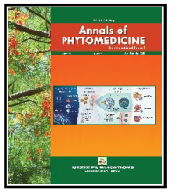
Annals of Phytomedicine 14(2): 430-439, 2025

DOI: http://dx.doi.org/10.54085/ap.2025.14.2.41

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Orginal Article : Open Access

Phytochemical, antioxidant and antibacterial effects of *Jatropha multifida* L.

P. B. Sruthy*◆, P. S. Sruthi*, R. Senthilkumar*, Vaisakh Venu, E. R. Ramdas* and Sreeja Puthanpura Sasidharan*****

** Department of Processing and Food Engineering, Kelappaji College of Agricultural Engineering and Food Technology, Tavanur-679 573, Malappuram, Kerala*

*** Department of Basic Engineering and Applied Sciences, Kelappaji College of Agricultural Engineering and Food Technology, Tavanur-679 573, Malappuram, Kerala*

**** Department of Botany, NSS College, Nemmara, Palakkad, Kerala, India*

Article Info

Article history
Received 6 July 2025
Revised 12 August 2025
Accepted 13 August 2025
Published Online 30 December 2025

Keywords
Anti-inflammatory
Antioxidative
Phytochemical
Therapeutic
Jatropha multifida L.

Abstract

Jatropha multifida L., commonly known as coral plant, is an evergreen shrub known for its medicinal activities for long time. This is generally valuable in managing chronic wounds owing to its antimicrobial, anti-inflammatory, antioxidant, and wound healing properties. The current study analysed the phytochemical, antioxidant and antibacterial characteristics of ethanolic and aqueous extracts from leaves and stems of *J. multifida* plant. Qualitative and quantitative examination of the phytochemicals was carried out using established methods. The quantity of amino acids, carbohydrates, flavonoids, and phenolic compounds were screened by phytochemical analysis. The antibiotic efficiency of the samples was assessed against the clinical isolates *Pseudomonas* sp., *Klebsiella* sp., and *Staphylococcus* sp. using well diffusion technique. The 2, 2- diphenyl 2, 2- 1 picryl hydrazyl diphenyl (DPPH) free radical scavenging test was used to evaluate the antioxidant potentials. The results signify that *J. multifida* demonstrate highest antibacterial activity against *Staphylococcus*, confirmed by the largest zone of inhibition. Our results reveal that leaf extract of *J. multifida* contains distinctive phytochemical components, along with antioxidant and antibacterial properties, suggesting that employing plant extracts as herbal bandages could be a novel approach. The GC-MS analysis of the plant sample identified phytol as the chief constituent of *J. multifida*. The present study confirmed that both the aqueous and ethanolic extracts of *J. multifida* leaves and stems consist of bioactive compounds that may contribute to their antibacterial property. This research can move forward towards the development of a natural bandage, following the completion of toxicological, allergy, animal, and clinical evaluation.

1. Introduction

Plants are the nature's gift, with a great therapeutic value. The compounds extracted from natural resources have gained popularity in the recent decades due to their vast chemical variety. This has resulted in an increase in demand for herbal medications over the last two decades to ensure the quality, safety, and efficacy of herbal treatments. Antibiotic resistance, particularly in food borne pathogens, is becoming a serious worldwide health issue, forcing men into ethnopharmacognosy (Canica *et al*., 2019). Traditional medicines for primary healthcare are the major choices which are deemed safer than synthetics which are considered hazardous to humans and the environment (Yohanna Musa *et al*., 2017). Phytochemicals are substances found naturally in the plants which are becoming increasingly popular due to their abundant medical applications. They are useful against a variety of ailments, including asthma, arthritis, and cancer. As phytochemicals treat illness without causing any harm to humans, they can be termed as "man-friendly medicines", which have been documented for their diverse biological actions, including anti-inflammatory, antioxidant, antibacterial, and antifungal properties (Durand Dah-Nouvlessounon *et al*., 2023).

In the current situation, there is a crucial requirement for the investigation and formation of a cheaper, more efficient novel plant based medication with additional bioactive potential and fewer side effects. As a result, there has been a new focus on plant derived bioactive extracts and chemicals for herbal medicine. Plant-derived antimicrobials have been shown to be successful in the management of infections with lesser side effects, better patient tolerance, and a lower cost. *J. multifida* belongs to the Euphorbiaceae family and is native to Barbados. It is known as coral plant or French physic nut. The leaves of this ornamental plant are used to treat scabies, itchy skin, eczema and neurodermatitis while the latex is applied to wounds and ulcers (Shu *et al*., 2008). The stems were utilized as chewing sticks for tooth care in Nigeria (Kayode and Omotoyinbo, 2008). *J. multifida* is used in tribal medicine to alleviate inflammation and decrease haemorrhage from cuts (Michael *et al*., 2014).

The bioactive chemical components in medical plants play a significant role in regulating host-microbe interactions in support of the hosts. As a result, it is important to recognize bioactive molecules in plants, isolate, purify, and characterize active components in crude extracts with a range of analytical approaches. The biopotential of plants as a source of novel medications is continuously being researched. The uncontrolled use of synthetic microbial antibiotics has resulted in a substantial and expanding risk to public health from the rise in multidrug-resistant strains of pathogenic bacteria.

Corresponding author: Dr. P.B. Sruthy
Department of Processing and Food Engineering, Kelappaji College of Agricultural Engineering and Food Technology, Tavanur-679 573, Malappuram, Kerala
E-mail: sruthypbaburaj@gmail.com
Tel.: +91-9778532025

Investigation on medicinal plants has improved extensively in an attempt to generate substitutes that can reduce the issues caused by antibiotic resistance (Ekangu Gerald and Alfonse, 2023).

J. multifida plant extract containing high levels of phenolic compounds and flavonoids exhibit significant antioxidant and antibacterial activity. The varied therapeutic capabilities of *J. multifida* may indicate the intricate phytochemical characteristics of the indigenous plant, featuring compounds such as alkaloids, flavonoids, terpenoids, and bioactive diterpenes (Devappa *et al*., 2011). These compounds exhibit diverse pharmacological properties including antimicrobial, anti-inflammatory, wound healing, antioxidant, and anticancer capabilities derived from *J. multifida* to explore new compounds for development of medicines (Aiyelaagbe *et al*., 2011). The present study intends to examine the plant's traditional use as a medicine for wound treatment and exploring its possibilities in addressing bacterial infections and oxidative damage by free radicals.

2. Materials and Methods

2.1 Collection and identification of sample

The fully maturated stems and leaves of coral bush (*J. multifida*) were collected from local area and were identified by Dr. Zereena Viji and Dr. P.S. Rekha, Assistant Professors in the Department of Botany, NSS College, Nemmara, Palakkad, Kerala and were used for the preparation of extracts. The Herbarium Number is NSS-2024-020.

2.2 Preparation of plant extracts

The leaves and stems of the plant, *J. multifida* were used to prepare hot and cold extract using water and ethanol in 1:10 proportion. Hot and cold extraction process was carried out for a period of 2 h and 3 days, respectively. The prepared extracts were allowed to filter through Whatman No. 1 filter paper and were used for further tests.

