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Performance Evaluation of Antimicrobial Activity of *Desmanthus* sp. Extracts against Selected Pathogenic Strains

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Abstract

This study evaluates and compares the antibacterial properties of water and ethanol crude extracts from Desmanthus sp. with commercial antibiotics. Plant samples were collected, processed, and pulverized into powder form. Using a G power of 80% and a 95% confidence level, the study was conducted with two groups and five samples. The first extraction utilized a cold maceration technique with water as the solvent, producing a novel crude extract. The extracts underwent comprehensive characterization, including Gas chromatography- Mass Spectrometry (GC-MS) analysis, to identify active biomolecules and assess the potential of antimicrobial activity. The ethanol extract was statistically analyzed using a two-tailed t-test, revealing a p-value of 0.035, demonstrating significant antimicrobial efficacy. The results highlighted the physiological significance of polyphenols, particularly flavonoids, for their antioxidant and anti-inflammatory properties. For the first time, unique crude extracts of *Desmanthus virgatus* were obtained through ethanolic solvent extraction using a cold maceration technique, yielding promising antimicrobial activity. GC-MS analysis and antimicrobial assays further validated the potential of these extracts as effective alternatives to commercial antibiotics.

Keywords: *Desmanthus virgatus*, GC-MS, crude extract, ethanol extract, Antimicrobial Activity.

Introduction

Desmanthus virgatus, commonly known as "Wild Tamarind" or "Virgin Mimosa," was first identified in coastal North Queensland. This fascinating plant species, native to various regions across North and South America, belongs to the Fabaceae family. Known by several local names, such as Brusca prieta, frijolillo, ground tamarind, langalet, and desmanto, *Desmanthus virgatus* is celebrated for its unique morphological characteristics, including pinnate leaves, delicate fern-like foliage, and slender stems, which contribute to its distinct and attractive appearance (1, 2).

Beyond its aesthetic appeal, *Desmanthus virgatus* has garnered attention for its diverse applications in environmental management, animal forage, and traditional medicine. Its most notable attribute is its antimicrobial activity, which has shown promise in combating various pathogens. Research indicates that extracts prepared from whole plant parts, *viz*. leaves, stems, and roots, are rich in active biomolecules, including alkaloids, saponins, tannins, and flavonoids. These phytochemicals exhibit various biological activities, particularly antimicrobial attributes, disrupting/lysing the cell membranes of microbes, and inhibiting disease-causing microorganisms growth (2, 3).

The traditional knowledge of indigenous communities has long recognized the medicinal value of *Desmanthus virgatus*, employing it to treat various ailments. However, the mechanisms underlying its pharmacological effects remain poorly understood, necessitating further scientific exploration. Recent studies have highlighted its potential as a natural source for developing novel antimicrobial agents,

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especially in the context of rising antibiotic resistance. With the growing demand for alternative treatments, *Desmanthus virgatus* offers an eco-friendly and sustainable solution to combat infections and diseases (4).

The present study's primary objective is to assess the efficacy of bioactive compounds extracted from *Desmanthus virgatus* using ethanol as a solvent against selected pathogenic organisms. A complete literature review in the ScienceDirect database revealed 208 related articles; however, none specifically addressed this plant's phytochemical profile and bioactive constituents. This gap in the literature underscores the novelty and significance of the current research. By characterizing the bioactive components and assessing their antimicrobial potential, this study focus on exploring the potential of *Desmanthus virgatus sp.* and to contribute to the growing knowledge of natural products as viable alternatives to synthetic antibiotics.

Materials and Methods

Plant collection and sample preparation

Plant samples of *Desmanthus virgatus* were collected from the university premises in October 2023. Freshly harvested leaves were cleaned thoroughly by washing with running tap water to remove surface dirt and debris, followed by a rinse with distilled water to ensure cleanliness and minimize contamination. The cleaned leaves were shade-dried for one week, a process known to preserve the integrity of heat-sensitive bioactive compounds by avoiding photodegradation (4, 5).

Once dried, the leaves were pulverised into a fine powder using a mechanical pulverizer. The powdered samples were then stored in refrigerated airtight containers to prevent moisture absorption and microbial contamination, ensuring their stability for subsequent analyses. This preparation method preserved the bioactive properties of the plant material, making it suitable for detailed characterization and extraction processes (6).

