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Antimicrobial Sensitivity of Plant Extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*

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Abstract

Background: In the recent era, biological treatment using therapeutic microbes or phytochemicals has proven more beneficial than conventional methods due to several reasons - permanent control of weeds, host-specific control, cost-effectiveness, and low health risk.

This study determined the antimicrobial sensitivity profile of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* against plants like *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa*. Given the importance and ease of using phytochemicals in modern Microbiology, this study has been carried out towards the approach of green synthesis of antimicrobial agents.

Methods: The primary purpose of this study was to determine the antimicrobial sensitivity of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using extracts from plants like *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa*. Antimicrobial properties of plant extracts were analyzed by determining the *Zone of Inhibition (ZOI)*. The antibiogram pattern of isolated *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and

Escherichia coli was observed to be Susceptible, Intermediate, and Slightly Resistant to *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa*.

Results: Phytochemical analysis indicates that the extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* have the potential for use in managing *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Further phytochemical analysis is required to identify the active components of plant extracts showing antimicrobial activity.

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Keywords: Antibiogram pattern; Phytochemical analysis; Zone of inhibition; Antimicrobial sensitivity; Plant extracts.

1. Introduction

The medicinal plants are widely used as sources of medicine. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Plants with medicinal value have been used for several years to conserve food and also to treat health disorders and prevent health-related diseases [1]. Undoubtedly, medicinal plants play a vital role in drug discovery, as they are rich in bioactive phytochemical constituents valuable in treating various diseases, particularly those posing recent threats to public health, including cancer, multidrug-resistant bacterial infections, diabetes mellitus, and chronic inflammatory systemic diseases [2][3]. The higher incidence of cancer and mortality rates [4], the emergence of bacterial resistance along with the decline in antibacterial research at several pharmaceutical companies [5], the significant prevalence and complications associated with diabetes mellitus [6], as well as the long-term suffering linked with chronic inflammatory systemic diseases such as rheumatoid arthritis and multiple sclerosis [7], are driving forces that motivate us to engage in the fight against these potential threats. Numerous studies exploring the application of computational tools in discovering naturally-derived drugs have been conducted [8][9].

Natural products have played an important role in drug and therapeutic discovery. Phytochemicals are small molecules with diverse chemical profiles and are more "drug-like" than synthetic compounds; hence, they are considered good candidates for developing drug leads [10]. In recent decades, the Fabaceae and Malvaceae families have been extensively studied due to their diverse chemical compositions [11][12]. In a study, methanolic and aqueous extracts (at a concentration of 50 mg/ml) of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* were found to potentially inhibit the growth of three bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The extracted parts were leaves and seeds, and both extracts showed moderate inhibitory activity against the

growth of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Furthermore, various extracts of the Magnoliopsida class containing tannins, flavonoids, and polyphenols exhibited potential antibacterial activities against multidrug-resistant pathogenic bacteria, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli* [13][14][15].

