

Antibacterial and Phytochemical Studies with Cytotoxicity assay of *Kalanchoe pinnata* leave extract against Multi-drug Resistant Human Pathogens Isolated from UTI

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ABSTRACT

UTI is one of the most common infections and more common in women than in men. Overuse of antibiotics has developed numerous defense mechanisms against chemical antimicrobial agents by bacterial pathogens; hence resistance to old and newly produced drugs is on the rise. The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Thus the present study was undertaken to investigate the antibacterial activity of different solvent (Methanol, Ethanol, Acetone, Petroleum ether and Water) extract of *Kalanchoe pinnata* leaves against multi-drug resistant UTI pathogens isolated from urine samples collected from patients suffering from UTI. Antimicrobial susceptibility test of 515 urinary isolates against 12 different antibiotics was performed to select multi drug resistant bacterial species and 7 different highly resistant bacterial species were selected for this study. *In-vitro* antibacterial activity was screened by well diffusion method against selected MDR bacterial species. All the selected extracts showed good antibacterial activity against all the selected MDR UTI pathogens in which water extract has greater antibacterial activity against all tested uropathogens. Preliminary phytochemical analysis showed presence of tannin, flavonoids, carbohydrates, protein, terpenoids and cardiac glycosides. Results of Cytotoxicity test performed by MTT assay and Viability assay showed less toxicity and more than 80% viability. Results of the present study showed that *K. pinnata* can be used as a potential source for drug development for the treatment of Urinary Tract Infection caused by multi drug resistant bacteria.

Key words: UTI, Antibacterial activity, Phytochemical analysis, *Kalanchoe pinnata*, Cytotoxicity assay

I. INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections worldwide which is more common in women than in men (Mihankhah *et. al.*, 2017). About 150 million people developed a urinary tract infection each year (Flores-Mireles, *et. al.*, 2015). UTI is defined as presence of bacteria in urine along with urinary symptoms like dysuria, frequency, urgency and occasionally suprapubic tenderness (Dash *et. al.*, 2013). Wide spectrums of organisms are implicated in its etiology but the most common being *Escherichia coli* and other gram negative bacteria, followed by gram positive organisms (Kamat *et. al.*, 2009). These bacteria cause UTI and if not treated, the infection will spread and cause serious damage to the patient (Singh *et. al.*, 2017).

UTI treatment with antibiotics is carried out usually before receiving microbiology test results. This therapy, without rational drug prescription occasionally leads to antibiotic resistance and treatment failure (Zone and Guide, 2017). Bacteria have the genetic ability to transmit and acquire resistance to drugs (Soulsby, 2005). Discovery of antibiotics was one of the greatest advances of modern medicine, but the availability and increased use of antibiotics gradually lead to microbial resistance to them (Gottlieb and Nimmo, 2011). Antimicrobial resistance is increasing around the world, especially in developing countries (Sadeghabadi *et. al.*, 2014). According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a serious threat to modern medicine (WHO, 2014). As uropathogens are increasingly becoming resistant to currently available antibiotics, it may be time to explore alternative strategies for managing UTI. Therefore researchers are increasingly turning their attention to folk medicine (Benkeblia, 2004). Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious and safe (WHO 1995).

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. According to World Health Organization (WHO), about 80% of the world population rely chiefly on plant based traditional medicine specially for their primary health care needs and there has been a worldwide move towards the use of traditional medicines due to concerns over the more invasive, expensive and potentially toxic main stream practices (WHO 1995).

The present study relates to preliminary phytochemical screening, antibacterial activity, phytochemical study and cytotoxicity assay of *Kalanchoe pinnata* leave extracts against multi-drug resistant (MDR) uropathogens isolated from urine sample of patients suffering from UTI.

II. MATERIALS AND METHODS

2.1. Collection of urine samples and Selection of MDR bacterial strain from urine:

Urine samples from the patients suffering from UTI were collected from various laboratories: Bhanumati laboratory and Parsi Hospital, Navsari and Advanced Diagnostic Laboratory, Surat. The isolated bacterial UTI pathogens were identified on the basis of gram staining, morphological and biochemical characteristics (Holt *et. al.*, 1994).

From the identified bacterial isolates, highly resistant bacterial strain was selected by performing antibiotic susceptibility test (Baris *et. al.*, 2005) using Pathoteq 'Bio-Disc-12' (Pathoteq Biological Laboratories, India), which includes 12 antibiotics (Ampicillin/Sulbactam, Co-trimoxazole, Ceftizoxime, Chloramphenicol, Cephalexin, Tetracycline, Ciprofloxacin, Nitrofurantoin, Sparfloxacin, Gatifloxacin, Norfloxacin and Ofloxacin) used for treatment of UTI. The zones of growth inhibition were then measured. The diameter of the zone is related to the susceptibility of the test microorganism and to the diffusion rate of the drug through the agar medium. The results were interpreted using the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2007).

