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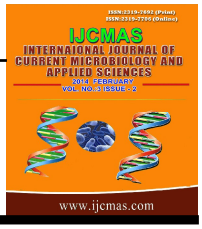

Original Research Article

In vitro inhibition of ESBL positive Multidrug resisting Uropathogenic bacteria using *Coleus forskohlii*

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Keywords

UTI; catheter; uropathogens; ESBL; *Coleus forskohlii*; Antimicrobial assay.

A B S T R A C T

Catheter associated Urinary Tract Infections (CAUTI) constitute the top priority concern in health care set up as they are complicated by drug resisting biofilm forming bacteria. The aim of this study was to observe the prevalence of catheter associated biofilm establishing drug resisting uropathogenic bacteria and to evaluate the in vitro efficacy of extracts of *Coleus forskohlii* (Indian Coleus) in controlling them. One hundred samples of tips of Foley (urinary) catheters were collected for a period of 5 months during January to May-2013 and processed for isolation of uropathogens using standard bacteriological methods. Twenty six isolates belonging to four different types of bacteria including *Escherichia coli* (42%), *Klebsiella pneumoniae* (23%), *Pseudomonas aeruginosa* (19%), *Staphylococcus aureus* (12%) and *Staphylococcus epidermidis* (4%) were obtained. The antibiogram of each isolate of these bacteria as recorded by disc diffusion method revealed that the multidrug resisting strains were very high among bacteria *E.coli* (72.7%) followed by *S.aureus* (66.7%), *P. aeruginosa* (60%) and *K.pneumoniae* (33.3%). The test on biofilm forming characteristics indicated that ten isolates from a total of 26 exhibit positivity with the bacteria *K.pneumoniae* (50%) predominating. The detection of Extended Spectrum Beta Lactamase (ESBL) by Double Disc Synergy test showed that its production was highest among the bacteria *E. coli* (75%) followed by *K. pneumoniae* (66.6%) and *P. aeruginosa* (50%). The ethanolic root extract of Indian Coleues when tested by Agar well diffusion method caused the inhibition of all the six strains of ESBL producing uropathogens while the aqueous extract was ineffective. Gas Chromatograph-Mass Spectroscopy (GC-MS) analysis of ethanolic extract revealed the occurrence of eight bioactive compounds corresponding to major peaks. As the root extract of Indian Coleus promises to be a potential drug, the need for exploring the pharmacological properties of its phytochemicals and possible application of the plant extract for treating UTI complicated by biofilm forming and multidrug resisting uropathogens has been suggested.

Introduction

The field of medicine although achieved tremendous progress in recent decades, infectious diseases caused by pathogenic microorganisms are still a major threat to

public health worldwide. The most alarming recent trend in the study of infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have now become less effective as a result of their indiscriminate use (Anwesa Bag *et al.*, 2012). Infections of urinary tract (UTI) is one of the most common diseases next to that of respiratory tract affecting people of all age worldwide (Kasi Murugan *et al.*, 2012). The commonest bacterial agent involved in causation of UTIs is *Escherichia coli*, being the principal pathogen both in the community as well as in the hospital set up (Jharna Mandal *et al.*, 2012). According to an estimation, about 150 million reports of urinary tract infections (UTIs) per annum were recorded worldwide and about 35% of those were of nosocomial origin (Monali Priyadarsini Mishra *et al.*, 2013).

The UTIs constitute 20-30% of health care associated infections requiring long-term care facilities (LTCFs) (CDC, 2012). Several researchers had addressed the thriving of multi drug resistant (MDR) bacteria in hospital environments impeding the attempts of ensuring successful health care. The condition of infections in catheterized patients could worsen if there is colonization of bacteria on medical equipment capable of resisting multiple drugs. The occurrence of extended spectrum beta-lactamase (ESBL) producing strains among such biofilm establishing bacteria confronts a high risk to the patients. Reports indicate that most of the ESBL producers are sensitive to Imipenem with 82.36% of them showing susceptibility to Amikacin. Hence these two antibiotics are the most preferred choice of drug against ESBLs (Aruna *et al.*, 2012).