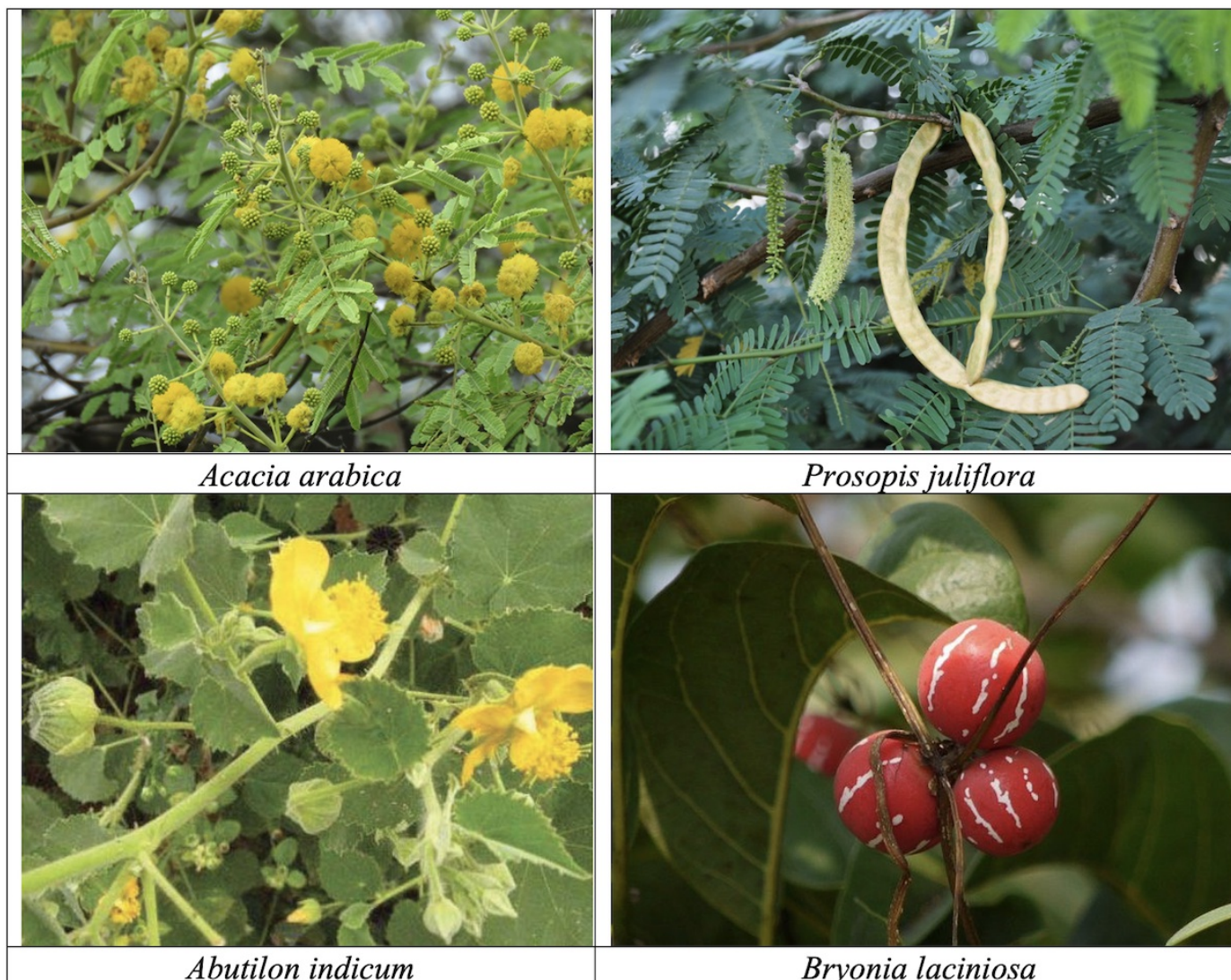
1.1. Vernacular Name

Languages	Plant Name			
	<i>Acacia arabica</i>	<i>Prosopis juliflora</i>	<i>Abutilum indicum</i>	<i>Bryonia laciniosa</i>
Hindi	Babul, Babur	Angaraji Babul, Kabuli Kikar, Vilayati Babul	Kanghi, Kakahi	Gargumaru, Ishwara lingi, shivalingi
English	Babul, Black Babul	Mesquite	Country mallow, Indian mallows	Indian bryony, Lollipop climber
Marathi	Babul	Vilayati Babul, Katkali	Mudra, Petari	Shivlingi, Vaduballi
Bengali	Babur	Baavlia	Petari	Shiva lingani
Malyalam	Karivelam	Mullan	Dabi, Uram	Neohmaka
Kannad	Karijali, Baunijali	Ballaari Jaali	Tutturubenda	Linga tondeballi, Lingatonde balli, Lingatonde, Shivalinga
Sanscrit	Barburah, Vavari	Gando baval	Atibala	Pastambhini, Bakapushpha, Shiva Mallika
Tamil	Karuvelei, Velampisin	Seemai Karuvel	Tutti, Paniara, Hutti	Iyaveli/Iyviralai

1.2. Taxonomic Classification

Taxonomic Rank	Taxon				Reference
	<i>Acacia arabica</i>	<i>Prosopis juliflora</i>	<i>Abutilum indicum</i>	<i>Bryonia laciniosa</i>	
Kingdom	Plantae	Plantae	Plantae	Plantae	[16]
Sub-kingdom	Tracheobionta	Tracheobionta	Tracheobionta	Tracheobionta	[16]
Superdivision	Spermatophyta	Spermatophyta	Spermatophyta	Spermatophyta	[16]
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida	Magnoliopsida	[16]
Subclass	Rosidae	Rosidae	Dilleniidae	Dilleniidae	[16]
Order	Fabales	Fabales	Malvales	Violales	[16]
Family	Fabaceae	Fabaceae	Malvaceae	Cucurbitaceae	[16]
Genus	Acacia	Prosopis	Abutilon	Bryonia L.	[16]
Species	arabica	juliflora	Abutilon Indicum	Bryonia laciniosa Linn.	[16]

1.3. Portrayal Representation of the Plants



1.4. Phytochemical Constitution

1.4.a. *Acacia arabica*

A. arabica is rich in many phytochemical constituents, including tannins, alkaloids, terpenoids, and flavonoids. Numerous studies have been conducted on it, resulting in evidence-based pharmacological data revealing the potential pharmacological activities of phytochemical compounds, including anticancer, antibacterial, antidiabetic, anti-inflammatory, and other activities. This makes the plant a promising source for the development of innovative, safe, and biodegradable drugs with significant potential [17].