Selected MDR bacterial isolates were also identified using VITECH system. Out of these MDR UTI pathogens, highly resistant bacterial strain of *K. pneumoniae* and *E. coli* (major cause of UTI) were further identified by 16s r-RNA analysis. In this method, DNA from the selected bacterial pathogens was isolated and quality was evaluated using agarose gel electrophoresis. The fragments of 16S rDNA gene were amplified by PCR using forward primer 8F and reverse primer 1492R. The DNA sequencing of purified PCR amplicon was carried out using BDT v3.1 cycle sequencing kit on ABI 7370x1 Genetic analyzer. BLAST of the 16S rDNA sequence was carried out with the nr databases of National Center Biotechnology Institute gene bank database and based on maximum identity score first ten sequences were selected. The sequencing of the bacterial strains was conducted by Saffron Life Sciences Pvt. Ltd.

2.2. Plant Extraction:

Kalanchoe pinnata was collected from Shree Bapalal Vaidya Botanical Research Centre, Veer Narmad South Gujarat University, Surat, India. Taxonomic identification of the plant was confirmed by Dr. B. K. Dhaduk, Horticulture Department, Agriculture University, Navsari. The leaves of plant material used for the study was washed under running tap water thoroughly, air dried and homogenized to fine powder and 10 g of powdered plant material was extracted using 150 ml five different solvents; methanol, ethanol, acetone, petroleum ether and water using Soxhlet extraction apparatus (Superfit Continental Pvt. Ltd.) for 8 h. The extracts were concentrated and extractive % yield was calculated (Kepam, 1986):

$$\text{Extractive \% yield} = \text{Weight of final extract} / \text{Weight of Powdered sample} \times 100.$$

The dried extracts were re-dissolved in minimum volume of DMSO and then preserved in refrigerator for further studies.

2.3. Preliminary phytochemical analysis:

All the plant extracts were subjected to preliminary phytochemical analysis to study the presence of phytoconstituents *viz.*, alkaloids, tannins, saponin, anthocyanide, phenolic flavonoids, flavonoids, carbohydrate, protein, terpenoids, cardiac glycosides, oil by standard methods described by Trease and Evans (1989).

2.4. Determination of antibacterial activity and MIC:

All the solvent extracts of *K. pinnata* were subjected to antibacterial screening test by well diffusion assay (Magaldi *et. al.*, 2004). The plates containing Muller Hinton Agar medium were inoculated with the selected MDR UTI pathogens and 6 mm wells were prepared. The wells were filled with 100 µl of respective solvent extract and control well with DMSO. Plates were then incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the selected UTI pathogens. The experiment was performed in triplicate and the mean of zone diameter was calculated. MIC was also determined by the same method.

2.5. Cytotoxicity assay:

The solvent extract of plant showing maximum antibacterial activity was selected for *in vitro* cytotoxicity assay to determine toxicity of the plant extract. Cytotoxicity assay (MTT and Viability Assay) of water extract of *K. pinnata* was performed on HeLa Cervical Cell line at Department of Toxicology GeneXplore Diagnostics and Research Centre Pvt. Ltd., Ahmedabad. % Inhibition and % viability was found by using following formula:

$$\% \text{ Inhibition} = 100 - \text{Abs (sample)} / \text{Abs (control)} \times 100$$

$$\% \text{ viability} = (\text{live cell count} / \text{total cell count}) \times 100$$

III. RESULTS AND DISCUSSION

3.1 Selection of MDR bacterial strain from urine:

In this study, out of 550 mid-stream urine samples, 543 uropathogens were recovered. Out of 543 isolates, 515 isolates were bacteria and 28 isolates were found to be *Candida albicans*. As the aim of the study was to check the antibacterial activity of medicinal plants, only bacterial isolates were used for further studies. The urinary isolates were identified based on morphological and biochemical characteristics. According to the current study, gram negative bacteria were responsible for 100% of UTIs.

This study reported *Escherichia coli* was the most predominant uropathogen with 85.40%, followed by *Pseudomonas aeruginosa* 5.04%, *Klebsiella pneumonia* 4.27%, *Proteus vulgaris* and *Enterobacter aerogens* 1.74%, *Acinetobacter baumannii* 1.55% and *Alkaligenes fecalis* 0.10% (Figure-3.1). It was found that 89% of infection was due to *Escherichia coli*, 3.7% due to *Klebsiella*, 1.2% due to *Proteus*, 1.2% due to *Citrobacter*, 1.2% due to *Staphylococcus* and *Enterococcus* 3.7%. Similar work was carried out by Ejaz *et. al.*, (2006). They collected 100 UTI samples, from those 48 samples from male and 52 samples from females with UTI. In their report they found UTI caused by gram negative bacteria. The most frequent causative agents of UTIs in this study were found to be *E. coli* and *Klebsiella* (each 37%) followed by *Pseudomonas* (23%). *Acinetobacter*, *Proteus* and *Staphylococcus aureus* were all 1% each. Studies carried out by the University of Florida, USA, with 81 patients having UTI.

