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Sensitivity of Uropathogenic Bacteria against some Bioactive Compounds of Kalmegha (*Andrographis paniculata*) and Chirayta (*Sweratia chirayta*)

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Abstract: ***Present study focused on the sensitivity of some Uropathogenic bacteria against the extracted compounds present in leaves of two most useful medicinal plants Kalmegha (Andrographis paniculata) and Chirayta (Sweratia chirayta),collected during Nov. -Dec 2015, from Rajpur forest (District Ambikapur, Chhattisgarh) located in eastern Surguja range of forest, Chhattisgarh. The Bacteria were isolated from the UTI infected patients and characterized by microscopic, staining, morphological and biochemical methods. Aqueous extracts from plants were prepared and then used to test their antibacterial activity against the bacteria isolated from UTI infected patients and the zone of inhibitions were compared with the zone of inhibition of standard antibiotics. The common antibiotics Including Ciprofloxacin, Chloramphenicol, Amikacin and Ofloxacin were (30µg/ml) serve as control. The Uropathogens includes three strains of E. coli (E.coli strain I, E. coli strain II and E. coli strain III), Enterobacter aerogens, Klebsiella spp., Proteus mirabilis, pseudomonas aeruginosa and Streptococcus faecalis showed their sensitivity against the compound present in aqueous extract.***

Keywords: Kalmegha (Andrographis paniculata) and chirayta (Sweratia chirayta), Antimicrobial activity and Uropathogens

1. Introduction

Ever since man has started his life, he suffers with never ending struggle. Some time he is busy with collections of food and other time from diseases inflicted on them, or their domestic animals. In their surrounding, the plants and other natural substances are available to cure the diseases. A few plants play an important role to control the pathogen are called medicinal plant. They always served to improve human health and play positive role in development of civilized population. Developing countries like India, about 80% population directly or indirectly depend upon the plants having medicinal value for their health problem. About 25% medicinal industries are growing through the plants and their products (**20,21,22,23,24,25,26,27&28)**. Multidisciplinary approaches are used to develop the medicines including pharmaceutical science, microbiology, biochemistry, biophysics, economics and engineering. Plants are synthesized various compound and producer for such biomolecules, having importance in curing diseases (**14)**. Medicinal plant can be used as such, their extract, slurry, powdered, or their herbal preparation to cure disease.

In this way, the Urinary tract infection (UTIs) is a common community born infection and account for one third population of the world. Urinary tract is considered as the second-most common site for infection. Infections of the lower urinary tract (urethra and bladder) are common among women and affecting as many as one in five women at some time during their lifetime. Although UTIs are not as common in men, they can indicate an obstruction in urinary tract or other associated problems such as a stone or enlarged prostate; thus, they are uncommon in men under age fifty. The UTI in this study refers to infections of the lower urinary tract, the bladder and urethra. UTIs can chronically recur 20 percent of women who have one infection will have a recurrence. Of this group, 30 percent will have a third occurrence, and of this group, 80 percent have additional recurrences. In other words, the more infections one has had, the more likely another will occur. Many women with chronic UTIs are on antibiotics more than off, running the risk of developing dysbiosis and antibiotic resistance.

2. Materials and Methods

For the present study, the leaves of Kalmegha (*Andrographis paniculata*) and chirayta (*Sweratia chirayta*), were collected in March 2015, from the Rajpur forest, Chhattisgarh India, where the plant occurred in natural habitat such as red and sandy soil with slightly alkaline pH. At the time of collection, only large sized leaves were collected in sterilized polythene bag and brought to laboratory for further analysis (**2,3,4,25**).

Dry weight

In laboratory, the sample of leaves were weighed and then spread over a plane surface and dried it on $28^0C \pm 2^0C$ in cool, dry and dark place for one to two weeks in shade. After drying the samples of leaves, again weighed. Dry weight of leaves was calculated by the following:

Dry weight of leaves (%) = $[(P_0-P_1)/P_0 \text{ X } 100]$

Where: P_0 is the initial weight of leaves and P_1 is the weight after drying **(25)**.