1.4.b. *Prosopis juliflora*

The various chemical agents present in it exhibit medicinal value that can alter certain physiological actions in the human body [18]. Several biochemicals present in the plant are terpenes, alkaloids, flavonoids, and phenolic compounds [19]. It also possesses a high calorific value. It contains a high flavonoidal content of 16%, which demonstrates antioxidant and

anticancer properties. The tannin and phenol content are very low, i.e., 0.33% and 0.66% respectively [20][21].

1.4.c. *Abutilon indicum*

Chemical compounds such as Undecane, Benzeneethanmine, Astaxanthin, Diosgenin, Vildagliptin, Isospathulenol, Ertugliflozin, 9-HCycloisolongifolene, 8-oxo, 7 Hydroxycadalene, Hentriacontane, and Tetratetracontane, which are highly active phytochemicals, were found in previous studies [22]. Various parts like leaves, bark, stem, and seeds contain different types of phytochemicals that have been studied for their antimicrobial, antioxidant, anticancer, and antifungal activities [23].

1.4.d. *Bryonia laciniosa*

The main chemical constituent present in the plant is Bryonin. The seeds of the plant contain saponin molecules, flavonoids, phenolic acids, sugars, punicic acid, goniothalamine, and glucomannan [24]. Polysaccharides and fatty acids were isolated from the pulp part of the plant. These polysaccharides include d-glucose, d-mannose, and L-arabinose in the ratio of 5:3:4. However, detailed studies on the phytochemical screening of the plant have not been reported yet [25].

1.5. Antimicrobial Properties

1.5.a. *Acacia arabica*

The antibacterial activity of *A. arabica* was attributed to the prevention of fatty acids and peptidoglycan biosynthesis, as well as the prevention of bacterial resistance to beta-lactam antibiotics [25]. The inhibitory activity of fatty acid biosynthesis was observed against various types of bacteria, including *Mycobacterium*, *Pseudomonas aeruginosa*, and *Vibrio cholera*. The antiviral activity was attributed to the action on toll-like receptor 9. The anti-HIV activity is due to the inhibition of the HIV integrase enzyme, while the anti-coronavirus activity is due to the coronavirus replicase polyprotein 1 ab enzyme [26].

1.5.b. *Prosopis juliflora*

The several alkaloid constituents present in *Prosopis juliflora* were assessed for their antibacterial property using the disc diffusion method on several Gram-negative and Gram-positive bacterial strains like *E. coli* and *Staphylococcus aureus*. The maximum antibacterial effect is exhibited by parts of the plant such as leaf, pod, and flower extracts, with MIC (*Minimum Inhibitory Concentration*) values ranging between 25 µg/ml and 100 µg/ml. The leaf extract showed the highest activity among all the other parts [27]. The most susceptible bacteria were Klebsiella, while Acinetobacter and Alcaligen were the least susceptible. These bacteria were affected by the alkaloidal extract of the plant, while they were not affected by the use of antibiotics [28]. The aqueous extract of the leaves demonstrated fungicidal activity, either when used in combination or alone. The aqueous extract exhibited maximum fungicidal activity at 24% concentration. Methanol and ethanol extracts showed highly significant antifungal activity [29].

1.5.c. *Abutilon indicum*

The seed oil of *Abutilon indicum* and *Abutilon muticum* demonstrated broad-spectrum activity as they were active against both Gram-positive and Gram-negative bacteria. The findings reveal that seeds of *Abutilon* species indigenous to Pakistan could be potentially valuable for oil production, drug delivery, and cosmetic active ingredients [30]. The highest larval mortality was found in the petroleum ether extract of *A. indicum* [31].

