Phytochemical Analysis and In-Vitro Antibacterial Activity of Some Medicinal Plants against Multi-Drug Resistant Urinary Isolates

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***Abstract*- Emerging antibiotic resistance is a worldwide problem that has led to the need for development of novel antimicrobials. Evaluation of natural products as safe and effective antimicrobial agents is one of the scientific strategies to combat the menace of drug resistant pathogens. In present study, methanol extract of four medicinal plants such as *Kalanchoe pinnata*(leaves), *Anethum graveolens*(leaves), *Crataeva nurvala*(stem bark) and *Boerhaaviya diffusa*(whole plant) were selected and analyzed for preliminary phytochemicals and *in-vitro* antibacterial activity against selected multi drug resistant urinary isolates by well diffusion assay and MIC values were also determined to find minimum effective concentration. All the extracts showed good antibacterial activity against highly resistant UTI isolates. The phytochemical analysis revealed that the plant extract contains the important phyto-constituents like tannin, flavonoid, carbohydrate, protein, terpanoid and cardiac glycoside indicating its potential activity for treatment of Urinary Tract Infection (UTIs).**

Key words: **Urinary Tract Infection (UTI), Anethum graveolens, Kalanchoe pinnata, Crataeva nurvala, Boerhaavia diffusa, anti-bacterial activity, phytochemical analysis.**

I. Introduction

Urinary Tract Infections (UTIs) are the most widespread microbial disease in India as per WHO. The common pathogenic bacteria causes UTI are *Escherichia coli, Klebsiella pneumoniae, Haemophilus influenza, Streptococcus pneumonia and Proteus vulgaris* [1]. Many of the antibiotics and synthetic drugs become resistant and show many undesirable side effects. So natural altrnative must be selected as they are more safe in biological system [2]. Plants and herbal medicines have important position in modern medicine, due to their chemical and medicinal contents found in natural form. They contain various secondary metabolites which work together and show wide range of antibacterial activities. Microorganisms may get mutated and become resistant to many antibiotics and so it generates a global health problem. These inspired scientists to search out new natural alternative to treat diseases [3].

A number of plants have been documented for their biological [4,5] and antimicrobial properties [6,7]. In an effort to expand the spectrum of antimicrobial agents from natural resores, four medicinal plants have been selected based on their traditional uses in India to assess their antibacterial activity against the 7 multidrug resistant urinary isolates.

II. Material And Methods

A. Collection of Plant Material

Four plants, shown in Table I, *Anethum graveolens, Kalanchoe pinnata, Crataeva nurvala and Boerhaavia diffusa* which were traditionally proved to have medicinal properties were selected. *Kalanchoe pinnata* was collected from Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat. *Anethum graveolens* was growen and other plant materials were collected from Botanical Garden, Vaghai. Selected plant material was washed with running water, dried in shade at room temperature, ground to powder and stored in dry air tight bag at low temperature. The picture of all the selected plants is shown in figure 1.

TABLE I
List of Plant and parts used

Scientific name	Common name	Family	Part used
Kalanchoe pinnata	Air plant	Crassualaceae	Leaves
Anethum graveolens	Sowa	Umbellifere	Leaves
Crataeva nurvala	Varuna	Capparaceae	Stem bark
Boerhaavia diffusa	Punarnava	Nyctaginaceae	Whole plant

Fig. 1. Selected Plants

Kalanchoe pinnata

Anethum graveolens

Crataeva nurvala

Boerhaavia diffusa

B. Extraction

The powdered samples were used for extraction using absolute methanol as a solvent by Soxhlet apparatus. After removing pigments, the solvents were evaporated and the residue was dissolved in DMSO. The % yield of different plant extract was calculated using following formula,
% yield = Weight of final extract × 100 / weight of powdered sample [8].

