International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

IN VITRO SCREENING ON *Andrographis paniculata* LEAF EXTRACT AGAINST MULTIDRUG RESISTANT UTI ISOLATES

Prabhu K [1] and Rajan S [2]

[1] *Dept. of biotechnology, Srimad Andavan Arts and Science College, (Autonomous), Trichy.*

[2] *Dept. of Microbiology, M.R.Govt Arts College, Mannargudi.Tamilnadu.*

E – mail :Prabhu@andavancollege.ac.in

ABSTRACT

Urinary tract infection (UTI) is mainly prevalent diseases among all age groups encountered in therapeutic practice today. Urinary tract pathogens have evolved frequent resistance mechanisms against different antimicrobial agents; for this reason resistance to old and recently produced drugs is on the climb. The aim of the study was to evaluate the antibacterial efficacy of *Andrographis paniculata* leaf extract against multidrug resistant *E. coli*. Aqueous and acetone crude of *Andrographis paniculata* was tested for antibacterial activity by disc diffusion method against MDR *E. coli*. It was concluded that *Andrographis paniculata* aqueous and acetone extracts has antibacterial activity against MDR *E. coli* from UTI. Qualitative phytochemical analysis demonstrated the presence phenolic compounds, flavonoids and other secondary metabolites act as bactericidal activity against the MDR *E. coli* .

Key words: UTI, A.paniculata, Phenolic compounds.

INTRODUCTION

Urinary tract infection was the second most common type of infection in the body. Accounting for about 8.1 million visits to health care provides each woman are especially prone to UTIs for anatomical reasons one factor is that a women´s uretha is shorter allowing bacteria quicker access to the bladder for women, the lifetime risk of having a UTI is greater than 50% (Amit kumar *et al.,* 2012). More than 95% of UTI are caused by single bacterial species *E.coli* which is the most frequently infecting organisms (Kebira *et al.,* 2009).

However many other bacteria can also because an infection for example *E.coli*, *Klebsiella, Pseudomonas, Enterobacteria, Proteus, Staphylococcus, Chlamydia, Serratia and Neisseria.* It is reported that about 35% of healthy women super symptoms of UTI and about 5% of women each year suffer with the problem of painful urination (dysuria) and

frequenchy (Hooten *et al.,* 2008). They occur most frequently between the age of 16 and 35 year. The treatment mainly involves use of antibiotics but the pathogenic bacteria are becoming increasingly resistant to antibiotics. *E.coli* is the head of the large bacterial family *Enterobacteriaceae*, the enteric bacteria, which are facultative anaerobic and gram-negative bacteria rods that live in the intestinal tracts of animals in health and disease (Jayachitra *et al.,* 2016).

In recent days various types of antibiotics were used to treat UTI for example ciprofloxain, fosfomycin, devoloxacin, nitrofurantion. These medicines are cure for the few days or weeks and it create the adverse effect of the peoples such as hives, weakness, paining, slow heart rate. Most of the organisms resist the antibiotic to withstand high doses antibiotics and pass that ability on to later generation. The indiscriminate use of antibiotic has led to evolution of multi drug pathogens.

Thus, the recent report of rapid spreading of resistant clinical isolates the need to find new antimicrobial agents was more importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al., 2002).* To overcome these problem investigators are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains (Braga *et al.,* 2005). For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. Some plants have the antimicrobial efficacy attributed in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru *et al.,* 1994). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *invitro* to have antimicrobial properties (Lewis *et al., 2*006*).* A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts (Bonjar *et al., 2004).* Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Brantner*, et al., 1994 and* Somchit, *et al., 2003).* Flavonoids isolated from natural dietary sources were also investigated in combination with antibiotics as a strategy against ESBL (Extended-Spectrum Beta-lactamase) producing clinical isolates of *Klebsiella pneumoniae*. Oregano essential oil, given in combination with fluoro quinolone was found to enhance the activity of the drugs

against ESBL-producing *Escherichia coli* These evidences contribute to support and quantify the importance of screening natural products.