1.5.d. *Bryonia laciniosa*

The ethanolic extract of the leaf, stem, seed, and fruit of *B. laciniosa* plant was examined for antimicrobial activity against various pathogenic microorganisms using the agar well diffusion method. The leaf and stem extracts of the plant exhibited antimicrobial activity against different gram-positive and gram-negative bacteria. A significant growth inhibitory effect of each extract was observed in *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus cereus*. The stem extract of the plant showed the minimum inhibitory effect against both gram-positive and gram-negative bacteria. This study concluded that *B. laciniosa* can be used as an antimicrobial agent [32]. The aqueous extract of the polysaccharide component isolated from the *B. laciniosa* leaf was examined for antibacterial activity against *Staphylococcus aureus*, *S. pyogenes*, *E. coli*, and *K. aerogenes* at dosages of 1.25 mg/ml, 3.12 mg/ml, 6.25 mg/ml, and 12.5 mg/ml. The extract showed antibacterial activity against *E. coli* at a minimum dosage of 6.25 mg/ml [33].

2. Material and Methods

2.1. Collection of Plant Samples

Healthy plant parts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* were collected from a grassy area near the Government Institute of Science, Aurangabad, Maharashtra, India (Latitude 19.912782°; Longitude 75.316327°).

2.2. Preparation of Plant Extracts

Collected leaves of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and seeds of *Acacia arabica*; *Bryonia laciniosa* were air-dried until completely dehydrated. They were then pulverized into a granular form by grinding in a mixer. Leaves of each species were pulverized into powder. Aqueous and ethanolic extracts were prepared separately for the tests. A weighed quantity of 50 mg/ml of each species was taken, and 50 g of powder of each species was treated with 1000 ml of 25% ethanol and water separately. Mixing and homogenization were performed by stirring and then placing the mixture into a boiling water bath for 15 minutes. After stirring, all extracts were filtered using Whatman filter paper no.1. The extracts were stored separately in screw-cap tubes and labeled clearly [34].



Figure 2.2. Labeled Screw-Cap Tubes of Extracts

2.3. Isolation and Identification of Bacterial Samples

Bacterial samples of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were obtained in pure culture form from the research laboratory of Government Institute of Science, Aurangabad. The selection of these microbial strains was based on two parameters: (i) ease of growth and availability, and (ii) current medical relevance. The cultures were then streaked onto Nutrient Agar plates and incubated at 37°C for 24 hours. Results were noted subsequently.

2.4. Antibioqram Pattern of Isolated *E. coli*, *P. aeruginosa*, and *S. aureus* to Plant Extracts

To test the antibiogram pattern of the selected bacterial species, instead of using the disk diffusion method, the antimicrobial activity of plant extracts was preliminarily screened using a plate diffusion assay. Vedpriya et al. [35] reported that the Kirby-Bauer agar diffusion method could be the best method for testing the antimicrobial effects of extracts, but the plate diffusion assay method is preferred. Nine Nutrient Agar (NA) plates were prepared. For each microbial species, three NA plates were differentiated as Aqueous, Ethanolic, and Control Plates. 100 µL of each bacterial strain was spread-plated as labeled. Wells of 6 mm diameter were formed in the agar plates. Three leaf extracts and two seed extracts were labeled as A, B, C, D, and E. On Ethanolic and Aqueous plates, selective extracts (400 µL) were pipetted into the wells, and on the control plate, 400 µL of ethanol and water were added for the control test. Further testing for antimicrobial activity was conducted. The plates were then incubated at 37°C for 24 hours. Subsequently, the plates were examined for the zone of inhibition (ZOI), and the diameter was measured in millimeters after subtracting the well diameter.

3. Results

3.1. Morphological Characteristics of Isolated Bacterial Samples

After 24 hours of incubation, distinct colony characteristics were observed and compared as mentioned in Table No. 2.3.

Colony Characters	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Appearance on NA	Off white or beige	Pearlescent	Faint Yellowish
Size of colony	Medium to large	Small	Small
Gram Nature	Gram-negative	Gram-negative	Gram-positive
Shape of colony	Circular	Circular	Circular
Cell shape	Rod	Rod	Round
Motility	Motile	Motile	Non-Motile
Margin	Entire	Entire	Entire and Rough
Texture	Shiny	Shiny	Shiny