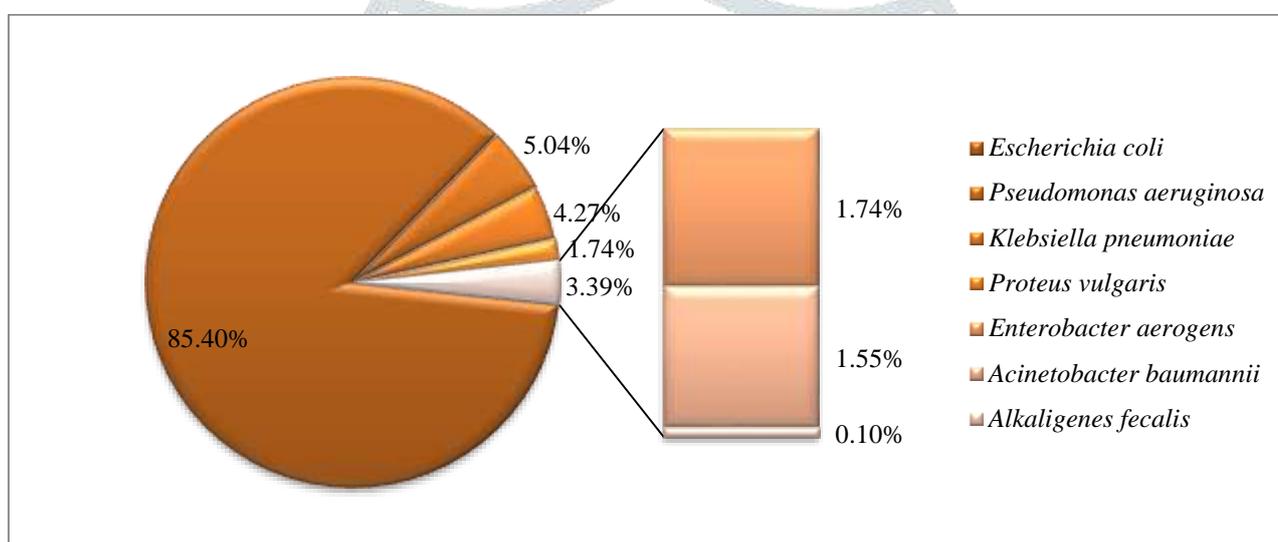


Figure 3.1: Percentage occurrence of bacterial pathogens in UTI

All the identified isolates were studied for antibiotic resistance profile to select MDR UTI pathogens. The results are shown in Table-3.1. *K. pneumoniae* showed maximum resistance, was sensitive to only 2 antibiotics, Nitrofurantoin and Gatifloxacin. *E. coli* showed sensitivity against three antibiotics i.e. Nitrofurantoin, Gatifloxacin and Chloramphenicol. *P. vulgaris* was also sensitive to three antibiotics; Nitrofurantoin, Sparfloxacin and Gatifloxacin while *A. baumannii* was sensitive to Co-trimoxazole, Chloramphenicol, Sparfloxacin and Gatifloxacin. Minimum resistance was observed with *E. aerogens* which showed sensitivity to all tested antibiotics except Co-trimoxazole and Norfloxacin followed by *A. fecalis* which was resistant to only four antibiotics i.e. Ampicillin/Sulbactam, Co-trimoxazole, Ceftizoxime and Tetracycline. Comparing the effect of all antibiotics on the tested seven bacterial isolates, Gatifloxacin was the only drug which was effective on all the selected UTI pathogens followed by Nitrofurantoin which was effective on all bacterial isolates except *A. baumannii* and Chloramphenicol which showed effectivity on all except *P. vulgaris* and *K. pneumoniae*.

Identification of these 7 different species, showing resistance against many antibiotics, was also confirmed by VITECH system and the results showed similarity with the results of biochemical identification. Highly resistant bacterial isolates (*K. pneumoniae* and *E. coli*) were also identified by molecular identification. On the basis of 16S rRNA sequences comparison with available sequences at NCBI GeneBank, sequences of *K. pneumoniae* showed 100% identity and 0% gaps with 16S rRNA partial gene sequence of *K. pneumoniae* strain CCFM83581. On the basis of 16S rRNA sequences comparison with available sequences at NCBI GeneBank, sequences of *E.coli* showed 100% identity and 0% gaps with 16S rRNA partial gene sequence of *E. coli* strain NRC111.

3.2 Extractive yield:

The quantity and composition of metabolite of an extract depends on type of extraction, time of extraction, temperature and nature, concentration and polarity of the solvent (Ncube *et. al.*, 2008). In the present study, powdered plant materials of leaves of *K. pinnata* was extracted individually with different solvent (methanol, ethanol, acetone, petroleum ether and water) by Soxhlet

extraction method showing extractive yield varied among different plant material and different solvent as the extractive values depends on the phytoconstituents present in plant and their solubility in a particular solvent.

