Glob. J. Med. Pharm. Sci.
ISSN PRINT: 2957-3610, **ISSN ONLINE**: 2957-3629
Website: https://globapc.com/
Open Access Journal

Global
Journal of Medical and
Pharmaceutical Sciences

ORIGINAL ARTICLE OPEN ACCESS

Aristolochia indica and Toddalia asiatica Aqueous and Ethanol Extracts' in vitro Antibacterial Activity against Multidrug-Resistant Bacteria

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OPEN ACCESS

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Received:11-05-2024
Accepted:16-06-2024
Available online:23-06-2024

ABS TRAC T

Bacteria have developed multidrug resistance to modern antimicrobial medications. Through infectious diseases, these multidrug-resistant microorganisms significantly increase human morbidity and mortality. Synthetic drugs not only have unfavorable side effects and are ineffective against bacteria that are resistant to several treatments, but they are also expensive and inadequate for treating ailments. Medicinal plants have the potential to have strong antibacterial qualities because they include phytochemicals such as alkaloids, flavanoids, tannins, and phenolic compounds. The purpose of the present study was to determine the antibacterial activity of medicinal plants against pathogens resistant to several medications. The Kirby-Bauer disc diffusion method was used to detect drug-resistant bacteria. The generation of β-lactamases, such as metallo β-lactamase, AmpC β-lactamase, and extended spectrum β-lactamases, was detected using the combination disc approach. The antibacterial activity of the aqueous and ethanol extracts of Toddalia asiatica and Aristolochia indica was assessed using the agar well diffusion test and minimum inhibitory concentration. Each of the bacteria used in this study shown resistance to three distinct antibiotics. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, and Vibrio cholerae were shown to produce β-lactamases. Aristolochia indica's ethanol extract shown greater antibacterial activity against drug-resistant pathogens than Toddalia asiatica. Ethanol extracts of Toddalia asiatica and Aristolochia indica showed minimum inhibitory concentration values of 100–200 µg/ml and 50–100 µg/ml, respectively, against multidrug-resistant bacteria. This study suggests that Aristolochia indica has a greater potential to treat drug-resistant bacteria than Toddalia asiatica.

Keywords: *Aristolochia Indica, Toddalia Asiatica, Combination Disc Technique, Minimum Inhibitory Concentration, Extended Spectrum B-Lactamases, and Multidrug-Resistant Bacteria.*

INTRODUCTION

Globally, infectious infections brought on by bacteria that are resistant to drugs are a leading cause of illness and death [1]. Multidrug resistance in pathogenic and opportunistic microorganisms has been reported more often in recent years [2]. Due to the unchecked use of wide spectrum antibiotics, the number of multidrug-resistant bacteria to existing antibiotics is constantly rising. These bacteria have also caused clinical treatment issues in cancer. In addition to being costly and insufficient for the therapy, synthetic antimicrobial medications have negative side effects. This circumstance made it necessary to look for novel antibacterial drugs derived from therapeutic plants [5]. Major multidrug resistant bacteria on a worldwide scale include Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), enterobacteriaceae bacteria that produce extended spectrum β-lactamases (ESBLs), and multidrug-resistant Mycobacterium tuberculosis (MDR-TB) [3, 4]. The decreased vulnerability Plant extracts may be utilized to treat infectious disorders brought on by drug-resistant microbes since they have a lot of promise as antimicrobial agents against microorganisms [6]. For generations, medicinal plant extracts have been utilized to treat a wide range of illnesses. Phytochemicals are what give plants their therapeutic qualities. Alkaloids, flavanoids, tannins, and phenolic compounds are the most significant bioactive substances [7]. In herbal medicine, phytochemicals were employed to treat