Extraction of crude extract

For this, 10g fresh leaves were taken in mortar and pestle, and made its slurry by addition with distilled water (Ratio up to 1:10). The material was taken in a separatory funnel, and gently shaken for 30 minutes then allowed to stand for next 30 minutes. Mixture was then filtered through double layered muslin clothe and clarified by centrifugation at 7000 rpm for 30 min. at 4^0C, and then reduced their volume in boiling water in a water bath. The crude extract was sterilized by filtration through 0.22 um sterile filter paper (Millipore, Bedford Massachusetts, USA) and stored at -20^0 C until it's used(**25,29,30,31**).

Extraction of oil

For extraction of oil from the leaves, 10 gram dried and powdered leaves were taken in a conical flask and shocked with 50 ml of di-ethyl ether for overnight. Then flasks were taken out and filtered. Clear solution obtained was contained the oil (**25)**.

Density at 30^0C

Density of extracted essential oils was calculated as the mass of the essential oil at 30^0 C temperature range and the mass/volume of distilled water at the same temperature

Antibacterial activity (MIC)

Antibacterial activity was evaluated by a quantitative microspectrophotometric assay. Growth inhibition was tested in 100 wells microtiter plates at 595 nm wave length. For this, 70 µl Nutrient Agar Media (HiMedia) and 100 µl of the extract to be taken in microtitre plates, and then add 30 µl of freshly harvested 17 hours old bacterial colony containing 2X10 ^{6} bacteria/ml in 0.1 percent tween-80solution, in each well for each micro-organism. The common antibiotics Including Ciprofloxacin, Chloramphenicol, Amikacin and Ofloxacin were (30µg/ml each) serve as control **(**1). The plates were incubated at room temperature for 30 min to allow the spore settlement in the well and then incubated in incubator for 24 hrs.. All experiments were done in duplicate. The MIC data were recorded by **spectrophotometric method** by a micro plate reader at 595 nm. MIC of different microorganism was computized by comparing the growth of bacteria in control wells and the growth of bacteria with plant extracts containing well (**32&33**).

Table 1: Physico-chemical properties of dry and extracted material

S no.	Plant extract	Dry weight of leaves	Density of oil	Crude* aqueous extract	Oil content (%)
1	Chirayta (*Sweratia chirayta*)	10 gm	0.89	100ml	3.142
2	Kalmegha (*Andrographis paniculata)*	10gm	0.85	100ml	1.169

***Note**: Crude aqueous extract- 10 gm dry leaves extracted by 100 ml and final volume reduced up to10 ml*

Table 2: Antimicrobial activity in the extracted oils and crude aqueous suspension of *Sweratia chirayita* and *Andrographis paniculata* leaves, against some bacterial Uropathogens

S.No	Name of test Bacteria	Minimal inhibitory concentration of aqueous extract and zone of inhibition in aqueous extract and extracted oil							
		Sweratia chirayta				*Andrographis paniculata*			
		Diluted aqueous extract			Oils	Diluted aqueous extract			Oils
		10%	100%	1000%	As such	10%	100%	1000%	As such
1	*E. coli strain I*	+	+	--	14mm	+	--	--	12mm
2	*E. coli strain II*	+	--	--	13mm	+	--	--	14mm
3	*E. coli strain III*	++	+	+	17mm	+	+	--	16mm
4	*Enterobacter aerogens*	+	--	--	12mm	+	+	--	13mm
5	*Klebsiella spp.*	+	+	+	12mm	+	+	--	14mm
6	*Proteus mirabilis*	+	--	--	15mm	+	--	--	15mm
7	*Proteus valgris*	+	--	--	12mm	+	+	--	14mm
8	*Pseudomonas aeruginosa*	+	--	--	15mm	+	--	--	18mm
9	*Streptococcus faecalis*	+	--	--	14mm	+	--	--	08mm