2.3 Qualitative analysis of phytochemicals

Coral bush (*J. multifida*) leaf and stem extracts were evaluated for secondary metabolites (alkaloids, amino acids, anthroquinones, carbohydrate, flavonoids, glycosides, terpenoids, tannins, phenols, and saponins) as per the procedure of Prashant Tiwari *et al*. (2011).

2.4 Quantitative determination of phytochemicals

The flavonoids, phenolic compounds, carbohydrates, and protein were quantitatively estimated as per the procedures of Chang *et al.* (2002). Gallic acid and quercetin were used as the standard for phenol and flavonoid, respectively.

2.5 Antimicrobial assay

The antimicrobial assay of the crude extracts of plant was carried out using the conventional agar well diffusion method using the clinical isolates *Pseudomonas* sp., *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus* sp. (Arundevi *et al*., 2010) which were collected from Polyclinic Pvt. Ltd, Thrissur. Bacterial cultures from overnight growth were standardized to the 0.5 McFarland standards and evenly spread onto Mueller–Hinton agar plates. Sterile cork borers created 6 mm wells, and the extracts were added to them. Ciprofloxacin acted as the positive control. Plates were incubated at room temperature for 30 min to facilitate diffusion, and then incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the diameter of inhibition zones (mm) using a Digital Caliper. All experiments were performed in triplicate, and average values were documented.

2.6 Antioxidant assay by (1, 1,-diphenyl-2-pierylhydrazyl) DPPH

Using the conventional protocols, DPPH was used to measure the capacity of each sample to scavenge free radicals (Siddartha Baliyan *et al*., 2022).

2.7 Thin layer chromatography

Thin layer chromatography was carried out on extracts of *J. multifida* using the mobile phase containing chloroform: ethanol (4:1). The chromatograms were observed under visible light and Rf value were calculated. For analysis of flavonoids, Iodine crystals were heated and chromatograms were placed in beaker containing evaporated iodine. Yellow brown spots were developed due to the evaporation of iodine.

2.7.1 Thin layer chromatography bioautography for antioxidant

Thin layer chromatography was carried on extracts of *J. multifida* using the mobile phase containing chloroform: ethanol (4:1) and was allowed it to dry. The chromatograms were sprayed with 0.004% of DPPH in ethanol. The chromatograms were observed under visible light. Yellow to pink colours in purple background was observed and the Rf values were calculated.

2.7.2 Thin layer chromatography bioautography for antibacterial

Direct bioautography using thin-layer chromatography (TLC-DB) was carried on extracts of *J. multifida* using the standard procedures. In this technique, TLC plates were developed to separate compounds and were sprayed separately with 24 h old culture of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.* It was allowed for incubation at 37°C for 24 h and the zone of inhibition was observed and the Rf values were calculated.

2.8 Antibacterial assay using wound dressing material impregnated with ethanol extracts

The wound dressing materials impregnated with ethanol extract were applied to the Muller Hinton Agar medium treated with *S. aureus* following the standard procedures and examined for zones of inhibition after incubation (Arundevi *et al*., 2010).

2.9 GC-MS analysis

GC-MS analysis was performed in Shimadzu GC-MS instrument, Model Number: QP2010S using 30 meter length Rxi-5Sil MS Column. Mass Spectroscopy was used to identify bioactive compounds in *J. multifida* ethanol extracts by comparing their retention indices and mass spectra fragmentation patterns to those stored in the computer library (NIST 11 and WILEY 8) as well as published literatures at the Kerala Forest Research Institute, Peechi Trissur.

2.10 Statistical analysis

Raincloud plot was used to statistically analyze the distribution of the antibacterial activity data for every treatment groups and the control against the bacterial strains. Kruskal-Wallis multiple

comparison test was used to examine the statistical significance across varying sample volumes against the bacterial strains. Post hoc statistical analysis was used to analyze which particular volumes are significantly different from one another after the Kruskal- Wallis test shows an overall difference.

3. Results

3.1 Phytochemical screening of *J. multifida*

3.1.1 Qualitative analysis of phytochemicals

J. multifida extracts revealed the presence of amino acids, carbohydrates, saponins, flavonoides, tannin and phenolic compounds. The qualitative analyses of phytochemicals are presented in the Table 1.

3.1.2 Quantitative analysis of phytochemicals

The stem ethanol extracts exhibited the presence of protein. The protein content was highest in leaf ethanol extract and was found to be 0.07 mg/ml. The carbohydrate content was highest in leaf ethanol extract and was found to be 0.05 mg/ml**.** Both leaf and stem extracts tested positive for phenolic compounds. The total phenolic content was highest in leaf ethanol extracts compared to stem extracts. The total phenolic content in leaf ethanol extract was found to be 0.05 mg/ml**.** In the present study, extracts from both the stems and leaves of *J. multifida* were known to contain flavonoids. Flavonoid content was highest in the leaf ethanol extract and was found to be 0.07 mg/ml**.** All experiments were performed in triplicate (n = 3) for each extract. The quantitative analyses of phytochemicals are presented in the Table 2.

Table 1: Qualitative analysis of phytochemicals

S. No.	Tests	Aqueous stem	Aqueous leaf	Ethanol stem	Ethanol leaf
1	**Test for alkaloids**				
	a Mayer's test	-ve	-ve	-ve	-ve
	b Wagner's test	-ve	-ve	-ve	-ve
2	**Test for amino acid**				
	a Xanthoproteic test	+ve	+ve	+ve	+ve
	b Ninhydrin test	-ve	-ve	-ve	-ve
3	**Test for carbohydrates**				
	a Molish's test	-ve	-ve	+ve	+ve
	b Benedict's test	+ve	+ve	+ve	+ve
4	**Test for saponins**				
	a Foam test	+ve	+ve	-ve	-ve
5	**Test for glycosides**				
	a Fehling's test	-ve	-ve	-ve	-ve
6	**Test for phenolic compounds and tannins**				
	a Ferric chloride test	-ve	+ve	+ve	-ve
7	**Test for flavonoids**	-ve	-ve	+ve	+ve
8	**Test for terpenoids**	-ve	-ve	-ve	-ve
9	**Test for anthroquinones**	-ve	-ve	-ve	-ve

Table 2: Quantitative analysis of phytochemicals

S. No.	Tests	Aqueous stem (mg/ml)	Aqueous leaf (mg/ml)	Ethanol stem (mg/ml)	Ethanol leaf (mg/ml)
1.	Protein	0.02	0.05	0.03	0.07
2.	Carbohydrates	0.01	0.04	0.02	0.05
3.	Phenolic compounds	0.01	0.04	0.03	0.05
4.	Flavonoids	0.02	0.06	0.03	0.07

3.2 Antimicrobial assay: Well diffusion

The leaf ethanol extracts showed highest antibacterial activity against Gram-positive organism where the zone of inhibition was highest against *Staphylococcus*. The leaf aqueous extracts showed highest antibacterial activity against Gram-negative organism where the zone of inhibition was highest against *Pseudomonas*. The stem extracts demonstrated weak activity, markedly lower than leaf extracts. The results are presented in Figures 1 and 2.