C. Isolation and Selection of Bacterial species

A total 500 Urine samples of the patient suffering from UTI were collected from Bhanumati laboratory and Parsi hospital at Navsari, Gujarat. The isolated bacterial species were identified by biochemical tests [9]. From all the identified causative agents, highly resistant species were selected by performing antibiotic susceptibility test against 12 different antibiotics which are Ampicillin (AS), Co-Trimoxazole (BA), Ceftizoxime (CI), Chloramphenicol (CH), Cephalexin (PR), Tetracyclin (TE), Ciprofloxacin (RC), Nitrofurantoin (FD), Sparfloxacin (DC), Gatifloxacin (GF), Norfloxacin (NX), Ofloxacin (ZN).

D. Anti-bacterial Assay

Anti-bacterial activity was determined by agar well diffusion assay against all selected bacterial species [10]. The zone of inhibition was measured in mm and Minimum Inhibitory Concentration (MIC) was also determined using 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 dilutions by the same method.

E. Phytochemical Evaluation

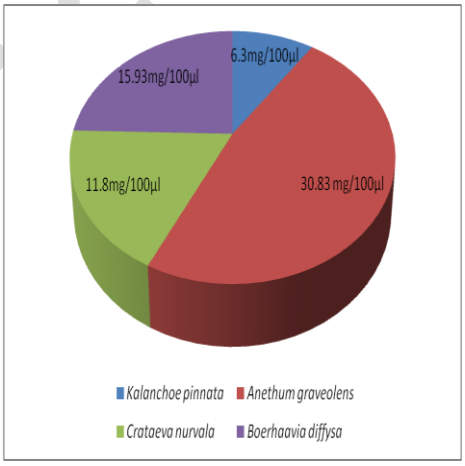
Preliminary phytovhemical analysis was carried out using standard protocol for determination of phytoconstituents like alkloids, tannins, saponin, aonthocyanide, phenolic flavonoids, flavonoids, carbohydrate, protein, terpenoids, cardiac glycoside and phlobatannins as directed by references [11], [12], [13] and [14].

III. Results And Discussion

A. Percentage Yield

The highest yield was obtained with methanol extract of *Anethum graveolens* followed by *Boerhaavia diffusa* followed by *Cretaeva nurvala*, while lowest yield was obtained with *Kalanchoe pinnata* (6.3mg/100µl). The extractive yield of selected plant is shown in Figure 2.

Fig. 2. Extractive Yield of Methanol Extract



B. Isolation and Selection of Bacterial species

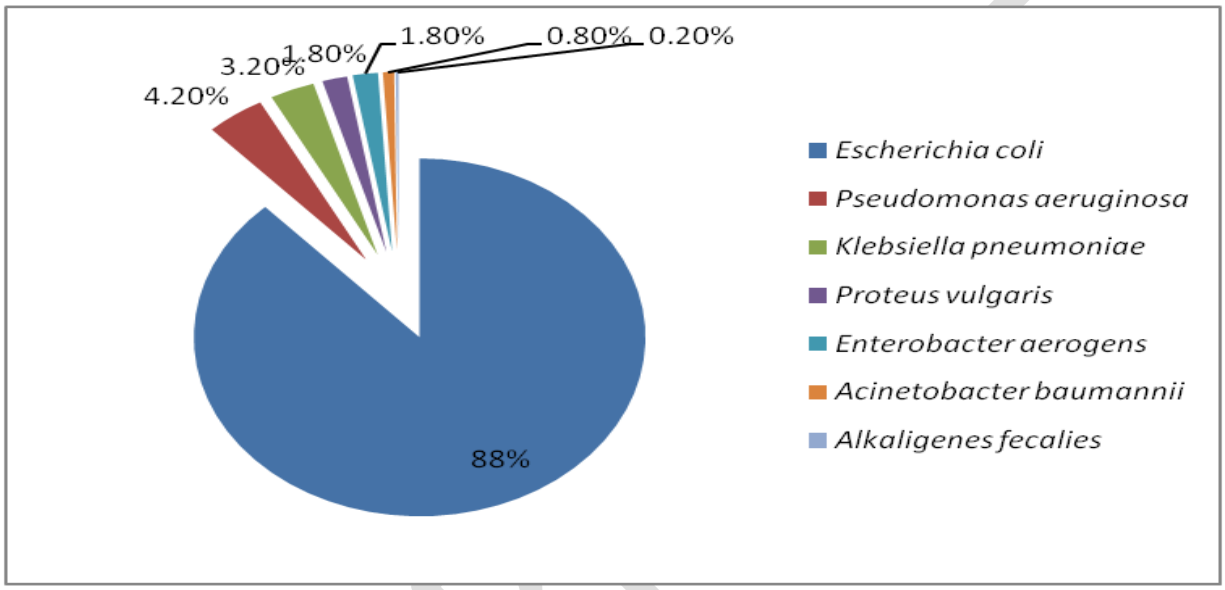
Out of 500 urine samples, the isolates belong to 7 different species. Of these *Escherichia coli is* predominant (88%); *Pseudomonas aeruginosa* (4.2%); *Klebsiella pneumoniae* (3.2%); *Proteus vulgaris* and *Enterobacter aerogens* (1.8%); *Acinetobacter baumannii* (0.8%) and *Alkaligenes facalies* (0.2%) were isolated. Frequency of microorganisms to cause UTI is given in figure 3. All the isolates were tested for antibiotic susceptibility and highly resistant specieses were selected. The results of antibiotic susceptibility test of selected species is shown in Table II.