infectious illnesses. One of the nations with the greatest flora of medicinal plants, with 120 families and 130,000 species, is India. In the ancient Indian medical system, around 2400 of these plants are used to treat wounds, leprosy, skin conditions, diarrhea, dysentery, jaundice, cough, and colds [8]. The antibacterial properties of aqueous and ethanol extracts of the entire plants of Toddalia asiatica and Aristolochia indica were assessed in this study against bacteria that produce β-lactamases and are resistant to drugs. The Post Graduate and Research Department of Microbiology and Biotechnology at Presidency College (Autonomous), Chennai, India, provided the multidrug-resistant bacterial strains. According to CLSI guidelines (CLSI, 2012), strains were tested for antibiotic susceptibility on Mueller Hinton Agar plates using the Kirby-Bauer disc diffusion method. The antibiotics used were amikacin, ampicillin, ciprofloxacin, gentamicin, doxycycline, tetracycline, cefotaxime, ceftazidime, imipenem, chloramphenicol, nalidixic acid, and co-trimoxazole (Himedia, Mumbai).

In accordance with CLSI recommendations, the combination disc technique was used to identify the synthesis of β-lactamases among gram negative bacteria [9]. The test isolates were cultured overnight and corrected to 0.5 McFarland's standard. Mueller Hinton Agar (MHA) plates were used to create lawn culture. On the agar surface, the cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/10 µg) discs were positioned 20 mm apart. Similarly, discs containing ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg) (Himedia Laboratories, Mumbai) were used to identify the synthesis of extended spectrum β-lactamase. A ≥5 mm increase in the zone of inhibition diameter was observed after an overnight incubation at 37°, and this was considered positive for ESBL formation. The quality control strains used in this investigation are K. pneumoniae ATCC 700603 as a positive control and E. coli ATCC 25922 as a negative control. Imipenem (10 µg) alone and in conjunction with EDTA (750 µg) were used to detect the synthesis of metello β-lactamase, while cefoxitin (30 µg) alone and in combination with cloxacillin (200 µg) were used to detect the formation of AmpC β-lactamase. The whole plants of A. indica and T. asiatica were gathered from the Nilagiris District of Tamil Nadu, India's Ooty hill regions. The whole plant was pulverized after being air dried. Twenty grams of whole plant powder were thoroughly dissolved in 100 milliliters of double-distilled water and ethanol, respectively, to create aqueous and ethanol extracts (ratio 1:5). A Seitz filter with 0.2 µm pores was used to filter the suspension. After that, the sterile extract was moved to a lyophilization flask and stored for four hours at -80° in a deep freezer. After that, the lyophilizer was filled with the frozen extract. After lyophilization, the powder was moved to sterile storage jars and kept for later use. According to Bauer *et al.*, [10], the antibacterial properties of plant extracts from A. indica and T. asiatica were investigated using the agar well diffusion technique. Dimethyl sulfoxide (DMSO) was used to generate a stock solution of the extracts (1 mg/ml) and dilutions of the stock solution containing 0.5, 1.0, 1.5, and 2.0 µg/ml. The McFarland standard 0.5 scale was used to prepare and alter the inoculum. Muller Hinton agar (MHA) plates were used to create lawn culture. On the swabbed plates, prepared extract was placed into a well and incubated for 48 hours at 37°. Following the incubation period, the zone of inhibition was measured in millimeters and contrasted with the conventional antibiotic discs. Mueller Hinton broth (MHB) was used in the microdilution technique [11] to determine the minimum inhibitory concentration (MIC). The extracts were produced in DMSO as a stock solution (1 mg/ml), and MHB was used to generate dilutions of the stock solution containing 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 µg/ml. In the microtitre plate, 100 µl of each dilution and 100 µl of MHB were placed into the corresponding wells for control. Each well was injected with a loop full of broth culture. The usual reference medications were gentamicin and ciprofloxacin (100 µg). For eighteen to twenty-four hours, the microtitre plates were incubated at 37°. Minimum inhibitory action was defined as the lowest dilutions that did not exhibit any growth. Every bacterium used in this investigation was resistant to at least three antibiotics. Table 1 displayed the bacteria's β-lactamase production and antibiotic resistance. Significant efficacy against both gram positive and gram negative bacteria is shown by the ethanol extract of A. indica, which is followed by the ethanol extract of T. asiatica. The obtained zone of inhibition was similar to that of conventional bacterial antibiotics. A. indica has superior effectiveness against both gram positive and gram negative bacteria in aqueous extracts compared to T. asiatica. Table 2 displayed the agar well diffusion findings. Compared to other extracts of both A. indica and T. asiatica, the ethanol extract of A. indica exhibits a greater MIC value of 50–100 µg/ml against the tested bacteria. MIC ranges for A. indica and T. asiatica in aqueous extracts were 100–400 µg/ml and 200–400 µg/ml, respectively. Figure 1 displayed the MIC values of A. indica and T. asiatica against microorganisms resistant to several drugs. The phytochemical components of these plants may be responsible for their antimicrobial action. According to Shafia *et al*., [12], the essential oil of A. indica shown strong antibacterial activity at 50 mg/ml against P. aeruginosa, B. substilis, S. aureus, and E. coli. Additionally, the ethanol extract of the whole A. indica plant shown antibacterial efficacy against all tested bacteria, ranging from 50 to 100 µg/ml.