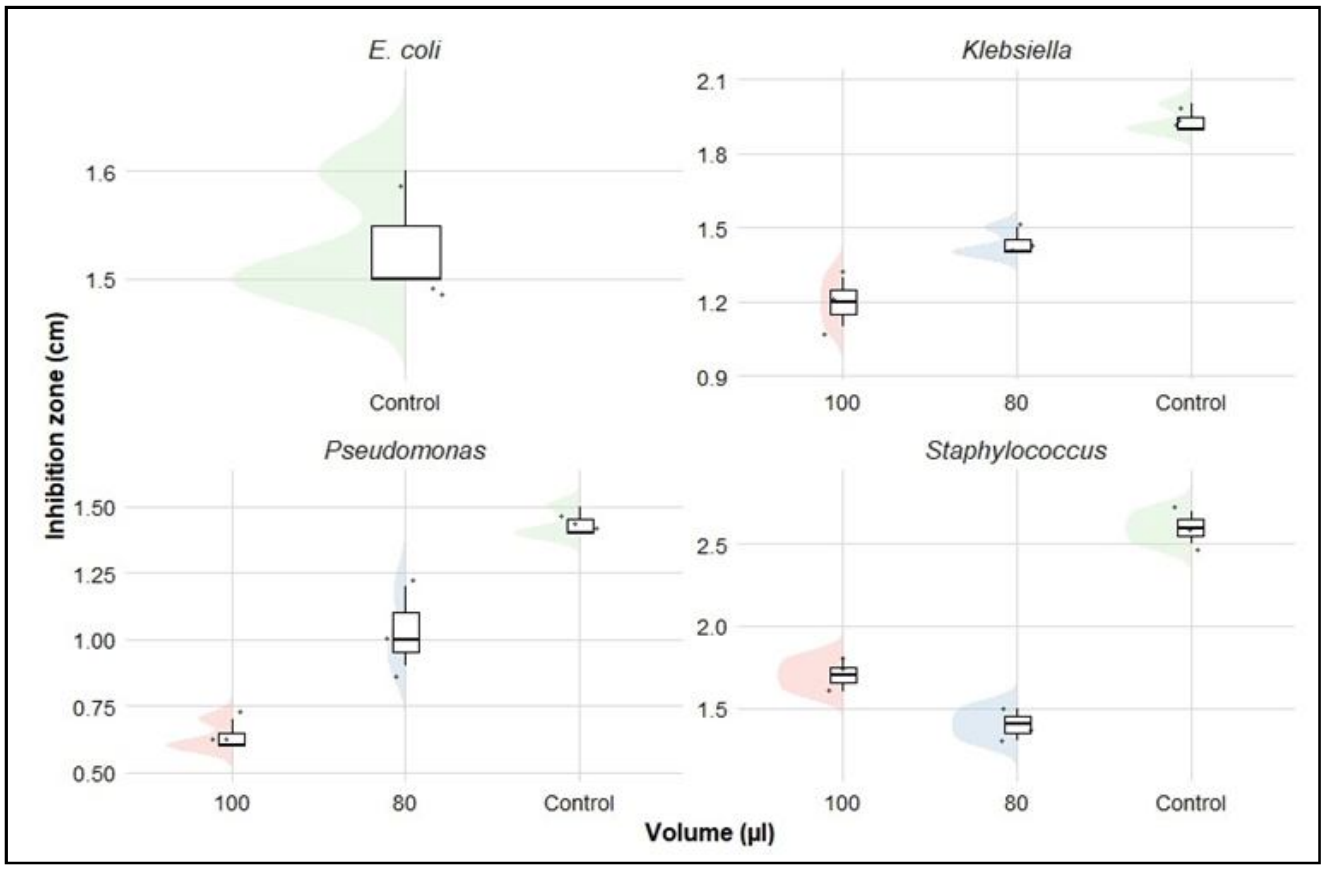


Figure 1: Raincloud plot of zone of inhibition by bacterial strain and volume of sample.

3.2.1 Leaf ethanol extract

According to Figure 1, strong antibacterial activity was shown by the sample (leaf ethanol extract) against all the bacterial strains except *E. coli*, in which only control (Chloramphenicol (80 µl (1 mg/10 ml)) was showing the zone of inhibition which indicates that only the antibiotic is effective. It was revealed that *Staphylococcus* demonstrated the highest zone of inhibition in a dose dependent manner, followed by *Klebsiella and Pseudomonas*. Chloramphenicol significantly outperforms the sample against all bacterial strains. In the case of *Klebsiella* and *Pseudomonas* dose effect is inverse (100 µl < 80 µl), possibly suggesting penetration limits at higher volume. In this case, treatment was showing dose-dependent inhibition, but it was less effective overall, especially at 100 µl.

Kruskal-Wallis test confirmed that that the antibacterial activity varied significantly across extract volumes (80 µl and 100 µl) against all the tested bacterial strains (*Staphylococcus*, *Klebsiella*, and *Pseudomonas*). Post hoc test was conducted in which a significant difference was observed between 80 µl and control. 80 µl extract significantly inhibits *Staphylococcus* compared to the control (p<0.05). However, no significant difference were noted between 100 µl vs 80 µl or 100 µl vs control, indicating that increasing the extract volume to 100 µl did not enhance the inhibitory effect beyond that observed at 80 µl. This could imply a non-linear dose-response.

100 µl significantly inhibits *Klebsiella* compared to the control (P<0.05), whereas the 80 µl extract did not differ significantly from control, suggesting that only the higher volume was effective. For *Pseudomonas*, a significant inhibition was observed only at 100 µl compared to control (p<0.05). The 80 µl treatment was not significantly different from the control.

Overall, these findings indicate that the 80 µl volume was effective only against *Staphylococcus*, while 100 µl was required for significant inhibition of *Klebsiella* and *Pseudomonas*. Moreover, there were no significant differences between 80 µl and 100 µl in any strain, suggesting that increasing the volume above 80 µl does not always yield additional antibacterial benefits and the effect is strain-specific.

3.2.2 Leaf aqueous extracts

Figure 2 illustrates a strong dose dependent inhibition by leaf aqueous extract against the bacterial strains *Pseudomonas* and *Staphylococcus*. In the case of *E. coli* and *Klebsiella*, only the control (antibiotic) was effective. It was revealed that the highest zone of inhibition was formed against *Pseudomonas* in a dose dependent manner followed by *Staphylococcus*. Antibiotic was showing lowest inhibition which implies that the tested treatment may be more effective than the antibiotic against *Pseudomonas*. In the case of *Staphylococcus,* 100 µl treatment is comparable or slightly better than the antibiotic.

Kruskal-Wallis test illustrates that sample shows a significant difference in antibacterial effect across the three treatment volumes (Control, 80 µl, 100 µl for both *Pseudomonas* and *Staphylococcus*. Post Hoc test suggest that leaf aqueous extract is strain-specific in its optimal dose. *Pseudomonas* responds significantly to 100 µl whereas *Staphylococcus* responds significantly to 80 µl.