Andrographis paniculata (Burm.f.) (FamilyAcanthaceae) (English name-King of Bitters, Tamil name-Nilavempu) is a herbaceous plant and is largely cultivated in Southern Asia, China and some parts of Europe. In traditional medicine, *A. paniculata* is used to get rid of body temperature, remove the toxins from the body; prevent common cold, upper respiratory tract infections including sinusitis and fever and as an antidote against poisons of snakes and insects[16]. The characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the arena of medicinal plants. The aim of the present study was to investigate the antibacterial and phytochemical activity of *Andrographis paniculata* extracts against UTI isolates.

2. Materials and Methods

2.1. Plant material – Selection and Collection

A. paniculata leaf was collected from Srirangam, Tiruchirapalli, and Tamilnadu. Collected leaves were dried in shade and ground into fine powder, stored in a closed container for further use.

2.2. Preparation of Aqueous extract

The powdered plant material (150gm) was mixed with water and extracted completely. The leaves powder was mixed with sterile water and kept for 72 hours and filtered with a muslin cloth and it was condensed in hot air oven at 50˚C. The aqueous extracts were stored in a sterile container and refrigerated for future use (Jonathan, 2009).

2.3. Preparation of Acetone extract

Acetone extract was collected by making use of soxhlet extraction. It was performed by placing 50gm plant material with 1:1 ethanol and methanol. Extraction was performed at 90˚C for 12 hours. The extracts were filtered under the vacuum through Whatman filter paper (No. 1) under gravity. Extract was dried under vaccum evaporator for removing the solvent. The remaining residues were stored in refrigerator till further use (Shi *et al.,* 2005).

2.4. Sample collection

Urine sample were collected in different patients from Government Hospital**,** Srirangam. About 30 samples were collected for a period of one month and were subjected to microbiological investigation. All samples were smeared on clean microscopic slide and were stained by using Gram's staining technique. Stained smears were observed under oil immersion microscope and also all the sample were subjected to isolation and identification of multi drug resistance *E.coli, Pseudomonas, Staphylococuus aureus* and *Streptococcus* spp

(Koneman *et al.,* 1994).

2.5. Confirmation of clinical isolates (Koneman *et al.,* 1994)

Selected colonies from selective and differential media were subjected to macroscopy, microscopy and biochemical tests for identification.

2.6. Assessment of antibiotic sensitivity pattern

All isolates were subjected into antibiotic sensitivity test according to Bauer *et al.,* 1966. The susceptibility of isolates was examined by a disc diffusion assay.

2.7. Determination of Antibacterial activity

Disc diffusion method was followed (Bauer *et al.,* 1966) to determine the anti bacterial activity. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains. By making use of template drawn discs were dispensed on the solidified Mueller Hinton agar with test organisms. This was incubated at 37°C for 24 hours in an incubator (Rands SBC). The test was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - Media). The resistance patterns were interpretated as per CDC recommendations.

2.8. Antibacterial study of plant extract

2.8.1. Preparation of disc with plant extracts

Known quantity of extracts of both aqueous and acetones were dissolved in DMSO: Methanol of 1:1 ratio. This in turn was diluted with equal volume of phosphate buffered saline (PBS pH 7). It was then filter sterilized by making use of sortorious syringe filter of pore size 0.22µm. Sterile discs of 6 mm diameter (Hi-Media) were loaded with 50µg - 250 µg / disc concentration of the extract and were dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and used as negative control. Oxytetracycline loaded Hi-Media discs were used as positive control.

2.8.2. Determination of Antibacterial activity

Disc diffusion method was followed (Bauer *et al.,* 1966) to determine the antibacterial activity of the leaves extract of *A. paniculata.* Petriplates containing 20 ml of Mueller-Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains. By making use of template drawn extracts and fractions loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms. Oxytetracycline antibiotic disc (30µg/disc) obtained from M/s Hi-Media laboratories Ltd, Mumbai was used as positive control for bacteria and solvent loaded discs were used as negative control. The plates were incubated at 37°C for 24 hours in an incubator (Rands SBC). The test was performed in

triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi – Media).

2.10. Phytochemical screening

Phytochemical analysis of secondary metabolites Powder material was extract with various solvent in the order of increasing polarity and the extract were subjected to phytochemical screening of secondary metabolites like alkaloids, flavonoids and steroids by standard methods.