Kar *et al*., [13] reported that the aqueous extract of stem bark of *T. asiatica* showed 7 and 5 mm of:

Table 1:

Escherichia coli	AMP, CTX, CAZ, COT, NA, TE, CPD, DO, CX
Klebsiella pneumoniae	AK, AMP, CTX, COT, GEN, NA, TE, CPD, IMP, CX ESBL and AmpC, ESBL, MBL and AmpC
Pseudomonas aeruginosa	AMP, CTX, COT, NA, TE, IMP MBL
Proteus mirabilis	AMP, CTX, COT, NA, TE ESBL
Enterobacter aerogenes	AMP, C, CTX, COT, NA, TE
Enterococcus faecalis	AMP, CTX, NA, TE
Vibrio cholerae	AMP, C, CTX, COT, NA, CX ESBL
Staphylococcus aureus	AMP, NA, TE
Staphylococcus epidermidis	AMP, CTX, COT
Acetobacter sp.	CTX, COT, NA, TE
Bacillus substilis	NA, TE, COT

Multidrug resistance and ESBLs, MBL and AmpC β-lactamases production of bacteria. AMP: Ampicillin, CTX: cefotaxime, CAZ: ceftazidime, COT: co-trimoxazole, NA: nalidixic acid, CPD: cepodoxime, DO: doxycycline, CX: cefoxitin, GEN: gentamicin, TE: tetracycline, C: chloramphenicol, AK: amikacin, IMP: imipenem, ESBLs: extended spectrum β-lactamases, MBL: metallo β-lactamase.

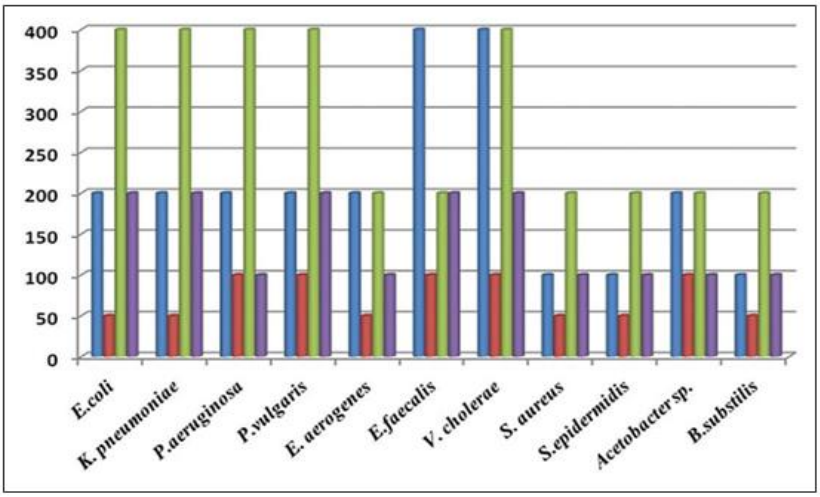

Fig 1: Minimum inhibitory concentration of aqueous and ethanol extracts of *Aristolochia indica* and *Toddalia asiatica* against multi drug resistant bacteria by microdilution

