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Phytochemical screening and antimicrobial activity of leaf extracts from *Pimenta dioica*

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ABSTRACT

Medicinal and aromatic plants have become a part of complementary medicine worldwide because of their potential health benefits, as raw materials for traditional as well as modern medicine. This study aimed to assess the effectiveness of crude solvent extracts, specifically ethyl acetate and chloroform extracts, as well as phytochemical extracts from the leaves of *Pimenta dioica*, a medicinal aromatic plant from South India, against pathogenic bacteria. The evaluation will be performed via a disc diffusion assay, and phytochemical analysis of the leaves of *P. dioica* has shown the presence of saponins, alkaloids, tannins, flavonoids, glycosides, terpenoids, polyphenols, and anthocyanins. The results indicated that chloroform extract has shown maximum inhibitory activity compared with ethyl acetate extract and that terpenoid extract has maximum inhibitory activity compared with the other phytochemical extracts.

Keywords: *Pimenta dioica*, Phytochemical analysis, South Indian Aromatic Medicinal plant, Secondary metabolites, antibacterial activity.

1. INTRODUCTION

Because of their potential health, medicinal and aromatic plants serve as a source of raw materials for traditional and modern medicine. These raw materials are secondary metabolites, considered valuable sources of novel compounds, and natural treasures possessing the potential to develop new pharmaceuticals. Traditional healers have led to the discovery of new antibiotics [1]. Approximately 80% of the global population depends on traditional medicines as their main source of primary healthcare [2]. The potential of lesser-known and under-explored medicinal and aromatic plants that can make major contributions to this need further research.

In India, roughly 3000 medicinal plants of both introduced and indigenous varieties are widely distributed based on reports of glossaries of Indian medicinal plants, out of which 2000 plants were registered in ethnomedicine [3]. The present study focuses on assessing the antibacterial activity of the crude solvent extracts, particularly the chloroform and ethyl acetate extracts, and also the phytochemical extracts of the *Pimenta dioica (P. dioica)* leaves because the review of the literature lacks studies concerning these extracts used in this study.

A review of literature unravels the multipurpose (traditional, domestic, industrial, and medicinal) uses of this medicinal aromatic plant in South Indian. *P. dioica (Linn.),* the Merill tree, belonging to the Myrtaceae family, is indigenous to the Caribbean islands and Central America. In the Indian subcontinent, this medicinal plant is widely grown in gardens in states like Orissa, Bihar, and Bengal. In Bangalore, it is reported to have a higher growth rate with more fruits. It was found that it can grow richly in the river valleys of Mysore (Karnataka, India) and also grows well in well-drained soil at a high plain altitude range of 1065 meters above sea level. The fact that it is used in folk medicine is justified by various traditional uses listed in Table 1. Allspice is aptly named due to its inherent fragrance derived from a blend of aromatic compounds found in spices like cinnamon, nutmeg, and cloves. Previous investigations have demonstrated that essential oils, crude preparations/extracts, and purified fractions derived from the plant P. dioica exhibit diverse biological activities.

The family is classified into two subfamilies, Myrtideae and Leptospermoideae. The genera Myrtoideae include *Myrtus* (100sp) *Psidium* (140sp), *Pimenta* (18 sp), *Eugenia* (1000 sp), *pseudo caryophyllus,* and *Syzigium* (Jambosa). It was observed that volatile oils and spices belong to this genus, e.g., pimento, eucalyptus oil, cajuput oil, and cloves. A study in Africa

emerged with a new concept of challenging the global problem of antibiotic resistance in pathogenic bacteria, which demands alternative strategies to combat bacterial infections using new antimicrobial compounds from a variety of sources, including medicinal plant extracts that can be used along with antibiotics as resistance-modifying agents. The concentration of eugenol was found to be 98.5 % in the leaf extract of *P. dioica* of Jamaican origin than those of Cuban leaf oil (54.26%), which is known to contain car-Caribbean leaf oils along with compounds like 1,8 -cineole, alpha-pinene, and caryophyllene. Leaf oil has a higher level of eugenol than the berry oil [4-7]. The methyl eugenol fraction in the oil extracted from berries was higher than the concentration of eugenol [8-9].

The studies assessing of the antibacterial activity of the different extracts from the different parts of *P. dioica* were performed using crude extracts [9-12]. 17 compounds were isolated from the essential oil extracted from berries, of which Eugenol (77%) was the major contributor [13]. The bioactive compounds extracted from different plant parts in the included studies are summarized in Table 1. The essential oil of *P. racemosa,* which is the other member of the same family is a rich source of chavicol and β -myrcene while the essential oil of *P. dioica* is rich in eugenol and methyl eugenol [14]· A recent study highlighted the potential benefit of Allspice essential oil as a safe and environmentally friendly biopesticide and larvicide [15].

Figure 1. Leaves of *P. dioica*

ACTIVITY	REF
Larvicidal	15,16
Biopesticide	15
Antiseptics	17
Slightly anesthetic	17
Anti-inflammatory	17,19
Antibacterial	16-24
Antifungal	18 -20
Anticandidal	20
Anti-quorum sensing	20
Antioxidant activity	21
Anticancer	21

Table 1. List of bioactive and medicinal activities in *P. dioica* extracts

2. MATERIALS AND METHODS

2.1. Plant material collection and authentication

Plant was obtained from Bethel Garden, Pazhani, Dindugal district, Tamil Nadu, India. This study was authenticated by medicinal plant expert from Siddha Ayurveda college, Arumbakkam, Chennai, India (Figure 1)

Kingdom	:	Plantae-Plants
Subkingdom	:	Tracheobionta-vascular plants
Superdivision	:	Spermatophyta-seed plants
Division	:	Magnoliophyta-Flowering plants
Class	:	Magnoliopsida-Dicotyledons
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae-MyrtleFamily
Genus	:	Pimenta

Species	:	dioica

Table 2. Botanical classification of *P. dioica*

2.2 Preparation of the Methanolic Extract

The fresh leaves of *P. dioica* were dried in a shaded area at room temperature for 5 days. Subsequently, the dried leaves were crushed using a mortar and pestle. Powdered leaves (10 g) were extracted using a solution of 80% methanol (known as methanolic extract) and 100 ml of water in a 100 ml conical flask. The conical flasks were sealed with a rubber cork and agitated at 120 rpm for 30 mins in a shaker incubator. Subsequently, they were left undisturbed at room temperature for 24 h. The extracts were filtered aseptically using Whatman no.1 filter paper. The samples were then centrifuged for 5 mins. The liquid portion, known as the supernatant, was transferred into a separate flask and utilized for the purpose of carrying out phytochemical assessment [22-23].