C. Anti-bacterial Assay

Methanolic extract of all the plants were tested for the antibacterial activity against all the selected highly resistant

bacterial species. Zone of inhibition was measured in mm which is shown in Figure 4. Methanol extract of *Anethum graveolens* showed maximum activity against all the selected isolates except *Klebsiella pneumoniae* while maximum activity against *Klebsiella pneumoniae* was shown by methanolic extract of *Kalanchoe pinnata* and *Crataeva nurvala*. No zone of inhibition was observed with methanolic extract of *Kalanchoe pinnata* and *Crataeva nurvala* against *Pseudomonas aeruginosa*. Formation of clear zone of inhibition surrounding the well clearly showed that all the methanolic extracts of selective plants were effective on all the selected multi drug resistant urinary isolates. This antibacterial activity might be due to presence of bioactive compounds in plant extract.

Fig. 3. Frequency of microorganisms in urine sample



MIC of the plant extracts was determined by well diffusion method by using different dilutions and results of MIC is shown in figure 5. Lowest MIC was shown by *Kalanchoe pinnata* (3.15 mg/100µl) against *Acinetobacter baumannii and* 6.3 mg/100µl against *Alkaligenes facalies*, *Enterobacter aerogens*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Escherichia coli.*

TABLE II

Antibiotic Susceptibility Test of Urinary Isolates

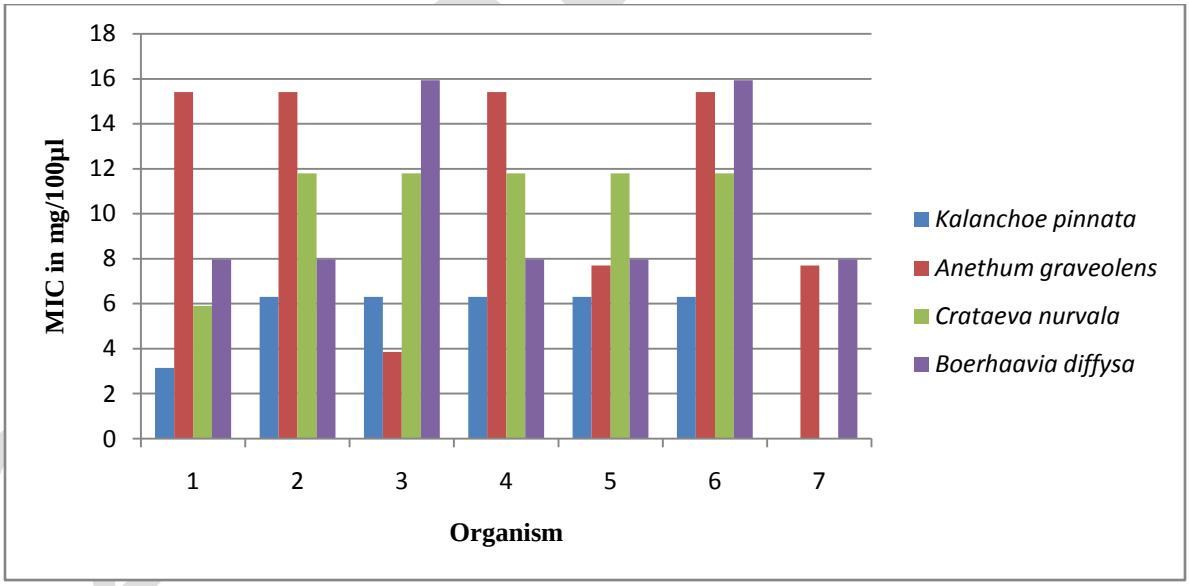
Sr. No.	Name of antibiotic	Number of organism						
		1	2	3	4	5	6	7
1.	Ampicillin	R	R	S	R	R	R	R
2.	Co-trimoxazole	S	R	S	R	R	R	R
3.	Ceftizoxime	R	R	R	R	R	R	R
4.	Chloramphenocol	R	S	S	R	R	S	S
5.	Cephalexin	R	S	S	R	R	R	R
6.	Tetracycline	R	R	S	R	R	R	S
7.	Ciprofloxacine	R	S	S	R	R	R	R
8.	Nitrofurantoine	R	S	S	S	S	S	S