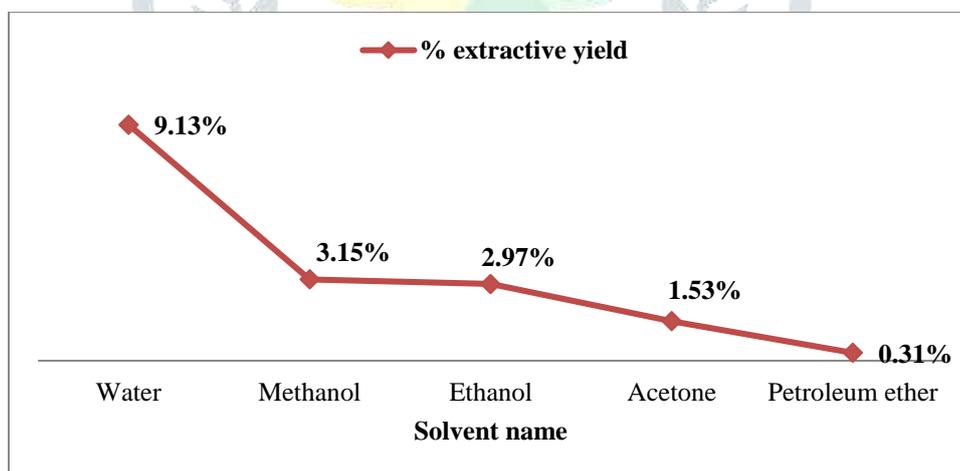
Table 3.1: Antibiotic resistance profile of urinary isolates

Sr. No.	Name of antibiotic	Diameter of zone of inhibition (mm)						
		<i>A.b.</i>	<i>A.f.</i>	<i>E.a.</i>	<i>P.v.</i>	<i>K.p.</i>	<i>E.c.</i>	<i>P.a.</i>
1.	Ampicillin/Sulbactam	00	00	27	00	00	00	00
2.	Co-trimoxazole	30	00	10	00	00	00	00
3.	Ceftizoxime	00	00	00	00	00	00	00
4.	Chloramphenicol	11	14	20	00	00	11	21
5.	Cephalexin	00	20	16	00	00	00	00
6.	Tetracycline	00	00	16	00	00	00	17
7.	Ciprofloxacin	00	19	10	00	00	00	00
8.	Nitrofurantoin	00	10	15	19	10	17	18
9.	Sparfloxacin	15	21	10	10	00	00	12
10.	Gatifloxacin	11	22	15	11	11	11	14
11.	Norfloxacin	00	17	00	00	00	00	00
12.	Ofloxacin	00	18	12	00	00	00	11

A.b.-*Acinetobacter baumannii*, *A.f.*-*Alkaligenes fecalis*, *E.a.*-*Enterobacter aerogens*, *P.v.*-*Proteus vulgaris*, *K.p.*-*Klebsiella pneumoniae*, *E.c.*-*Escherichia coli*, *P.a.*-*Pseudomonas aeruginosa*.

The extractive % yield of different solvent extracts of *K. pinnata* is shown in Figure-3.2. Maximum % extractive yield was obtained with water extract followed by and minimum with petroleum ether extract. Solvent wise rank can be given from high to low % extractive yield in following order:

Water > Methanol > Acetone > Ethanol > Petroleum ether

Figure 3.2.: % Extractive yield of *Kalanchoe pinnata* with different solvent extract

3.3. Preliminary phytochemical analysis:

Phytochemical screening of different solvent extract of the *K. pinnata* showed variations in presence of phytoconstituents such as tannin, flavonoids, carbohydrates, protein, terpenoids and cardiac glycosides. Saponin was present only in water extract and tannin in petroleum ether extract. The results are summarized in Table-3.2. Tannin and cardiac glycoside were present in all the solvent extracts while anthocyanide, phenolic flavonoids and oil were absent in all the solvent extracts. Petroleum ether extract showed minimum phytoconstituents i.e. alkaloid, tannin and cardiac glycoside.

According to this study, the ethanol extract of *K. pinnata* showed presence of tannin, flavonoids, carbohydrates, proteins, terpenoids and cardiac glycoside while, Akinnibosun and Edionwe, (2015), reported the presence of saponin, flavonoids,

alkaloids, steroids, tannins, cardiac glycosides and reducing sugars. According to Biswas *et. al.*, (2011), alkaloids, glycosides, steroids, gums, flavonoids, saponin, reducing sugars and tannins were present. Acetone extract of fresh leaves of *K. pinnata* showed presence of tannin, flavonoids, carbohydrates, proteins, terpenoids and cardiac glycoside while according to Akinnibosun and Edionwe, (2015), saponin, flavonoids, steroids, alkaloids, cardiac glycosides and reducing sugar were present.