2.3. Phytochemical Analysis

A fresh preparation of the methanol extract of *P. dioica* was prepared and analyzed for its numerous chemical components [22-23]. In the assay method for phytochemical analysis, we tested the presence or absence of constituents like terpenoids, plant alkaloids, glycosides, anthocyanins, polyphenols, and tannins (Figure 2).

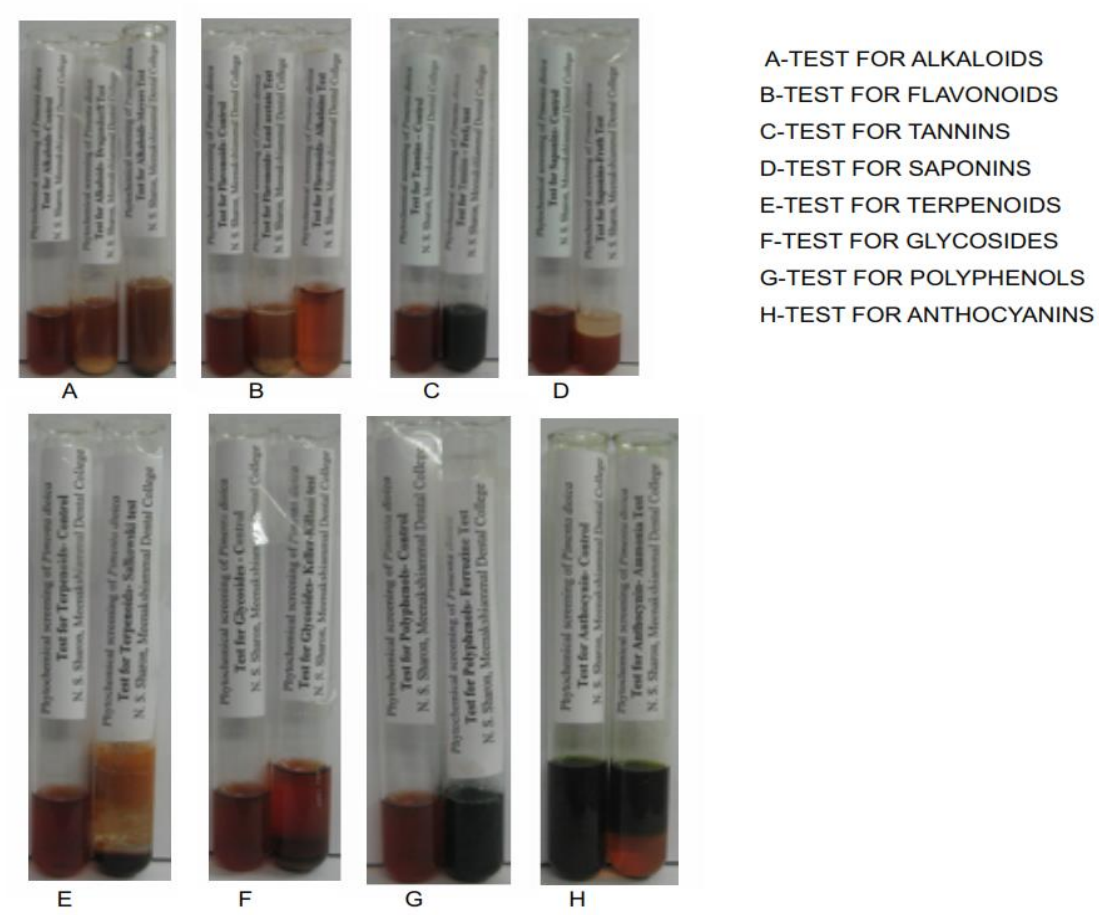


Figure 2. Phytochemical screening of the leaf extract of *P. dioica*

2.4. Bacterial Strains

The test organisms chosen for the study were three gram-negative bacterial strains viz., *Escherichia coli, Klebsiella species,* and *Pseudomonas aeruginosa,* and the one-gram positive strain *Staphylococcus aureus (*Figure 3)

