

# Phytochemical Insights and Antibacterial Efficacy of *Andrographis paniculata* from Chhattisgarh Against UTI Pathogens

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**Abstract**—Urinary tract infections (UTIs) are among the most common infections worldwide, increasingly complicated by rising antimicrobial resistance. Approximately 40% of women and 12% of men experience symptomatic UTIs at least once in their lifetime. However, the growing resistance of UTI-causing bacteria to conventional antibiotics presents a significant challenge in managing this condition. The implications of antibiotic resistance include increased mortality and morbidity, posing serious risks to patients, particularly in cases of severe or chronic infections. This study investigates the phytochemical composition and antimicrobial activity of *Andrographis paniculata* against UTI-causing pathogens. Leaf extracts of *Andrographis paniculata*, obtained using chloroform, methanol, and distilled water as solvents, were evaluated for antibacterial efficacy against both Gram-positive and Gram-negative pathogens. The results revealed that the plant exhibited notable antibacterial activity, indicating its potential as a natural antimicrobial agent. These findings suggest that *Andrographis paniculata* holds promise for the development of natural compounds that could lead to the creation of innovative pharmaceuticals. Such advancements would address the critical therapeutic needs posed by antibiotic-resistant UTIs, offering a valuable and sustainable solution for treating this widespread health issue in the future.

**Index Terms**—Antimicrobial activity, pathogens, *Andrographis paniculata*, chloroform, methanol, Urinary tract infection.

## I. INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, impacting millions of individuals each year. These infections are primarily caused by uropathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. UTIs manifest in varying degrees of severity, from mild discomfort to recurrent, debilitating episodes. Their widespread occurrence contributes to significant morbidity and places a considerable financial strain on healthcare systems worldwide [1]. Currently, the primary approach to managing UTIs involves antibiotic therapy. While antibiotics are effective in eradicating bacterial infections, the increasing prevalence of antibiotic resistance among uropathogens poses a significant global health challenge [2]. Antibiotic resistance not only complicates treatment regimens but also heightens the risk of treatment failure and recurrence. This alarming trend has spurred a growing interest in exploring alternative therapeutic approaches, including the use of medicinal plants renowned for their antimicrobial properties. Medicinal plants have played a crucial role in healthcare systems for centuries, particularly within traditional medicine practices. These plants are a rich reservoir of bioactive compounds with diverse therapeutic potentials [3]. Unlike synthetic antibiotics, plant-based antimicrobials often exert their effects through multiple mechanisms, thereby reducing the likelihood of resistance development. As such, they represent a promising avenue for addressing the challenges associated with multidrug-resistant (MDR) pathogens

[4]. One medicinal plant that has garnered significant attention in this context is *Andrographis paniculata*, commonly known as the "King of Bitters." This traditional herb, extensively used in Ayurveda and Unani medicine, is native to South and Southeast Asia. *A. paniculata* is renowned for its broad spectrum of pharmacological properties, which include anti-inflammatory, antiviral, antioxidant, and antimicrobial activities. The therapeutic potential of this plant is largely attributed to its rich array of bioactive compounds, such as andrographolide, flavonoids, and diterpenoid [5]. These compounds have been extensively studied for their ability to combat bacterial infections, making *A. paniculata* a promising candidate for addressing the growing threat of MDR uropathogens. The study focuses on the antimicrobial efficacy of *Andrographis paniculata* plant on the urinary tract infection causing organisms which may lead to the possibility to develop more effective and sustainable strategies for managing UTIs, ultimately improving patient outcomes and alleviating the burden on healthcare systems.

## II. METHODOLOGY

### A. Collection of plant material

The *Andrographis paniculata* plants were sourced from the Jagdalpur area in Chhattisgarh, India. The leaves were carefully washed under running tap water and then dried in the shade. Once dried, the leaves were finely ground into a powder using a grinder and stored in airtight containers at room temperature for future use.

### B. Collection of Test Microorganisms

Bacterial samples, including *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 12011), and *Staphylococcus aureus* (MTCC 10787), were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh.

### C. Preparation of Plant Extracts

The powdered leaf and stem samples were individually extracted using different organic solvents, including methanol, distilled water, and chloroform, in a Soxhlet apparatus for a 7-hour cycle

at 60°C. The extracts were then filtered and evaporated, and the resulting crude extracts were stored in a refrigerator for future use [5].