Experimental design and statistical analysis

The experimental design was structured based on G-Power software, with a power of 80% and a confidence level of 95%, to determine the appropriate sample size and grouping. Statistical analyses, including significance testing and error estimation, were performed using IBM SPSS Statistics (version 21), ensuring robust and reliable data interpretation (7). The antimicrobial activity measured using varying concentrations of crude extract obtained using water was analyzed for a zone of inhibition using an independent sample t-test; here, the independent variable was absorbance, and the dependent variables were the concentration of crude extract and the percentage of a zone of inhibition.

Extraction procedure

The cold maceration technique, a recognized clean and sustainable extraction method, prepared crude extracts from *Desmanthus virgatus* plant material. For the aqueous extract, 16.5 g of pulverised plant material was taken and soaked in 150 mL of ultra-pure double-distilled water (procured from SRL) in a sterile container. The prepared sample mixture was allowed to macerate for 72 hours at room temperature with intermittent stirring. After maceration, the processed sample was filtered using Whatman No. 1 filter paper and a Büchner funnel to separate the liquid extract from the plant residue (8, 9).

Similar procedure was followed for preparing the ethanolic extract. In this process, 16.5 g of pulverised plant material was added with 150 mL of ethanol and subjected to the same cold maceration process. The resulting mixture was filtered to obtain the crude extract. The filtered extracts were processed using hot air oven at 50°C for 30 minutes to remove excess solvent while preserving heat-sensitive phytochemicals (10).

The crude extracts were then transferred to airtight containers wrapped in

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aluminum foil to prevent light-induced degradation and stored at room temperature for subsequent analyses. This method minimized the loss of bioactive compounds and ensured the integrity of the extracts, making them suitable for detailed characterization and evaluation (11).

GC-MS analysis

The volatile compounds and its chemical composition present in the water and ethanolic crude extracts of *Desmanthus virgatus* was determined using a Shimadzu GC-QP2010 Gas Chromatography-Mass Spectrometer (GC-MS) (Tables 1 & 2). The system was equipped with an HP5MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). for optimal separation efficiency. A constant flow rate of 1 mL/min of helium gas was allowed to pass. Further, the temperature of oven was set to 50°C with an increment of 5°C per minute until it reached 250°C, ensuring proper volatilization and separation of compounds (12, 13).

The obtained GC-MS scan spectra of the crude extracts of selected solvents were analyzed using the National Institute of Standards and Technology (NIST) library to identify compounds by matching spectral data with known profiles. This approach enabled the extract's detection and characterization of bioactive volatile compounds, as summarized in (Table 1). Similarly, the ethanolic crude extract was subjected to the same GC-MS analysis conditions. The spectra were compared against the NIST library database to identify and confirm the volatile compounds present. This method ensured high precision in compound identification, providing valuable insights into the phytochemical profile of the extracts (14).

Antimicrobial activity

The disc diffusion method evaluated the antimicrobial activity of *Desmanthus virgatus* crude extracts (water and ethanol). Mueller-Hinton agar (MHA). was prepared, sterilized in an autoclave, and poured into previously sterilized Petri dishes under aseptic conditions. After solidification, the plates were inoculated with clinical microbial isolates (i.e. gram-negative bacteria) *viz*. *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* sp., and *Shigella* sp. (15, 16). The clinical isolates were collected from Anna University - BIT Campus, Tiruchirappalli, and identified through standard microbiological methods.

Commercial standard antibiotic discs were impregnated with the water crude extract at varying concentrations with an incremental range of 20 µg/disc, from of 20 µg/disc up to 100 µg/disc and placed on the inoculated MHA plates. The respective petri plates were kept for incubation for 24 hours at 37°C, followed by the zone of inhibition was measured to assess antimicrobial activity (17).

Similarly, discs impregnated with the ethanolic crude extract at identical concentrations of 20 µg/disc with an increment of 20 µg/disc up to 100 µg/disc, were placed on microorganism-inoculated MHA plates. The inhibition zones were measured and recorded after incubation under the same conditions (37°C for 24 hours). The observed inhibition zones indicated the antimicrobial efficacy of the crude extracts against the tested pathogens, demonstrating a concentration-dependent activity pattern (18).