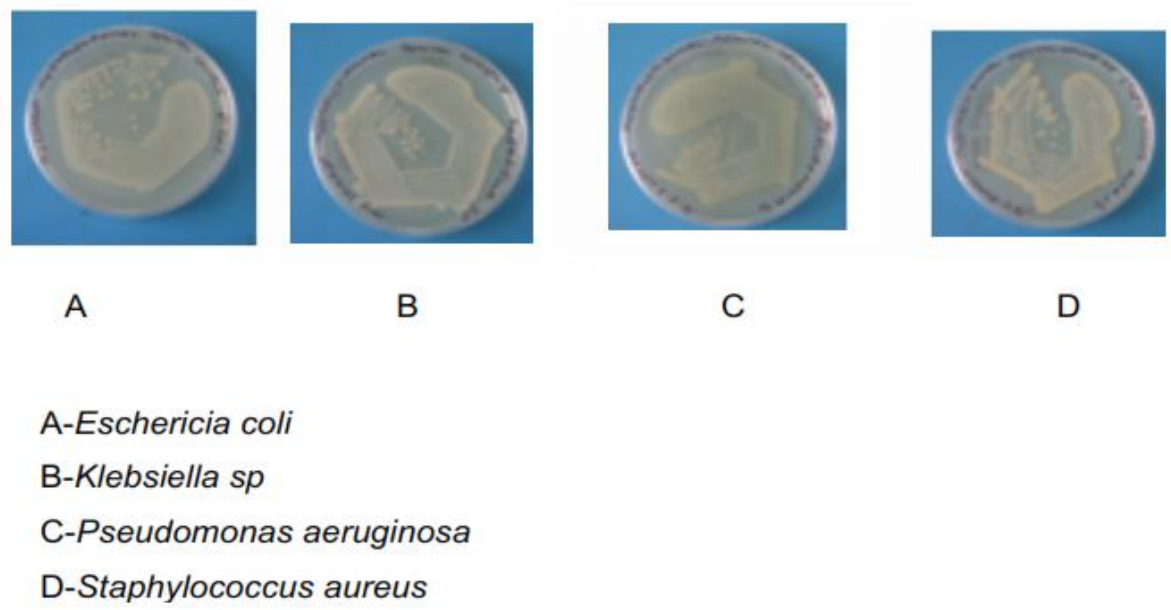


Figure 3. Organisms used in the study

2.5. Antibacterial activity of the crude extracts and phytochemicals of *P. dioica* was determined using the disc diffusion method

We conducted a study to assess the antibacterial properties of a plant extract. The disc diffusion method [24-25] was used, and the bacteria used were *Staphylococcus aureus*, *Klebsiella species*, *Escherichia coli,* and *Pseudomonas aeruginosa*. These bacteria were cultured overnight on Mueller Hinton (MH) agar plates. Five initial colonies of each organism were combined in 5ml of sterilized saline solution, and the concentration was adjusted to approximately 3×10^{8} colony forming units (CFU). To remove the excess fluid, we used a sterile cotton swab dropped into the inoculum suspension which was repeatedly moved by applying pressure inside the tube walls. The MH agar plates were dried on their surface by swab test over the surface of the agar and maintaining constant rotation to the plates at approximately 90°C to ensure that inoculum was evenly distributed.

The different solvent extracts of the leaves of the plant (crude extract with concentration of 1mg in 1 ml of 7% methanol) were mixed in various concentrations (5, 10, 15, and 20 µg/ml of the methanol) to separate a sterile paper disc of 5mm diameter for the four plates and allowed to evaporate water into dryness. The disks were gently tapped down in the agar to ensure uniform contact continuity. The plates were incubated at 37 °C for 24 h; After the incubation, clear zones of inhibition were measured (in diameter), expressed in mm of the

zone with no bacterial growth around the filter paper disc impregnated with the extracts. The assay was performed in triplicates with samples. For all the data, the average of the three determinations was calculated and reported as mean SD ± (n=3).

2.6 Extraction of phytochemicals from *P. dioica* leaves

2.6.1 Extraction of alkaloids

We used chilled distilled methanol and swirled it to extract alkaloids from the powdered leaves. The sample was then filtered and solvent removed using reducing pressure at 40 ^{O}C, to minimize head-induced damage to the extracts. The neutral and acidic materials were removed from the crude alkaloid mixture by extraction with aqueous acetic acid (CH_3COOH) followed by treatment with dichloromethane. Finally, a crude alkaloid extract was obtained from the layer of dichloromethane by basification of the aqueous solution [26].

2.6.2 Extraction of terpenoids

The terpenoids were extracted from ground leaves by treatment with 95% ethanol at 60 ºC. Following by filtration of the extract, the green solvent was dried by evaporation at low pressure in incubator temperature at 40ºC. Further, the residue was separated between CHCL and H_2O. The dark green syrup was obtained by separating of the organic layer. The extract was separated between hexane and 10% aqueous methanol and the CHCL. Finally, the terpenoid extract from the aqueous methanol step was used for antibacterial activity studies [27].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Phytochemical analysis of an aqueous extract of the leaves of *P. dioica* revealed the presence of terpenoids, flavonoids, saponins, alkaloids, polyphenols, glycosides, anthocyanins, and the tannins, the details of the test and the results tabulated (Table.3)

PHYTOCHEMICAL	**TEST**	**OBSERVATION**	**Leaf extract of *P. dioica***
Alkaloids	Dragendorff's test	Orange / red precipitate	Present
	Mayer's test	Yellowish precipitation	
Flavonoids	Alkali reagent tests	Intense yellow	Present
Tannins	FeCl3	Brownish-green coloration	Present
Saponins	Frothing test	Foam	Present
Terpenoids	Salkowski test	Reddish-brown colouration of the interface	Present
Glycosides	Keller-killani test	Formation of the brown-ring interface	Present
Polyphenols	PotassiumFerricyanide test	Blue	Present
Anthocyanins	Ethyl acetateextract-ammonia test	Yellow	Present

Table 3. Phytochemical analysis of *P. dioica*

3.2. Antibacterial activity of different solvent and phytochemical extracts by disc diffusion method

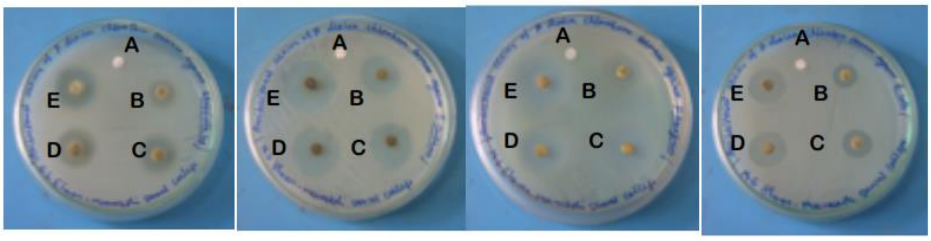
The mean values for the zones of inhibition were tabulated for the antibacterial activity of the different solvent extracts and phytochemical extracts, and histogram charts were plotted with these values. The chloroform and ethyl acetate and extracts of leaves from *P. dioica* were screened for anti-bacterial activity, and the inhibition zones were tabulated (Table 3, Figure 4, Figure 8, Figure 9 A and B).

All four tested bacteria were found to be sensitive to the extracts of chloroform and ethyl acetate extracts. Both ethyl acetate and chloroform extracts of *P. dioica* leaves exhibited

good antibacterial activity against the tested organisms. Compared with the two extracts chloroform extract had higher antibacterial activity than the ethyl acetate extract.

