WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 7, Issue 2, 922-929 Research Article ISSN 2278 – 4357

INVITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* AGAINST UROPATHOGENS

Murugesan Rajeswari*, Sigamani Ajithadevi, Sengodan Kalaivani and Raja Vinodhini

Department of Microbiology, Shri Sakthikailaassh Women's College, Military Road, Ammapet, Salem- 636003, Tamil Nadu, India.

Article Received on
24 Nov. 2017,

Revised on 16 Dec. 2017,
Accepted on 06 Jan. 2018,

DOI: 10.20959/wjpps20182-10876

***Corresponding Author**
Murugesan Rajeswari
Department of Microbiology, Shri Sakthikailaassh Women's College, Military Road, Ammapet, Salem- 636003, Tamil Nadu, India.

ABSTRACT

The *invitro* study was undertaken to identify the antibacterial activity and phytochemical analysis of ethanolic extract of *Andrographis paniculata* against uropathogens. A total of 100 urine samples were collected from the suspected patients and were examined for the identification of bacteria by standard procedures. Of the 100 urine samples, 47 isolates were identified (gram positive and gram negative bacteria) *viz.*, *Escherichia coli* 21(44.68%), *Klebsiella pneumonia* 16(34.04%), *Pseudomonas aeruginosa* 5(10.63%), *Proteus mirabilis* 3(6.38%) and *Staphylococcus aureus* 2(4.25%). The antibacterial activity of crude ethanolic extract of *Andrographis paniculata* was screened by disc diffusion method. The ethanolic extract shown maximum inhibitory activity against *Escherichia coli* (21 mm), *Klebsiella pneumonia* (23 mm), *Pseudomonas aeruginosa* (19 mm) and *Staphylococcus aureus* (26 mm) but plant extract was fail to show the inhibitory activity against *Proteus mirabilis.* The results of antibacterial activity of *A. paniculata* against selective resistant isolates were compared with the results of standard antibiotics. The study result revealed that the antibacterial properties of *A. paniculata* might be associated with the presence of phytochemical constituents such as, flavonoids, steroids, terpenoids phenols, tannin, oils and resins.

KEYWORDS: Urine, *Andrographis paniculata*, Phytoconstituents, Antibacterial activity, Disc diffusion method.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common microbial diseases encountered in medical practice affecting people of all ages.[1] Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*.[2] Worldwide, UTIs' prevalence was estimated to be around 150 million persons per year.[3] Although, most UTIs are self-limiting, improving without treatment even when culture is positive, other poses dangerous health risk which if left untreated may tend to spread up through the ureters, into the kidneys resulting in pyelonephritis.[4] While not generally considered a cause of significant mortality, UTI do represent an important cause of morbidity. Typical symptoms associated with UTI include the triad of dysuria (painful urination), urgency (the enhanced desire to void the bladder) and frequency (increased frequency of urination.[5] Although this infection affects both genders, women are the most vulnerable may be due to their anatomy and reproductive physiology. The prevalence also increases with advancing age, catheterization, sexual activity, menopause and prostate problems.[6]

Antimicrobial resistance of urinary tract pathogens has increased worldwide. Almost all of the synthetic drugs cause side effects and also most of the microbes developed resistant against the synthetic drugs.[7] To alleviate this problem, medicinal plants are considered as an important of potentially useful structure for the development of new chemotherapeutic agents. Medicinal herbs are widely used with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases.[8] *Andrographis paniculata*, commonly known as 'King of Bitter, is a small, annual, branched and erect plant belongs to the family *Acanthaceae*. It grows abundantly in south eastern Asia including India, Sri Lanka, Java, Pakistan, Indonesia and Malaysia. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, waste lands and dry or wet lands.[9] Various medicinal properties like choleretic, antidiarrhoeal, immunostimulant and antiinflammatory have been attributed to this plant in the traditional system of Indian medicine.[10] Further reported activities are hepatoprotective, antimalarial, antihypertensive, antipyretic, antithrombotic and antidote for snake bites.[11] Therefore the present study has

been extensively evaluated for the antimicrobial activity of *Andrographis paniculata* against pathogenic organism isolated from the urine samples.

MATERIALS AND METHOD

Sources of microbial strains

The urine samples were collected from the Government Hospital, Salem, Tamil Nadu. The collected isolates were examined for the identification of bacteria by the standard procedures.

Collection of plant materials

The whole plants of *Andrographis paniculata* was collected were collected from in and around Salem District, Tamil Nadu. The plant material were cleaned with distilled water and shade dried at room temperature. The shade dried plant material was powdered by using electric blunder.

Extraction of Plant Materials

The plant materials were shade dried and pulverized. About 250g of powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8h using 450ml of ethanol as solvent. All the extracts were concentrated under vacuum and dried in a dessicator.

