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Efficacy of Plant Extracts Against Multi-Drug Resistant *Escherichia Coli* from Urinary Tract Infection

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Abstract

Introduction: Urinary tract infections (UTI) are among the most commonly prevalent infections in clinical practice. *E. coli* is the causative agent in about 85% of community acquired urinary tract infections, followed by Klebsiella that accounts for 6 to 17% of such infections. **Objectives**: Present study was conducted to evaluate the antibacterial potential of five plants used against *E.coli* causing UTI **Methods**: A total of 40 pregnant women were screened for significant a symptomatic bacteriuria. All the subjects were clinically identified to have no signs and symptoms of UTI. Clean catch midstream urine samples were collected for both groups. Urine samples were examined microscopically and cultured and incubated at 24 hrs at 37°C. Isolated organisms were identified and characterized on the basic of morphological, cultural and biochemical characteristics. **Results**: A total of 40 urine sample were analyzed for isolation of multidrug resistance E. coli. Out of which 20 samples showed significant bacteriuria. The bacteria identified using biochemical characteristic as *E. coli*. In general, over all 100% of isolates were resistant to Rifampicin, 90% of isolates to Ampicillin, 85% of isolates to Nalidixic acid, 75% to Gentamycin, and 65% to Oflaxacin**Conclusions**: The use of plants extracts with known antimicrobial properties can be of great significance for therapeutic treatments. Results of antimicrobial susceptibility test showed marked differences among bacterial isolates in their susceptibility and resistance patterns to a particular antibioticbasis.

Keywords: Urinary tract infections, Escherichia coli, multidrug resistant, plant extracts

Introduction

Urinary tract infection represents one of most common diseases occurring from one neonate to the geriatric age groups encounters in medical practice today (Ammon and Wahi, 1991)and .It is estimated that about 35% of healthy women suffer symptoms of urinary tract infections at some stages in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency (Hootan, 2003). The incidence of urinary tract infections is greater in women as compared to men which may be either due to anatomical predisposition or urothelial mucosal adherence to mucopolysaccharide lining or other host factors (Shokeen et al, 2009). The ESBL producing bacteria has been identified in members of enterobacteriaceae, are increasingly causing urinary tract infections both in hospitalized patients and out patients (Morris and Masteron, 2002). The increasing drug resistance among these bacteria has made therapy if urinary tract infection difficult and has led to greater use expensive broad-spectrum drugs. This resistance problem needs a renewed effort resulting in searching effective antibacterial agents against current antibiotics (Schaeffer et al, 2001).is a series health problem affecting millions of people in year.

Infections of urinary tract are common (only respiratory infections occur more often). Women have a higher risk of developing UTI than men; approximately 50% to 70% of women will have UTI during their lifetimes, and 20% to 30% of women will have recurrent episodes (Gupta et al, 2001). Pregnant women are at increased risk for UTI (starting in week 6 through week 24), because uterus sits directly on top of bladder and displaces it. Shift in position of urinary tract and hormonal changes during pregnancy make it easier for bacteria it travels up urethras to the kidneys. For these reasons, many doctors recommended periodic testing of urine. In many cases, however, pregnant women with positive urinary tests have no symptoms of UTI. Presence of bacteria in urine without symptoms is known as asymptomatic bacteriuria and presence of leukocytes in urine is called pyuria. Asymptomatic bacteriuria is common during pregnancy. Its average prevalence is 6%. It is an important risk factor for low birth weight and prematurely. Therefore, if causative bacteria are detected in urine, pregnant women will be treated even in symptoms are not present.

Women's with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or low birth-weight infants and have a 20 to 30 fold increased risk of developing pyelonephritis during pregnancy compared with women without bacteriuria. The presence of bacteria in a properly collected urine specimen from a person without symptoms or signs of UTI characterizes as asymptomatic bacteriuria. Antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographic locates and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of critical importance of changing rate of antibiotic resistance has a large impact on empirical therapy of UTIs (Velickovic et al, 2003).

Plants and plant products have been used extensively throughout history to treat medicinal problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity (Soulsby, 2005). Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants have been tested many reports show the effectiveness of traditional herbs against microorganisms as a result, plants are one of the bedrocks for medicine to attain new principles (Evans et al, 2002). In this regard, plants have given western pharmacopoeia about 7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, e.g. qunine, artemisinin, taxal, camptothecin etc. Until natural products have been approved as new antibacterial drugs; there is an urgent need to identify novel substances active towards highly resistant pathogens (Sakagami et al, 2001).