3.2. Plate Diffusion Assay

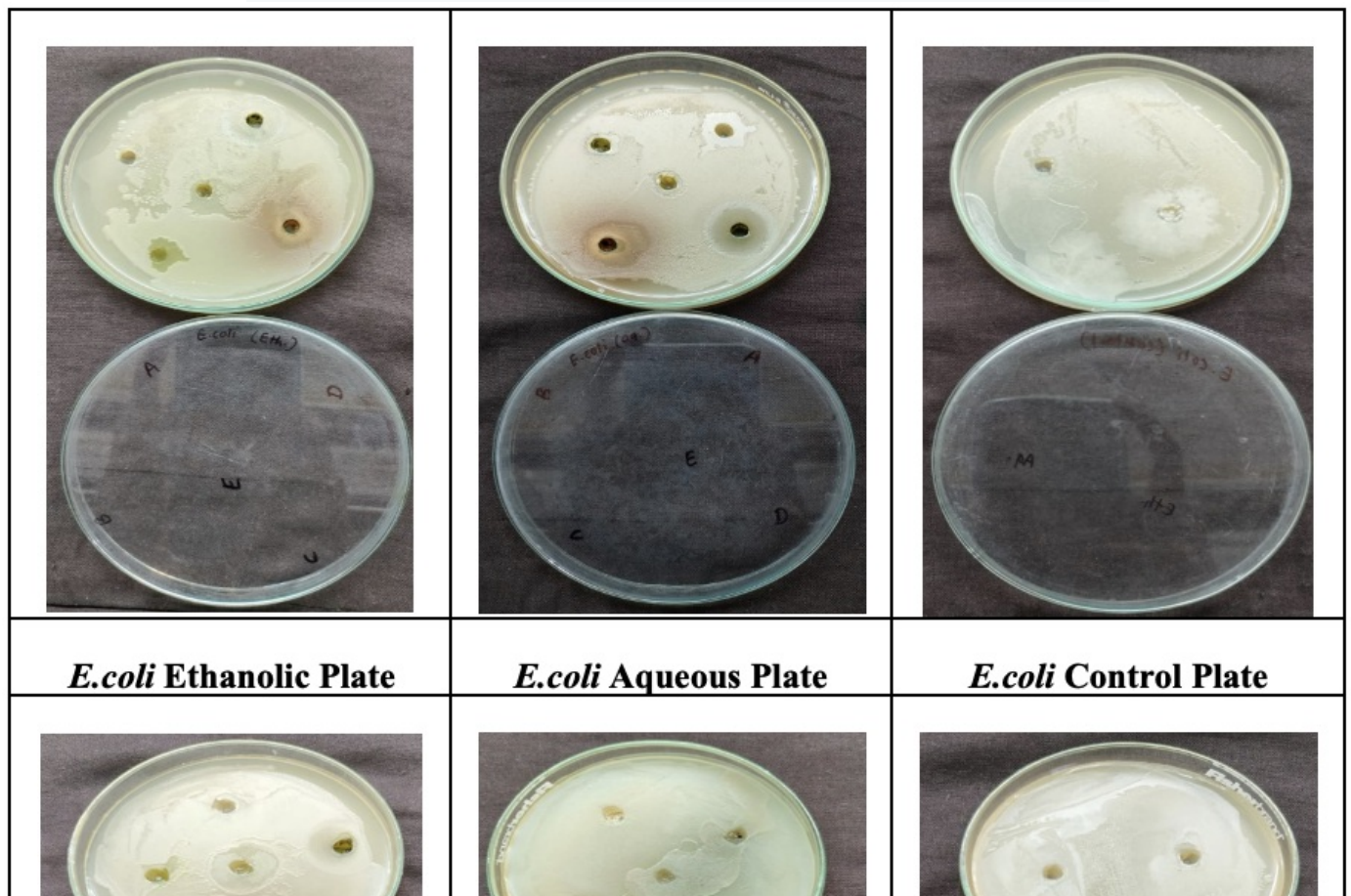
Preliminary screening of the selected four plant extracts against *E. coli*, *P. aeruginosa*, and *S. aureus* was performed using the plate diffusion method. A zone of inhibition greater than 4 mm in diameter was considered to have significant activity against a particular bacterium. Both the aqueous and ethanolic extracts showed significant antimicrobial activity against all three bacterial species. The leaf extract of *A. arabica* exhibited the highest ZOI against *E. coli*, i.e., 4 mm with aqueous and 5 mm with ethanolic extract. The lowest ZOI for *E. coli* was shown by the seed extract of *B. laciniosa*, i.e., 1.1 mm with aqueous and 3 mm with ethanolic extract. Similarly, for *P. aeruginosa*, the leaf extract of *A. arabica* showed the highest ZOI among the other extracts, i.e., 1.5 mm with aqueous and 2.2 mm with ethanolic extract. However, the seed extract of *A. arabica*, *B. laciniosa*, and the leaf extract of *A. indicum* did not show any ZOI against *P. aeruginosa*. For *S. aureus*, the ethanolic seed extract of *B. laciniosa* and the aqueous leaf extract of *P. juliflora* exhibited the highest ZOI, i.e., 5 mm and 2.5 mm, respectively. Comparative results with the control are mentioned in Table No. 3.4.