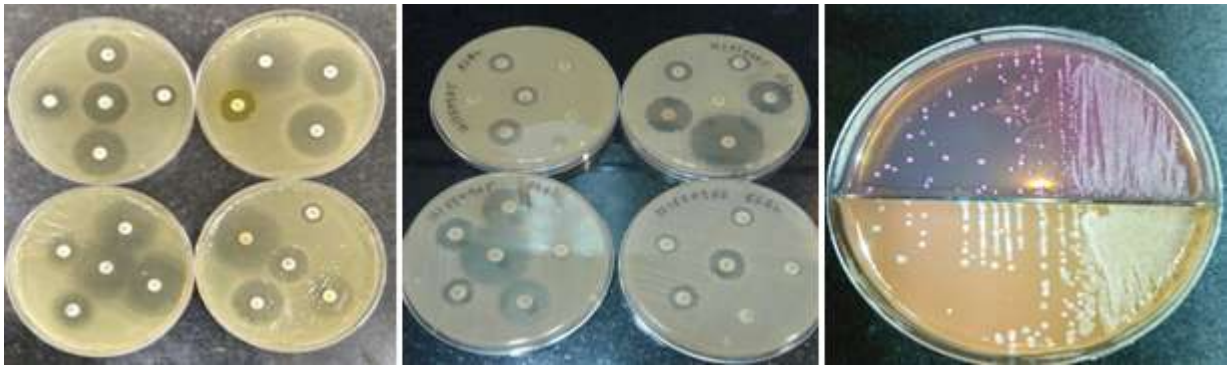
Table 3: Antimicrobial activity of some common antibiotics against some bacterial uropathogens (Used as control)

S.no.	Name of organism	Ciprofloxacin	Chloramphenicol	Amikacin	Ofloxacin
1	*Escherichia coli strain I*	12 mm (R)	O6 mm (R)	16 mm (S)	14 mm (S)
2	*E. coli strain II*	12 mm (R)	04 mm (R)	14 mm (S)	10 mm (R)
3	*E. coli strain III*	12mm(R)	06mm (r)	14mm (R)	12 mm (S)
4	*Enterobacter aerogens*	15 mm (S)	04 mm (R)	17 mm (S)	17 mm (S)
5	*Proteus mirabilis*	15 mm (S)	06 mm (R)	16 mm (S)	14 mm (S)
6`	*Proteus valgris*	06 mm (R)	04 mm (R)	14 mm (S)	14 mm (S)
7	*Pseudomonas aeruginosa*	18 mm (S)	06 mm (R)	16 mm (S)	12 mm (S)
8	*Klebsiella sp.*	14 mm (S)	04 mm (R)	18 mm (S)	12 mm (S)
9	*Streptococcus faecalis*	16 mm (S)	14 mm (S)	26 mm (S)	16 mm (S)

3. Result & Discussion

Kalmegha and Chirayita play an important role in clinical practices to cure the diseases and dis-order ([15,16,17 18]). Ayurveda contributes the knowledge of about 5000 plant species with having characteristic medicinal value. In this way, Kalmegha and Chirayita are able to synthesize and store various bioactive compounds as their secondary metabolites ([6,7,8,9,10]) including Xanthones, Amarogentin, Gentianine, Swertiamarin, Swerchirin, various phenolic compounds, flavonoids and antioxidant etc ([11,19]), that are capable of inhibiting the microbial growth and development in various way. However, many bacteria can cause an infection on urinary tract for example *Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia and Neisseria spp*. In literature, many workers reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria). The incidence of UTI is greater in women as compared to men. Several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI difficult.

Bacteria have the genetic ability to transmit and acquire resistance to drugs (39). Essential extracts of certain plants have been shown to have antimicrobial effects and produce flavor. The synergistic effect of the mixture of phytochemical plays an important role to use plant extracts as an antimicrobial agents. It has been suggested that volatile essential oils, either inhaled or applied to the skin, act by means of their lipo-proteino-philic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of calcium and phosphate ion channels. The antimicrobial and other biological activities of the plant extracts varied depending upon the origins and occurrence of cultivars.