RESULT AND DISCUSSION

UTI is one of the most common bacterial infections affecting all age groups across the life duration. UTI are the second most common type of health problem in the body, accounting for about 8.1million visits to health care providers each year. Mostly women are easily affected to UTIs for anatomical reference reasons. For women, the lifetime risk of having a UTI is greater than 50 percent UTIs in men are not as common as in women but can be serious when they occur. More than 95% of UTI are caused by single bacterial species *E. coli* which is the most frequently infecting organisms.

In most cases bacterial infection if not treatment travel to the urethra and multiply causing kidney infection .Urinary tract bacterial infection are common in women because they have a shorter urethra than men.The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of these families include *E. coli, Klebsiella , Enterobacter* and *Proteus*. Also Gram positive *Stapylococcus* sp. plays a role in the infection. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections (Zahira, 2011).

Table 1: Prevalence of urinary tract isolates

S. No	SAMPLE	Clinical isolate 1	Clinical isolate 2	Clinical isolate 3	Clinical isolate 4
1	Male (12)	8(66%)	1(8.4%)	2(16%)	1(8.4%)
2	Female(18)	15(83%)	2(11%)	1(5%)	1(5%)

Four clinical isolates were identified based on growth pattern, microscopic nature and biochemical parameters. The 30 samples were collected from both male and female patients. The possible pathogens were isolated from urine samples based on the colony morphology observed in selective media.

Table.2. Identification of Clinical Isolates

S.No	Test	*1*	*2*	*3*	*4*
1	Simple Staining	Rod	Rod	Rod	Cocci
2	Gram staining	-	-	-	+
3	Motility	+	-	+	-
4	Indole	+	-	-	-
5	MR	+	-	-	+
6	VP	-	+	-	-
7	Citrate	-	+	+	+
8	TSI agar test	A/A	A/A	K/K	NP
	Gas	+	+	-	NP
	H_2S	-	-	-	NP
9	Urease	-	-	-	+
10	Nitrate	+	+	+	+
11	Catalase	+	+	-	+
12	Oxidase	-	-	+	+
13	Phenylalanine deaminase	-	-	-	NP
14	Lysine decarboxylation	+	+	-	NP
15	Arginine decarboxylation	+	-	+	NP
16	Ornithine decarboxylation	+	-	-	NP
17	Esculin hydrolysis	-	+	-	+

Table.2.shows the identification of the clinical isolates it clearly indicates the pathogens like *E.coli, Klebsiella, Staphylococcus* and *Pseudomonas* were isolated. Out of 12 sample collected from male patients the *E.coli* (66%) was predominantly present in sample out of others. 18 samples were collected from female patients in different ages *E.coli* is majorly present than others. When compared to other pathogens *E.coli* present in 80% and *Staphylococcus* 5% and *Pesudmonas* was 5% present out of 18 samples. Our result also indicated in table.1. In present study we collected urine sample from both male and female, mostly female was affected the UTI because of the anatomy of the urethra. Priyadharshani *et al.,* 2014 already revealed that above statements.

Out of 30 samples, four different bacterial strains were isolated from urine samples. Identification was done based on XLD agar, EMB agar, Blood agar, Hektoen enteric agar, Baired parker agar and Cetrimide agar (Table.2). Isolates were subjected to microscopic methods and also subjected to biochemical characterization. Biochemical reactions and colour of the colony on different medium indirectly indicates the genomic nature of the isolates. These features were compared with standard and the test organism was identified as

E .coli. In this study*, E. coli* was the most frequently isolated uropathogen (80 %). This finding was in close association with other studies (Das *et al.,* 2006).

ANTIBIOTIC SENSITIVITY

Commercially available antibiotics disc Ampicillin 10µg, Chlorophenical 10µg, Erythromycin 30µg, Kanamycin 30µgm, Streptomycin 25 µg, Neuomycin 30 µg were used. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National committee for clinical laboratory standards.

Table 3: Antibiotic sensitivity assay

S. No	**Antibiotic and it concentration**	***E.coli***
1	Kanamycin(K) -30µl/ disc	Resistant
2	Neuomycin (n) -30µl/ disc	Resistant
3	Chloromphenical -30µl/ disc	Resistant
4	Erythromycin(E) -15µl/ disc	Resistant
5	Ampicillin -10µl/ disc	Sensitive

Table 3 shows the test organism *E.coli* was highly resistant to all antibiotics. Walters *et al.,* (2012) reported that 76.5% of the community acquired infections are due to multidrug resistant microorganisms. They reported that pathogens like *E. coli* 100% resistance to ciprofloxacin, cephalosporin, cefodoxime and are 98% resistant to erythromycin and bacitracin and 93% to novobiocin and tetracycline. Most of the pathogens showed multiple virulent factors (Tchesnokova *et al.,* 2011). The clinical isolate used in this study was also found to be multidrug resistant isolates (Table .3.).