Table 3.2: Preliminary phytochemical analysis of *K. pinnata* solvent extracts

Sr. No.	Phytochemicals	Methanol	Ethanol	Acetone	Petroleum ether	Water
1.	Alkaloid	-	-	-	+	-
2.	Tannin	+	+	+	+	+
3.	Saponin	-	-	-	-	+
4.	Anthocyanide	-	-	-	-	-
5.	Phenolic flavonoids	-	-	-	-	-
6.	Flavonoids	+	+	+	-	+
7.	Carbohydrate	+	+	+	-	+
8.	Protein	+	+	+	-	+
9.	Terpenoids	-	+	+	-	-
10.	Cardiac glycoside	+	+	+	+	+
11.	Oil	-	-	-	-	-

“+” = Present and “-” = Absent.

The petroleum ether extracts of the powdered leaves of *K. pinnata* showed presence of alkaloid, tannin, gum, saponin, reducing sugar and glycoside while the study of Chowdhury *et. al.*, 2011, showed presence of only alkaloid, tannin and cardiac glycoside. Water extract of *K. pinnata* showed phytochemicals like, tannin, saponin, flavonoids, carbohydrate, protein and cardiac glycoside. These results are in accordance with the results obtained by Jhon and Ojewole, (2005) and Anjoo and Kumar, (2010), while according to Akinnibosun and Edionwe, (2015), saponin, flavonoids, alkaloids and reducing sugars were present in aqueous extract of *K. pinnata*. According to Chowdhury *et. al.*, 2011, alkaloid, glycoside, steroid, saponin and tannin were present in aqueous extract of *K. pinnata*. This difference in the result may be environmental and climatic conditions of the plant and the choice of extraction method (Zhongying *et. al.*, 2006).

3.4 Determination of antibacterial activity:

Antibacterial activity of *K. pinnata* is represented in Table-3.3. All the selected MDR urinary isolates was inhibited by all solvent extracts of *K. pinnata* except, *P. aeruginosa* which was resistant to petroleum ether extract. Inhibition zone was not observed with control.

Table 3.3: Antibacterial activity of *Kalanchoe pinnata* leaves extract

Sr. No.	Solvent	Diameter of zone of inhibition in mm						
		<i>A.b.</i>	<i>A.f.</i>	<i>E.a.</i>	<i>P.v.</i>	<i>K.p.</i>	<i>E.c.</i>	<i>P.a</i>
1.	Methanol	16	14	13	10	10	13	12
2.	Ethanol	16	11	12	10	14	12	11
3.	Acetone	16	12	12	11	15	13	13
4.	Petroleum ether	17	15	11	11	12	10	00
5.	Water	17	17	15	14	15	14	12
6.	Control	00	00	00	00	00	00	00

A.b.-*Acinetobacter baumannii*, *A.f.*-*Alkaligenes fecalis*, *E.a.*-*Enterobacter aerogens*, *P.v.*-*Proteus vulgaris*, *K.p.*-*Klebsiella pneumoniae*, *E.c.*-*Escherichia coli*, *P.a.*-*Pseudomonas aeruginosa*.

Among five extracts of *K. pinnata*, water extract showed maximum zone of inhibition (17 mm) against *A. baumannii* and *A. fecalis*, 15 mm against *E. aerogens*, and *K. pneumoniae*, 14 mm against *P. vulgaris* and *E. coli* and minimum zone 12 mm was obtained with *P. aeruginosa*. Compare to all selected urinary pathogens, *P. aeruginosa* showed minimum sensitivity to all extracts; methanol (12 mm), ethanol (11 mm), acetone (13 mm), water (12 mm) and not inhibited by petroleum ether extract. In the present study, *E. coli* (14 mm), predominant cause of UTI and resistant to nine antibiotics tested and *K. pneumoniae* (15 mm), highly resistant urinary isolate among seven selected MDR UTI pathogens, were found sensitive to water extract.

Results showed that water extract has greater antibacterial activity among all except *P. aeruginosa*. The zone of the inhibition noted against *E. coli* was more than Chloramphenicol (11 mm) and Gatifloxacin (11 mm) but less than Nitrofurantoin (17 mm). Similarly, the zone of inhibition obtained against *K. pneumoniae* (17 mm) was approximately 1.5 times higher than Nitrofurantoin (10 mm) and Gatifloxacin (11 mm).

In the experiment performed by Biswas *et. al.*, (2011), the ethanol extract of *K. pinnata* Linn. showed significant sensitivity to the five of the test organisms. The highest zone of inhibition (8.2 ± 0.22 mm) was recorded against *E. coli*. *In vitro* antibacterial activity of chloroform/aqueous extract of leaves of *Kalanchoe pinnata* (lam) was evaluated against microbial flora organisms MTCC78pBR322 in *E. coli*, MTCC227 *C. albicans*, MTCC265 *Rhodococcus rhodochrous* and MTCC2682 *Arthrobacter protophormial*. Amoxycillin 10 mg/ml was used as standard compound. The aqueous extract of plant leaves 15 mg/ml of water of injection showed potent 12 mm zone inhibition of the bacterial MTCC78pBR322 *E. coli* compared with standard compound (Raj *et. al.*, 2012). Two novel flavonoids; 5' methyl 4',5,7 trihydroxyl flavones and 4',3,5,7 tetrahydroxy 5 methyl 5' propenamine anthocyanidines, identified by Okwu and Nnamdi, (2011), showed potential antimicrobial activities against *P. aeruginosa*, *K. pneumonia*, *E. coli*, *S. aureus*, *C. albicans* and *A. niger*. The experiment performed by Akinnibosun and Edionwe, (2015), the ethanolic extract of *K. pinnata* Linn. showed significant sensitivity to the test organisms. The highest zone of inhibition (17.3 ± 1.2 mm) was recorded against *S. aureus* and 12.7 ± 0.9 mm against *E. coli*.