According to WHO herbal medicines serve the health needs of about 70% of the world's population, especially for millions of people in the vast rural areas of developing countries (Jiofack, 2010**).** Although many of the plants are reputed in the indigenous systems of medicine owing to their antimicrobial activities, a lion share of them yet remain to be scientifically established (Poovendran *et al*., 2011; Jasmine *et al*., 2013). Traditionally the urinary tract ailments have been treated with certain common herbs including those of *Vacciniumma crocarpon* (Cranberrry), *Hydrastis canadensis* (Goldednseal), *Agathosma betulina* (Buchu), *Arctostaphylosuva-ursi* (Bearberry), *Echinaceae purpurea* (Cone flower) and *Equisetum arvense* (Horse tail) (Geetha *et al*., 2011).

Coleus forskohlii is a perennial, branched, aromatic herb belonging to the order Lamiales and the family of Lamiaceae. The plant is native to areas of India, Myanmar, Nepal and Sri Lanka and is distributed mainly in warm temperate regions. All the species of *Coleus* have four didynamous, dedinate stamens, and the filaments of the stamens unite at their base to form a sheath around the style. The species name *forskholii* was given to commemorate the Finnish Botanist, Forskel (Kavitha *et al.*, 2010). Coleus has long been used in traditional Indian (Ayurvedic) medicine and commonly called as Indian Coleus in English. It possess a characteristic strong camphor-like odor and a chemical extract of the root (forskolin) has been demonstrated to exhibit drug potential for treatment of asthma, bronchitis, glaucoma, congestive heart failure, etc. (Mariya Paul *et al.*,

2013). Research studies have shown significant reduction of UTI through synergistic enhancement of activity of antibiotics (Soman Abraham *et al*., 2010) by this extract to destroy *Escherichia coli*, the bacteria that causes most bladder infections and control of kidney stone diseases (Kumkum Agarwal and Ranjana Varma, 2012).

Antibiotic use has been increasing steadily in recent years. Between 2005 and 2009, the units of antibiotics sold increased by about 40% (ICMR, 2012). Antibiotic resistance is a continually evolving and threatening issue that requires immediate attention especially when implicated with nosocomial infections, thus could be viewed as a global health crisis. Control of ESBL producing bacteria endowed with the ability of colonizing medical equipments needs top priority concern as the deviation of which would be disastrous. Since the efficacy of current therapies is waning and conventional antibiotics are only a temporary fix to bacterial multi-drug resistance, now there is a renewed interest on indigenous medicine as a savior. This study demonstrates the antimicrobial characteristics of the extract of *Coleus forskohlii* against the uropathogens isolated from foley (urinary) catheters, hoping to identify a reliable pharmacological compound that could be used to treat complicated urinary tract infections.

Materials and Methods

Sample collection and Isolation of bacteria

One hundred tips of foley catheters were collected for a period of five months (January to May – 2013) from patients complaining of kidney ailments and undergoing treatment at R.G. Stone Urology and Laproscopy Hospital Chennai, India. The tips were aseptically cut, placed in sterile screw cap tubes and transported to the laboratory under cold chain. Ringer solution (5 mL) was injected into each screw cap tube and shaken well. A loop full of sample from this was taken and streaked on to solid culture media such as Cysteine lactose electrolyte deficient Agar, Nutrient Agar, MacConkey Agar and Blood Agar (HiMedia laboratories, India) and incubated at 37^{o}C for 24 hrs for growth of colonies. Standard bacteriological procedures as described in Bergey's Manual of Determinative Bacteriology (1994) and Mackie & McCartney Practical Medical microbiology rise (1996) were followed to identify the bacterial isolates.

Test on Antibiogram of bacteria

Antibiotic sensitivity pattern of isolated bacteria were determined following the disc diffusion method (Kirby and Bauer *et al*., 1985). Antibiotic discs used for this purpose were Ciprofloxacin, Gentamicin, Ampicillin, Oxacillin, Erythromycin, Imipenem, Nalidinic acid, Co-Trimoxazole, Vancomycin, and Cefepime (HiMedia laboratories, India).

Identification of Biofilm forming bacteria

The bacterial isolates were then subjected to the test prescribed by Subramanian Pramodhini *et al*. (2012) to identify their virulence characteristic of establishing biofilm. The nutrient broth culture of each isolate was inoculated into test tubes containing Brain Heart infusion broth (HiMedia laboratories, India) and incubated at 37^{o}C for 48 hrs. At the end of

incubation the broth culture in each tube was decanted and tubes were washed with distilled water thrice. The tubes were air dried and stained with 0.1 % safarnin (HiMedia laboratories, India) solution. Excess stain was washed. The tubes were then dried in inverted position. The positive result for biofilm formation was confirmed with the appearance of lining the inner wall of the test tube (Fig. 1).

