



To the Investigation of the Antibacterial Effects of Plant Extracts

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DOI: <https://doi.org/10.5281/zenodo.17661412>

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Abstract

Antimicrobials made from medicinal plants provide an additional weapon in the battle against illnesses, especially because bacteria and other organisms have learned to resist pharmaceuticals. The leaves were then dried using a Rota evaporator. As an anti-inflammatory and anti-diarrhea therapy, the plant *Teucrium polium* finds value in medicine. A variety of medicinal uses exist for herbal antimicrobials. There is an innate resistance to many medicines in the Gram-negative bacterium *Pseudomonas aeruginosa*. In order to identify and assess the antibacterial activity of plant extracts against antibiotic-resistant bacteria, the primary goal of the research was to analyze their phytochemical composition. Prior to testing against antibiotic-resistant bacteria, plant extracts were first tested against MTCC cultures obtained from IMTECH, Chandigarh. Through the use of Vitek 2 and DNA sequencing, the antibiotic-resistant microbes found in the local hospital were identified.

Keywords: Antibacterial, Plant, Extracts, Antimicrobials and antibiotic

1. Introduction

Recently, there has been a great deal of attention paid in medical treatments to plant extracts and compounds with biological features, because of the resistance and side effects that the micro-organisms of pathogens have shown in the face of antibiotics. The anti-bacterial compounds of plants are a significant medical resource. As a result of the spread of infectious diseases, exploration of more of these compounds will be useful. Anti-bacterial compounds with herbal sources have a wide range of therapeutic use. These compounds are not only efficient for the treatment of infectious diseases, but also concurrently diminish existing side effects via their anti-bacterial compounds. *Escherichia coli* is a pathogenic bacteria transmitted by infected food. Worldwide epidemics of this disease have been reported. The signs and symptoms of *E. coli* exposure include diarrhea, nausea, ulcerative colitis, abdominal pain and, in some cases, kidney disorders or death, especially among children. The third hospital-infection factor after *Escherichia coli* and *Staphylococcus aureus* is *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is a Gram-negative bacterium that has shown an innate resistance to many antibiotics. However, it has shown sensitivity to various antibiotics,

such as piperacillin, ciprofloxacin, tobramycin and imipenem. *Pseudomonas aeruginosa* is a factor for urinary tract infections and lung diseases, including cystic fibrosis is an herb in the polygonaceae family. Its leaves and flowers have already been used for lividness and biting treatments, and as an efficient anti-venom. Its impact on the treatment of peptic ulcers in animals has also been demonstrated. The herb *Teucrium polium* is used as anti-inflammatory and as an anti-diarrhea treatment. In traditional medicine, it is used as an anti-bacterial. *Tragopogon gramineus* is an herb from the Asteraceae family that grows at elevations above 1400 m in the area of the Zagros mountains.

The antibacterial effect of various antibiotics is based on distinct processes. Bactericidal penicillin inhibits cell wall synthesis; bacteriostatic tetracyclines inhibit protein synthesis; carbapenems inhibit cell wall synthesis; bactericidal quinolones inhibit DNA replication; macrolides and bacteriostatic sulfonamides both block protein synthesis and function as competing inhibitors. Antimicrobial compounds derived from medicinal plants provide an alternative strategy for combating infections caused by bacteria and other microbes that have developed resistance to antibiotics. Pharmacognosy medication development is getting more and more attention from academics due to the

fact that plant-based antibacterial drugs have less adverse effects.

The "Report of the Task Force on Conservation & Sustainable use of Medicinal Plants" from 2002, released by the Government of India's planning commission, states that plant variety is a well-known trait of India. An incredible array of plant and animal species may be found here in India's many regions since the country experiences all kinds of climates. India is home to a great diversity of habitats, each having its own flora and wildlife. For example, the country is home to the coldest and hottest desert regions, as well as areas with considerable rainfall, such as Cheerapunji and Unbar Valley, and Rajasthan, respectively. The majority A considerable percentage of the total population resides in rural regions relies on traditional medicine, namely home medicines made from plants, to treat and prevent illness. Local Health Tradition is the name given to this autonomous system. When compared to systems like Ayurveda, Sidha, and Unani, which use only about 2000 plants for illness treatment, the LHT's utilization of 4500 to 5000 plant species is far larger.