Results

GC-MS analysis

The representative GC-MS spectra of the aqueous leaf extract of *Desmanthus virgatus* are displayed (Figure 1). The analysis revealed the presence of multiple bioactive phytochemicals, which were identified based on reverse search matching (RSI) against the National Institute of Standards and Technology (NIST). library. The characteristics of these phytochemicals, including retention time, molecular weight, and compound identification, are summarized in (Table 1). According to NIST guidelines, RSI values between 800–900 are considered suitable matches, while values above 900

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Table 1: List of compounds present in the water-based crude extract was determined using Gas Chromatorgraphy – Mass Spectrometry (GC-MS) analysis

S.No.	Retention Time	Name of the Compounds	Formula	Mol. Wt.	Area %
1	4.817	Heptane, 2,4-dimethyl-	C_9H_{20}	128.2551	1.61
2	8.893	Decane, 4-methyl-	$C_{11}H_{24}$	156.3083	1.81
3	9.651	Decane, 3,7-dimethyl-	$C_{12}H_{26}$	170.3348	11.14
4	10.573	Decane, 3,7-dimethyl-	$C_{12}H_{26}$	170.3348	2.01
5	11.809	Silane, cyclohexyl dimethoxy methyl-	$C_9H_{14}O_2Si$	182.2918	1.96
6	12.800	Undecane, 2,5-dimethyl-	$C_{13}H_{28}$	184.3614	1.21
7	13.760	Tetradecane, 5-methyl	$C_{15}H_{32}$	212.4146	2.64
8	14.080	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	198.3880	7.72
9	14.923	2,6,10-Trimethyl Tridecane	$C_{16}H_{34}$	226.4412	2.00
10	16.234	Tetradecane	$C_{14}H_{30}$	198.3880	2.49
11	16.447	Tridecane, 2,5-dimethyl-	$C_{15}H_{32}$	212.4146	3.36
12	16.625	Eicosane	$C_{20}H_{42}$	282.5475	3.14
13	17.150	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296.5741	1.72
14	17.344	2,6,10-Trimethyl Tridecane	$C_{16}H_{34}$	226.4412	4.89
15	17.940	Eicosane	$C_{20}H_{42}$	282.5475	9.30
16	18.682	Eicosane	$C_{20}H_{42}$	282.5475	1.41
17	19.725	Hexadecane	$C_{16}H_{34}$	226.4412	0.88
18	22.286	Eicosane	$C_{20}H_{42}$	282.5475	6.31
19	26.564	Eicosane	$C_{20}H_{42}$	282.5475	5.26
20	29.645	Eicosane	$C_{20}H_{42}$	282.5475	1.85
21	29.866	Eicosane	$C_{20}H_{42}$	282.5475	4.36
22	17.805	Eicosane	$C_{20}H_{42}$	282.5475	4.57
23	34.028	5,5-Diethyl Pentadecane	$C_{19}H_{40}$	268.5209	2.81
24	35.146	Lupeol	$C_{30}H_{50}O$	426.7174	8.90
25	36.095	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5026	6.63

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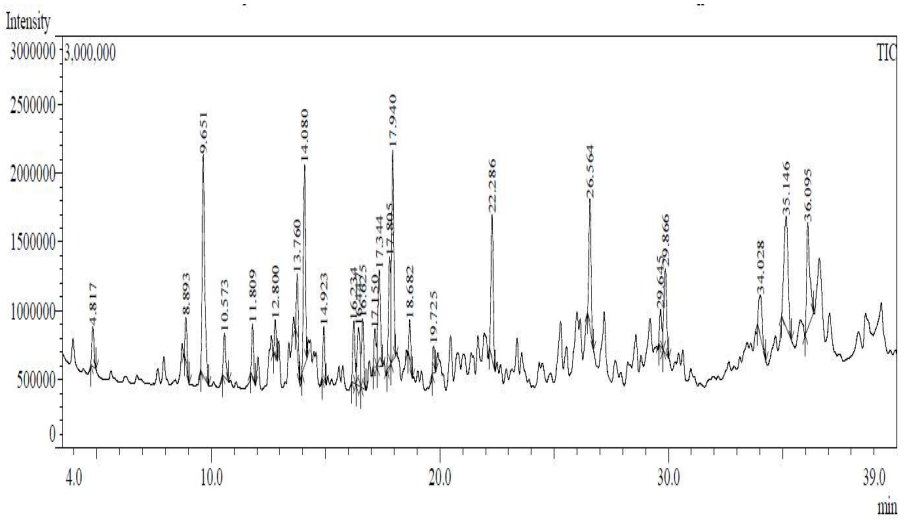
Table 2: List of compounds present in the ethanol-based crude extract was determined using GC-MS analysis