All the phytochemical extracts exhibited good antibacterial activity (Table 4, Figure 5, Figure 6, Figure 7, Figure 8, and Figure 9 C, D, E. The phytochemical extracts evaluated for antimicrobial activity in this study include alkaloid extract (Table 4, Figure 6, Figure 9 C), Terpenoid extract (Table.4 Figure 7, Figure 9 D), and phenolic extracts (Table 4 Figure 8, Figure 9 E). Terpenoid extract showed comparatively higher antibacterial activity. The efficacy of the Ethyl Acetate, chloroform, alkaloid, terpenoid, and phenolic extracts against *Escherichia coli* (Figure 10 A), *Klebsiella sp*. (Figure 10B), *Pseudomonas aeruginosa* (Figure 10 C) and *Staphylococcus aureus* (Figure 10 D) was compared. The antibacterial potential of the extracts against individual organisms reflected the sensitivity of the individual organisms to the different extracts.

Based on the literature collected, leaf oil possessing the richest concentration of eugenol ultimately indicates our choice of the source to be the leaves, which certainly a better source of choice, holding good medicinal value. This study is the first to evaluate the antibacterial potential of phytochemical leaf extracts.



Eschericia coli *Klebsiella sp* *Pseudomonas aeruginosa* *Staphylococcus aureus*

A-0 µl CONTROL(NEGATIVE CONTROL)

B-5 µl - 5 µg/ml OF CHLOROFORM EXTRACT

C- 10 µl-10µg/ml OF CHLOROFORM EXTRACT

D- 15 µl -15µg/ml OF CHLOROFORM EXTRACT

E-20ul -20ug/ml OF CHLOROFORM EXTRACT

Figure 4 Antibacterial activity of the chloroform leaf extract of *P. dioica* against pathogens using the disc diffusion method

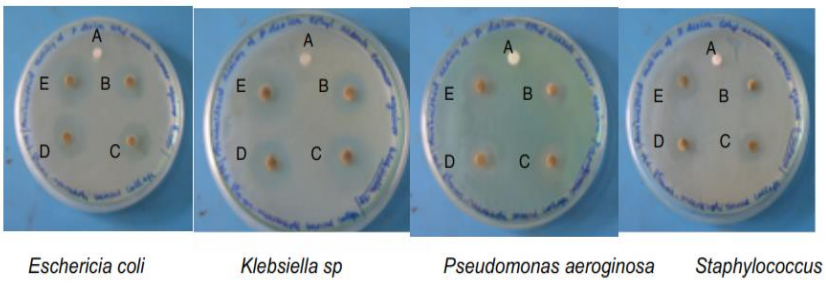


Figure 5. Antibacterial activity of the ethyl acetate leaf extract of *P. dioica* against pathogens using the disc diffusion method

Different Solvent extract	*Escherichia coli*				*Klebsiella*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Chloroform extract	8.3±0.55	10.5±0.81	14.3±0.50	17.5±0.73	7.5±0.85	9.4±0.81	12.6±0.90	14.2±0.75
Ethyl acetate	8.0± 0.74	10.2±0.85	13.1± 0.80	16.1±0.90	7.6±0.61	9.4±0.77	12.3±0.65	13.3±0.65
	Pseudomonas aeruginosa				*Staphylococcus aureus*			
Chloroform extract	7.3±0.70	11.5±0. 83	14.5±0.6	17.3±0.61	9.8±0.55	11.4±0.65	14.4±0.83	17.6±0.62
Ethyl acetate	7.6±0.65	10.5±0.85	12.4±0.65	15.5±0.65	7.4±0.65	10.5±0.76	13.5±0.73	15.4±0.55

Table 3. Antibacterial activity of chloroform and ethyl acetate extracts from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method

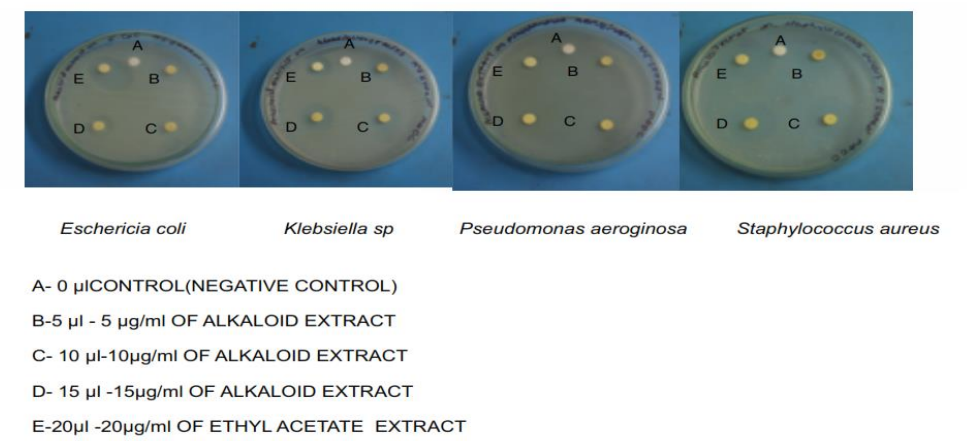


Figure 6. Antibacterial activity of alkaloid extract from *P. dioica* leaves against pathogenic bacteria using the disc diffusion method