### D. Phytochemical Analysis

Standard methods will be used to identify the presence of phytochemicals in the extracts [6].

### E. Antimicrobial Susceptibility Testing

#### 1. Agar Well Diffusion Method

The agar well diffusion method was carried out following the standard procedure described by [7]. The antibacterial activity of the crude leaf and stem extracts was evaluated against bacterial strains. Wells of 6 mm diameter were prepared in agar plates, and the bacterial strains were swabbed onto the plates using a sterile well cutter. Subsequently, 100 µL of various concentrations of crude plant extracts prepared in different solvents was added to the wells using a micropipette. An antibiotic served as the positive control, while plates containing only bacterial cultures served as the negative control. The plates were then incubated at 37°C for 24 hours.

#### 2. Minimum Inhibitory Concentration: -

The broth dilution method was used to determine the minimum inhibitory concentration (MIC) values, following the protocol outlined by [8]. Test organisms were cultured in a nutrient broth medium. A volume of 100 µL of different concentrations of plant extracts was mixed with sterile nutrient broth, followed by the addition of 100 µL of bacterial suspension that had been cultured overnight in the broth medium. The tubes were incubated at 37°C for 18 hours. Bacterial growth inhibition was assessed by measuring absorbance at 600 nm using a spectrophotometer. The MIC was defined as the lowest concentration of the extract that inhibited bacterial growth.

## III. RESULT AND DISCUSSION

In the present study, preliminary phytochemical screening was conducted on various extracts of the leaves and stems of *Andrographis paniculata*. The presence of key phytochemicals, including alkaloids, flavonoids, saponins, tannins and phenolic compounds, was observed and recorded (Table 1).

Table 1. Qualitative analysis of phytochemicals

Plant sample	Solvent used	Phytochemicals present in the crude extracts of leaves of <i>A. paniculata</i>				
		Flavonoids	Alkaloids	Tannins	Saponins	Phenolic compounds
Leaves of <i>A. paniculata</i>	Methanol	+	-	-	-	+
	Chloroform	+	+	+	+	+
	Distilled water	+	-	+	+	+

A. Antimicrobial activity by Agar well diffusion method

Results of antimicrobial susceptibility test showed marked differences among bacterial isolates in their susceptibility and resistance patterns to a particular antibiotic. The test was done by Agar well Diffusion and the antibacterial effect of plants extract was different according to the type of plant extracts and their concentrations. The results revealed that the pathogens were susceptible to different crude extracts of leaves of *A. paniculata*. The chloroform extract of

leaves of Jagdalpur region showed effective antibacterial activity against the pathogens at 1mg/ml, 5mg/ml and 10mg/ml concentrations whereas the *E. coli* was sensitive towards the distilled water extract at 1mg/ml and 10mg/ml. It was investigated that pathogens all the bacterial pathogens were highly resistant to the methanol extracts. (Table.2) (Fig1). All the pathogens showed sensitivity against the antibiotic Norfloxacin which was taken as positive control.