There are no adverse effects from the phytochemicals that plants are packed with. Tannins, quinines, and terpenoids are among the phytochemicals that plants are making. Aromatic compounds, such as phenols or oxygen derivatives of them, may be synthesized by plants. We are now in the midst of a mad dash to create the most advanced medical facilities so that patients may recuperate more quickly and effectively. Since herbal-derived medicines are beneficial and increase immunity throughout life, their discovery is the primary focus of research at the moment.

2. Literature Review

"Cock, I.E., Blonk, B., Cheesman, M.J., and Ilanko, A. (2017) ^[1]" Aesthetic and medicinal uses of plants date back to prehistoric times. Their therapeutic qualities are derived from phytochemical substances, which are abundant in these foods and have no negative side effects. On top of that, negative effects might occur with antibiotic usage over an extended period of time. Acute toxicity, carcinogenic consequences, and environmental dangers are adverse effects of using synthetic drugs to treat microbiological infections. An alternative option for treating pathogens that have developed resistance to many antimicrobials is herbal medications.

Published in 2016 by Katz and Baltz There is a vast array of nutritional and medicinal uses for natural plant products. There are more negative effects associated with synthetic antibiotics than with the natural drive. Some of the many beneficial medical effects of phytochemicals found in plants include their ability to reduce inflammation, strengthen the immune system, and even fight cancer.

In Garcia-Grijalva, E.P., López-Martínez, L.X., Contreras-Angulo, L.A., Elizalde-Romero, C.A., and Heredia, J.B. 2020 ^[3], Plants produce substances with an alkaline pH. Chemicals such as ephedrine, muscarine, and alkaloids like hordenine include A nitrogen-containing heterocyclic ring. Some chemicals, such as piperine, are heterocyclic but basic; betadine is acidic; and hordenine, muscarine, and ephedrine are amino alkaloids; these compounds do not have heterocyclic but basic nature. The medicinal uses of alkaloids are extensive.

In, Eldahshan and Singab 2023 ^[4] This includes β -carotenoid, α -carotenoid, Lycopene, β -cryptoxanthin, and lutein, which are pigmented chemicals that cause various fruits and plant colors. They help plants absorb light, which is essential for photosynthesis, and they shield chlorophyll from harmful light rays. It is possible to convert β -carotene, a pro-vitamin, into vitamin A. Additionally, it boosts the immune system with a boost in NK and CD4 cell numbers. The β -carotene has several impacts on the body's defense mechanisms, including preventing auto-oxidation of phagocytic cells, increasing the number of macrophages, enhancing the efficacy of effector T cells, and increasing the production of interleukins.

Bansal, M., Singh, N., Pal, S., Dev, I. and Ansari, K.M., 2018 ^[5], Cyanate groups or carbon-incyclicornoncyclic confirmations connect Sulphur atoms in organo-sulfur compounds. Broccoli, Brussels sprouts, cauliflower, garlic sprouts, and onions are examples of cruciferous vegetables that naturally contain sources. A study found that an extract of aged garlic reduced oxidative stress and increased lung capacity.