The aim of our study to investigate the hidden antimicrobial potential of the essential oils and aqueous extract of powdered leaves of Kalmegha and Chirayita were tested against some common bacterial uropathogens causing Urinary Tract infection in man and woman (table 1,2&3). Most of the bacterial Uropathogenic species showed their susceptibility and sensitive to oils and crude extract. Their growth reduced up to various degrees and inhibited due to the compounds present in the extract in various concentration (**5,6,12,13,19**). Minimum inhibitory concentration (MIC) of aqueous extracts and crud essential oils were range between moderate to low level. On the other hand, the sensitivity against essential oils were found satisfactory and ranged between 12mm to 18mm zone of inhibition in case of ***E. coli*** strain III. Most common etiological agents are being gram-negative bacilli. Including *Escherichia coli* (*E. coli*) and accounts for 80 percent of UTIs, while other gram-negative bacilli, including *Klebsiella pneumonia*, *Proteus mirabilis*, and *Enterobacter aerogens* contribute somewhat less to incidence. Gram-positive cocci account for fewer UTIs than gram-negative bacilli. Among the organisms involved are *Staphylococcus saprophyticus* (responsible for 10-15 percent of UTIs in young-age women), *Enterococci*, and *Staphylococcus aureus* (most common in individuals with stones or who have been catheterized). Because the majority of UTIs are bacterial in origin, they are most commonly treated conventionally with antibiotics during the acute episode. While antibiotics are used to treat and prevent recurrent urinary tract infections, frequent antibiotic use can result in vaginal and intestinal dysbiosis as well as antibiotic resistance. Thus, it is desirable to seek alternative methods of prevention and treatment of UTIs.

In literature, the use of cranberry in folk medicine for the treatment of urinary tract infection was common in some country. It was once thought to benefit UTIs because hippuric acid in cranberries has the potential to acidify the urine. However, a more complete understanding of the pathogenesis of UTIs has led to a greater understanding of the mechanisms of action of cranberry in prevention and treatment – as an anti-adhesion agent. Cranberries have been found effective in the form of pure juice, sugared cocktail, and capsules and tableted extracts. Similarly in India, a large number of tribal medicines are evolved but they are not recorded in literature including: Kalmegha (*Andrographis paniculata*) and chirayta (*Sweratia chirayta*) can be use as an anti-adhesion agent.

Although, the strains of *E. coli*, those are most common pathogen associated with UTIs, has been studied extensively to determine virulence factors. One factor essential to its infective potential is the ability to adhere to epithelial cells of the urinary tract. Both *E. coli* and *Proteus* attach to uroepithelial cells by proteinaceous appendages called fimbriae (**35**). ***E. coli*** adheres to uroepithelial cells via type 1 pili actually it is long, hairy-surface organelles with a mannosebinding Fim H, which is a protein component at the fimbrae end that acts as an adhesive. Bacterial attachment results in a cascade of events involving elaboration of interleukin-6 and interleukin -8, which associated with leukocyte infiltration **(35,36,37).**These two herbal medicines are involved in preventing the bacterial adhesion on uroepithilial cells.

4. Conclusion

From the above results we can conclude that extracts of plants origin has remarkable antimicrobial activity as compare to antibiotic activity. We know that organisms are gaining resistance day by day towards the antibiotics, keeping this view in mind, some natural products should be tried to overcome these antibiotic resistant organisms. The plants produce including extracts or oils have no side effect produce one of our choices because it contains hydrophobic liquid which can be easily extracted by the simple process of distillation. Plant products or oils contain volatile aromatic, flavonoids and other biochemical's which show the antimicrobial activity. More over plants can be grown easily and their products are sophisticated than antibiotics. Expense on these material is bearable than antibiotic. The present study has been conducted to identify effective herbal medicines to control UTI caused by bacterial because of herbal medicines are available in our environment and safe

to human consumption and do not cause any side effects. On the basis of these important properties of plants we can say that natural medicine can replace the antibiotics in future.

The bioactive compounds extracted from Kalmegha and Chirayita have considered as a number of beneficial medicinal effects for different types of dis-order including UTIs, without any harms and side effects. These bioactive compounds are very cheaper than other allopathic medicines. Proper protection is required to conserve these species of plants and promote its medicinal value for pharmaceutical purpose, because herbal medicine has very high value in international market. In India, less attention paid in the development of our natural resources. On other hands, China, Korea, Malaysia and Indonesia exploited their intellectual properties and fully use their resources to earn foreign exchange and generate employment and established big industries.

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