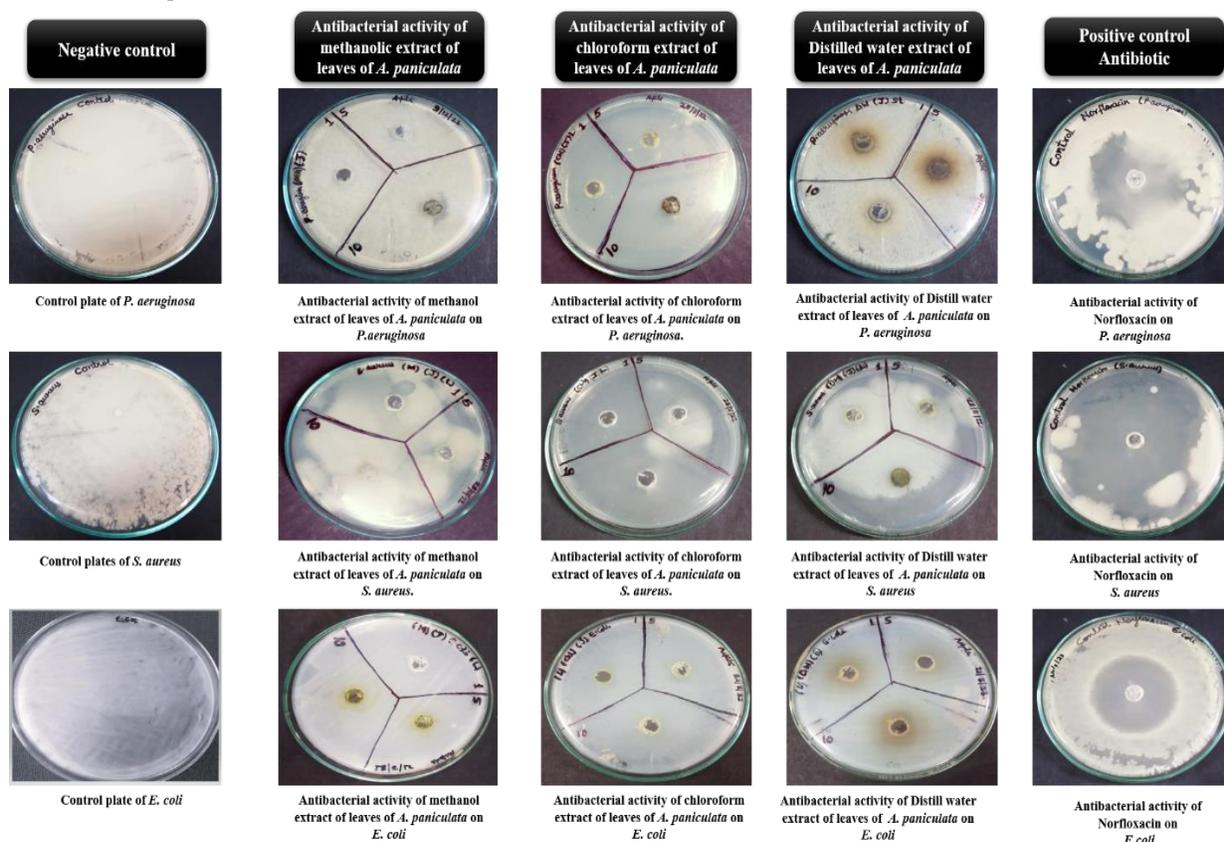


Fig 1. Antimicrobial activity of different crude extracts of leaves of *Andrographis paniculata* of Chhattisgarh at the concentrations of 1mg/ml, 5mg/ml and 10mg/ml along with the antibiotics Norfloxacin on *P. aeruginosa*, *S. aureus* and *E. coli*.

Table 2. Antibacterial activity of crude extracts of leaves parts of *Andrographis paniculata* along with antibiotic Norfloxacin on *P. aeruginosa*, *S. aureus* and *E. coli*.

UTI causing pathogens	Activity of crude extracts of leaves of <i>A. paniculata</i> at different concentrations in mg/ml									Positive control (Norfloxacin)
	Methanol			Chloroform			Distill water			Norfloxacin
	1	5	10	1	5	10	1	5	10	
1. <i>Pseudomonas aeruginosa</i>	-	-	-	+	+	+	-	-	-	55mm + 50mm
2. <i>Staphylococcus aureus</i>	-	-	-	+	-	+	-	-	-	
3. <i>Escherichia coli</i>	-	-	-	+	+	+	15mm	-	20mm	

- = Resistant  
 + = Sensitive  
 Zone of Inhibition in mm

**B. Minimum Inhibitory Concentration (MIC)**

Minimum Inhibitory Concentration was performed using the broth dilution method. The results revealed that the different extracts of the leaves of *A. paniculata* showed MIC against both gram positive and gram-negative organisms, the determination of MIC values was carried out with methanol, chloroform and distilled water extracts of leaves of *A. paniculata* of Jagdalpur area. MIC values of the chloroform extract of leaves were found to be effective at 3mg/ml and 1mg/ml respectively against

*P. aeruginosa*. *E. coli* was sensitive at 1, 2 and 4mg/ml concentration whereas *S. aureus* was sensitive at 3,4 and 5mg/ml. The distilled water extract of leaves showed effective results against pathogens at the concentration of 1, 3,4 and 5mg/ml. MIC values of the methanolic extract of the leaves were not effective. The result from the present study indicated that the chloroform and distilled water extract showed potential MIC against the pathogens. (Table.3).