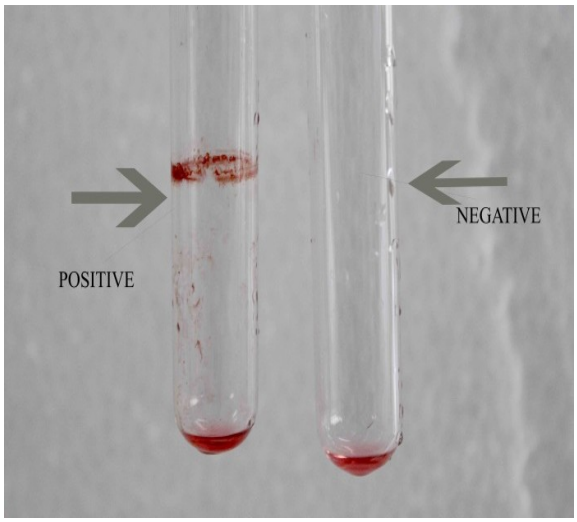

Fig.1. Results of test for Biofilm

Detection of ESBL by Double Disc Synergy Test (DDST) (Subramanian Pramodhini *et al*., 2012; Mahuasinha *et al*., 2007)

Broth culture of each bacterial isolate was prepared and standardized equivalent to that of McFarland 0.5 turbidity standard in nutrient broth. After swab inoculating each culture on Mueller-Hinton agar (MHA) (HiMedia laboratories, India) plate, disks containing cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg) were placed at 25mm distance (from center-center) around an amoxicillin-clavulanic acid disk (30 and 10µg, respectively) (HiMedia laboratories, India). The plates were incubated at 37^{o}C overnight. The synergetic action (combination of zones of growth inhibition) caused by antibiotics with the edge of the zones appear to expand or enhance, was considered as indicative to determine the stains producing ESBLs. The prevalence of ESBL producing strains among the bacteria isolated was calculated by the following formula:

$$\% \text{ of ESBL Producing stains} = \frac{\text{No. Isolates producing ESBL}}{\text{Total no. of isolates}} \times 100$$

Preparation of Solvent extracts of Plant material

Dry fragments of root of *Coleus forskohlii* were collected from the local market located in Salem city (Tamil Nadu, India) and brought to the laboratory of department of Microbiology, Asan Memorial College of Arts and Science, Chennai, India. It was further shade dried and then subjected to blending at 3000 rpm for 15 min. The resultant fine powder of the root (5g) was dissolved in an aliquot of 25mL of double distilled water, boiled for 30 min and left at 4^{o}C for 72 hrs, with intermittent stirring.

The ethanolic extract of root of coleus was prepared by soaking 5g of powdered plant material in an aliquot of 25mL of 80% ethanol (Sisco Research laboratory, India) for 72 hrs with usual hand-shankings and filtration through Whatman paper no.1. The filtrate was kept in a water bath at 40^{o}C till obtaining a sticky mass from which 100 mg was then weighed and dissolved in 1 mL of 10% dimethyl sulfoxide (DMSO) (Sisco Research laboratory, India). Extracts were stored at

4°C until further use.

Assay of Antibacterial activity of extract by well diffusion method

(Shakti Rath *et al.,* 2012)

The broth culture of each bacterial isolate was swab inoculated on to sterile MHA and three wells each measuring 1.5mm dia were made on agar medium. Each well was filled with 30µl of plant extract. Plates were then incubated at 37°C for 24 hrs and examined for growth inhibition. The size (diameter in mm) of the zone of growth around each well was measured and recorded. The extract causing zone of growth inhibition for > 8mm was considered as toxic for bacterial growth.