Preliminary phytochemical screening

The phytochemical studies were performed as described by Wagner and Harborne. The presence of starch, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids and flavonoids were analysed.[12,13]

Screening of antimicrobial activity- Disc diffusion method

Kirby-Bauer method was followed for disc diffusion methods.[14] *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The extracts were loaded on 5 mm sterile individual discs and placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. Negative control was prepared using respective solvent. Tetracycline (10µg/disc) was used as

positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

RESULTS

Totally 100 non-repetitive urine samples were collected from the suspected patients of Government Hospital, Salem, Tamil Nadu. The collected isolates were examined for the identification of bacteria, based on microscopy, biochemical and cultural characters, 47 gram positive and gram negative bacterial isolated were identified as *Escherichia coli* 21(44.68%), *Klebsiella pneumonia* 16(34.04%), *Pseudomonas aeruginosa* 5(10.63%), *Proteus mirabilis* 3(6.38%) and *Staphylococcus aureus* 2(4.25%) from these urine samples (Table 1).

Table 1: Prevalence of bacterial isolates from urine sample.

S.no	Bacterial types	Urine
		Total no. of isolates (%)
1	*S. aureus*	2(4.25)
2	*E.coli*	21(44.68)
3	*K. pneumonia*	16(34.04)
4	*P. aeruginosa*	05(10.63)
5	*P. mirabilis*	03(6.38)
	Total	**47**

Phytochemical screening was done qualitatively using the ethanol extracts of *A. paniculata*. The ethanolic extracts of this plant were subjected to various qualitative tests to identify the phytoconstituents such as Flavonoids, Steroids, Terpenoids Phenols, Tannin, Oils and Resins (Table 2).

Table 2: Preliminary Phytochemical analysis of *A. paniculata* (Ethanol).

Sl. No	Phytoconstituents	*A. Paniculata*
		Ethanolic extract
1	Tannins	+
2	Phenol	+
3	Terpenoids	+
4	Steroids	+
5	Flavonoids	+
6	Oils and resins	+

The antibacterial activity of crude ethanolic extract of *A. paniculata* were screened by disc diffusion method against microorganisms was presented in Table 3. The results of antibacterial activity of *A. paniculata* against selective resistant isolates were compared with the results of standard antibiotics.

Table 3: Antibacterial activity of ethanolic extract of *A. paniculata.*

Sl. No.	Microorganisms	Zone of inhibition (mm in diameter)		
		Standard antibiotics(mcg/disc)	Zone in mm	*A. paniculata*
1	*S. aureus*	Amoxycillin/Clavulanic acid (30)	40	26
2	*E. coli*	Ciprofloxacin (5)	30	21
3	*K. pneumonia*	Chloramphenicol (30)	30	23
4	*P. mirabilis*	Lomefloxacin (10)	25	-
5	*P. aeruginosa*	Lomefloxacin (10)	30	19

DISCUSSION

Urinary tract infections (UTIs) are one of the most common microbial diseases encountered in medical practice affecting people of all ages.[1] The ever-increasing bacterial resistance to antibiotics is one of the most challenging tasks of all the medical issues which are being faced by us today.[15] Medicinal plants are the prime sources of new medicines and may constitute an alternative to the usual drugs. The involvement of gram negative enteric organisms that commonly cause urinary tract infections, such as, *E. coli*, *Klebsiella* spp. and *Proteus* spp. [16] Similarly, in the present study prevalence of gram negative bacteria [*Escherichia coli* 21(44.68%), *Klebsiella pneumonia* 16(34.04%), *Pseudomonas aeruginosa* 5(10.63%), *Proteus mirabilis* 3(6.38%)] was higher than that of gram positive bacteria [*Staphylococcus aureus* 2(4.25%)].

A study from India, it was found that *E. coli* was the most commonly grown organism (64.3%), followed by *S. aureus* (21.4%) and *K. pneumoniae* (14.3%).[17] In the present study among four different gram negative bacterial isolates, *E. coli* (44.68%) was identified as a predominant spp. followed by *K. pneumonia* (34.04%), *P. mirabilis* (6.38%) and *P. aeruginosa* (10.63%). Similarly, a study by Janifer *et al*. reported that, *Escherichia coli* (71%), *Klebsiella* spp.(13.5%), *Pseudomonas* spp.(9%), *Enterobacter* spp.and *Citrobacter* sp p. (2%) and non fermenting gram negative bacilli and the *Proteus* spp. (1%) from the UTI patients.[18]

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. In this study ethanolic extract of *A. paniculata* revealed the presence of phytoconstituents such as flavonoids, steroids, terpenoids phenols, tannin, oils and resins. Likewise, a prior study revealed that, the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside in the ethanolic extract of *A. paniculata*.[19]

In the present study ethanolic extract of *A. paniculata* showed the inhibitory activity against *E. coli*, *K. pneumonia*, *P. aeruginosa* and *S. aureus* but fail to show the activity against *P. mirabilis.* Comparatively**,** ethanolic extract of leaves exhibited antibacterial activity against *E. coli*, *S. aureus* and *B. subtilis* and antifungal activity against *C. albicans*, *A. niger* and *A. flavus*.[19] Contrarily, another study revealed that, methanolic extracts of *A. paniculata* at the highest concentration showed the strongest bacterial inhibitory activity of other extracts.[20] Mishra *et al.* reported that 75% methanol extract of *A. paniculata* leaves was found to be active against *S. aureus*, *E. faecalis* and *M. tuberculosis*.[21]

CONCLUSION

The ethanolic plant extract of *A. paniculata* have great potential for the presence of inhibitory compounds against bacteria. Therefore, the plant extract of *A. paniculata* can be used to treat the urinary tract pathogens without any side effects. So, the present study result affirmed that ethanolic extract of *A. paniculata* has antibaterial property. However, further studies needed to ascertain the antimicrobial activity based on clinical trials.