3. Research Methodology

We used the bore-well approach to examine plant leaf extracts in light of their antimicrobial efficacy against a number of MTCC diseases. *Bacillus subtilis* (MTCC 10619), *Staphylococcus aureus* (MTCC-740), *Escherichia coli* (MTCC-40), *Klebsiella pneumonia* (MTCC-109) *Pseudomonas aeruginosa* (MTCC-424), and *Micrococcus luteus* (MTCC-6164) were among the pathogens that were detected. Minimal changes were made to the typical CLSI approach

4. Data Analysis

4.1 Botanical essences' antibacterial capabilities

Tables exhibit the findings of the antimicrobial activity tests conducted on the plant extracts against MTCC cultures obtained from IMTECH Chandigarh. In this investigation, DMSO did not demonstrate antimicrobial activity against any of the examined bacteria. The goal of the statistical analysis was to find extracts that had strong antibacterial effects. A descriptive analysis In Table 1, you can see the average inhibition zone and standard deviation (SD) for plant extracts. The biggest inhibitory zone was seen in the crystalline extract of *A. vasica*, followed by *W. fruticosa* and *R. communis*.

Bambusa aqueous extract, in contrast, had the least potent inhibitory effect against the examined microbes. The outcomes of the data-driven two-way analysis of variance (ANOVA) may be seen in Table 2. The findings demonstrated that different types of plants have significantly different antibacterial activities. Thanks to Tukey's post-hoc test ($p < 0.05$), the findings were further validated. Every plant extract was classified according to the results of Tukey's Post Hoc analysis.

It was shown that the *A. vasica* plant's leaf extracts, which were categorized as 'a', had antibacterial characteristics that vary significantly from those of other plant extracts. The 'ab' classification for *R. communis* indicates that it outperformed all other plant extracts in terms of antibacterial activity, which is equivalent to that of *A. vasica*. The *W. fruticosa* plant was categorized as 'abc' due

to its higher zone of inhibition, which is comparable to *A. vasica* and *R. communis*. The antibacterial activity of bamboos was also the lowest when measured in terms of zone of inhibition, and were therefore classified as 'g'.

Table 1: Descriptive statistics of mean of zone of inhibition against MTCC pathogens

S. N.	Plant*	Aqueous Extract	Hydrocarbon Refinement	Extract from Methyl ethanol
1	<i>M. indica</i> ^{fs}	7.20±1.70	17.67±3.10	23.60±3.68
2	<i>Bambusa</i> ^g	10.07±2.68	11.13±2.10	16.40±2.15
3	<i>R. communis</i> ^{ab}	40.20±3.49	49.60±3.17	61.53±3.71
4	<i>T. indica</i> ^{defg}	17.53±3.38	27.53±4.00	34.27±3.06
5	<i>W. fruticosa</i> ^{abc}	25.93±2.99	54.13±4.24	70.00±3.54
6	<i>C. bonplandianum</i> ^{bcd}	22.93±2.39	35.20±3.59	43.93±4.10
7	<i>L. leucocephala</i> ^{bcd}	27.43±3.48	41.33±2.85	43.67±2.62
8	<i>A. vasica</i> ^a	49.33±3.09	59.93±3.06	72.87±3.25
9	<i>C. procera</i> ^{bcd}	26.80±2.47	41.20±3.17	45.27±2.76
10	<i>T. peruviana</i>	18.93±2.39	26.73±3.51	30.13±2.73
11	<i>G. robusta</i> ^{bcd}	25.47±3.42	38.13±2.90	37.00±3.16
12	<i>T. grandis</i> ^{def}	22.83±2.83	36.60±3.48	29.40±2.88
13	<i>Cymbopogon</i> ^{defg}	15.59±1.92	34.47±2.59	38.67±3.43
14	<i>A. calamus</i> ^{bcd}	27.67±2.69	33.00±2.79	41.00±3.18
15	<i>A. ficoidea</i> ^{bcd}	27.13±2.22	45.07±3.06	37.20±2.74
16	<i>C. oppositifolia</i> ^{bcd}	29.47±1.73	37.67±2.76	41.27±3.18
17	<i>R. ellipticus</i> ^{efg}	16.93±1.55	18.27±2.59	25.93±3.39
18	<i>A. conyzoides</i> ^{defg}	16.47±2.22	24.47±2.22	32.33±2.96
19	<i>P. longifoliace</i> ^{def}	25.60±2.44	36.53±3.64	36.60±3.45
20	<i>R. cathartica</i> ^{bcd}	19.33±2.47	36.80±3.86	44.53±4.20
21	<i>M. hexandra</i> ^{def}	24.80±2.36	32.53±2.76	36.07±3.36
22	<i>F. religiosa</i> ^{defg}	16.40±2.18	28.20±2.73	42.20±3.85