Antibacterial activity test

The antibacterial activity was carried out against *E.coli*. The disc diffusion method was followed for antibacterial assay. Antibacterial activity of the leaves extract using disc diffusion method (Bauer *et al.,* 1966). The sterilized disc was soaked in the different concentration of the leaf extract.

20 ml of sterilized nutrient agar medium for *E.coli* were poured into the each sterile petridish. After solidification, the sterile cotton swab dipped into the culture or broth of *E.coli* . The entire agar surface of each plate was inoculated with this swab first in a horizontal direction and then in a vertical direction, which ensure the distribution of organism over the agar surface. The extracts was mix with DMSO solvent then added prepared disc .the disc

was prepared for various concentration 50µl, 100µl, 200µl, 400µl and 800µl. The disc was placed in the agar plate. The plate was incubated at 37 ° C at 24 hours .The antibacterial activity was recorded by measuring the width of the clear zone around each disc.

Table.4. Antibacterial activity of *Andrographis paniculata* acetone extract.

S.No	Acetone extract	Concentration	Sensitive
1	Leaf	50µl	8mm
2	Leaf	100µl	10mm
3	Leaf	200µl	12mm
4	Leaf	400µl	15mm
5	Leaf	800µl	20mm

Table 4 shows the antibacterial activity of *Andrographis paniculata* leaf acetone extracts showed maximum zone of inhibition (20mm) for *E .coli* and the minimum zone of inhibition (8mm).

Table.5. Antibacterial activity of *Andrographis paniculata* aqueous extract.

S. No	Aqueous extract	Concentration	Sensitive
1	Leaf	50µl	5mm
2	Leaf	100µl	7mm
3	Leaf	200µl	8mm
4	Leaf	400µl	12mm
5	Leaf	800µl	15mm

The activity of leaf extract of aqueous showed maximum zone of inhibition (15mm) for *E .coli* and the minimum zone of inhibition (5mm) for *E .coli* (Table.5.). That acetone based leaf extract was more effective and best when compared with aqueous extracts. That 800µl was optimum for all the test cultures and it was found to have more activity for *E .coli* (20mm).

Table.6. Phytochemical analysis

S.No	Test	Reagent	Observation	Acetone extract	Aqueous extract
1	Alkaloids	Mayer's	Creamy white precipitate	Positive	Negative
2	Steroids	Acetic anhydride	Reddish brown precipitate	Negative	Positive

3	Terpenoids	Chloroform	Reddish brown precipitate	Negative	Negative
4	Flavonoids	Ethyl Acetate Test	Yellow colour	Positive	Positive
5	Saponins	Extract + boiled	Foam formed	Negative	Negative
6	Tannins	Ferric Chloride Test	Brownish green of Blue Black	Negative	Negative
7	Phenolic compounds	Alcohol + Ferric Chloride	Bluish green	Negative	Positive

In the present investigation, preliminary phytochemical testing has been done in both the aqeuous and acetone extracts of *Andrographis paniculata* leaves. It shows the presence and absence of some phytochemicals in the extracts. These secondary metabolites are involved in antibacterial activity. One of the previous studies indicated the presence of steroids, terpenoids, flavonoids, and phenolic compounds were present in *Andrographis paniculata* (Priyanka *et al.,*2014). Thus, extract containing the specific compound may serve as a potential source of bioactive compound act against the UT infecting *E.coli.*.

REFERENCE

[1] Amit Kumar Bharti, Umar Farooq, Sudhir Singh, Navdeep Kaur, Raees Ahmed, Komal Singh "Incidence of Enterococcal Urinary Tract Infection and it's Sensitivity Pattern among Patients Attending Teerthanker Mahaveer Medical College and Research Centre, Moradabad, India" *International Journal of Scientific Study*, 2016(3), 115-119.

[2] Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, 45(4), 493.