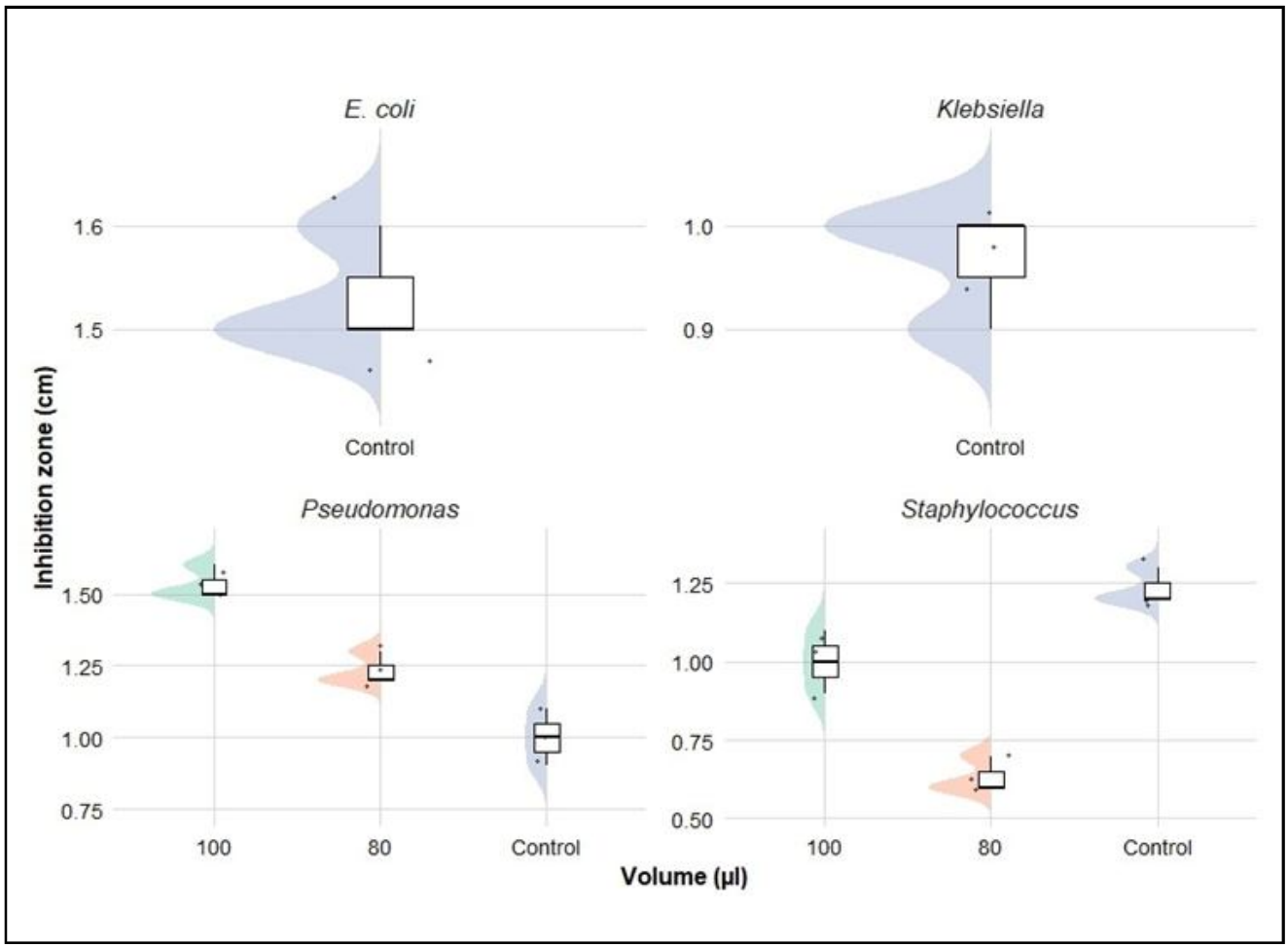


Figure 2: Raincloud plot of zone of inhibition by bacterial strain and volume of sample.

3.3 Thin layer chromatography

Thin layer chromatography of the plant extracts revealed the presence of different compounds with Rf values ranged from 0.29 to 1.00 which are presented in Table 3.

Table 3: Thin layer chromatography of compounds

S. No.	Aqueous leaf	Aqueous stem	Ethanol leaf	Ethanol stem	Leaf	Stem
1	0.45	1.00	0.98	1.00	0.90, 0.29, 0.46	0.37

The aqueous leaf extract showed a single prominent spot with an Rf value of 0.45, whereas the aqueous stem extract exhibited a higher Rf value of 1.00, indicating differences in polarity and compound migration. Similarly, the ethanol leaf extract demonstrated a spot at 0.98, while the ethanol stem extract also produced a spot at 1.00, suggesting that ethanol efficiently extracted compounds of lower polarity from both leaf and stem tissues.

Multiple spots were observed in the leaf extract fraction, with Rf values of 0.90, 0.29, and 0.46, signifying the presence of diverse phytoconstituents with varying affinities to the solvent system. In contrast, the stem extract showed a comparatively lower number of bands with a single Rf value of 0.37, reflecting a lower chemical diversity than the leaf. Overall, TLC profiling demonstrated that leaf extracts (both aqueous and ethanol) contained more phytochemical diversity than stem extracts, indicating leaves as a richer source of bioactive compounds in *J. multifida*.

3.4 Antioxidant activity by DPPH radical scavenging assay

The total antioxidant activity which was measured by DPPH method and the results are presented in Table 4.

Table 4: Percentage inhibition of antioxidants (DPPH assay, *n*=3)

S.No.	Extract type	Antioxidant activity (%)
1.	Aqueous leaf	84.0 ± 0.4
2.	Aqueous stem	54.0 ± 0.4
3.	Ethanol leaf	28.0 ± 0.4
4.	Ethanol stem	23.0 ± 0.2

The DPPH assay revealed that the aqueous leaf extracts exhibited the highest percentage of inhibition (84%), followed by the aqueous stem extract (54%). In contrast, the ethanol extracts showed comparatively lower activity, with 28% for ethanol leaf and 23% for ethanol stem. These findings indicate that aqueous extracts, particularly from leaves, possess stronger antioxidant potential than ethanol extracts.

3.5 Bioautography for antioxidant

Bioautography of the leaf extract developed on TLC and treated with DPPH revealed distinct yellow zones against a purple background, indicating the presence of antioxidant-active compounds. The clear and intense band observed in the aqueous leaf extract suggests a strong radical scavenging potential.

3.6 Bioautography for antibacterial against ethanol leaf extract

After the TLC-direct bioautography analysis, spots appear on the bioautogram corresponding to molecules exhibiting an antimicrobial activity. Antimicrobial activity against *Staphylococcus, Klebsiella, E. coli* and *Pseudomonas* revealed the occurrence of different bioactive clusters of components indicated by their Rf values potentially responsible for the antimicrobial activity of *J. mutifida* (Figures 3 and 4).