According to Tukey's post-hoc test ($p < 0.05$), the inhibition zone signifies the average departure from the mean. If there is a superscript with different letters, it means that difference.

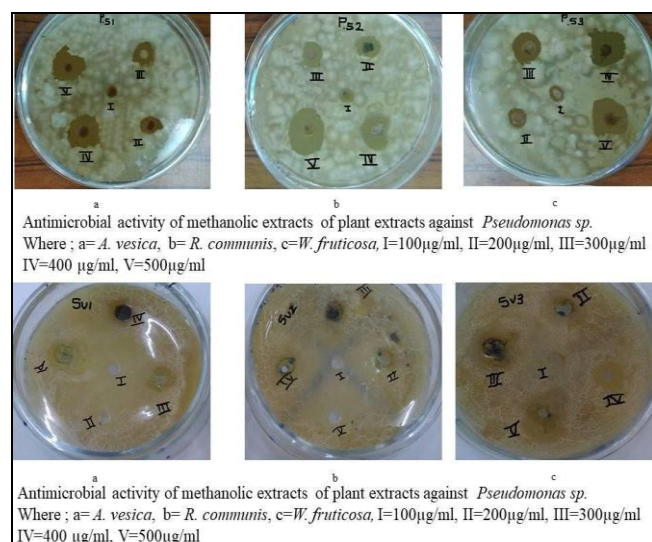


Fig 1: Antimicrobial activity of plant extracts

Table 2 displays the results of doing an ANOVA with a single independent variable for each of the plant extracts. A substantial difference ($p < 0.05$) was found when comparing all of the plant extracts, indicating that there was a

considerable variation in antibacterial activity. Figure 2 shows that the three the plant extracts that have the strongest antibacterial action against MTCC strains are fruticosa, *A. vasica*, and *R. communis*. The solvent level underwent further investigation in order to determine the most effective the three plant species that showed the most potent antibacterial activity-*A. vasica*, *R. communis*, and *W. fruticosa*-extracted from them. Based on the zone of inhibition for all three tested extracts, the methanolic extract had the strongest antibacterial effect (Table 3 and Figure 3.). The ethanolic and water-based fractions were the others. In addition, there was no statistically significant difference between the groups in terms of the effectiveness of the methanolic extract of the chosen plant in inhibiting the development of antibiotic-resistant bacteria and one another.

Table 2: One way ANOVA test of plant extracts comparing antibacterial potential.

P-value	<0.0001		
Are means signif. Different? ($p < 0.05$)	Yes		
Number of groups	22		
F	13.35		
R squared	0.8643		
ANOVA Table 4.	SS	df	MS
Treatment (between columns)	67500	21	3214
Residual (within columns)	10600	44	240.8