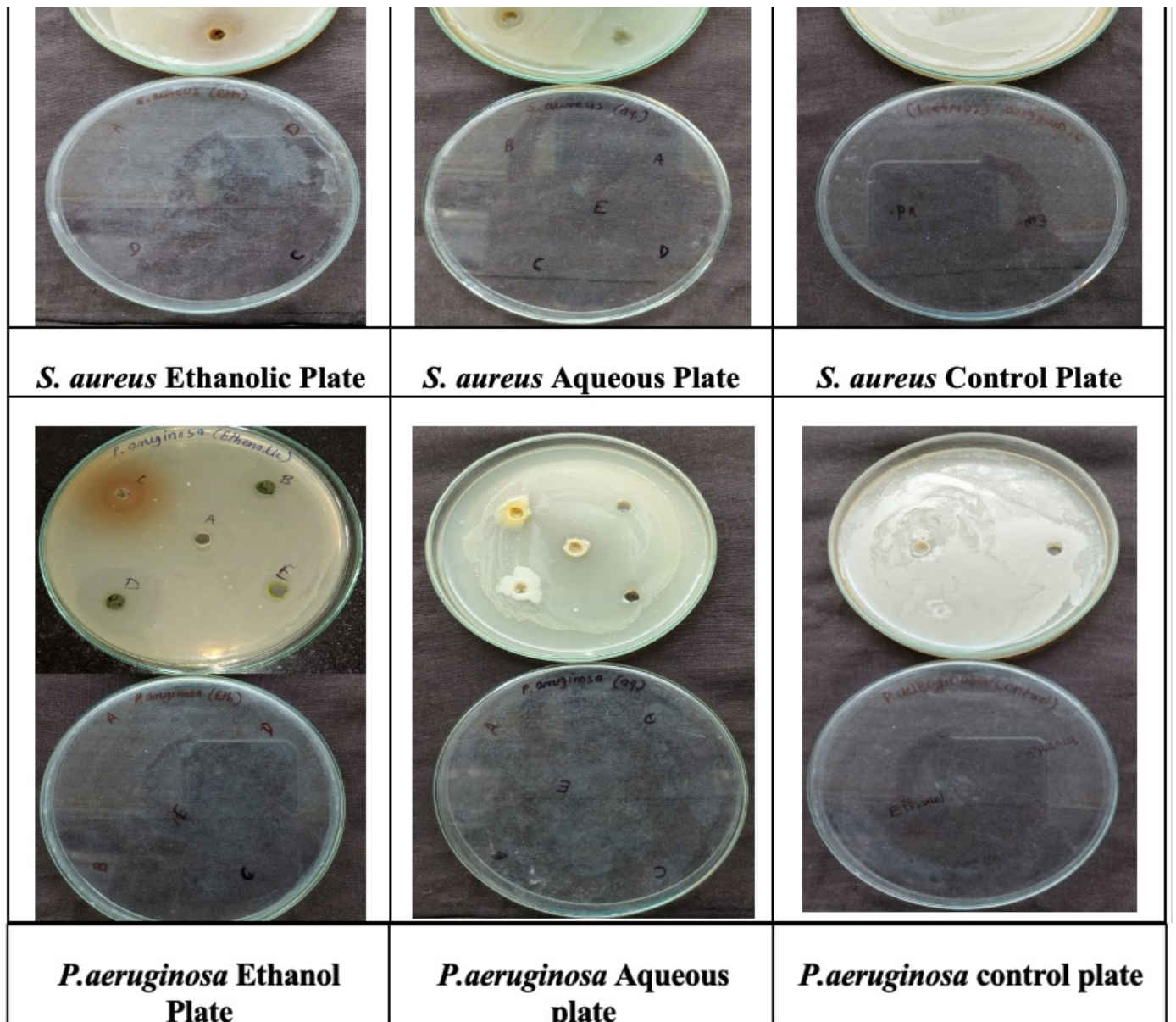
Table 3.4. Zone of Inhibition against Extracts

Plant Extract	Solvent	Bacterial Species (Zone of Inhibition in mm)		
		<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
AALE	Aqs	4	1.5	NZ
	Eth	5	2.2	1.5
AASE	Aqs	3	NZ	NZ
	Eth	1	NZ	1.1
PJLE	Aqs	3	NZ	2.5
	Eth	4.2	3.1	3
AILE	Aqs	2.1	NZ	NZ
	Eth	2.3	NZ	2.2
BLSE	Aqs	1.1	NZ	NZ
	Eth	3	NZ	5
Control	Aqs	NZ	NZ	NZ
	Eth	NZ	1.3	1.1