Table 3. Minimum Inhibitory Concentration of crude extracts of leaves part of *Andrographis paniculata* at different concentrations on *P. aeruginosa*, *S. aureus* and *E. coli*.

UTI causing pathogens	Minimum Inhibitory Concentration of crude extracts of leaves of <i>A. paniculata</i> at different concentrations in mg/ml				
	Chloroform extract				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
<i>Pseudomonas aeruginosa</i>	0.055833±0.002857	0.202467±0.00607	0.0492±0.015431	0.1887±0.007264	0.113633±0.0169
<i>Staphylococcus aureus</i>	0.035767±0.00317	0.03426±0.00190	0.002133±0.000289	0.0027±0.00017	0.0064±0.00017
<i>Escherichia coli</i>	0.0073±0.00025	0.0119±0.00012	0.0459±0.00137	0.0181±0.00048	0.0251±0.00300
	Methanol extract				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
<i>Pseudomonas aeruginosa</i>	0.0178±0.0002	0.0265±0.0020	0.0163±0.0004	0.0139±0.0002	0.0126±0.0004
<i>Staphylococcus aureus</i>	0.0600±0.0036	0.0514±0.0002	0.0635±0.0003	0.0439±0.0003	0.0291±0.0008
<i>Escherichia coli</i>	0.0065±0.0009	0.0061±0.0009	0.0166±0.0004	0.0154±0.0002	0.0156±0.0004
	Distill water extract				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml

<i>Pseudomonas aeruginosa</i>	0.1230±0.0026	0.2267±0.0173	0.0600±0.0032	0.0800±0.0032	0.0633±0.0751
<i>Staphylococcus aureus</i>	0.1007±0.0751	0.2310±0.0165	0.0610±0.0017	0.1489±0.0002	0.1238±0.0033
<i>Escherichia coli</i>	0.0065±0.00045	0.0647±0.04562	0.0435±0.00050	0.0183±0.00055	0.0247±0.00416

Qualitative analysis of *Andrographis paniculata* has revealed the presence of a wide variety of phytochemicals, including alkaloids, flavonoids, terpenoids, saponins, tannins, and phenolic compounds, which are responsible for its extensive pharmacological activities. Simple qualitative assays such as the Wagner's test for alkaloids, Shinoda test for flavonoids, and foam test for saponins have been employed to confirm their presence in extracts of the plant. Studies such as those by Mishra et al. (2007) and Akbar (2020) have extensively documented the qualitative phytochemical profile of *A. paniculata*, emphasizing its rich content of andrographolide, a diterpenoid lactone, which is a key bioactive compound known for its anti-inflammatory and hepatoprotective effects.[9],[10]. The presence of flavonoids and phenolics has also been associated with the plant's antioxidant and antimicrobial properties, as highlighted by Niranjana et al. (2010). These secondary metabolites not only contribute to the plant's therapeutic properties but also provide a basis for further quantitative and bioactivity-guided fractionation studies [11]. Variability in the phytochemical composition, influenced by geographical and environmental factors, has been reported by Roy et al. (2018), underscoring the need for standardized cultivation and extraction practices [12]. Overall, qualitative analysis serves as a foundational step in exploring the pharmacological potential of *A. paniculata* and supports its traditional use in herbal medicine. Therapeutic plants are increasingly utilized in medicine to combat drug-resistant bacteria. The agar well diffusion method is commonly employed to evaluate the antimicrobial efficacy of plant extracts against various pathogens. Ethanolic extracts of *Curcuma longa* (turmeric) have demonstrated inhibitory effects on multi-drug-resistant bacteria, as highlighted by Gunes et al. (2013) [13]. Additionally, *Punica granatum* (pomegranate) peel extracts have been found to significantly inhibit the growth of fungal pathogens like *Candida albicans*, according to a study by Abd El-Hamid et al. (2024). These findings emphasize the