Gas Chromatograph - Mass Spectroscopy (GC-MS) analysis

The sample was fitted with a split splitless injector connected to an MS Polaris Q-Quadrupole Ion Trap (Thermo Electron) fused silica column VB5 (5% phenyl, 95% methylpolyxiloxane, 30 m with 0.25mm i.d. film thickness 0.25 µm) (J & W Scientific Fisons, Folsom, CA). The injector and interface were opened at 250 and 300°C, respectively. The oven temperature was programmed as follows: 50°C raised to 250°C (4°C/min) and held for 3 min. Helium was used as the carrier gas at 1 ml/min. The sample (1µl) was injected in split mode (1:20). MS conditions followed were ionization voltage El of -70 eV and mass range of 10-350 amu. The chromatograph showing peaks corresponding to different compounds was obtained and interpreted.

Results and Discussion

Isolation Bacteria from Foley Catheters

Morphological and biochemical characterization of isolates obtained on agar media indicated the occurrence of four types of bacteria in the urinary catheter. A total of 26 isolates identified to be of *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were obtained (Table 1). Among these the bacteria *E.coli* was the most predominant bacteria with a prevalence rate 42% and the least occurring was *S. epidermidis* (4%) (Fig.2).

Antibiogram of Uropathogens

The analysis on antibiotic susceptibility pattern of different catheter associated bacteria revealed varying responses with respect to their resistance/sensitivity to the antibiotics (Table 2). Among the 26 isolates belonging to different groups of bacteria tested for their antibiogram, eleven isolates showed resistance to multiple drugs. Based on the results of present study the occurrence of MDR strains were very high among bacteria *E.coli* (72.7%) followed by *S.aureus* (66.7%), *P. aeruginosa* (60%), and *K.pneumoniae* (33.3%) (Fig.3).

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Biofilm formation by Uropathogens

Among the 26 isolates subjected for test on biofilm forming characteristic ten of them showed positivity (Table 3). While substantial amount of biofilm establishment was noted with *K. pneumoniae* (50%), the only isolate of *S. epidermidis* did not show positivity to the test and hence it was excluded from further studies.

Detection of ESBL in Uropathogens

Ten uropathogenic isolates which demonstrated the virulence characteristics

Table.1 Isolation of Catheter associated bacteria on CLED agar

Sl. No.	Type of Colony	No. of isolates	Bacteria Identified
1	Type – 1	11	*E.coli*
2	Type – 2	6	*K.pneumoniae*
3	Type – 3	5	*P.aeruginosa*
4	Type – 4	4	*Staphylococcus sp.*