(AALE: *Acacia arabica* leaves extract; AASE: *Acacia arabica* seed extract; PJLE: *Prosopis juliflora* leaves extract; AILE: *Abutilon indicum* leaves extract; BLSE: *Bryonia lacinosa* seed extract; Aqs: aqueous; Eth: ethanolic; NZ: No Zone)

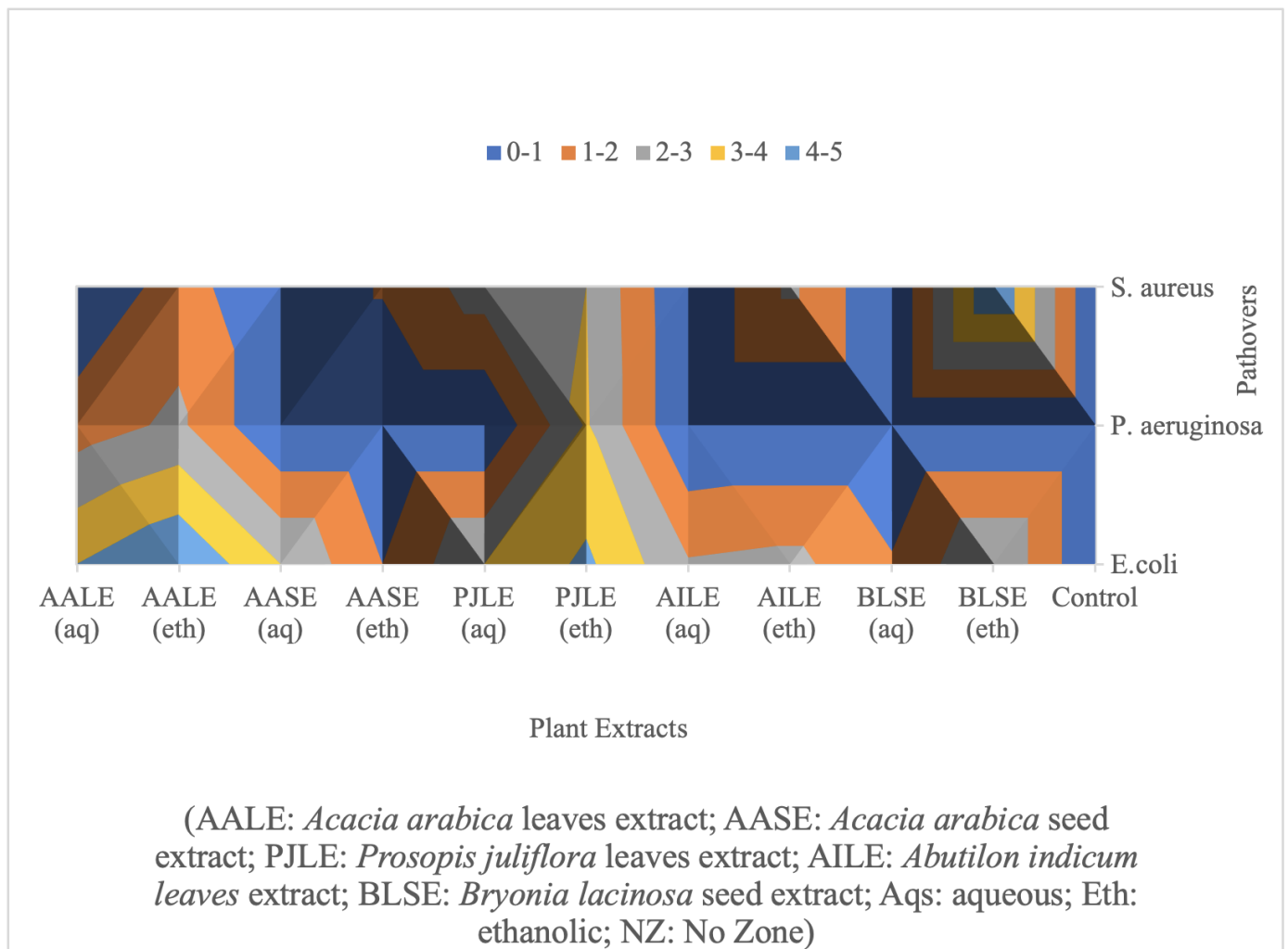
3.3 Portrayal Representation of ZOI on Nutrient Agar Plates