Methanol extract of *K. pinnata* showed MIC of 1.57 mg/ml against *A. baumannii*, 3.15 mg/ml against all selected urinary isolates except *P. vulgaris* and *K. pneumoniae* which were inhibited at higher concentration 6.3 mg/ml. Ethanol extract showed MIC of 0.74 mg/ml against *A. baumannii*, 1.48 mg/ml against all selected urinary isolates except *P. vulgaris* which was inhibited at higher concentration 6.3 mg/ml. Acetone extract showed MIC of 1.27 mg/ml against *A. baumannii* and *K. pneumoniae*, whereas MIC of 2.55 mg/ml was found against other urinary isolates. Petroleum ether extract showed MIC of 0.38 mg/ml against *A. baumannii*, 0.77 mg/ml was found against other urinary isolates except *E. coli* (1.55 mg/ml). Water extract showed MIC of 1.42 mg/ml against *A. baumannii*, *A. fecalis*, *E. aerogens* and *K. pneumoniae*, whereas 2.85 mg/ml concentration was needed to inhibit other urinary isolates.

3.7 Cytotoxicity assay:

MTT assay is a Colorimetric assay which is based on the capacity of Mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically (Wilson, 2000). Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death

The results of water extract of *Kalanchoe pinnata* are represented in Figure-3.3. The minimum concentration of *K. pinnata* water extract required to inhibit the growth of *E. coli* and *K. pneumoniae* was 2.85 mg/ml and 1.42 mg/ml respectively while the cytotoxicity assay with HeLa cell line showed inhibition at such required concentration is < 3.0% indicating the tested extract was safe and can be used for the treatment. Acute and Sub-Acute toxicological assessment of aqueous leaf extract of *K. pinnata* (Lam.) in Sprague-Dawley rats was performed by Raymond *et. al.* in 2010 and the results suggest that aqueous leaf extract of *K. pinnata* was safe and can be used for the medicinal purposes at doses as high as 2 g/ kg.

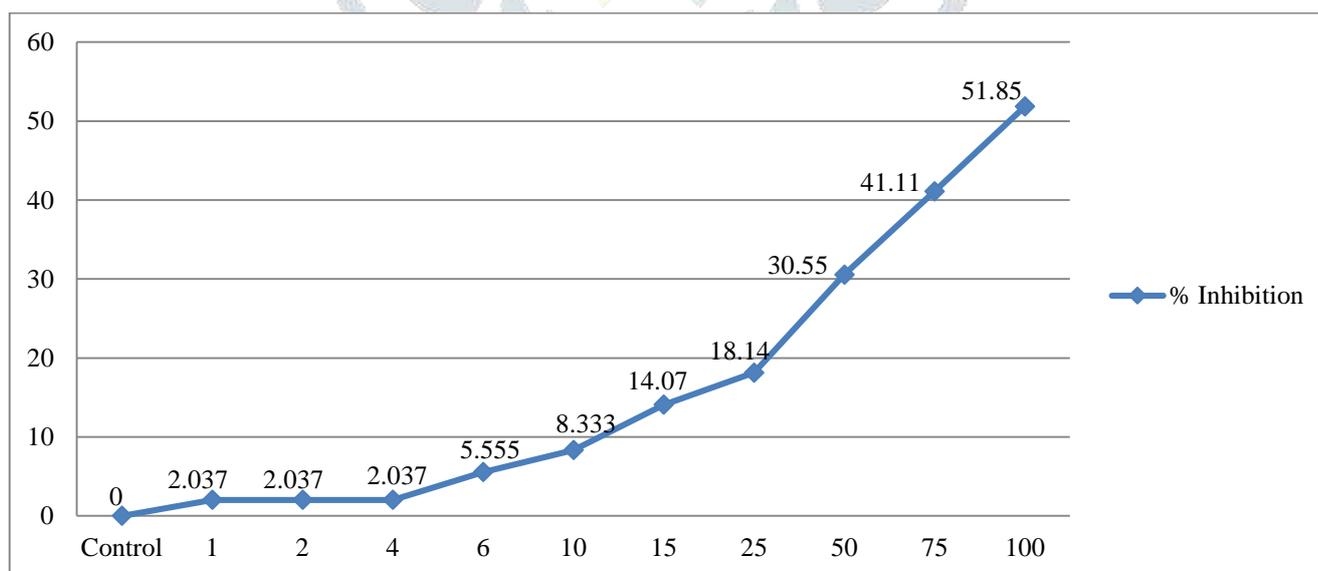


Figure 3.3: MTT assay of *K. pinnata* water extract in different concentration

Trypan Blue is a blue acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan Blue is an essential dye, use in estimating the number of viable cells present in a population (Phillips and Terryberry, 1957).