Fig.2 Prevalence of Cather associated uropathogens

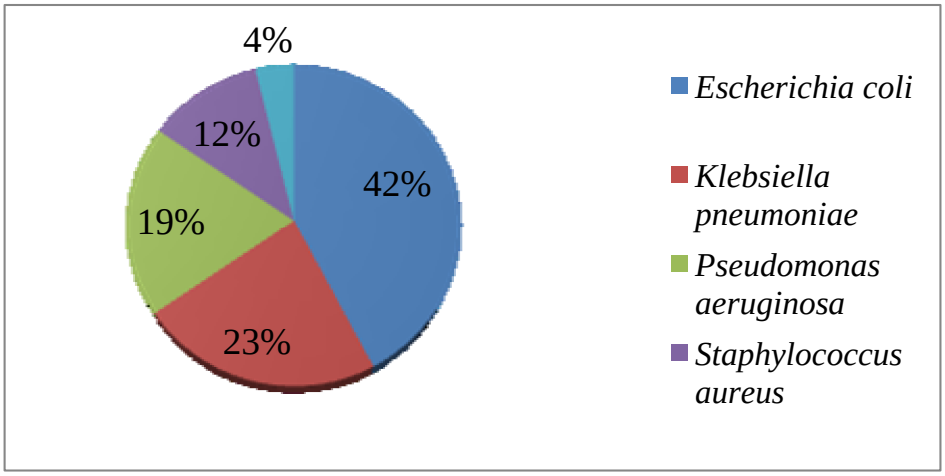

Fig.3 Prevelance of MDR uropathogens in Foley catheters

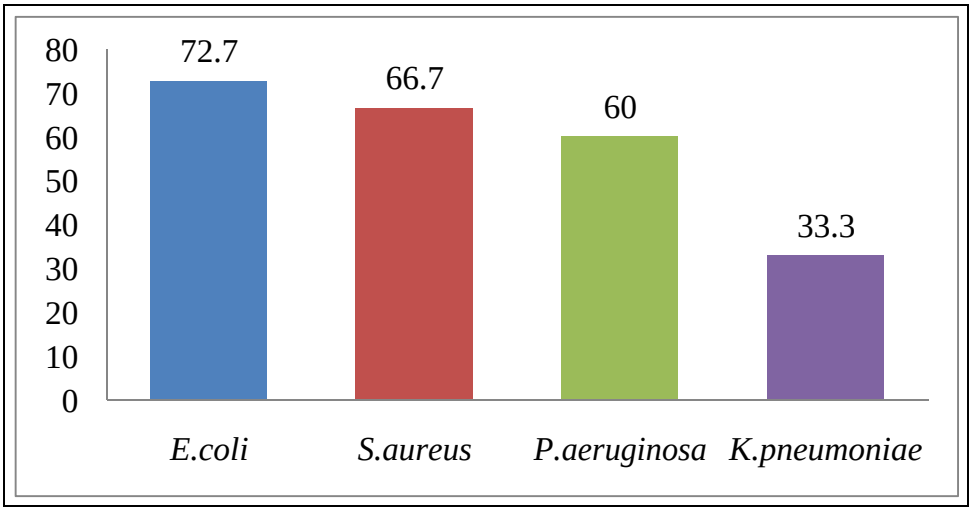

Table.2 Antibiogram of Catheter associated bacteria

Sl No.	Name of the Bacteria	Isolate Number	Response to the Antibiotics										% of Resistance
			1	2	3	4	5	6	7	8	9	10	
1.	*Escherichia coli*	S1	S	R	S	R	R	M	R	R	R	R	70 %
		S3	S	R	S	S	S	S	S	S	R	S	20%
		S6	S	S	S	S	R	S	R	S	S	S	20%
		S9	R	R	R	R	R	R	R	R	R	R	100%
		S10	S	S	S	S	S	S	S	S	R	S	10%
		S11	S	S	R	S	R	S	S	S	R	S	30%
		S15	R	R	R	R	S	R	R	R	R	R	90%
		S16	S	S	S	S	R	S	S	S	S	S	10%
		S19	S	S	R	S	S	S	S	S	S	S	10%
		S25	R	M	S	S	S	R	S	S	S	S	20%
		S26	M	S	S	S	S	S	S	S	S	S	0
2.	*Klebsiella pneumoniae*	S2	S	R	R	M	R	R	R	M	S	S	50%
		S7	M	S	S	S	S	S	M	S	S	S	0
		S13	S	S	S	S	M	S	S	S	S	M	0
		S18	S	S	S	S	S	S	R	S	S	S	10%
		S20	R	M	S	S	S	S	S	S	S	S	10%
		S22	M	R	R	S	R	R	S	R	R	S	60%
3.	*Pseudomonas aeruginosa*	S4	S	S	S	S	M	S	S	S	S	S	0
		S5	S	S	S	S	M	S	M	S	S	S	0
		S8	R	R	R	R	R	S	R	R	R	S	80%
		S12	S	R	S	R	S	S	R	S	S	S	30%
		S23	R	S	M	S	S	S	S	R	S	S	20%
4.	*Staphylococcus aureus*	S17	M	S	S	R	S	S	R	S	S	S	20%
		S21	R	S	R	R	R	M	S	S	S	R	50%
		S24	S	S	S	S	M	S	M	S	S	S	0
5.	*Staphylococcus epidermidis*	S14	S	S	S	S	S	S	S	S	S	S	0

Antibiotic discs : 1. Ciprofloxacin, 2. Gentamicin, 3. Ampicillin, 4. Oxacillin, 5. Erythromycin, 6. Imipenem, 7. Nalidinic acid, 8. Co-Trimoxazole, 9. Vancomycin, 10. Cefepime.

of multidrug resistant and biofilm establishment when subjected to Double Disc Synergy test six of them showed positivity. The results of ESBL production exhibited by individual isolates are depicted in the table 4. The analysis on ESBL production among the isolated strains showed that it was highest among the bacteria *E. coli* (75%) followed by *K. pneumonia* (66.6%) and *P. aeruginosa* (50%) (Fig.4).

Antimicrobial activity of plant extracts against ESBL producing uropathogens

The results of test on sensitivity of six

isolates confirmed to be ESBL producers against aqueous and ethanolic extracts of Indian Coleus were recorded and tabulated (Table 5). The ethanolic extract was observed to be efficacious by causing complete inhibition of all the bacterial isolates tested.