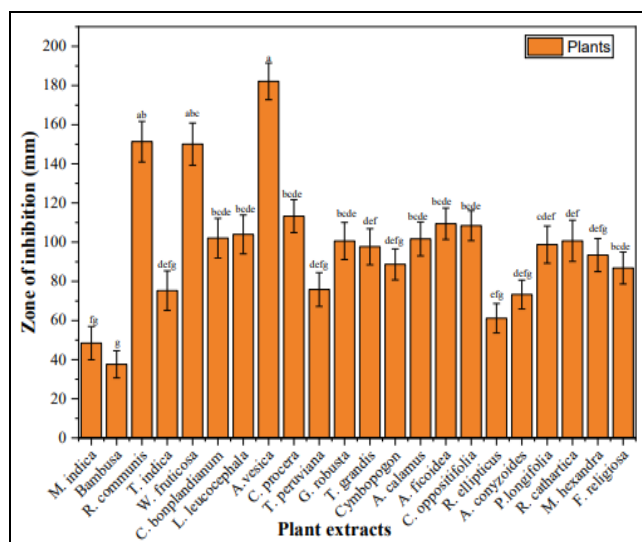


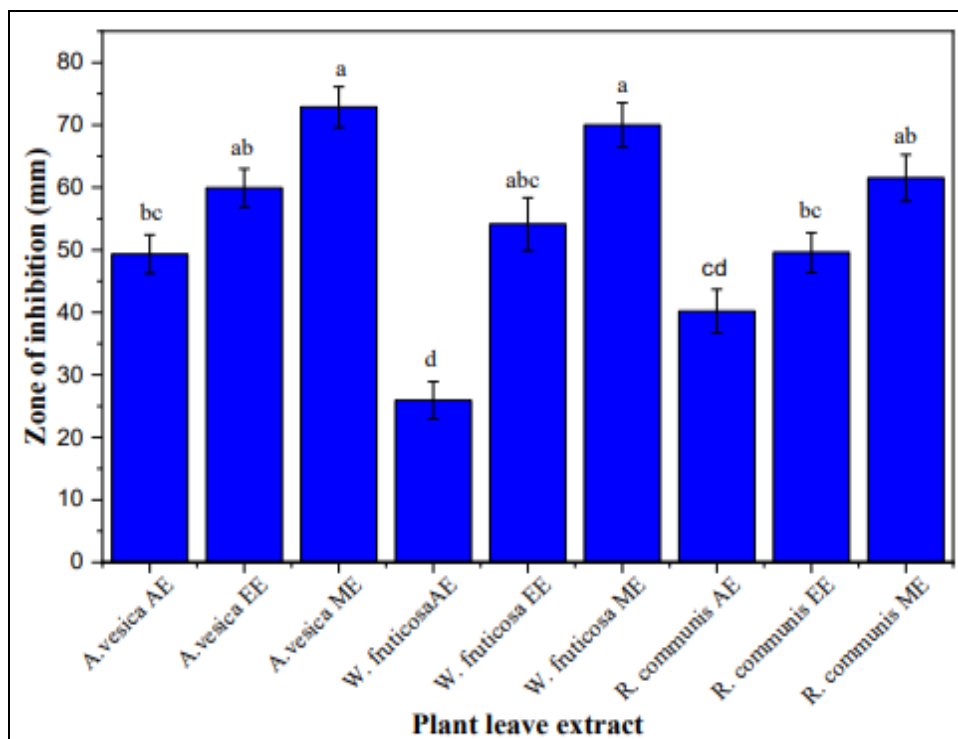
Fig 2: Mean of zone of inhibition of various plant extracts

Variables superscripted with different letters indicate a statistically significant difference as determined by Tukey's post hoc test ($p < 0.05$)

To discover the most effective extract, researchers conducted further analyses at the solvent level on the plant extracts of *A. vasica*, *R. communis*, and *W. fruticosa*, which had the greatest antibacterial activity. Table 1 and Figure 3 demonstrate that, when compared to extracts in water and alcohol, the methanolic extract exhibited the highest zone of inhibition, indicating the highest antibacterial potential. When tested against bacteria that are resistant to antibiotics, the non-methylated extract of the chosen plant showed no statistically significant change in antibacterial effectiveness.

Table 3: One way ANOVA analysis of plant extracts with respect to solvent.