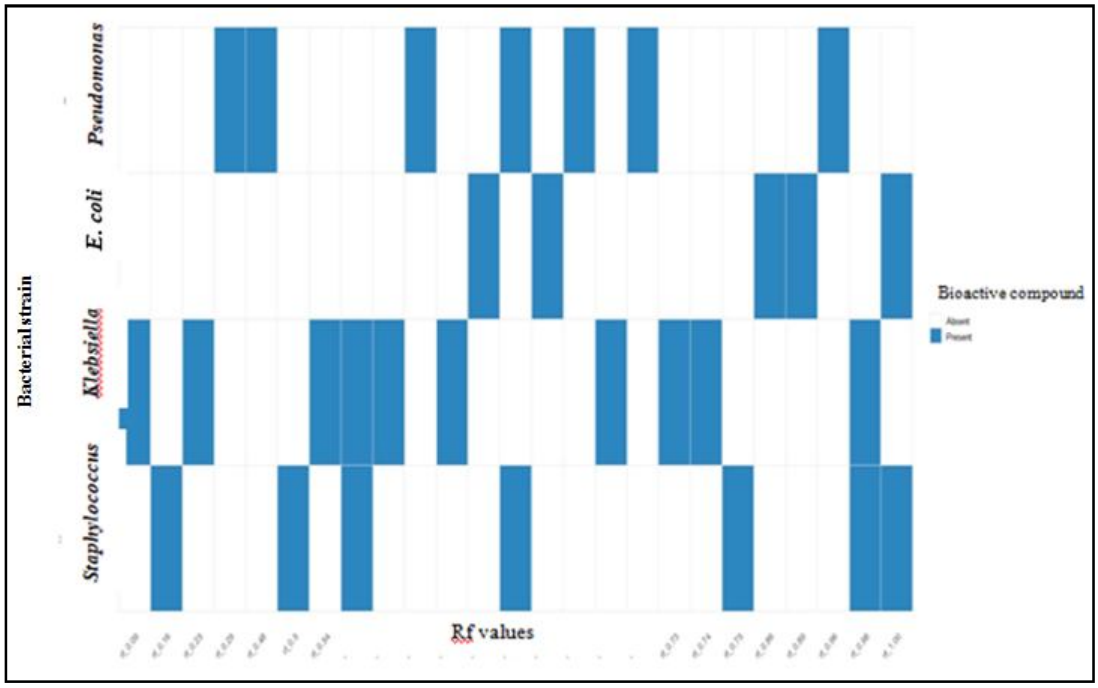


Figure 3: Bioautography for antibacterial.

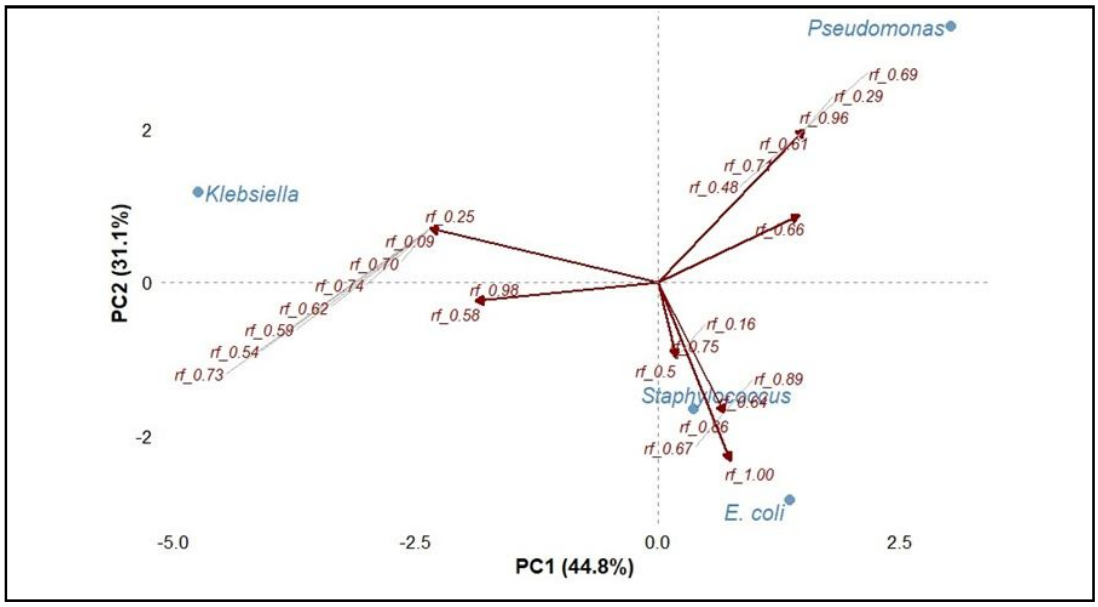


Figure 4: Principal component analysis of antibacterial activity by bioautography.

Principal component analysis (Figure 4) was carried out to discover the patterns of antibacterial activity across different components separated by bioautography, characterized by their Rf values. The first three principal components (PC1, PC2, and PC3) accounted for 100% of the total variation in the dataset, with PC1 explaining 44.78%, PC2 contributing 31.08%, and PC3 accounting for the remaining 24.14%. This pointed out that the dimensionality of the data can be efficiently reduced to three components without loss of information. The PCA scores revealed clear distinctions among the bacterial strains.

PCA study assisted to recognize the key Rf values (bioactive compounds) connected with each bacteria. Compounds with an Rf value of 0.66 were suggestive of strong antibacterial efficacy against *Pseudomonas*. Rf values 0.98 and 0.56 be further associated with *Klebsiella*. Component with Rf value 1.00 was strongly correlated to both *Staphylococcus* and *E. coli*.

On the whole, the PCA revealed that *Pseudomonas* has an exclusive sensitivity pattern which was resistant to a different group of antibacterial components compared to other bacteria. The bacterial strains *Staphylococcus* and *E. coli* established a strong association in their responses. In contrast, *Klebsiella* demonstrated an exclusive pattern owing to it active response to more polar materials. The effectiveness of PCA in identifying the relationship between bioactive compounds and strains were highlighted by these techniques, approving its use in generating antibacterial agents from natural sources.

3.7 Antibacterial assay using wound dressing material impregnated with ethanol leaf extracts

The antimicrobial activity was highest against *Staphylococcus aureus* and the zone of inhibition was found to be 2.8 cm. The results are tabulated in Table 5.

Table 5: Antimicrobial activity of wound dressing materials impregna ted with ethanol extracts

S. No.	Organisms	Sample zone of inhibition (cm)	Antibiotic zone of inhibition (cm)
1	*S. aureus*	2.8	3.5
2	*Klebsiella*	0.5	2.4
3	*E. coli*	0	2.2
4	*Pseudomonas*	1.9	2.0

3.8 GC-MS analysis of ethanol leaf extract

GC-MS analysis was conducted on the ethanol extract of the leaf, as it exhibited the highest phytochemical diversity and strongest biological activity among the tested extracts. Each dominant peak in the chromatogram was identified by matching its mass spectrum with reference spectra in the database, allowing determination of the corresponding compounds. The GC-MS analysis of *J. multifida* leaf extract (Figure 5) showed that the major compounds present include phytol, neophytadiene, palmitic acid .beta-monoglyceride, 2 methoxy-4-vinylphenol, hexadecanoic acid, 11-dodecyn-1-ol acetate, vitamin E, and n-hentriacontanol-1. The main chemical compositions of *J. multifida* extract are presented in Table 6.