Retention Time	Name of the Compound	Formula	Mol. Wt.	Area%
5.328	Propanoic acid, 2-oxo-, methyl ester	C4H6O3	102.0886	1.63
5.539	Methylazoxymethanol acetate	$C_4H_8N_2O_3$	132.12	6.16
22.934	4-O-Methylmannose	C7H14O6	194.18246	63.51
29.366	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296.5310	23.71
35.878	Tetratetracontane	C44H90	619.1854	1.08
37.015	Eicosane	C20H42	282.5475	1.61
38.265	Eicosane	C20H42	282.5475	2.30



(* X-axis depicts time in minutes; Y-axis depicts intensity in counts per second (cps)

Fig. 1: Depicts GC-MS spectra of novel crude extracts identified from *Desmanthus virgatus*

indicate exceptional similarity (19). In the current investigation, the RSI values of identified compounds ranged between 800 and 900, demonstrating reliable identification of bioactive components (12–14).

A total of 25 peaks were observed in the GC-MS chromatogram of the aqueous leaf extract, indicating the complexity and richness of the phytochemical profile. These peaks' mass spectral fragmentation patterns were compared with known compounds in the NIST library. The analysis identified several bioactive compounds, including flavonoids, alkaloids, phenolic acids, and terpenoids, recognized for their antimicrobial, anti-inflammatory, and antioxidant properties (4).

Notable bioactive components detected in the extract are listed in (Table 1),

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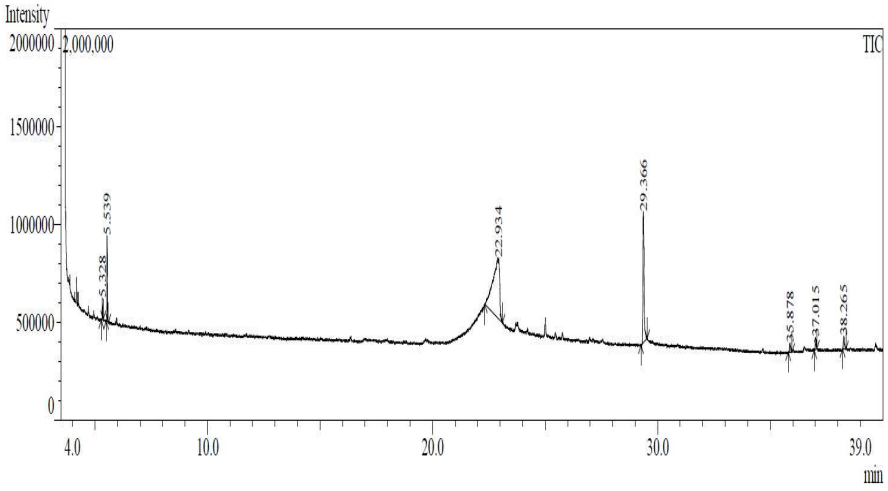
which have been previously reported to exhibit significant pharmacological activities. For instance, terpenoids and phenolic compounds are well-documented for their ability to scavenge free radicals and inhibit microbial growth, making them potential candidates for therapeutic applications. Identifying these compounds aligns with the plant's traditional use in folk medicine, where *Desmanthus virgatus* has been widely used to treat various medical ailments like inflammatory conditions and infections. The diverse bioactive molecules in the aqueous extract support the hypothesis that the plant has significant potential for developing natural remedies or pharmaceutical agents.

The GC-MS analysis underscores the phytochemical richness of *Desmanthus virgatus*, particularly its aqueous leaf extract. The observed bioactive compounds validate their antimicrobial properties, as demonstrated in previous studies where similar phytochemicals were shown to disrupt microbial cell membranes and inhibit enzyme activities (20). The RSI values obtained in this study suggest a robust and reliable identification process, ensuring confidence in the reported findings.

This study provides the details of chemical compounds and its composition of *Desmanthus virgatus* leaf extract. It highlights its potential for further bioactivity-guided fractionation to isolate individual compounds with specific therapeutic properties. Future scope of the present work could focus on exploring the mechanism-of-action in detail and *in vivo* assessments to fully explore its pharmacological potential (21).