GC-MS of Ethanolic extract of Indian Coleus

A total of eight major peaks and several minor peaks at different retention times were obtained on GC-MS chromatogram. These peaks were deduced with standard chromatogram for the identification of principal chemical compounds. The compounds identified corresponding to the major peaks were recorded (Table 6; Fig.5).

The contamination of invasive medical equipment with drug resisting bacteria is of serious concern as it constraints the medical assistance provided to the patients with focally weakened immunity. The serious threat to health caused by catheter associated UTI had been addressed by several researchers (Rahul Mittal *et al*., 2009; Kathleen *et al.,* 2011). The primary objective of this study was to observe the prevalence of catheter associated uropathogenic bacteria. The analysis indicated the occurrence of four types of bacteria such as *K.pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* with the preponderance of *Escherichia coli* (42%). This is in concordance with the reports of many research studies conducted elsewhere which depict the dominant etiological nature of *E.coli* in UTI (Deepti Rawat *et* al., 2010; Surbhi Leekha *et al*., 2011). In contrast to the observations of the present study few researchers had reported the occurrence of certain other uropathogenic bacteria *Proteus sps*. (Jacobsen *et al.,* 2008), *Acinetobacter* (Nadia Kazemi Pour *et al.,* 2011) and *Serratia* (Rajdeep Saha *et al.,* 2012). The absence of these bacteria from the samples of the present study could be attributed to their less efficient nature of establishing biofilm on foley catheters.

The astounding development of multidrug resistance among the uropathogens during the recent years had been demonstrated by several studies (Anwesa Bag *et al.,* 2012; Monali Priyadarsini Mishra *et al.,* 2013; Tiruvayipati Suma Avasthi *et al.,* 2011). The investigation of antibiogram of isolates of the present study portrayed significant association of multidrug resistance with *E. coli*, followed by *S. aureus, P. aeruginosa* and *K. pneumoniae*. Concomitant with the present study the occurrence in the catheter tips of multidrug resistant *E. coli* **(**Mohammad Yousef Alikhani *et al.,* 2013**)**, *S. aureus* (Jadwiga Handzlik *et al.,* 2013), *K. pneumoniae* (Jesus Silva-Sanchez, 2011) and *P. aeruginosa* (Ahmed Bakr Mahmoud *et al.,* 2013) had been reported elsewhere. As these bacteria colonize together to establish biofilm on foley catheters the possibility of exchange of drug resistance characteristics cannot be voided. It is noteworthy from the present study that although the bacteria *K. pneumoniae* possessed dominant biofilm forming ability (50%), it exhibited comparatively lesser multidrug resistance (33.3%)(Table 3; Fig.3). However, it may be inferred that the development of multi drug resistance is still taking place at an alarming rate.

The emergence of ESBL producing bacteria pose further obstacle in treating UTI as these bacteria could survive well in the presence of third generation antibiotics (Ann *et al*., 2013; Timothy *et al*., 2002).

Ever since the first report on ESBL positivity exhibited by *Klebsiella* sp. several studies had been reporting the emergence of ESBL producing uropathogenic bacteria across the world (Sanchez *et al*., 2013). Out of the ten multidrug resisting biofilm forming bacterial isolates tested in the present study six of them showed positivity for ESBL production (Table 4). The bacteria *E.coli* could produce the ESBL to an extent of 75% followed by *K. pneumoniae* and *P. aeruginosa*. It is generally acknowledged that the gram negative bacteria predominate over their counterpart in ESBL production. In agreement with this statement the biofilm forming *S. aureus* isolated in the present study did not show any positivity for ESBL production. The ESBL are coded by certain plasmid borne gene namely CTX-M, TEM and SHV (Aruna *et al*., 2012; Sasirekha, 2012). Therefore the transmission of resistance among the bacteria colonizing to form biofilm need greater attention to be paid as the plasmids are known for their high efficiency transfer by natural genetic mechanisms.