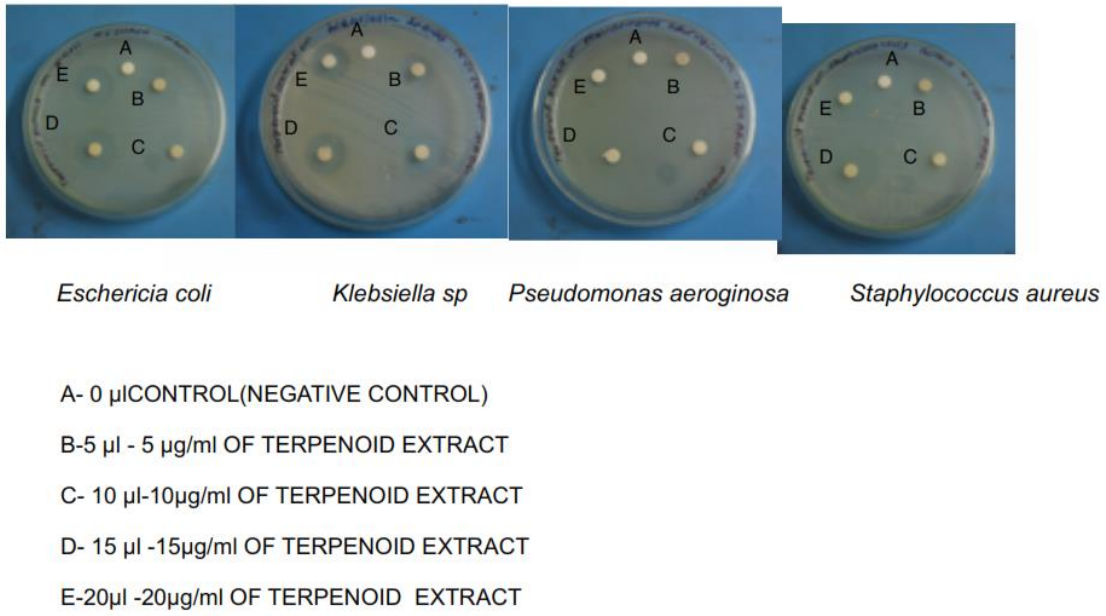
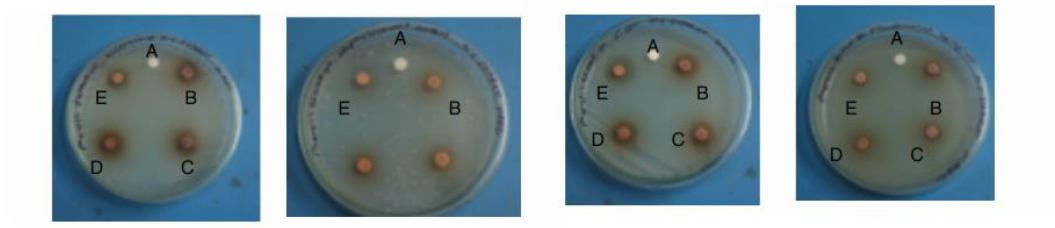


Figure 7. Antibacterial activity of the terpenoid extract from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method



Eschericia coli *Klebsiella sp* *Pseudomonas aeroginosa* *Staphylococcus aureus*

A- 0 µlCONTROL(NEGATIVE CONTROL)

B-5 µl - 5 µg/ml OF PHENOLIC EXTRACT

C- 10 µl-10µg/ml OF PHENOLIC EXTRACT

D- 15 µl -15µg/ml OF PHENOLIC EXTRACT

E-20µl -20µg/ml OF PHENOLIC EXTRACT

Figure 8. Antibacterial activity of the phenolic extract from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method

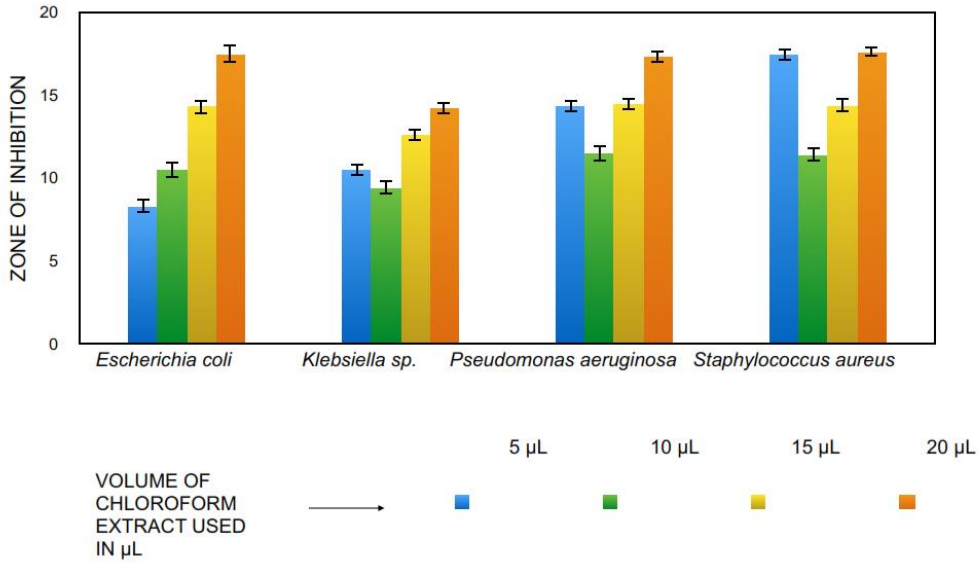
Different Solvent extract	*Escherichia coli*				Klebsiella			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Alkaloid extract	8.7±0.42	11.6±0.57	13.6±0.40	16.7±0.50	8.9 ±0.907	10.8 ± 0.458	14 ± 0.5	17.5 ±0.816
Terpenoid extract	9.8±0.50	12.8±0.6	15.8±0.32	18.8±0.60	9.6±0.61	11.7±0.5	15.4±0.45	18.8±0.6
Phenolic extract	8.7±0.503	11.5±0.4	10.3 ±0.47	16.6±0.404	8.9±0.6	10.8±0.458	13.4±0.450	16.4±0.458
	Pseudomonas aeroginosa				*Staphylococcus aureus*			
Alkaloid extract	9.6±0.62	11.8±0.50	14.6±0.40	17.4±0.35	9.7±0.45	12.6±0.45	15.6±0.3	18.5±0.50
Terpennid extract	10.6±0.60	13.8±0.55	16.6±0.50	18.8±0.50	10.8±0.72	14.6±0.46	17.7±0.36	20.6±0.66
Phenolic extract	7.4±0.451	10.8±0.551	13.8±0.503	15.6±0.450	8.5±0.503	11.5±0.4	14.7±0.321	16.6±0.404