■ Aqueous extract of *A. indica*, ■ ethanol extract of *A. indica* ■ aqueous extract of *T. asiatica* ■ ethanol extract of *T. asiatica*

Table 2: Zone of Inhibition of Aqueous and Ethanol Extracts of *Aristolochia Indica* and *Toddalia Asiatica*

Organisms	***Aristolochia indica* (zone of Inhibition in mm)**									***Toddalia asiatica* (zone of inhibition in mm)**								
	Aqueous extract (mg)					**Ethanol extract (mg)**				**Aqueous extract (mg)**					**Ethanol extract (mg)**			
	0.5	**1**	**1.5**	**2**		**0.5**	**1**	**1.5**	**2**	**0.5**	**1**	**1.5**	**2**		**0.5**	**1**	**1.5**	**2**
Escherichia coli	11	13	14	15		13	15	16	18	-	-	10	11		12	13	15	16
Klebsiella pneumoniae	09	10	12	13		12	14	16	17	-	-	9	10		09	11	13	15
Pseudomonas aeruginosa	10	12	13	14		15	16	18	19	-	-	8	10		09	10	12	14
Proteus mirabilis	10	11	13	14		13	14	15	17	-	-	-	11		12	14	16	17
Enterobacter aerogenes	11	13	15	16		14	16	17	18	-	-	-	10		09	11	13	15
Enterococcus faecalis	11	12	14	15		13	15	15	16	-	-	9	11		10	11	13	14
Vibrio cholerae	09	11	13	14		12	14	15	15	-	10	11	12		11	13	14	15
Staphylococcus aureus	13	14	15	16		13	15	16	19	9	11	12	13		13	14	15	16
Staphylococcus	11	12	14	16		14	15	17	18	8	10	11	13		10	12	14	15

epidermidis																			
Acetobacter sp.	13	15	17	15		13	14	16	18		-	9	10	12		13	15	16	17
Bacillus substilis	09	11	13	15		14	15	16	18		10	11	13	14		13	14	16	16

Zone of inhibition of aqueous and ethanol extracts of whole plant of *Aristolochia indica* and *Toddalia asiatica* against multidrug-resistant bacteria by Agar well diffusion method zone of inhibition against E. coli and S. aureus, respectively. When tested against S. aureus and E. coli, ethanol extract demonstrated 10 and 6 mm, respectively. At a concentration of 2 mg/ml, the whole plant ethanol extract of T. asiatica demonstrated 13 and 11 mm against S. aureus and E. coli, respectively, while the aqueous extract shown 16 mm against both. According to Ali *et al.*, [14], A. indica's phytochemical, pharmacological, and toxicological characteristics shown antibacterial efficacy against Enterococcus faecalis. When it comes to E. faecalis, it has strong antibacterial action. The ethanol extract of A. indica had the highest antibacterial activity of 19 mm against clinically isolated multidrug-resistant Enterococcus faecalis, according to Gopinath and Prakash [15]. The ethanol extract of A. indica demonstrated 18 mm against multidrug-resistant E. faecalis in our investigation. T. asiatica shown encouraging action against tested bacteria, according to Yadav and Khan [16]. Compared to gram negative bacteria, the evaluated plant extracts were more effective against gram positive microorganisms. T. asiatica shown more antibacterial activity against gram positive bacteria than gram negative bacteria in this investigation as well. Multidrug resistance bacterial infections are mostly caused by unsanitary circumstances and unchecked antibiotic usage in hospital and community settings. Antibiotics are being transformed into useless chemicals by these bacteria [17]. The potential of plant extracts as antibacterial agents against germs that are resistant to many drugs is enormous. The significance of A. indica and T. asiatica is highlighted in this work, and active chemicals extracted from these plants may be used as potent antibacterial agents against bacteria that produce β-lactamases and are resistant to several drugs.

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