The results of viability assay of *Kalanchoe pinnata* water extract on *HeLa* cell line is described in Figure-3.4 which indicate that with increase in concentration of the exposed dose, number of viable count decreases. 90% cell viability was obtained at lowest exposed dose i.e. 5 while with highest exposed dose (20%), 81% viability was obtained. % viability with positive control was 73% which is less than maximum exposed dose of tested extract (20%) i.e. 81%.

Results of MTT and Viability Assay of water extract of *K. pinnata* showed less cytotoxic effect which indicates that this extract can be used as a remedy against urinary tract infections caused by multi drug resistant pathogenic bacteria.

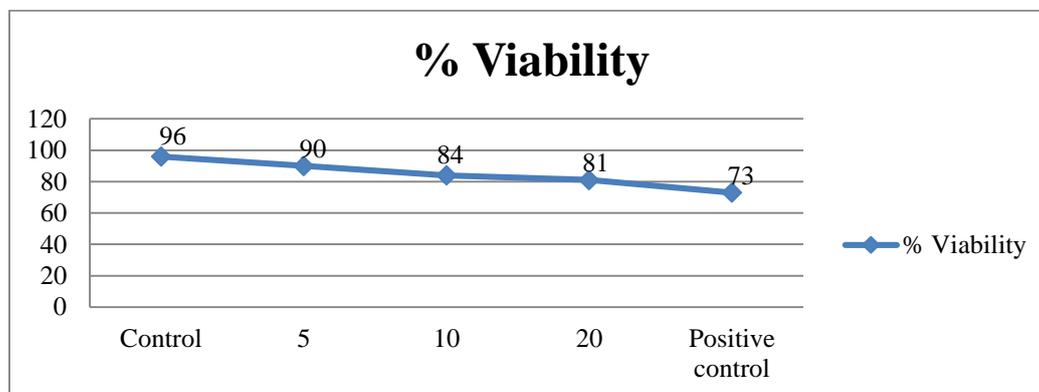


Figure 3.4: Viability assay of *Kalanchoe pinnata* water extract exposed in different concentration

IV. CONCLUSION

Kalanchoe pinnata water extract has remarkable antibacterial activity as compare to synthetic antibiotics. It may be considered as a potential source of new chemotherapeutic drugs because of their diverse phytochemicals and little or no toxic effect. This has introduced the plant as a potential material for drug development for the treatment of Urinary Tract Infection caused by multi drug resistant bacteria. However there would be the need of further studies whether any single or combination of pure active metabolites would be better, safer and more efficient in treating UTI than the crude extract of whole plant

V. SUGGESTIONS FOR FURTHER RESEARCH

Further work can be focused on extraction, purification and identification of bioactive phytochemicals and their mode of action on these bacterial pathogens as well as *in vivo* stability, toxicity and efficacy of the bioactive phytoconstituents in the management of UTI caused by such multi drug-resistant bacteria.

REFERENCES

- Akinnibosun, F. I. and Edionwe, O. (2015). Evaluation of the Phytochemical Antimicrobial potential of the leaf extracts of *Bryophyllum pinnatum* L. and *Citrus aurantifolia* Sw. and their Synergy. *J Appl Sci Environ Manage*, 19(4), 611-619.
- Anjoo, K., & Kumar, S. A. (2010). Microscopical and Preliminary Phytochemical Studies on Aerial Part (Leaves and Stem) of *Bryophyllum Pinnatum* Kurz. *Pharmacognosy Journal*, 2(9).
- Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., and Ozkan, H. (2005). Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan. (Asteraceae). *Turk J Biol*, 30, 65-73.
- Benkeblia, N. (2004). Antimicrobial activity of essential oil extract of various onions (*Allium sativum*). *Lebensm-Wiss-u-Technol*, 37, 263-268.
- Biswas, S.K., Chowdhury, A., Das, J., Karmakar, U. and Chandra Shill, M. (2011). Assessment of cytotoxicity and antibacterial activities of ethanolic extracts of *Kalanchoe pinnata* Linn. (Family: Crassulaceae) leaves and stem. *International Journal of Pharmaceutical Sciences and Research*, 2(10), 2605-2609.
- Chowdhury, A., Biswas, S. K., Das, J., Karmakar, U. K., Shill, M. C., & Dutta, N. (2011). Investigation of cytotoxicity and antifungal activities of petroleum ether and aqueous extracts of leaves and stems of *Kalanchoe pinnata* L.(Crassulaceae). *Asian Journal of Plant Sciences*. 10, 274-277.