P-value	<0.0001		
P value summary	***		
Are means signif. Different? ($p<0.05$)	Yes		
Number of groups	9		
F	18.12		
R squared	0.8896		
ANOVE Table 4.	SS	df	MS
Treatment (between columns)	5071	8	633.8
Residual (within columns)	629.5	18	34.97

**Fig 3:** Solvent wise zone of inhibition of selected plant extracts

For example, AE stands for aqueous extract, EE for ethanolic extract, and ME for methanolic extract. Variables superscripted with different letters indicate a statistically

significant difference as determined by Tukey's post hoc test ($p<0.05$).

Table 4: The effectiveness of methanolic leaf extracts as antibacterial agents against microorganisms that are resistant to antibiotics

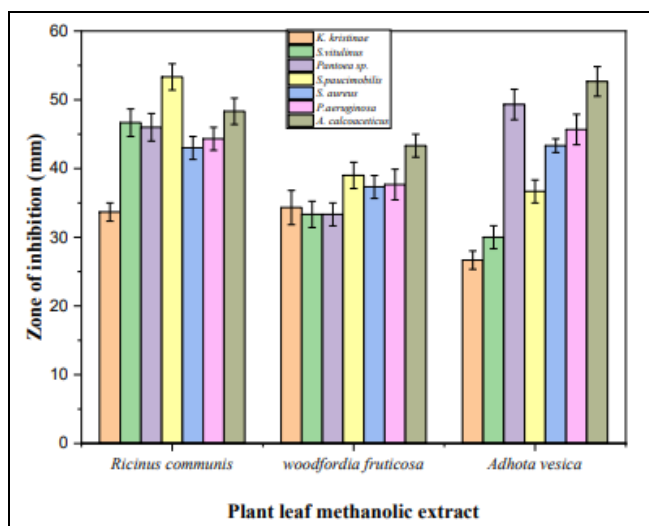
Plant Name	Conc.	KK	SV	PS	SP	SS	PSp	AS
<i>A. vesica</i>	100µg/ml	0.00±0.00	0.00±0.00	2.67±0.58	2.67±0.58	2.00±0.00	2.00±0.00	4.67±0.58
	200µg/ml	2.33±0.58	2.67±0.67	6.33±1.53	4.67±0.58	5.33±0.58	5.67±1.15	7.67±0.58
	300µg/ml	5.33±0.58	5.33±0.58	10.67±0.58	7.33±0.58	8.67±0.58	9.67±1.53	10.33±0.58
	400µg/ml	7.67±0.58	8.67±0.58	13.33±0.58	10.67±0.58	12.33±0.58	13.67±0.58	14.00±1.00
	500 µg/ml	11.33±0.58	13.22±0.58	16.33±0.58	12.33±0.58	14.67±0.58	14.67±0.58	16.00±1.00
<i>R. communis</i>	100µg/ml	2.00±0.00	3.33±0.58	2.67±1.15	3.67±0.58	2.33±0.58	2.67±0.58	4.33±0.58
	200µg/ml	5.33±0.58	6.67±1.15	5.33±0.58	7.67±0.58	5.33±0.58	6.33±0.58	7.00±1.00
	300µg/ml	6.67±0.58	9.67±0.58	9.33±0.58	10.33±0.58	8.33±0.58	9.33±0.58	9.33±0.58
	400µg/ml	8.33±0.58	12.33±0.58	13.33±0.58	14.67±0.58	11.67±0.58	11.67±0.58	12.33±0.58
	500µg/ml	11.33±0.58	14.67±0.58	15.33±0.58	17.33±0.58	15.33±0.58	14.33±0.58	15.33±0.58
<i>W. fruticosa</i>	100µg/ml	2.67±0.58	3.00±0.58	2.33±0.58	2.33±0.58	2.33±0.58	2.33±0.58	2.33±0.58
	200µg/ml	4.33±0.58	6.25±0.58	4.33±0.58	5.33±0.58	4.33±0.58	5.00±1.00	5.33±0.58
	300µg/ml	7.00±0.58	8.50±0.58	6.33±0.58	7.33±0.58	7.33±0.58	7.67±0.58	8.67±0.58
	400µg/ml	9.00±0.58	12.00±1.00	8.67±0.58	10.33±0.58	9.67±0.58	9.33±0.58	11.67±0.58
	500µg/ml	11.33±0.67	13.25±0.58	11.67±0.58	13.33±0.58	13.67±0.58	13.33±1.15	15.33±0.58