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the report of WHO, 80% of the world's populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances (Vermani and Garg, 2002). The microorganisms have developed resistance against many antibiotics due to indiscriminate use of antimicrobial drugs (Ahmad et al, 1998). This creates problems in the treatment of infectious diseases (Davis, 1994). Furthermore, antibiotics are sometimes associated with side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Nascimento et al, 2000). Tribal people of India have been using several types of plants as medicine since the ancient time which has not been studied extensively. Although screening of Indian medicinal plants has revealed varying degrees of antimicrobial activity against pathogenic and opportunistic micro organisms (Ahmad and Beg, 2001), but there is still a lack of experimental scientific studies confirming the possible antimicrobial properties of a great number of these remedies. Hence the present study was conducted to evaluate the antibacterial potential of five plants used against UTI causing E.coli.

Research method

Urine sample collection:A total of 40 pregnant womens were screened for significant a symptomatic bacteriuria [colony count > 1, 00, 000 cfu / ml]. All the subjects were clinically identified to have no signs and symptoms of UTI. Clean catch midstream urine samples were collected for both groups. Urine samples were examined microscopically and cultured by placing on EMB, Mac Conkey Agar and incubated at 24 hrs at 37°C. Isolated organisms were identified andcharacterized on the basic of morphological, cultural and biochemical characteristics and were identified with the help of Bergeg's Mannual of systematic Bacteriology.

Antibiotic susceptibility Testing:Antibiogram of the UTI isolates was ascertained on Mueller Hinton Agar using disc diffusion method. Five antibiotics most commonly used for the treatment of UTI were employed i.e, Ampicillin, Gentamycin, Rifamycin, Nalidixic acid, Oflaxacin. The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as Resistant or Sensitive based on the Standard interpretation chart.

Plant materials: A total of five plants parts VIZ. Corriendar Sativum [seed], Terminalia chebula [dry fruit], Allium Sativum [clove], Emblica officinalis [fruit], and Curcuma longa [dry rhizomes] were collected based on ethno medical importance.

Extraction of plant material: Plant materials were washed with distilled water, dried in shade, grined to tine powder and stored in airtight container at room temperature in the dark until used. The powdered samples were subjected [Acetone, ethanol] in addition to water.

Antibacterial activity of plant extract: Susceptibility of UTI isolated to the extracts was determined by the disc diffusion assay. Petriplates containing Mueller Hinton Agar medium were seeded with 24 hrs old culture of bacterial strains. The inoculum size was adjusted to achieve a final concentration of 10 cfu /ml by making with 0.5 McFarland Nephlometer standards. The sterile Whatman filterpaper disc [5mm in diameter] impregnated with plants extract [40mg/0.1ml] were placed on the surface of the culture plates. And incubated at 37°C for 24 hrs and diameter of zone of inhibition were measured in mm. Discs with acetone, ethanol, and distilled water were used as control.

Measurement of Total phenolics and Tannins using Folin –ciocalteu method

The method for total phenol is useful in order to know the efficiency of extraction of phenolics in solvents. This method can be with the use of insoluble matrix. [Binds tannin-phenolics] for measurement of tannin. The results

can be expressed as tannic acid equipment. The nature of tannic acid varies from one commercial source to the other.

Folin ciocalteu reagent [1N]: Dilute commercially available folin-ciocalteu reagent [2N] with an equal volume of distilled water. Transfer it in a brown bottle and store in a refrigerator [4◦ C]. It should be golden in colour. Do not use it if it luins olive green. Sodium Carbonate [20%]: weigh 40g sodium carbonate [X10 water] dissolve it in about 150ml distilled water and make up to 200ml with distilled water.

Standard tannic acid solution [0.1mg/ml]: Dissolve 25mg tannic acid [TA] obtained from merck in 25ml distilled water and then dilute 1;10 in distilled water [always we a freshly prepared solution].

Analysis of Total phenols: Take suitable aliguots of the tannin containing extract [Initially try 0.02, 0.05] with distilled water, add 0.25ml of the Folin ciocalteu reagent and then 1.25ml of the sodium carbonate solution. Vortex the tubes and record absorbance at 725nm after 40min.

Analysis Results

A total of 40 urine sample were analyzed for isolation of multidrug resistance E. coli. Out of which 20 samples showed significant bacteriuria. The bacteria identified using biochemical characteristic as E. coli (Table 1)

Antimicrobial Susceptibility: Result of antimicrobial Susceptibility test showed marked difference among bacterial isolates in their susceptibility and resistance pattern to a particular antibiotic. The bacterial isolates were generally resistance to Rifampicin and Ampicillin followed by Nalidixic acid, Gentamycin and Oflaxacin. In general, over all 100% of isolates were resistant to Rifampicin, 90% of isolates to Ampicillin, 85% of isolates to Nalidixic acid, 75% to Gentamycin, and 65% to Oflaxacin. (Table 2).