- Clinical, L.S.I. (2007). Performance Standards for Antimicrobial susceptibility testing, Nineteenth Informational Supplement. *CLSI Document*, 27.
- Dash, M., Padhi, S., Mohanty, I., Panda, P., & Parida, B. (2013). Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India. *Journal of family & community medicine*, 20(1), 20-26.
- Ejaz, H., Zafar, A., Anwar, N., Cheema, T. A., & Shehzad, H. (2006). Prevalence of bacteria in urinary tract infections among children. *Biomedica*, 22, 139-142.
- Flores-Mireles, A.L., Walker, J.N., Caparon, M. and Hultgren, S.J. (2015). "Urinary tract infections: epidemiology, mechanisms of infection and treatment options." *Nature reviews. Microbiology*. 13(5), 269–84.
- Gottlieb, T., & Nimmo, G. R. (2011). Antibiotic resistance is an emerging threat to public health: an urgent call to action at the Antimicrobial Resistance Summit 2011. *Med J Aust*, 194(6), 281-3.
- Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). In: *Bergey's Manual of Determinative Bacteriology*, ninth ed. Williams and Wilkins Pub., MD, USA.
- Jhon, A.O., and Ojewole (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnata* leaf extract. *Journal of Ethnopharmacology*, 99(1), 13-19.
- Kamat, U. S., Fereirra, A., Amonkar, D., Motghare, D. D., & Kulkarni, M. S. (2009). Epidemiology of hospital acquired urinary tract infections in a medical college hospital in Goa. *Indian journal of urology: IJU: journal of the Urological Society of India*, 25(1), 76.
- Kepam, W. (1986). Qualitative organic analysis (Spectrochemical techniques). Ed. II. McGraw Hill, London, 40-58.
- Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M., and Olaizola, C. (2004). Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*, 8, 39-45.
- Mihankhah, A., Khoshbakht, R., Raeisi, M., & Raeisi, V. (2017). Prevalence and antibiotic resistance pattern of bacteria isolated from urinary tract infections in Northern Iran. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 22,108.
- Ncube, N.S., Afolayan, A.J., and Okoh, A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, 7 (12), 1797-1806.
- Okwu, D.E., and Nnamdi, F.U. (2011). Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial Activity. *Pharmaceutical Chemistry Journal*, 3(2), 1-10.
- Phillips, H.J. and Terryberry, J.E. (1957). Counting actively metabolizing tissue cultured cells. *Cell Research*, 13, 341-347.
- Raj, V., Kumar, A., Singh, V., Kumar, P., and Kumar, V. (2012). *In vitro* Antimicrobial activity of *Kalanchoe pinnata* leaf. *Int J of Curr Phar Res*, 4(3), 70-73.
- Raymond, I.O., Sylvester, E.I. and Glory E.T. (2010). Acute and Sub-Acute Toxicological Assessment of Aqueous Leaf Extract of *Bryophyllum Pinnatum* (Lam.) in Sprague-Dawley Rats. *American Journal of Pharmacology and Toxicology*, 5 (3), 145-151.
- Soulsby, J. (2005). Resistance to antimicrobials in humans and animals. *Braz J Med*, 331, 1219- 1220.
- Sadeghabadi AF, Ajami A, Fadaei R, Zandieh M, Heidari E, Sadeghi M, *et al*. Widespread antibiotic resistance of diarrheagenic *Escherichia coli* and *Shigella* species. *J Res Med Sci* 2014;19 Suppl 1:S51-5.
- Trease, G.E. and Evans W.C. (1989). *A Textbook of Pharmacognosy*. 13th edition; 83, 685. Brailliere Tiridall Ltd., London, ISBN: 0702013617.
- Singh, V., Jaryal, M., Gupta, J., & Kumar, P. (2017). Antibacterial activity of medicinal plants against extended spectrum beta lactamase producing bacteria causing urinary tract infection. *International Journal of Drug Research and Technology*, 2(3), 4.
- Veitch, N.C., and Grayer, R.J. (2007). *Natural Product Reports*, 21, 539-573.
- WHO (1995). Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila.

World Organization Health. Antimicrobial Resistance: Global Report on Surveillance. World Health Organization; 2014.

Wilson, A.P. (2000). Cytotoxicity and Viability Assays in Animal Cell Culture: A Practical Approach. 3rd ed., Oxford University Press: Oxford Vol. 1.

Zhongying, L., Lan, D., Hanqi, Z., Xiuli, H., and Fengquan, B. (2006). Comparison of the Different Extraction Methods of Flavonoids in *Epimedium Koreanum Nakai* by HPLC-DAD-ESI-MS. *J. Liquid Chromatogr Relat Technol*, 29(5), 719–731.

Zone, C.P. and Guide, S. (2017). Antimicrobial resistance and urinary tract infections in the community. *Signs*, 6,3531-93.

