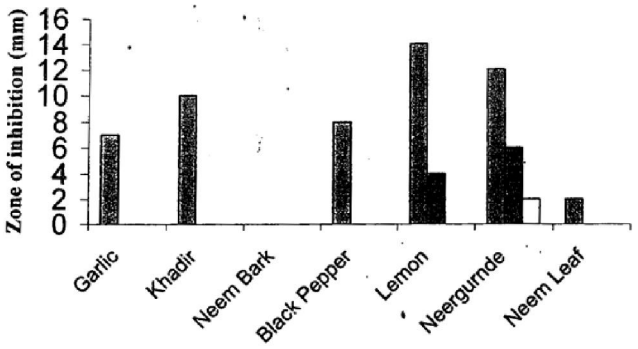

Figure 4 : Effect of different herbal extracts on *Pseudomomas aerogenosa* CCSUB94.

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Similarly, garlic has also been shown to have antimicrobial activity against many disease causing organism including those associated 'with infection *E.cdli*, *Proteus* sp. *Klebsiella pneumonia*, *Streptococcus* sp.

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