In this context, KK can mean *K. kristinae*, SV can mean *S. vitulinus*, PS can mean *Pantoea* sp., SP can mean *S. paucimobilis*, SS can mean *Staphylococcus* sp., PSp can

mean *Pseudomonas* sp., and AS may mean *Acinetobacter* sp.

Table 5: One way ANOVA analysis of methanolic plant extract of *A. vasica*, *R. communis*, and *W. fruticosa*.

P-value	0.2049		
P value summary	ns		
Are means signif. different? ($p < 0.05$)	No		
Number of groups	3		
F	1.734		
R squared	0.1615		
ANOVE Table 4.	SS	Df	MS
Treatment (between columns)	191.9	2	95.96
Residual (within columns)	996.3	18	55.35

**Fig 4:** Antibacterial activity shown by methanolic extract against antibiotic-resistant bacteria.

The minimum concentration of a powerful extract being inhibited

The methanolic extracts were used for microbiological susceptibility testing on isolated microbes. Table 6 displays the minimum inhibitory concentration (MIC) of *A. vasica* as well as the inhibition of *K. kristinae* growth that was found. *Staphylococcus vitulinus* and *S. aureus*.

Table 6: Half-life of *A. vasica* methanolic leaf extract against antibiotic-resistant bacteria.

	A. vasica (µg/ml)											
	256	128	64	32	16	8	4	2	1	0.50	0.25	0.12
K. kristinae	-	-	+	+	+	+	+	+	+	+	+	+
S. vitulinus	-	-	+	+	+	+	+	+	+	+	+	+
Pantoea sp.	-	-	-	+	+	+	+	+	+	+	+	+
S. paucimobils	-	-	-	+	+	+	+	+	+	+	+	+
Staphylococcus sp.	-	-	-	+	+	+	+	+	+	+	+	+
Pseudomonas sp.	-	-	+	+	+	+	+	+	+	+	+	+
Acinetobacter sp.	-	-	-	+	+	+	+	+	+	+	+	+

Where += growth of bacteria observed, - = no an increase in sp. being $256\mu\text{g/ml}$ in concentration, at $64\mu\text{g/ml}$, in the same way as *Pantoea* sp., *S. paucimobils*, *Staphylococcus* sp., and *Acinetobacter* sp. According to Table 7, *R. communis* had a minimum inhibitory concentration (MIC) of $64\mu\text{g/ml}$ against *K. kristinae*, *S. vitulinus*, *S. paucimobils*, and *Staphylococcus* sp. However, at $32\mu\text{g/ml}$, it inhibited the growth of *Pantoea* sp. and *Acinetobacter* sp.

Table 7: MIC of methanolic leaf extract of *R. communis* against antibiotic-resistant bacteria

	<i>R. communis</i> ($\mu\text{g/ml}$)											
	256	128	64	32	16	8	4	2	1	0.50	0.25	0.125
<i>K. kristinae</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>S. vitulinus</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Pantoea</i> sp.	-	-	-	-	+	+	+	+	+	+	+	+
<i>S. paucimobils</i>	-	-	-	+	+	+	+	+	+	+	+	+
<i>Staphylococcus</i> sp.	-	-	-	+	+	+	+	+	+	+	+	+
<i>Pseudomonas</i> sp.	-	-	-	+	+	+	+	+	+	+	+	+
<i>Acinetobacter</i> sp.	-	-	-	-	+	+	+	+	+	+	+	+