Table 4. Different phytochemical-extracted samples from the *P. dioica* leaves were tested for antibacterial activity against pathogenic bacteria using the disc diffusion method

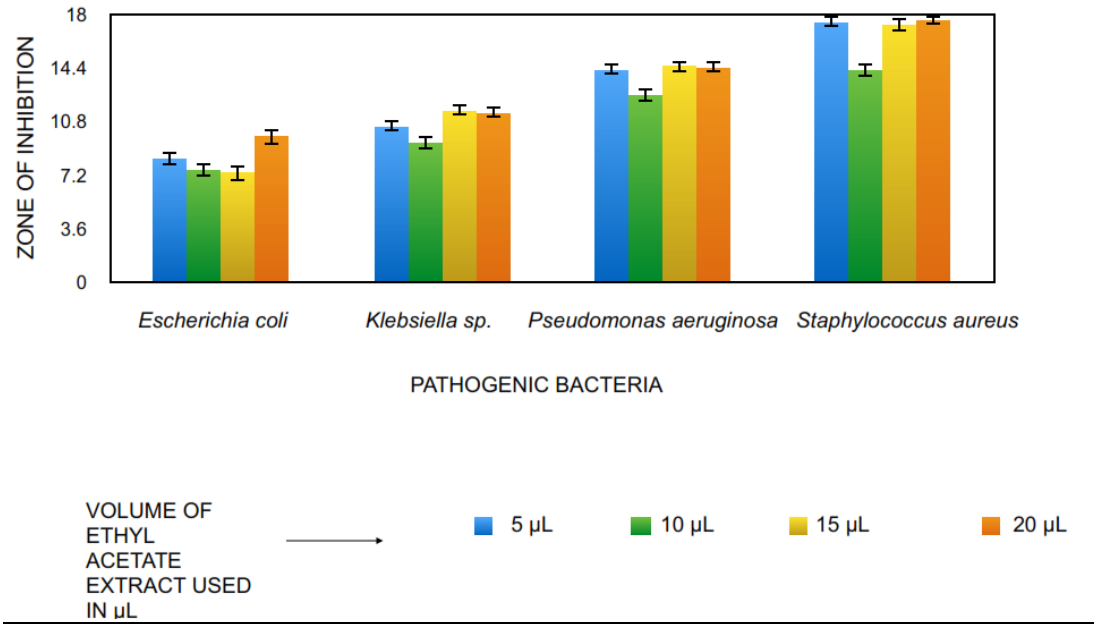
Figure 9: Antibacterial activity of different extracts from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method

A. Chloroform extract
B. Ethyl acetate extract
C. Alkaloid extract
D. Terpenoid extract
E. Phenolic extract

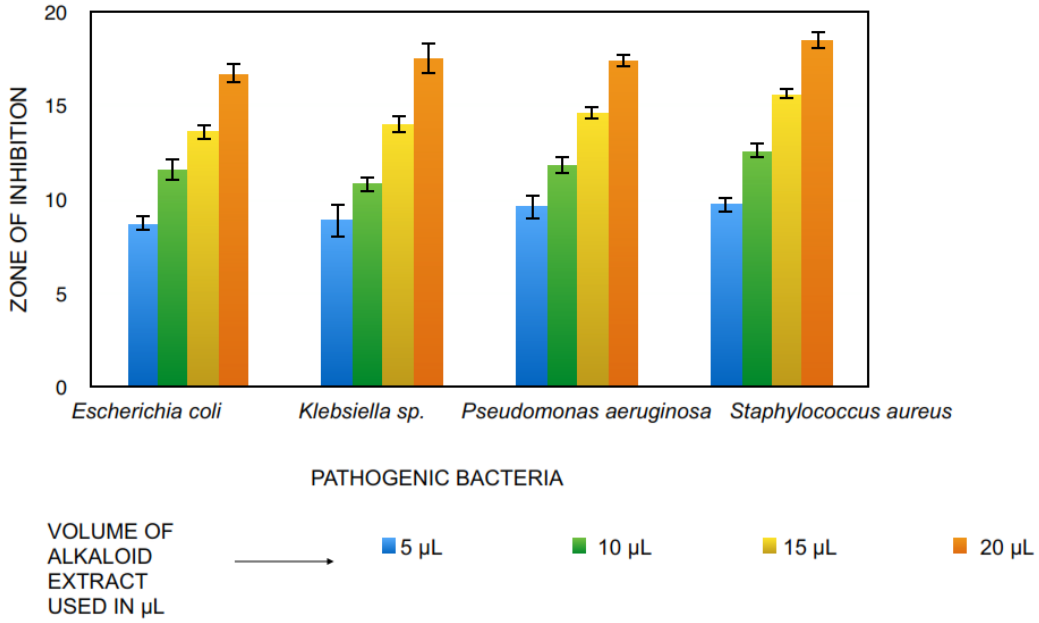
A. Antibacterial activity of chloroform extract from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method.