Antibiotic sensitivity test: Results of antibiotic susceptibility showed that nearly all the isolates were resistant against most of the antibiotics tested during the present investigation. The isolated E. coli strain showed Multidrug resistance. Out of 20, 12 strains were showed antibiotic resistance pattern of Amp- Gen – Rif – Na-Ofl, 2 strains were Rif, 2 were Amp- Gen –Rif- Na, 2 were Amp – RIF – Na , 1 was Amp – Gen – Rif, 1 was Amp – Rif – Na – Ofl resistance. Eighteen resistance patterns were observed in enteric pathogens for the nice antimicrobial agents tested. Resistance to Ampicillin, Amoxicillin, Tetraglycine, Cotrimoxazole, Sulfanilamide [AM – AL – Te – SX – Su] was the most frequent pattern, observed in 73 % of the isolates of S. dysentriae type 1. The most common multiple resistance pattern was resistance to Ampicillin, Amoxicillin, Tetracycline, Colrimoxoazole, Sulfanilamide, and Chloramphenicol. Multiple resistances were particularly high in urinary pathogens. E. coli has a 58 % resistance rate to at least four of ten antimicrobial agents [Ahmed et al., 2000] while K. pneumoniae and P. miralrilis had 70 % and 82 % resistance rates respectively. E.coli [21 %] K. pneumoniae [52 %] and P. miralrilis [60%] were resistance to at least five antimicrobial agents.

Growth inhibition: Different concentration of E. coli, A. sativam and T. chebula were used to identify the percentage of inhibitory activity against E. coli, A. sativam and T. chebula showed potential effect (80-90% inhibitory activity) in the concentration 25 µl, 50 µl of A. sativum and T. chebula showed 90-100% inhibitory activity against E. coli. E. coli showed less inhibitory activity compared to T. Chebula and A. sativum. (Table 3).

Plant extract: Out of 20 isolates, five multidrug resistant E.coli were selected for further works. The antibacterial activity of plants was observed against selected five MDR E.coli. Totally five plant parts selected for the antibacterial activity. Out of 5 plants [T. chebula, C. longa, E. officinalis, C. sativum, A. sativum]. 3 plants [A. sativum, E. officinalis, and T. chebula] showed the potential antibacterial activity against E.coli isolated from urinary tract infected pregnant women. The highest antibacterial activities observed in T. chebula, followed by A. sativum and E.officinalis. Other C.longa and C.sativum did not show any antibacterial activity. Ethanol extract of Turmeric and Chebulic myrobalan, Fresh extract of Garlic and Amla, Aqueous extract of Coriander were used for this study. In well diffusion assay, Turmeric chebula showed mm range of 25-27 against five MDR E.coli. A.sativum showed maximum zone of inhibition (32mm) followed by E.officinalis against MDR E.coli . In disc diffusion assay, plant extract showed lower mm of zone of inhibition when compared to well diffusion assay (Table 4)

Inhibitory concentration (mic) detetmination: The plant extracts were further subjected to the broth murodilution method to determine the MIC. 30 mg/ml was the initial concentration for E.officinalis and A.sativum. E.officinalis showed the minimal inhibitory activity even in the concentration of 0.9 mg/ml. A.sativum showed MIC of 1.9 mg/ml. T.chebula showed very potential effect in the low concentration of 0.07 mg/ml. In the presence study, T.chebula having good efficiency to inhibit the MDR E.coli at the lower concentration compared to garlic and amla (Table 5).

Percentage of growth inhibition by E. officinalis, A. sativum and T. chebula

Turmeric extract was not effective against Enterobacter aerogens, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus aureus and had very little effect on proteus vulgaris, Bacillus subtilis and Listeria monocytogenes. In general, turmeric extract was exhibited the lowest antimicrobial activity as compared to the other plant extracts tested (Table 6).

Conclusion

Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4, 00,000 plant species on Earth only a small number has been systematically investigated for their antimicrobial activities. Additionally, there is a rich local ethno botanical knowledge and bibliography describing the species most frequently used by population to cure various diseases (Taneja et al, 2002). However there has been seldom effective collaboration between the traditional and western medical therapeutics, largely due to the perception that the use of traditional and herbal medicines has no scientific basis. According to world health organization (Vermani and Garg, 2002).medicinal plants would be the best source to obtain a variety of drugs. Therefore such plants should be investigated to better understand their properties, safety and efficiency (Neu, 1992). Bacterial infection is one of the most serious global health issues in 21st century (Morris and Masteron, 2002). The emergence of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems. Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and were being (Iwu et al, 1999). The use of plants extracts with known antimicrobial properties can be of great significance for therapeutic treatments.