agar well diffusion method's reliability and reproducibility in identifying plant-based antimicrobial agents, offering promising alternatives for treating resistant microbial strains [14]. A study by Kumar et al. (2023) highlighted the significant antibacterial activity of both ethanol and aqueous extracts of *Andrographis paniculata* against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The antimicrobial effectiveness was found to be directly related to the concentration of the extract, underscoring its potent potential [15]. Additionally, Kumar et al. (2022) confirmed the antifungal efficacy of *Andrographis paniculata* against *Candida albicans*, a key cause of urinary tract infections and other fungal diseases [16]. The Minimum Inhibitory Concentration (MIC) method is a vital tool for assessing the antimicrobial activity of medicinal plant extracts by determining the lowest concentration required to inhibit visible microbial growth. *Eucalyptus globulus* leaf extracts have shown significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, with MIC values as low as 0.78 mg/mL (Ahmad et al., 2022).[17]. Similarly, *Azadirachta indica* (neem) has exhibited potent antibacterial effects against a range of bacterial strains, with MIC values ranging from 1 to 4 mg/mL for both Gram-negative and Gram-positive bacteria (Anwar et al., 2021). [18]. Additionally, Prakash et al., 2023, reported that *Cinnamomum zeylanicum* (cinnamon) demonstrated antifungal properties, particularly against *Candida albicans*, with MIC values of 0.5 to 1 mg/mL [19]. Singh et al. 2023, reported significant antibacterial activity of the ethanolic extract of *Andrographis paniculata* against *Escherichia coli* and *Staphylococcus aureus*, with MIC values ranging from 0.5 to 1 mg/mL, highlighting its broad-spectrum potential [20]. Furthermore, these findings align with Srivastava et al. (2023), which highlighted the effectiveness of *Andrographis paniculata* against pathogens like *P. aeruginosa*, *S. aureus*, *E. coli*, and *B. licheniformis* [21]. Additionally, studies by Shahwal et al. (2023)

and Verma et al. (2023) have investigated the antimicrobial efficacy of other plant species, such as *Cassia tora* and *Curcuma* species, showcasing their potential as alternatives to conventional antibiotics [22],[23]. Srivastava and colleagues (2021) reviewed and highlighted the phytochemical compounds and medicinal properties of *Swertia chirayita*, emphasizing its significant therapeutic potential [24]. Similarly, Sur (2020) conducted a comparative study on the antimicrobial activity of fennel (*Foeniculum vulgare*) seed oil against bacteria and fungi, revealing that fennel oil was more effective against bacterial strains [25]. Verma et al. (2015) reported the potent antimicrobial properties of clove oil against both bacterial and fungal species [26]. In another study, Srivastava et al. (2021) explored the pharmacological benefits of *Tinospora cordifolia*, emphasizing its diverse phytochemical and medicinal properties [27]. Their work also reviewed the therapeutic significance of *Butea monosperma*, highlighting its medicinal value [28]. Bacterial infections continue to pose one of the most pressing global health challenges, exacerbated by the alarming rise in antibiotic resistance. This growing threat underscores the urgent need for novel antibiotics with innovative mechanisms of action to address these challenges effectively. Plants, with their diverse bioactive compounds, remain a rich source of potential therapeutic agents, contributing significantly to human health. Incorporating such natural compounds into therapeutic practices offers a sustainable and effective strategy for combating the escalating issue of antibiotic resistance, paving the way for innovative and impactful healthcare solutions.

#### IV. CONCLUSION

Plants have long been recognized as a rich source of bioactive compounds that could serve as valuable precursors for the development of novel chemotherapeutic agents. The first critical step in this process is conducting *in vitro* studies on plant extracts to assess their biological activities. Recent research has highlighted the antimicrobial potential of *Andrographis paniculata*, particularly its leaf extracts, which have demonstrated significant activity against both Gram-positive and Gram-negative bacteria responsible for urinary tract infections (UTIs). These findings offer a promising avenue for exploring

natural alternatives to synthetic antibiotics, especially given the increasing concerns over antibiotic resistance. The current study sheds light on the intersection between traditional healing systems, such as Ayurveda and Siddha, and modern microbiology. *Andrographis paniculata*, a plant with a longstanding history of medicinal use in these ancient systems, has shown considerable antimicrobial properties that align with its traditional use for treating infections. By demonstrating its efficacy against UTI-causing pathogens, this research paves the way for further exploration of the plant's therapeutic potential in the context of modern medicine.

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