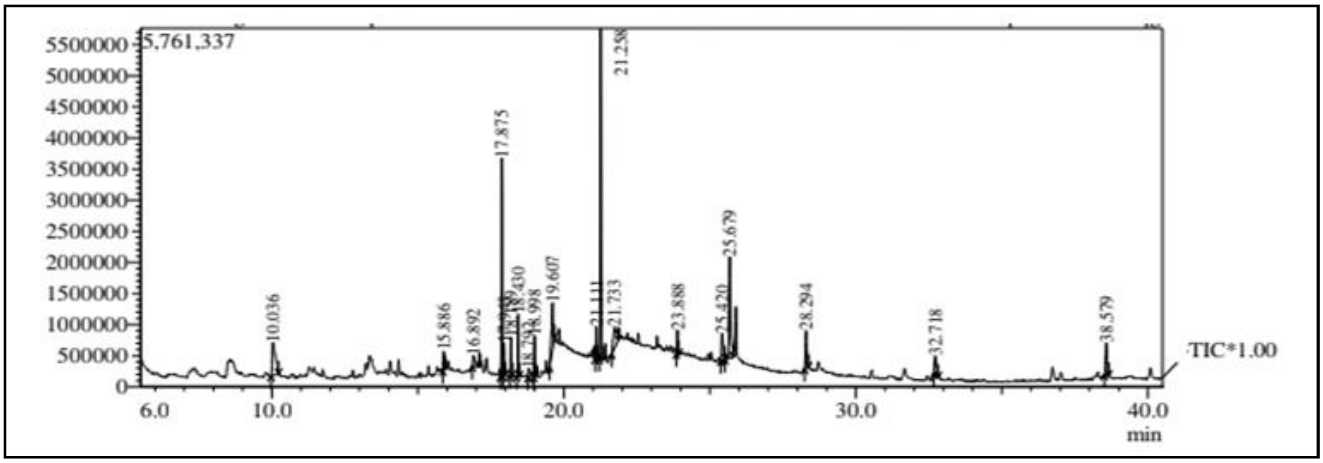


Figure 5: Chromatogram of ethanolic leaf extract of *J. multifida*.

Table 6: Bioactivity of compounds in the gas chromatogram

S. No.	Compound name	R. time (minute)	Area %	Bioactivity
1	2-Methoxy-4-vinylphenol	10.036	8.45	Anti-inflammatory and anticancer effects
2	3-Buten-2-one, 4-(4-hydroxy-2,2, 6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl)-	15.886	1.29	-
3	9-Octadecenoic acid (Z)-	16.892	1.81	Antioxidant, anti-inflammatory, and anticancer properties

4	Neophytadiene	17.875	12.34	Anti-inflammatory, antimicrobial, anticancer, and neuroprotective properties
5.	2-Pentadecanone, 6,10,14-trimethyl-	17.943	2.18	Hypocholesterolemic, anti-inflammatory, antibacterial, anti-nociceptive, antioxidant, and lubricating effects
6.	9-Eicosyne	18.189	2.30	-
7.	Phytol, acetate	18.430	3.86	Antioxidant, anti-inflammatory, and antimicrobial properties
8.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca -6,9-diene-2,8-dione	18.792	1.32	Radical scavenging and anti-inflammatory properties
9.	Hexadecanoic acid, methyl ester	18.998	2.42	Antimicrobial, anti-inflammatory, and antioxidant properties
10.	Hexadecanoic acid	19.607	6.39	Antioxidant, antibacterial, and anti-inflammatory properties
11.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	21.111	2.12	Anti-inflammatory, antidiarrheal, antibacterial, and antileishmanial properties
12.	Phytol	21.258	21.67	Antioxidant, anti-inflammatory, and antimicrobial effects
13.	11-Dodecyn-1-ol acetate	21.733	5.53	Role in insect pheromones
14.	4,8,12,16-Tetramethylheptadecan-4 -olide	23.888	1.55	Antimicrobial, antioxidant, and anticancer properties
15.	Trichloroacetic acid, pentadecyl ester	25.420	3.56	Anti-inflammatory properties
16.	Palmitic acid beta-monoglyceride	25.679	12.10	Role in lipid metabolism, inflammation, and potentially in modulating HIV infection.
17.	1-Heneicosanol	28.294	4.00	Antifungal and potential antidermatophytic properties
18.	N-Hentriacontanol-1	32.718	2.90	Antimalarial, and antibacterial effects
19.	Vitamin E	38.579	4.20	Fat-soluble antioxidant

4. Discussion

In the current study, phytochemical analysis was conducted on extracts from leaves and stems of *J. multifida*, enlightening the presence of various secondary metabolites well-known for their therapeutic potential. These results support with those stated by Agban *et al.* (2020). Qualitative analysis of phytochemicals in the *J. multifida* extracts revealed the presence of amino acids, carbohydrates, saponins, flavonoids, tannin and phenolic compounds. This suggests that the solvent is effective in isolating potential biological compounds owing to their significant polarity. All *J. multifida* extracts revealed the presence of terpenoids. In the pharmaceutical industry, terpenoids such as diterpenes, triterpenes and sesquiterpenes are known for their antibiotic, insecticidal, antithelmintic, and antiseptic properties (Parveen *et al.*, 2010).

Generally, proteins function as antibiotic as well as antimicrobial agents. Plants utilize various defense mechanisms, including the production of small molecular weight antimicrobial proteins, to protect themselves against microbial pathogens. Although the use of *J. multifida* in traditional medicine is well established, there is a lack of researches that supports its application and guarantees its safety in medical use (Daniglayse *et al.*, 2020). Extracts from the *J. multifida* plant contain phytochemicals such as alkaloids, flavonoids, phenols, saponins, tannins, and carbohydrates, which exhibit antimicrobial properties (Gerald and Alfonse, 2023).

Phenolic compounds are aromatic secondary metabolites that contribute to color and flavor while being linked to health benefits, including a lower risk of heart as well as cardiovascular illnesses. Phenolic compounds are primarily responsible for the antioxidant activities found in plants (Aliyu *et al.*, 2009). There is growing evidence that consuming a wide range of phenolic compounds found in natural foods may reduce the risk of serious health issues due to the antioxidant properties of these compounds.

Flavonoids are part of the polyphenolic compound family and are extensively recognized for their health-promoting properties, like anti-allergic, anticancer, anti-inflammatory, antimicrobial and antioxidant properties (Aiyelaagbe and Osamudiamen, 2009). There is a positive relationship among higher intake of flavonoids as well as a reduced threat of cardiovascular and cancer-related illnesses and they are typically found all over the plant kingdom. As stated by Aransiola *et al.* (2014), the antibacterial properties of flavonoid lead to membrane lysis, which consequently causes cell death. Therefore, the significant phytochemical characteristics discovered in the current investigation using extracts from *J. multifida* leaves and stem will be useful in the therapy of a variety of diseases.