(A: *Acacia arabica* seed extract; B: *Abutilon indicum* leaves extract; C: *Prosopis juliflora* leaves extract; D: *Acacia arabica* leaves extract; E: *Bryonia lacinosa* seed extract)

3.4. Graphical Representation of ZOI on Nutrient Agar Plates



4. Discussion

In the panel of test organisms, a Gram-positive bacterium (*S. aureus*) and two Gram-negative bacteria (*P. aeruginosa*, *E. coli*) strains were used for antibacterial testing. Chandankar [36] prepared extracts from *Acacia arabica* leaves using different solvents, showing varying degrees of antimicrobial activity against the organisms selected for the study. Among the extracts prepared using different solvents, the methanol extract was found to be effective against all the organisms except *Escherichia coli*, while the acetone extract and fresh juice were found to be effective against all the selected organisms except *Pseudomonas aeruginosa* and *Escherichia coli*. The water extract and chloroform extract showed inhibition only against *Staphylococcus aureus* and *Bacillus subtilis*, respectively. According to the studies of Saeed Tajbakhsh et al. [37], in the antibacterial activity assay, a concentration of 2.5 mg/ml of *P. juliflora* extract exhibited activity against *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. They reported the Minimum Inhibitory Concentration (MIC) of the extract as 0.312 mg/ml and 0.078 mg/ml for *S. aureus* and *S. epidermidis*, respectively, while the MIC was 1.25 mg/ml for both *E. coli* and *P. aeruginosa*. In comparison to their results, based on the findings of our study, the Gram-positive organism (*S. aureus*) was less susceptible to the extract of *P. juliflora* than the Gram-negative organism (*E. coli*). The lower susceptibility of Gram-negative bacteria to antibacterial substances in such studies may be associated with their outer membrane and lipopolysaccharide molecules, which provide a barrier against the easy penetration of certain

antimicrobial molecules. Gram-positive bacteria do not possess this type of outer membrane and cell wall structure [38][39][40].

Poonkothai [41] reported the growth inhibition produced by the leaf extracts of *Abutilon indicum*, showing activities that can be categorized as either less, moderate, or highly active based on the zone of inhibition. The leaf extract of *Abutilon indicum* was found to be highly active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* (25, 25, 17, 18mm). According to the studies of Sujata et al. [42], the antimicrobial activity was performed using the Agar disc diffusion method at concentration levels of 2.5, 5.0, 7.0, 10µg/ml respectively. The *Abutilon indicum* leaf extract showed high activity against *Staphylococcus aureus* at a very low concentration (2.5µg/ml) compared to *E. coli*. Based on the Zone of Inhibition (ZOI), Bonyadi Rad et al. [43] found the effect of ethanol extract of different parts of *B. laciniosa* using the well diffusion method. There were positive responses of the organisms to the leaf and stem extracts compared with standard antibiotics, while the test organisms *S. aureus*, *Micrococcus luteus*, *Bacillus cereus*, *P. aeruginosa*, and *E. coli* did not show any susceptibilities to fruit and seed extracts. The present study showed the antibacterial effect of the seed extract of *B. laciniosa*. The ZOI was found to range from 1-5mm in diameter for *S. aureus* and *E. coli*, while there was no ZOI for *P. aeruginosa*.

The current research has revealed the greater potential of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* plants compared to previous experiments. In the present study, it has been observed that the extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* demonstrate impressive antimicrobial activity when compared with the ethanol and water controls. This investigation confirms that *Acacia arabica* leaves extract exhibits remarkable antimicrobial susceptibility towards all three bacterial strains, while relatively lower inhibitory activity was recorded by *Acacia arabica* seed extract. Based on the results, it is indicated that the extracts of *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* have the potential to be used in the management of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*-related diseases. Further phytochemical analysis is required to identify the active components of plant extracts displaying antimicrobial activity.

5. Conclusion

The present study concludes that *Acacia arabica*, *Prosopis juliflora*, *Abutilon indicum*, and *Bryonia laciniosa* exhibit potential antimicrobial activities among the investigated species. These findings offer ample opportunities for plant-based drug design due to their significant role in ethnomedicine, high effectiveness against various microbial pathogens, and their notable phytoconstituents.

Ethics Approval and Consent to Participate

Not applicable.

Human and Animal Rights

No animals/humans were used for studies that form the basis of this research.

Availability of Data and Materials

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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