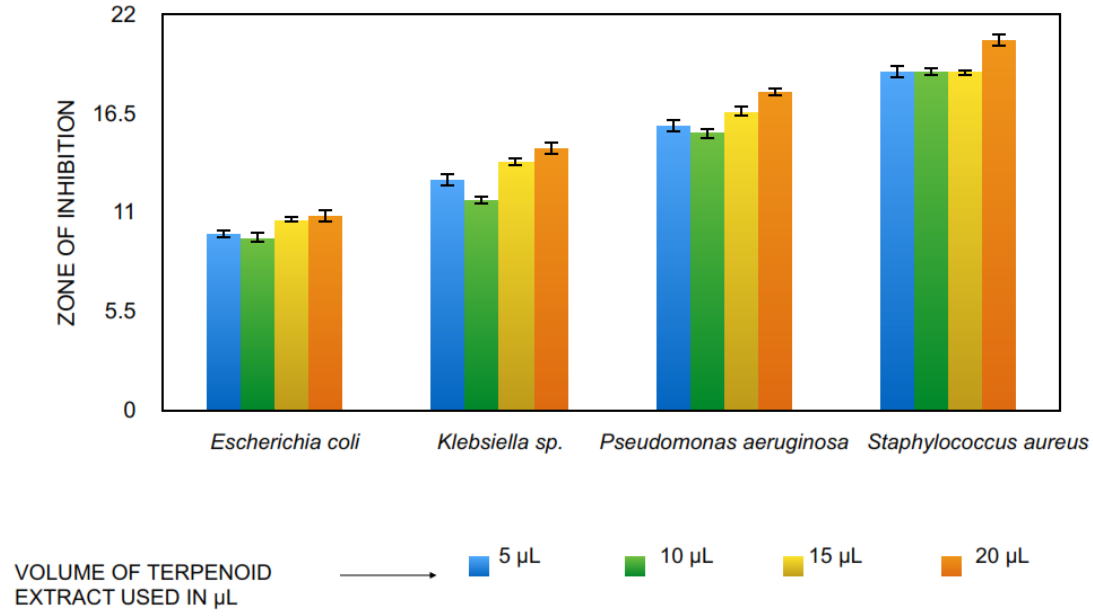
B. Antibacterial activity of ethyl acetate extract from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method.



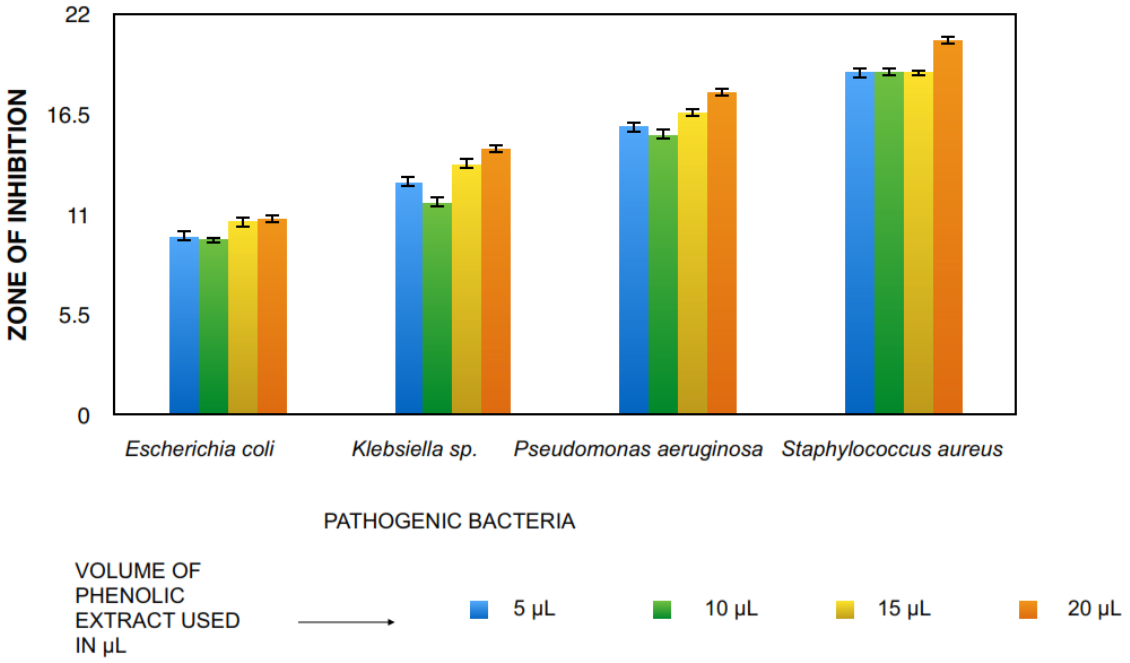
C. Antibacterial Activity of alkaloid extracts from *P. dioica* leaves against pathogenic bacteria using the disc diffusion method.



D. Antibacterial Activity of terpenoid extracts from *P. dioica* leaves against pathogenic bacteria using the disc diffusion method.



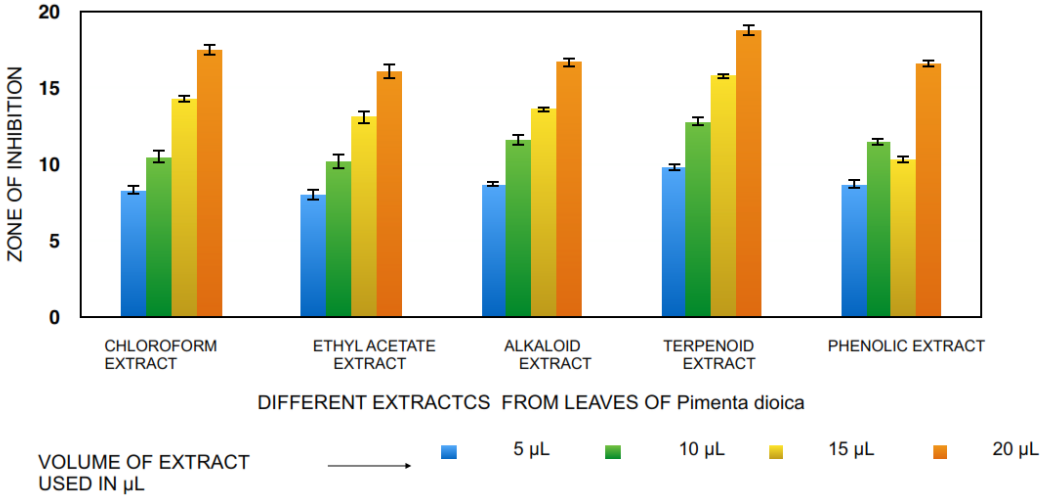
E. Antibacterial activity of the phenolic extract from the leaves of *P. dioica* against pathogenic bacteria using the disc diffusion method.