Where += growth of bacteria observed, - = no growth was observed

The antimicrobial activity of the methanolic leaf extract of *A. vasica*, which contains the active alkaloid vasicine, was found to be significantly higher than that of the standard antibiotics used in the study, which include amphotericin B, ofloxacin, nystatin, and ciprofloxacin. The bacteria tested included both Gram-positive and Gram-negative types, as well as *Escherichia coli*, *Staphylococcus pyogenes*, *Staphylococcus marcescens*, *Staphylococcus aureus*, *P. aeruginosa*, and *K. pneumoniae*. The antimicrobial effectiveness of *A. vasica* methanol leaf extracts was investigated by against many bacteria that might potentially cause UTIs. The antibacterial capabilities of the extract were shown to be effective against a variety of bacteria, including *Enterobacter*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Proteus fluorescens*. investigated the antibacterial growth inhibition activity of *A. vasica* leaf extracts in aqueous, N-hexane, and methanol against pathogens associated with skin infections, UTIs, and GIT illnesses.

Micrococci had the lowest antibacterial activity, whereas *Streptococcus mitis* showed the highest. When tested against several bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and others, found that the extract from the leaves of *A. vasica* had strong antibacterial properties. The plant extracts included phytochemicals. It is closely related to their work as the present research employed the same microorganisms to evaluate the antibacterial potential of the plant extract. The presence of numerous phytochemicals tested by both the conventional technique and GCMS analysis was then shown in the plant extract.

Several phytochemicals were measured in these preparations. Methanolic extract has the highest content of phytochemicals compared to the other extracts. Water, methanol, and ethyl acetate were among the solvents investigated by for their antioxidant and anticancer properties, as well as their effects on the antibacterial activity of *A. vasica* plant extracts from the leaves. The extracts shown strong antibiotic effectiveness against many bacteria, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus mutans*, and *Staphylococcus aureus*. The antioxidant properties and phytochemical content were also validated by the extracts. Further, when tested on HCC-827 cell lines, the methanolic extract showed a high cytotoxic impact.

The presence of several phytochemicals in methanolic extracts was also shown in this study. In the methanolic extract, compounds such as 6,9,12-octadecatrienoic acid phenylmethyl ester (Z, Z, Z) were found by gas chromatography mass spectrometry (GCMS). These compounds have anti-inflammatory, hepatoprotective, and anti-cancer properties found that *A. vasica* leaf extracts were efficient against microbes. They also verified the existence of several phytochemicals, which add to the medicinal value of plants. Antimicrobial activity against antibiotic resistant bacteria was investigated in relation to *R. communis*, a secondary botanical component. The plant exhibited antimicrobial activity against *Acinetobacter* sp. and *K. kristinae* at a concentration of 100µg/ml, with an average zone of inhibition of 4.33mm±0.58mm and 2.00mm±0.00 mm, respectively. An inhibitory zone of 17.33mm±0.58mm against *S. paucimobilis* and an inhibitory zone of 11.33mm±0.58mm against *K. kristinae* were noted at a concentration of 500µg/ml, respectively.

Conclusion

The phytochemical components found in plant extracts have a wide variety of medical applications, one of which is an antibacterial effect. An alternate tactic in the fight against illnesses caused by bacteria and other germs that have developed a mechanism to resist antibiotics is antimicrobials produced from medicinal plants. The major objective of the study was to analyze the phytochemical makeup of plant extracts in order to discover and evaluate their antibacterial activity against antibiotic-resistant pathogens. *Bacillus subtilis* (MTCC 10619), *Staphylococcus aureus* (MTCC 740), *Escherichia coli* (MTCC-40), *Klebsiella pneumonia* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-424), and *Micrococcus luteus* (MTCC 614) were among the numerous bacteria tested for antibacterial properties in the aqueous, ethanolic, and methanolic variants of plant leaf extracts. All of the plant extracts were statistically tested using a One-Way ANOVA test to find out which one had the best antibacterial function.

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