There is a remarkable interest in recent years among the researchers in finding an effective drug of natural origin to combat UTI associated bacteria. *Coleus forskohlii* commonly known as Indian Coleus has now become a priority choice for this purpose by virtue of its significant medicinal property (Paul *et al*., 2013; Madhavi Jagtap *et al*., 2011). Soman Abraham (2010) had investigated the application of Indian Coleus as an augmenter for the control of uropathogenic bacteria using the antibiotics amoxicillin, ciprofloxacin, levofloxacin, sulfamethoxazol, etc. The ethanolic root extract of Indian Coleus employed in the present study has been proved to be more efficacious over aqueous extract in inhibiting all the six ESBL strains tested (Table 5). Therefore the ethanolic root extract of Indian Coleus was selected as potential extract and was further analysed to explore its bioactive compounds. The short of antibacterial activity of aqueous root extract could be due to non-extractable nature of phytochemical constituents conferring antimicrobial characeristics to Coleus.

The GC-MS analysis of potential extract revealed the occurrence of 8 different phytochemicals corresponding to the peaks obtained at different retention times including Terephthalic acid, Mercaptophenol, Gluco-heptulosan, Galacto-heptulofur, Heptadecene, Acetamide, etc. (Table 6). The compound 2,7-Anhydro-l-galacto-heptulofur was observed to occur predominantly with a peak area of 36.09%. The least occurring compound namely 2,6-Dimethyl-3,5-di(4-methoxyphenyl) was obtained with the peak area of 1.13%. Similar studies conducted on ethanolic extract of root of Indian Coleus depicted the occurrence of different chemical compounds numbering eleven (Manoharan Sivananthan *et al.*, 2013). Although most of the compounds

Table.3 Determination of Biofilm forming characteristics of uropathogens

SL No.	Name of bacteria	Isolates No.	Results of biofilm formation	Percentage of prevalence
1	*Klebsiella pneumoniae*	S2,S22,S13	+	50%
		S7,S18,S20	-	
2	*Pseudomonas aeruginosa*	S8,S12	+	40%
		S4,S5,S23	-	
3	*Escherichia coli*	S1,S9,S15,S3	+	37%
		S6,S10,S11,S16,S19,S25,S26	-	
4	*Staphylococcus aureus*	S17	+	35%
		S21,S24	-	
5	*Staphylococcus epidermidis*	S14	-	0

Table.4 Occurrence of ESBL producers among uropathogens

Sl No.	Name of bacteria	Isolate number	ESBL production	% of prevalence
1.	*Escherichia coli*	S1,S9, S15	Positive	75%
		S3	Negative	
2.	*Klebsiella pneumoniae*	S2,S22	Positive	66.6%
		S13	Negative	
3.	*Pseudomonas aeruginosa*	S8	Positive	50%
		S12	Negative	
4.	*Staphylococcus aureus*	S17	Negative	0

Fig.4 Prevalence of ESBL producing uropathogens

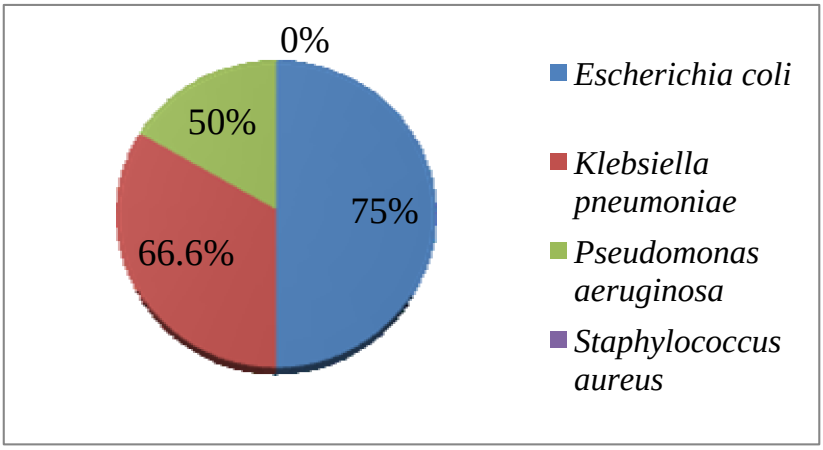

Table.5 Antimicrobial activity of plant extracts against ESBL producing uropathogens.