Figure 2 depicts the GC-MS spectra of *Desmanthus virgatus* ethanolic leaf extract. The analysis revealed a complex profile of bioactive phytochemicals, with distinct peaks identified. The characteristics of these compounds, including retention times, molecular weights, and structural identities, are detailed in (Table 3). Based on the mass spectral fragmentation patterns, chemical compounds were identified with those in the National Institute of Standards and Technology (NIST) library. According to NIST library guidelines, RSI values between 800–900 indicate suitable matches, while values



(The x-axis denotes time in minutes; Y-axis denotes intensity in counts per second (cps)

Fig. 2: Depicts GC-MS spectra of extracts identified from *Desmanthus virgatus* using ethanol as solvent.

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Table 3: Comparison of the Independent sample T-test values for crude extract

Independent Samples Test Levene's test for Equality of Variances				t-test for equality of means						
		F	Sign.	t	df	Significant two-tailed	Mean Difference	Standard Error Difference	95 % Confidence Interval of the difference	
									lower	upper
Microbial growth	Concentration of water extract of *Desmanthus* sp.	2307	0.146	2.320	18	0.032	0.08025	0.03459	0.00758	0.15292
	Zone of Inhibition by crude water extract			2.320	13.747	0 .036	0.08025	0.03459	0.00594	0.15457

above 900 signify exceptional similarity (19, 22). In this study, the RSI values ranged between 800 and 900, ensuring reliable validation of the chemical compounds present in the ethanolic extract.

The detected phytochemicals included terpenoids, alkaloids, flavonoids, phenolic acids, and saponins, widely recognized for their pharmacological properties. Among these, compounds listed in (Table 4) have been associated with antimicrobial, antioxidant, and anti-inflammatory activities in previous studies. For example, flavonoids and phenolic acids scavenge free radicals, inhibit bacterial growth, and modulate inflammatory responses by targeting microbial cell walls and oxidative stress pathways (23, 24).

Bioactive molecules found in the ethanolic extract support the medicinal use of *Desmanthus virgatus*, particularly in treating infections and inflammatory conditions. Ethanol as a solvent is well known for its potential in eluting a gamut of polar and non-polar phytochemicals, which may account for the diversity of compounds detected in this extract compared to water-based extractions using water as solvent (4).

The spectra obtained from GC-MS analysis provides compelling evidence of the phytochemical richness of *Desmanthus virgatus* ethanolic extract. The identified compounds exhibit various biological activities, supporting their potential for pharmaceutical applications. The ethanolic extract demonstrated a more complex phytochemical profile than aqueous extracts, highlighting the influence of solvent polarity on the extraction efficiency of bioactive compounds (25).

These findings align with prior research emphasizing the antimicrobial and antioxidant potential of *Desmanthus* species. For instance, the detected terpenoids and saponins are known to disrupt microbial cell membranes, while phenolic acids and flavonoids contribute to free radical scavenging and oxidative stress mitigation. The RSI values obtained in this study confirm the robustness of the GC-MS methodology and provide a strong basis for the reported phytochemical profile. Future studies should focus on isolating individual compounds from the ethanolic extract to assess their specific bioactivities and elucidate their mechanisms of action. Additionally, in vivo studies could

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Table 4: Comparison of the T-test values between the groups

Independent Samples Test Levene's test for Equality of Variances				t-test for equality of means						
		F	Sign.	t	df	Significant two-tailed	Mean Difference	Standard Error Difference	95 % Confidence Interval of the difference	
									lower	upper
Microbial growth	Concentration of ethanol extract of *Desmanthus* sp.	0.061	0.808	1.741	18	0.099	0.07540	0.04331	-01559	16640
	Zone of Inhibition by crude ethanol extract			1.741	17.226	0.100	0.07540	0.04331	-01589	16669

validate the therapeutic potential of these compounds and pave the way for their development into natural remedies or pharmaceutical agents (25–27).

Antibacterial Activity

The antibacterial activity of the crude extracts of *Desmanthus virgatus* leaves prepared from water and ethanol was evaluated against selected bacterial strains, and the results are presented in the corresponding figures (Figs. 3 & 4). The aqueous crude extract demonstrated significant antibacterial activity, with *Escherichia coli* at a concentration of 160 mg/mL exhibiting the largest zone of inhibition (19 mm), followed by *Pseudomonas aeruginosa* (18 mm). The minimum inhibitory concentration (MIC) for *Pseudomonas aeruginosa* was evaluated to be 2 mg/mL, indicating the extract's effectiveness even at lower concentrations. These findings suggest that the bioactive compounds present in the aqueous extract, such as flavonoids and phenolic acids, contribute to its antibacterial potency by microbial cell membranes disruption and essential metabolic pathways inhibition (4).