ACKNOWLEDGEMENTS

The authors are grateful to **Mr. K. KAILASAM**, Chairman, Shri Sakthikailassh Women's College, Salem, **Mr. K. SENTHIL KUMAR,** Correspondent, Shri Sakthikailassh Women's College, Salem, and **Dr. S. JAYANTHI,** Principal, Shri Sakthikailassh Women's College, Salem District, Tamil Nadu for providing all the facilities for our research work.

REFERENCE

1. Kunin CM. "Chemoprophylaxis and suppressive therapy in the management of urinary tract infections". J Antimicro Chemother, 1994; 33 Sup(A): 51-62.
2. Ana LFM, Jennifer NW, Michael C, Scott JH. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol, 2015; 13(5): 269-84.
3. Gupta K, Sahm DF, Mayfield D, StammWE. "Antimicrobial resistance among uropathogens that cause communityacquired urinary tract infections in women: a nationwide analysis". Clin Infect Dis, 2001; 33(1): 89-94.
4. Lane DR, Takhar SS. Diagnosis and management of urinary tract infection and pyelonephritis. Emerg med clinics of North America, 2011; 29(3): 539-52.
5. August SL, De Rosa MJ. Evaluation of the Prevalence of Urinary Tract Infection in Rural Panamanian Women. PLoS ONE, 2012; 7(10): e47752.

6. Gould CV, Umscheid CA, Agarwal RK, Kuntz G, Pegues DA. Guideline for prevention of catheter-associated urinary tract infections 2009. Infect Control Hosp Epidemiol, 2010; 31(4): 319-26.
7. Geetha I, Catherine P, Alexander S. Antibacterial activity of *Andrographis paniculata* Extracts. The Pharma Inno J, 2017; 6(5): 1-4.
8. Swati P, Dibyajyoti S, Srikanta C. Pharmacognostic Studies on Aerial Part of Methanolic Extract of *Mimosa Pudica*. Asian J. Pharm. Tech., 2012; 2(3): 101-3.
9. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A hand book of medicinal plants, A complete source Book. Agrobios, Jodhpur, India, 2003; 45-6.
10. Mukherjee PK. Quality control of herbal drugs. 1st ed. New Delhi: Business Horizons Pharmaceutical Publishers, 2002; 701.
11. Mishra US, A. Mishra R, Kumari P, Murthy N, Naik BS. Antibacterial Activity of Ethanol Extract of *Andrographis paniculata*. Indian J Pharm Sci, 2009; 71(4): 436-38.
12. Wagner H, Bladt S, Zgainski EM. Plant drug analysis 1984. Springer-Verlag:B erlin, 1984; 298-334.
13. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. New York: Chapman and Hall, 1998; 1-150.
14. Bauer AW, Kirby MDK, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol, 1966; 45: 493-96.
15. Misra P, Pal NL, Guru PY, Katiyar JC, Srivastava V, Tandon JS. Antimalarial activity of *Andrographis paniculata* (Kalmegh) against *Plasmodim berghei* NK 65 in Mastomys natalensis. Int J Pharmaco and Phytochem, 1992; 30: 263-74.
16. Bova JG, Potter JL, Arevalos E, Hopens T, Goldstein HM, Radwin HM. Renal and perirenal infection: to the role of computerized tomography. J Urol, 1985; 133: 375-8.
17. Goswami R, Bal CS, Tejaswi S, Punjabi GV, Kapil A, Kochupillai N. Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. Diab Res Clin Pract, 2001; 53: 181-6.
18. Janifer J, Geethalakshmi S, Satyavani K, Viswanathan V. Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol, 2009; 19(3): 107-11.
19. Rajalakshmi V, Cathrine L. Phytochemical screening and antimicrobial activity of ethanolic extract of Andrographis paniculata. J of Pharmaco and Phytochem, 2016; 5(2): 175-77.

20. Baby SV, Sriman NJ. Antibacterial activity of Andrographis paniculata Nees against selective human pathogens. Afr J Microbiol Res, 2015; 9(16): 1122-27.
21. Mishra PK, Rahul Kunwar S, Anamika G, Adya C, Rahul P, Shree Prakash T *et al*. Antimicrobial activity of *Andrographis paniculata* (Burm.f.) wal ex Nees leaves against clinical pathogens. J Pharm Res, 2013; 7: 459-62.