[3] Bonjar, G. S. (2004). Evaluation of antibacterial properties of Iranian medicinal-plants against *Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae and Bordetella bronchoseptica. Asian J. Plant Sci,* 3(1), 82-86.

[4] Braga, L. C., Leite, A. A., Xavier, K. G., Takahashi, J. A., Bemquerer, M. P., Chartone-Souza, E., & Nascimento, A. M. (2005). Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. *Canadian journal of microbiology*, 51(7), 541-547.

[5] Brantner, A., & Grein, E. (1994). Antibacterial activity of plant extracts used externally in traditional medicine. *Journal of ethnopharmacology*, 44(1), 35-40.

[6] Coates, J. & Rogers, B. L., (2002). Food-based safety nets and related

programs. Washington, DC: World Bank Social Protection Discussion Paper, 223.

[7] Das, T., Pal, A. K., Chakraborty, S. K., Manush, S. M., Chatterjee, N., & Apte, S. K. (2006). Metabolic elasticity and induction of heat shock protein 70 in *Labeo rohita* acclimated to three temperatures. *Asian Australian journal of animal sciences,* 19(7), 1033.

[8] Hooton, T. M. & Lichtenberger, P.,(2008). Complicated urinary tract infections. *Current infectious disease reports,* 10(6), 499-504.

[9] Jayachitra, A & Nithya, B.,(2016). Imrpoved antibacterial and antobiofilm activity of plant mediated gold nanoparticles using *Garcinia cambogia*. *Int J Pure App Biosci*, 4(2), 201-210.

[10] Jonathan Y. Phytochemical analysis and Antimicrobial activity of *Scoparia dulcis* and *Nymphaea lotus. Aus J Basic and Appl Sci,* 3(4): 3975-3979 (2009).

[11] Kafaru, C. J., Keay, M. B., & Roper, Z. F. (1994). Flavonoids: antibacterial constituents of *Vitex doniana. J. Nat. Sci,* 61, 80-81.

[12] Kebira, A. N., Ochola, P., & Khamadi, S. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections. *Journal of Applied Biosciences*, 22, 1320-1325.

[13] Koneman EW, William MJ, Stephen DA, Schreeken B and Washington CW. Laboratory and clinical diagnosis of infectious diseases. In: Introduction to diagnostic Microbiology. J.B. Lippincott Company, Philadelphia; pp. 1 – 19 (1994).

[14] Lewis, K., & Ausubel, F. M. (2006). Prospects for plant-derived antibacterials. *Nature biotechnology,* 24(12), 1504.

[15] Priyadharsini, M., Bhardwaj, S., & Sheeba, E. (2014). Isolation, identification of microbial isolates from urinary tract infection patients and evaluation of antimicrobial activity using plant extracts. *Int. J. Curr. Microbial. Appl. Sci,* 3(4), 153-160

[16] Priyanka Das, Alok Kumar Srivastav (2014).”Phytochemical Extraction and Characterization of the Leaves of *Andrographis paniculata* for Its AntiBacterial, Anti-Oxidant, Anti-Pyretic and AntiDiabetic Activity”. *International Journal of Innovative Research in Science, Engineering and Technology*. 3(8).15177-15184

[17] Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y and Jiang Y. Extraction of polyphenolics from plant material for functional foods-engineering and technology. *Food Rev Int* ; 21: 139-166 (2005).

[18] Tchesnokova, V., Aprikian, P., Kisiela, D., Gowey, S., Korotkova, N., Thomas, W., & Sokurenko, E. (2011). Type 1 fimbrial adhesin FimH elicits an immune response that enhances cell adhesion of *Escherichia coli*. *Infection and immunity*, 79(10), 3895-3904.

[19] Walters S, Chelsea Lane M, Patrick D. Vigil, Sara N. Smith, Seth T.Walk, and Harry L. T. Mobley (2012). Kinetics of Uropathogenic *Escherichia coli* Metapopulation Movement during Urinary Tract Infection". *mBio*. 3(1): e00303-11.

[20] Zahira M, F El-Sayed, Fadwa M Al-Sharif (2011). Urinary Tract Infection with *Escherichia coli* and Antibacterial Activity of Some Plants Extracts. *Int. J. Microbiol. Res.* 2 (1): 01-07.