Similarly, the ethanol crude extract showed notable antibacterial activity. Among the tested organisms, *Klebsiella pneumoniae* exhibited the largest zone of inhibition (19 mm). at 160 mg/mL, followed by *Pseudomonas aeruginosa* (18 mm). and *Klebsiella pneumoniae* (17 mm). The MIC for *Pseudomonas aeruginosa* was also observed at 2 mg/mL, consistent with the aqueous extract results. The ethanol extract's enhanced activity against *Klebsiella pneumoniae* may be ascribed to its capability to isolate a wide range of bioactive compounds, including terpenoids and saponins known for their antimicrobial properties (28).

The observed zones of inhibition and MIC values highlight the potential of *Desmanthus virgatus* extracts as natural antibacterial agents. The ethanol extract's slightly higher activity against certain strains, such as *Klebsiella pneumoniae*, aligns with previous studies emphasizing the role of solvent polarity in extracting diverse and

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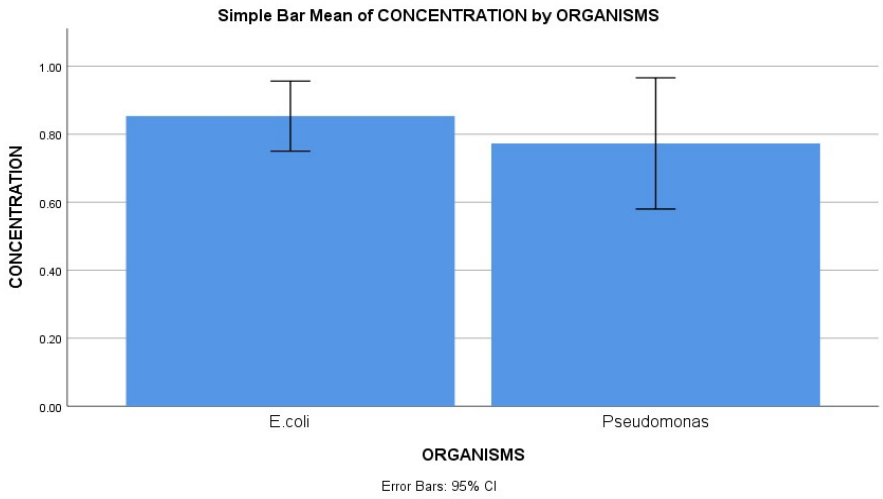
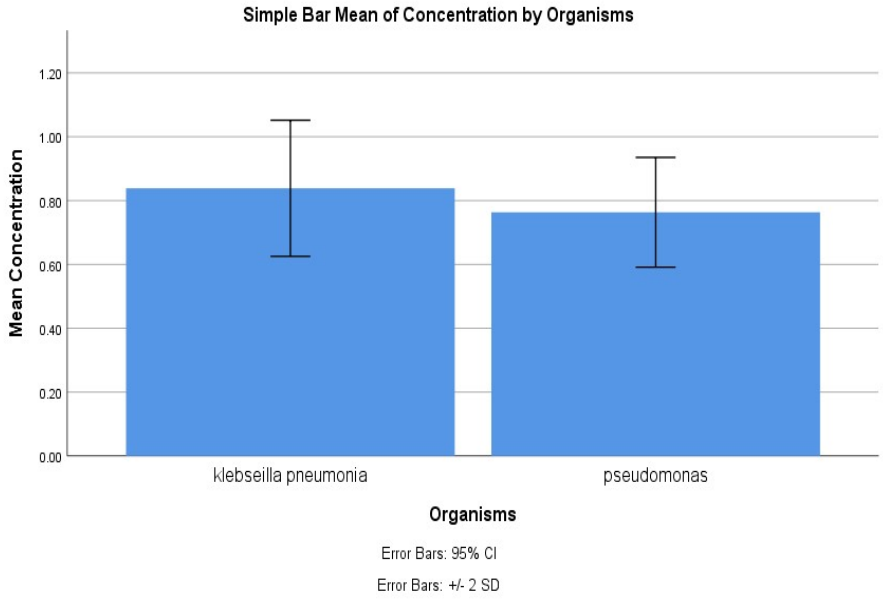


(CI: 95%; SD +/-1)

Fig. 3: Depicts the antimicrobial activity of crude extract isolated using water solvent from *Desmanthus virgatus* plant leaf sample.