Sl No.	Extract of Indian Coleus	*E.coli*			*K.pneumoniae*		*P.aeruginosa*
		S1	S9	S15	S2	S22	S8
1.	Aqueous extract	-	-	-	-	-	-
2.	Ethanolic extract	+	+	+	+	+	+

+ Inhibition of growth - No inhibition

Fig.5 GC-MS chromatogram of ethanolic extract of *C. forskohlii*

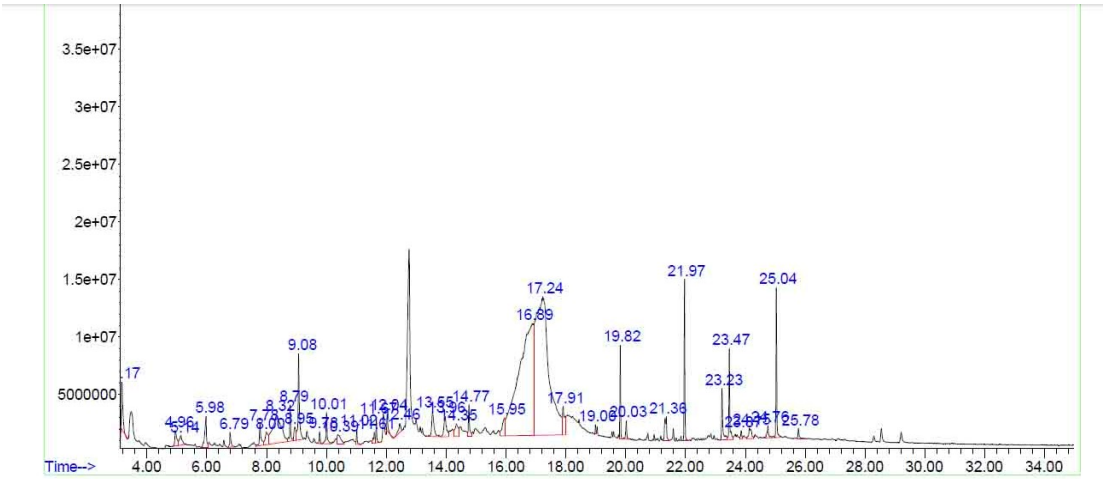

Table.6 Identification of Bio active compounds of *Coleus forskohlii* by GC-MS

Sl No.	Retention time	Compound identified	Peak area (%)
1.	21.9	Acetamide [N-(2, 5-dimethoxypheny)-2-phenylthio and Terephthalic acid, but-3-enyl undecyl ester]	2.18
2.	9.07	5-Hydroxymethylfurfural 4-Mercaptophenol	2.18
3.	16.88	2,3,4,5-Tetrahydroxypentanal d-Gluco-heptulosan	31.04
4.	17.24	2,7-Anhydro-l-galacto-heptulofur 1-Nitro-1-deoxy-d-glycero-l-mann	36.09
5.	19.82	3-Heptadecene, (Z) 3,7-Nonadien-2-one, 4,8-dimethyl	1.20
6.	23.23	2,6-Dimethyl-3,5-di(4-methoxyphenyl) 1H-Naphtho[2,1-b]pyran-7-carboxy	1.13
7.	23.47	1,3a-Ethano(1H)inden-4-ol, Octahedral-N-Acrylonitryl-2,2,6,6-tetramethyl	1.51
8.	25.04	3-Buten-2-ol, 2-methyl-4- (1,3,3-Spiro[4.5]decan-7-one) 1,8-dimet Benzo	2.20

isolated in the present study have been known for various biological activities, the compound Acetamide (N-(2, 5-dimethoxypheny)-2-phenylthio) has been demonstrated to exhibit significant pharmacological effects such as antibacterial, anti-tumor, antiviral etc. Exploring the pharmaceutical potential of each of these compounds by both *in vitro* and *in vivo* studies would help identifying the principal antimicrobial compound of Indian Coleus.

The substantial occurrence of biofilm forming multidrug resisting uropathogenic bacteria in the urinary catheters as shown in the present study warrants the priority intervention to conduct a comprehensive investigation to render proper health care to the patients. As these patients are afflicted with underlying urinary ailments the occurrence in them of these kinds of biofilm forming drug resisting agents could be devastating. Although the sanitation and personal hygiene are advocated as top priority preventive measures in hospital set up, minor slapdash and eventual contamination by drug resisting pathogens of invasive medical devices could lead to serious complications. As show cased by the present study, the root of Indian Coleus could be considered as a promising future drug for treating UTI superimposed with ESBL producing uropathogenic bacteria.

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