From this study, the antimicrobial tests signified that extracts of *J. multifida* were ineffective against *E. coli* strains but showed effectiveness against *Staphylococcus* strains. These results align with those of Gerald and Alfonse (2023), which reported that ethanolic plant extracts were effective against Gram-positive bacteria due to their outer cell membranes being permeable to hydrophobic compounds. On the other hand, Gram-negative bacteria, with their outer membrane made of phospholipids, impede the passage of phytomolecules, predominantly hydrophilic substances (Soundararajan *et al.*, 2012).

The TLC analysis of all plant extracts indicated the presence of various metabolites, including alkaloids, flavonoids, phenols, and tannins. Hidayati and Hardani (2024), noted that out of the four

solvents (chloroform, acetone, methanol, and water), ethanol was the most effective in extracting the greatest number of secondary metabolites. TLC profiling of all extracts in the present study yielded impressive results that suggest the presence of multiple phytochemicals.

Due to the toxicity and high cost of synthetic antioxidants, there has been a growing interest in natural antioxidants within research studies. In this study, the DPPH assay was employed to evaluate the antioxidant potential of plant extracts, indicating that the total antioxidant activity, as assessed by the DPPH method, ranged from 24-84%. For instance, Sultana *et al*. (2009) reported that aqueous extracts of various medicinal plants showed higher antioxidant activity compared to ethanol and methanol extracts. They attributed this to the greater solubility of polar phytochemicals in water. Similarly, Iqbal and Bhanger (2006) demonstrated that leaf extracts contained higher total phenolic content and exhibited stronger radical scavenging activity than stem or seed extracts. A study conducted by Nilima and Hande (2011) found that extracts from all plants showed some capability of scavenging the radical. Antioxidants that show DPPH radical scavenging activity are capable of contributing hydrogen to free radicals, mainly lipid peroxides or hydroperoxide radicals, which are primary agents in the chain autoxidation of lipids, leading to the development of non-radical species and accordingly inhibiting the propagating phase of lipid peroxidation (Bamforth *et al*., 1993).

The TLC bioautography assay provided a direct means of detecting antioxidant-active compounds in *J. multifida*. Distinctive yellow zones against the purple DPPH background, predominantly in the leaf extracts, confirmed the presence of potent free radical scavengers. This finding associate with the high percentage of inhibition recorded in the spectrophotometric DPPH assay, suggesting that the antioxidant potential of the plant is strongly linked with phenolic and flavonoid components. Similar patterns have been reported in other medicinal plants where TLC-DPPH bioautography effectively localized phenolic-rich fractions accountable for antioxidant activity (Sultana *et al*., 2009). The antibacterial bioautography assay further revealed clear inhibition zones on TLC plates, signifying the presence of compounds with antimicrobial activity. The intensity and clarity of these inhibition zones support the hypothesis that *J. multifida* possesses metabolites with therapeutic potential, consistent with its traditional use in treating infections. Similar results were acquired in previous studies with *Punica granatum*,

Acacia senegal, and *Mangifera indica*, where TLC bioautography localized bioactive bands directly on chromatograms (Samrat *et al*., 2016). The combination of chromatographic separation with biological assays strengthens the evidence for the therapeutic potential of this plant and supports the need for further bioassay-guided fractionation and compound characterization.

Utilizing plant extracts as herbal bandages could be an innovative approach, as our findings demonstrate that the leaf extract of *J. multifida* contains specific phytochemical constituents and possesses antioxidant and antibacterial properties. Antimicrobial agents like silver, povidone-iodine, and poly hexamethylene biguanide are sometimes added to dressings to manage or prevent infections. According to Joyjit *et al*. (2020), samples treated with aqueous extracts of *Cynodon dactylon* and *Mikania micrantha* demonstrated notable antimicrobial properties which were signified by prominent zones of inhibition against both Gram negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*). This result was credited to the occurrence of phytochemicals such as alkaloids, flavonoids, steroids, tannins, and saponins in their aqueous extracts.

The GC-MS analysis of *J. multifida* extract showed that the major compounds present include phytol, neophytadiene, palmitic acid beta-monoglyceride, 2 methoxy-4-vinylphenol, hexadecanoic acid, 11-dodecyn-1-ol acetate, vitamin E, and n-hentriacontanol-1. This finding aligns with the assertion made by Asish and Rajpratab (2013), indicating that phytol is a type of diterpenoid and triterpenoid compound frequently found in seaweed with known microbial properties. Triterpenoids are vital in plant defense mechanisms, safeguarding plants from insects and environmental pressures, whether these defenses are naturally present or triggered (Shukla *et al*., 2021). The leaf extract of *J. curcas* revealed major compounds such as hexadecanoic acid, hexadecanoic acid methyl ester, and phytol, which exhibit antifungal activity (Francis *et al*., 2021). The antimicrobial and antioxidant properties documented in the *J. multifida* extract can be credited to the compounds recognized through the GC-MS analysis carried out in the present study.

Plant extracts possess significant promise as antimicrobial agents. The collective impact of antibiotics and plant extracts on resistant bacteria opens up new alternatives for treating infectious diseases. Our research has demonstrated that the crude extract from the leaves and stems of *J. multifida* exhibit phytochemical, antioxidant, and antibacterial properties. This research could be advanced by developing a natural bandage, following toxicity assessments, allergy tests, animal trials, and clinical studies.

5. Conclusion

This study demonstrates that *J. multifida* possesses significant phytochemical, antioxidant, and antibacterial potential. Phytochemical analysis demonstrated the presence of amino acids, flavonoids, carbohydrates, and phenolic components, while DPPH evaluation revealed prominent free radical scavenging action. Antibacterial evaluation with the agar well diffusion technique pointed out that *Staphylococcus aureus* was the most susceptible organism, exhibiting the largest inhibition zones, particularly when exposed to cotton crepe bandages impregnated with ethanolic extract. According to the GC-MS examination report phytol was identified as the major bioactive component. These findings emphasized the potential of *J. multifida* extracts for the developing herbal bandages with antimicrobial and antioxidant potential. Further investigation can be carried out in the nearby future to isolate and characterize the active components and elucidate their mechanism of action.

Acknowledgements

The authors would like to thank Kerala Forest Research Institute, Peechi Trissur, Kerala for performing the GC-MS analysis of our samples in their Institute. No specific funding has been provided for the present study.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Agban, A.; Gbogbo, K.A.E.; Amana, K.; Tegueni, K.; Batawila, K.; Koumaglo, K. and Akpagana, K. (2020). Evaluation des activités antimicrobiennes de *Tridax procumbens* (Asteraceae), *Jatropha multifida* Linn (Euphorbiaceae) et de *Chromolaena odorata* (Asteraceae). Eur. Sci. J., **9**:278-290.

Aiyelaagbe, O.O. and Osamudiamen, P.M. (2009). Phytochemical screening for active compounds in *Mangifera indica*. Plant Sci. Res., **2**:11-13.