(CI: 95%; SD +/-1)

Fig. 4 Antimicrobial activity of ethanol extract isolated using water solvent from *Desmanthus virgatus* plant leaf sample.

Antimicrobial Activity of Desmanthus sp. Extracts

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potent bioactive compounds (4, 5). The comparable MIC values (2 mg/mL) across both extracts for *Pseudomonas aeruginosa* underscore the robustness of the plant's antimicrobial properties.

The findings suggest that *Desmanthus virgatus* extracts could be a basis for developing novel antibacterial agents, particularly in light of rising antibiotic resistance. Future scope of the present research study should focus on isolation and characterization of the specific bioactive compounds responsible for the observed antibacterial activity. Furthermore, in vivo studies and toxicity analysis are essential to validate the therapeutic potential of the selected solvent extracts.

Statistical analysis

The statistical analysis of the antibacterial activity of *Desmanthus virgatus* aqueous and ethanolic crude extracts against selected pathogens was conducted to evaluate the significance of the observed differences in the zone of inhibition at varying concentrations. The mean, standard error of the mean (SEM) and standard deviation (SD) for the both crude extracts at different concentrations are detailed in (Table 1). For the ethanolic crude extract, the corresponding data are presented in (Table 4). These descriptive statistics provide an overview of the variability and precision of the results obtained for each concentration.

Independent Sample T-Test Analysis

An independent sample t-test was performed to differentiate the effects of varying concentrations of the crude extracts on bacterial inhibition. Levene's test for equality of variances and the t-test for equality of means were used, with results summarized in (Tables 3 & 4). The aqueous crude extract's t-value was 2.320, and the p-values were 0.00758 and 0.15292 for different test conditions. Similarly, a t-value of 2.320 was observed for the ethanolic crude extract, with the p-values also reported as 0.00758 and 0.15292.

The aqueous extract's results were significant at $p < 0.05$, indicating that the observed differences in antibacterial activity across concentrations were statistically significant. This finding suggests a concentration-dependent antibacterial effect of the aqueous crude extract. In contrast, while some results were substantial for the ethanolic crude extract, others exceeded the significance threshold, suggesting potential variability in the extract's efficacy against specific pathogens (29).

The t-test results underscore the efficacy of the crude extracts in inhibiting bacterial growth at specific concentrations. The significant p-values for the aqueous extract highlight its consistent and reliable antibacterial activity, particularly at higher concentrations. The ethanolic extract showed similar trends but slightly less consistent, as reflected in the p-values exceeding 0.05 in some cases; this could be due to the differential extraction and concentration of bioactive compounds in the ethanol and aqueous solvents, as solvent polarity plays a critical part in estimating the range and efficacy of extracted phytochemicals (5).

The t-value of 2.320 across both extracts suggests a moderate effect size, supporting the observed activities' biological relevance. These findings align with prior research demonstrating that plant-derived crude extracts can exhibit statistically significant antimicrobial effects, often mediated by their bioactive components such as flavonoids, terpenoids, and phenolic acids (30). Future studies should explore a broader range of bacterial strains and include additional statistical approaches, such as ANOVA, to simultaneously compare extract efficacy across multiple pathogens. Furthermore, standardizing the extraction process and ensuring batch-to-batch consistency would enhance the reproducibility and reliability of these findings (31).

Limitation and future scope

The crude aqueous extract of *Desmanthus* sp. is highly hygroscopic.

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Special care has to be taken while holding the sample for future use. The crude extracts of *Desmanthus* sp. and its bioactive compounds require further investigation based on their exceptional results in this study.

Conclusion

Desmanthus virgatus emerges as a promising plant with significant medicinal properties and notable potential in antimicrobial research. Its natural antibacterial properties, attributed to its diverse bioactive phytochemicals, make it a strong candidate for future pharmaceutical and therapeutic innovations. The demonstrated antimicrobial efficacy highlights its efficacy as a promising natural substance towards alternative synthetic antibiotics, particularly in combating resistant pathogens. With its wide-ranging therapeutic characteristics and inherent medicinal qualities, *Desmanthus virgatus* continues to attract scientific interest. Future research focusing on the isolation of bioactive compounds and its characterization cum clinical validation could pave the way for groundbreaking advancements in medicine and therapy. This positions *Desmanthus virgatus* as a valuable resource for developing novel antimicrobial agents and underscores its relevance in the ongoing search for effective and sustainable healthcare solutions.

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Conflict of interest

No conflicts of interest.

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