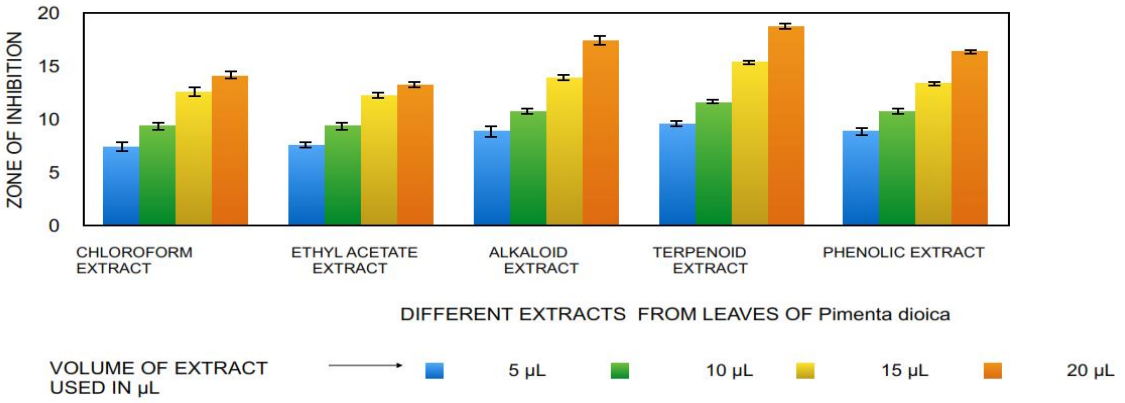
A. Figure 10. Graphs comparing the antibacterial activity of different extracts from *P. dioica* leaves against pathogenic bacteria using the disc diffusion method.

A. Escherichia coli
B. Klebsiella sp.
C. Pseudomonas aeruginosa
D. Staphylococcus aureus

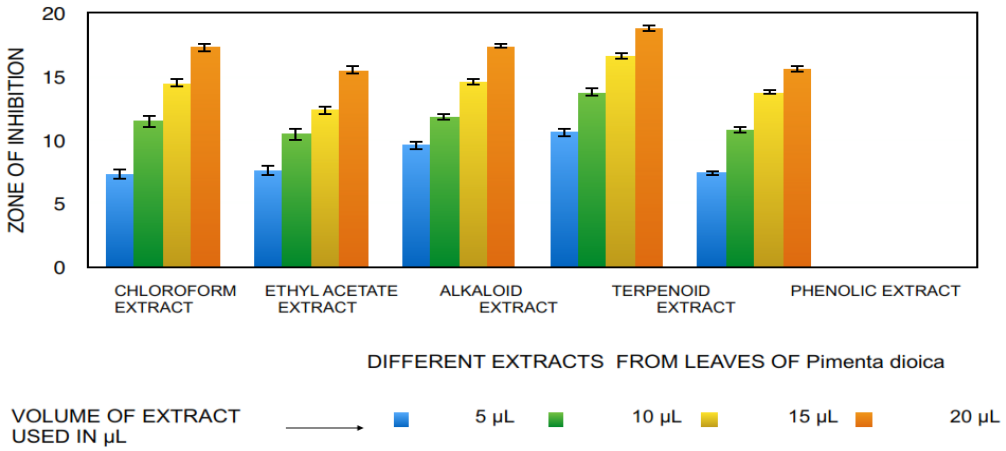
A. Antibacterial efficacy of different extracts of *P. dioica* leaves against *Escherichia coli*



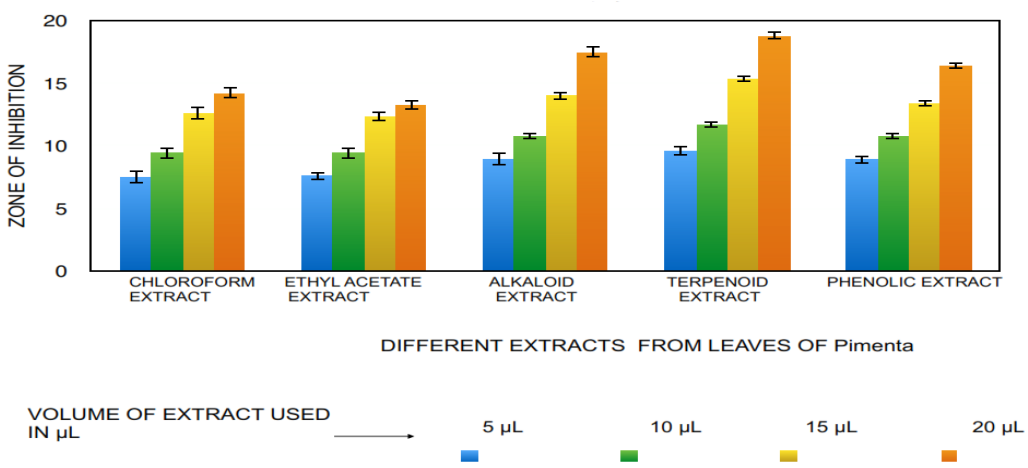
B. Antibacterial efficacy of different extracts of *P. dioica* leaves against *Klebsiella sp.*



C. Antibacterial efficacy of different extracts of *P. dioica* leaves against *Pseudomonas aeruginosa*



D. Antibacterial efficacy of different extracts of P. dioica leaves against *Staphylococcus aureus*



4. CONCLUSION

The result of this study demonstrated that the leaf extract of *P. dioica* effectively inhibits all four pathogenic bacteria. The observed antibacterial activity may be attributed to the presence of bioactive secondary metabolites. The traditional use of plants reveals the therapeutic properties of water-based and alcohol-based extracts.

Previous studies on crude extracts with the solvents like methanol, water, and ethanol proved that extracts from bark and leaves have good antibacterial activity. According to a recent study, the essential oil derived from Allspice can be to be used as a non-harmful substance for kill

larvae and pests. The current study demonstrated the antibacterial efficacy of ethyl acetate and chloroform extracts, as well as phytochemical extracts (alkaloid, terpenoid, and phenolic) from *P. dioica* leaves. The terpenoid extract was found to have good antibacterial activity. Hence, further extensive studies exploring the potency of bioactive compound will reveal whether plants have the potential to be a suitable candidate for the production of bioactive substances that may be utilized as a treatment for diseases caused by pathogenic bacteria.

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