9.	Sparfloxacine	S	S	S	S	R	R	R
10.	Gatifloxacin	S	S	S	S	S	S	S
11.	Norfloxacin	R	S	R	R	R	R	R
12.	Oflixacin	R	S	S	R	R	R	S

1-*Acinetobacter baumannii*, 2-*Alkaligenes facalies*, 3-*Enterobacter aerogens*, 4-*Proteus vulgaris*, 5-*Klebsiella pneumoniae*, 6-*Escherichia coli*, 7-*Pseudomonas aeruginosa*. R= Resistant, S=Sensitive

Fig. 4.Antibacterial activity of Methanol extract of different plants

1-*Acinetobacter baumannii*, 2-*Alkaligenes facalies*, 3-*Enterobacter aerogens*, 4-*Proteus vulgaris*, 5-*Klebsiella pneumoniae*, 6-*Escherichia coli*, 7-*Pseudomonas aeruginosa*.

Fig. 5. MIC of different plant extract against different organisms



1-*Acinetobacter baumannii*, 2-*Alkaligenes facalies*, 3-*Enterobacter aerogens*, 4-*Proteus vulgaris*, 5-*Klebsiella pneumoniae*, 6-*Escherichia coli*, 7-*Pseudomonas aeruginosa*.

D. Phytochemical Evaluation

The phytochemical analysis reveals presence of alkaloid, tannin, flavonoid, carbohydrate, protein, terpanoid and cardiac glycoside etc..Their presence in extract indicate its effectiveness in inhibition of microbial growth. The results of phytochemical constituents is given in table III .

Table III

Preliminary Phytochemical Screening of Metanol extract of Different Plants

Sr. No.	*Phytoconstituent*	*Kalanchoe pinnata*	*Anethum **graveolens***	*Crataeva nurvala*	*Boerhaavia diffusa*
1.	Alkaloid : 1)Dragendroff'	+	-	+	+
	2)Hagner's	+	-	+	+
2.	Tannin : 1) $FeCl_3$ test	+	+	+	+
	2) Gelatin test	+	+	+	+
3.	Saponin	-	+	+	-
4.	Aonthocyanide	-	-	-	-
5.	Phenolic flavonoides	-	-	-	-
6.	Flavonoides : 1)NaOH	+	+	+	+
	2) NH_3	+	-	-	+
7.	Carbohydrate	+	+	+	+
8.	Protein : 1)Millon's	-	-	-	+
	2)Biuret	+	+	-	-
9.	Terpanoid	-	+	+	+
10.	Cardiac glycoside	+	+	+	+
11.	Phlabatannin	-	-	-	-
12.	Oil	-	-	+	-