Antibacterial susceptibility: Results of antimicrobial susceptibility test showed marked differences among bacterial isolates in their susceptibility and resistance patterns to a particular antibiotic. The bacterial isolates were generally resistance to Ampicillin, Cephataxime followed by Tetracycline and Chloramphenicol. The antimicrobial resistance patterns are valuables as a guide to empirical therapy and as an indicator of the dissemination of antimicrobial resistance determinations (Delappe et al, 2003).Lactams is the most widely used antibiotics and lactamase are the greatest source of resistance to them. An understanding of extended spectrum lactamase defection is therefore valuable. Recent studies conducted by (Hansotia et al, 2003). On ASBL production in members of Anterobacteriaceae isolated from clinical specimens showed 9-50% ASBL producers. There is marked difference in present finding. Only 42.4% of isolated uropathogens were found to be lactams positive, belonging to P. pseudomalleii, E.coli, Anterobacter, Klebsiella and Proteus species. A study from north India on ASBL production in uropathogens showed 26.6% ASBL producers which belonged to Klebsiella, E.coli, Anterobacter, proteus and Citeobacter species (Khanna and Nag, 1973).

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Authors' contributions

Wilda participated in the design of the study; carried out and personally financed the studies; SJK performed the statistical analysis; and drafted the manuscript. STK participated in the design of the study; EON assisted in the supervision on the theoretical and practical work. JM participated in the design of the study; and supervised on the molecular genetic studies and the statistical analysis. All authors have read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests

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Tables

Table 1:Biochemical characterization of E. coli

S. No	Biochemical Tests	E. coli
	Gram Staining	Gram Negative Bacilli
	Motility	Motile
	Indole	Positive
	Methyl red	Positive
	Vogus Proskaur (VP	Positive
	Citrate	Negative
	Catalase	Negative
	Urease	Negative
	Oxidase	Negative
	TSI	A / A, Gas

Table 2: Antibiotic resistance pattern

S. No	Antibiotic pattern	No. of Resistant Antibiotics	No. of Strain
1	Amp-Gen-Rif	3	1
2	Amp-Rif-Na	3	2
3	Amp-Gen-Rif-Na	4	2
4	Amp-Gen-Rif-Na-Ofl	5	12
5	Rif	1	2
6	Amp-Rif-Na-Ofl	4	1

Table 3: Antibiotic sensitivity test (ast) using plant extracts

S.No	PLANT EXTRACTS USED	Antimicrobial Activity of Plant Extracts (mm)				
		E. coli 11	*E. coli 13*	*E. coli 16*	*E. coli 19*	*E. coli 20*
1	*Emblica officinalis*	23	-	-	22	24
2	*Curcuma longa*	-	-	-	-	-
3	*Allium sativum*	19	32	-	19	20
4	*Coriandrum sativum*	-	-	-	-	-
5	*Terminalia chebula Retz*	26	27	-	26	25

Table 4: Minimal inhibitory concentration (mic) determination for *Emblica officinalis* and *Allium sativum*

DILUTION (mg)	PLANT PRODUCTS									
	1. Emblica officinalis (30 mg)					**2. Allium sativum** (30 mg)				
	E.coli 11	E.coli 13	E.coli 16	E.coli 19	E.coli 20	E.coli 11	E.coli 13	E.coli 16	E.coli 19	E.coli 20
30	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-
7.5	-	-	-	-	-	-	-	-	-	-
3.8	-	-	-	-	-	-	-	-	-	-
1.9	-	-	-	-	-	-	-	-	-	-
0.9	-	-	-	-	-	-	G	-	-	G
0.04	-	-	G	-	-	G	G	-	-	G
0.02	-	-	G	-	-	G	G	-	-	G

G → GROWTH. - → NO GROWTH.

Table 5: Minimal inhibitory concentration (mic) determination for *Terminalia chebula*

DILUTION	PLANT PRODUCTS				
	Terminalia chebula Retz (10mg)				
	E.coli 11	E.coli 13	E.coli 16	E.coli 19	E.coli 20
10.00	-	-	-	-	-
5.00	-	-	-	-	-
2.50	-	-	-	-	-
1.25	-	-	-	-	-
0.63	-	-	-	-	-
0.31	-	-	-	-	-
0.16	-	-	-	-	-
0.07	-	-	-	-	-

Table 6: Percentage of growth inhibition *by E. officinalis, A. sativum* and *T. chebula*

S. No	Strain no	Percentage of inhibition (µl)								
		Emblica officinalis			*Allium sativum*			*Curcuma longa*		
		25	50	100	25	50	100	25	50	100
1	11	79	88	100	87	90	100	83	100	100
2	13	75	81	100	82	92	100	83	100	100
3	16	73	84	100	85	95	100	82	100	100
4	19	75	83	100	83	96	100	81	100	100
5	20	75	84	100	80	97	100	85	100	100