IV. Conclusion

The present study shows that all the selacted plant extracts had successfully inhibited all the selected multi-drug resiatant isolates of UTIs. Methanolic extract of Kalanchoe pinnata showed least MIC value against selected pathogens. All the selected plants showed presence of various ptytoconstituents which might be effective to inhibit microbial growth. These results suggest that methanolic extract of these plants may be serve as a good source of natural treatment of UTI.

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REFERENCES

[1]. Anita, P., Anthoni Samy, A. and Raj, J. S., (2011). *In Vitro* Antibacterial Activity of *Aegiceras corniculatum* and *Burguiera cylindrica* Against Isolated Bacterial Urinary Tract Infections : IJPRD, 3(11): 120-125.

[2]. Atal, CK., (1985). Chemistry of Some Activity Indian Medicinal Plants: Proc Ind Nat Sci Acad, 48: 99-121.

[3]. Khoobchandani, M.,Ojeswi, B.K., Ganesh, N., Srivastava, M.M., Gabbanini, S., Matera, R., Iori, R. and Valgimigli, L., (2010). Antimicrobial Properties and Analytical Profile of Traditional *Eruca satavia* seed oil: Comparision with VariousArial and Root Plant Extracts: Food Chem.,120: 217-224.

[4]. Grover, J.K., Yadav, S.V., Vats V., (2002). Medicinal Plants of India with Anti-diabetic potential: J Ethanopharmacol, 81: 81-100.

[5]. Gajera, H.P., Patel, S.V., Golakiya, B.A., (2005). Antioxidant Properties of some Therapeutically Active Medicinal Plants-an Overview: JMAPS, 27: 91-100.

[6]. Arora, D.S., (1998). Antimicrobial Activity of Tea (*Camellia sinesis*): Antibiot Chemother, 2: 4-5.

[7]. Polambo, E.A., Semple, S.J., (2001). Antibacterial Activity of Traditional Australian Medicinal Plants: J Ethanopharmacol, 77: 151-157.

[8]. Janjua, S., Shaid, M. and Abbas, F., (2013). Phytochemical Analysis and *In Vitro* Antibacterial Activity of Root Peel extract of *Raphanus sativus* L. var niger: Advancement in Medicinal Plant Research, 1(1): 1-7.

[9]. Holt, J.G., Kreig, N,R., Sneath, P.H.A., Staley, J.T. amd Williams, S.T., (1994). In: Bergy's Manual of DeterminativeBacteriology, ninth ed. Williams and WilkinsPub., MD, USA.

[10]. Ettebong, E. andNwafor P., (2009). *In Vitro* Antibacterial Activities of extracts of *Carpolobia lutea* root: Pak J Pharm Sci, 22(3): 335-338.

[11]. Trease, G.E., Evans W.C.,(1978). Pharmacognosy.11th ed.BrailliarTiridel andMacmillian Publishers, London.

[12]. Harborne, J.B. and Harborne, A.J.,(1998). Phytochemical methods: Aguide to Modern Techniques of Plant analysis. Kluwer Academic Publishers, London, UK.

[13]. Kaur, G.J. and Arora, D.S., (2009). Antibacterial and Phytochemical Screeningof *Anethum graveolens, Foeniculum vulgare* and *Trechyspermum ammi*: BCM Complement Altern Med 9: 30.

[14]. Kumar, K.A., Narayani,M., Subanthini, A. andJaykumar, M., 920110. Antibacterial Activity and Phytochemical Analysis of Citrus fruit peels Utilization of Fruit waste: Int J Environ Sci Tecj, 3: 5415-5421.