Aliyu, A.B.; Musa, A.M.; Ibrahim, M.A.; Ibrahim, H. and Oyewale, A.O. (2009). Preliminary phytochemical screening and antioxidant activity of leaf extract of *Albizia chevalieri* Harms (Leguminoseae-Mimosoideae). Bayero. J. Pure Appl. Sci., **2**(1):149-153.

Aransiola, M.N.; Ehikhase, C.; Mmegwa, J.C. and Wahab, I.O. (2014). Antibacterial and antifungal activities of *Jatropha multifida* (Ogege) sap against some pathogens. IOSR J. Pharm. Biol. Sci., **9**(4):53-57.

Arundevi, R.; Sudhakar, S. and Lipton, A. P. (2010). Assessment of antibacterial activity and detection of small molecules in different parts of *Andrographis paniculata*. J. Theor. Exp. Biol., **6**(34):235-241.

Asish, K. Bhattacharya and Rajpratab Babanrao Kshatriya. (2013). Antimycobacterial agent, (E) - phytol and lauric amide from the plant *Lagascea mollis*. Indian J. Chem., **52**(7):901-903.

Ayuba Yohanna Musa.; Okoro Benedict Chidiogor.; Abubakar Muhammad Nazif and Ilesanmi Esther. (2017). Phytochemical constituents, thin layer chromatography and antimicrobial activity of methanol extract of the stem and leave of *Citrus limon* (L). Int. J. Biochem. Biophys. Mol. Biol., **2** (4):31-35.

Bamforth, C.W.; Muller, R.E. and Walker, M.D. (1993). Oxygen and oxygen radicals in malting and brewing: A review. J. Am. Soc. Brew. Chem., **51**(3):79-88.

Caniça, M.; Mansageiro, V.; Abriouel, H.; Moran-Gilad, J. and Franz, C.M.A.P. (2019). Antibiotic resistance in food borne bacteria. Trends Food Sci. Technol., **84**:41-44.

Chang, C.C.; Yang, M.H.; Wen, H.M. and Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., **10**:178-182.

Daniglayse Santos Vieira.; Fabianny Torres de Oliveira.; Jorge Andrés Garcia Suarez.; Davi Porfírio da Silva.; Thais Honório Lins Bernardol and Maria Lysete de Assis Bastos. (2020). Biological activities: anti-infectious, antioxidant and healing of the vegetable species *Jatropha multifida*. Rev. Bras. Enferm., **74**(2):e20200451.

Durand, Dah-Nouvlessounon.; Michaelle Chokki.; Essé A Agossou.; Jean-Baptiste Houédanou.; Martial Nounagnon.; Haziz Sina.; Romana Vulturar.; Simona Codruta Heghes.; Angela Cozma.; Jacques François Mavoungou.; Adriana Fodor.; Farid Baba-Moussa.; Ramona Suharoschi and Lamine Baba-Moussa. (2023). Polyphenol analysis *via* LC-MS-ESI and potent antioxidant, anti-inflammatory, and antimicrobial activities of *J. multifida* L. extracts used in Benin Pharmacopoeia. Life (Basel). **13**(9):1898.

Francis, M.; Chacha, M.; Ndakidemi, P.A. and Mbega, E. (2021). Phytochemical analysis and *in vitro* antifungal evaluation of *Jatropha curcas* against late leaf spot disease on groundnut. J. Anim. Plant Sci., **47**(1):8358-8371.

Gerald Ekangu and Opio Alfonse. (2023). A comparative study of the effects of *J. multifida* and *Euphorbia hirta* and their mixture on pathogenic growth rate. Eng. Proc., **37**(1):63.

Hidayati, H. D. and Hardani. (2024). Phytochemical screening of secondary metabolite compounds in ethanol extract of castor bean (*Jatropha multifida* L.) leaf. Indones. J. Pharm. Res., **1**(2):61-72.

Iqbal, S. and Bhanger, M. I. (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J. Food Compos. Anal., **19**(6-7):544-551.

Isabelle, A. Kaga and Michael, D. Flythe. (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. J. Vis. Exp., **85**:e51411.

Joyjit, Ghosh.; Redwanul, Islam M.D. and Amit Chakrabortty. (2020). A qualitative analysis of different types of water repellent agent used on cotton fabric. Eur. Sci. J., **16** (6).

Kayode, J. and Omotoyinbo, M.A. (2008). Ethnobotanical utilization and conservation of chewing sticks plants species in Ekiti State, Nigeria. Res. J. Bot., **3(3):**107-115.

Michael, Niyi Aransiola.; Charles, Ehikhasc.; Joy, Mmegwa. C. and Idris, Olayinka Wahab. (2014). Antibacterial and antifungal activities of *Jatropha multifida* (Ogege) sap against some pathogen. IOSR J. Pharm. Biol. Sci., **9**(4):53-57.

Nilima, S. Rajurkar and Hande, S.M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian J. Pharm. Sci., **73**(2):146-51.

Parveen, M.; Ghalib, R.M.; Khanam, Z.; Mehdi, S.H.; Ali, M. (2010). A novel antimicrobial agent from the leaves of *Peltophorum vogelianum* (Benth.). Nat. Prod. Res., **24:**1268-1273.

Prashant, Tiwari.; Bimlesh, Kumr.; Mandeep, Kaur.; Gurpreet, Kaur and Harleen, Kaur. (2011). Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, **1**(1):98-106.

Rajendra, Prasad Gujjeti and Estari, Mamidala. (2013). Phytochemical analysis and TLC profile of *Madhuca indica* inner bark plant extract. Int. J. Sci. Eng. Res., **4** (10):1505-1510.

Samrat, A.V.; Sahiti, K.; Raji, P.; Rohan, B.D.; Kumar, D. and Sharma, K. (2016). TLC bioautography guided identification of antioxidant and antibacterial activity of *Acacia senegal*. Der. Pharmacia. Lettre, **8**(9):41-47.

Shu, M.F.S.; Bingtao, L.; and Gilbert, M.G. (2008). Jatropha. Fl. China, **11**:268-269.

Shukla, Abha.; Choudhary, Anchal.; Shukla, Rishi Kumar and Kaur Amanpreet. (2021). Isolation and identification of two triterpenoids from ethyl acetate extract of bark of *Boehmeria rugulosa*. Res. J. Pharm. Technol., **14**(6):2919-2923.

Siddartha, Baliyan.; Riya, Mukherjee.; Anjali, Priyadarshini.; Arpana, Vibhuti.; Archana, Gupta.; Ramendra, Pati Pandey and Chung-Ming Chang. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules, **27**(4):1326.

Soundararajan, V.; Zuraini, Z.; Yeng, C.; Lachimanan, Y.L.; Jagat, R.K. and Sreenivasan, S. (2012). The Antimicrobial efficacy of *Elaeis guineensis*: Characterization, *in vitro* and *in vivo* studies. Molecules, **17**:4860-4877.

Sultana, B., Anwar, F. and Przybylski, R. (2009). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. Food Chemistry, **104**(3): 11061-114.

Citation **P. B. Sruthy, P.S. Sruthi, R. Senthilkumar, Vaisakh Venu, E.R. Ramdas and Sreeja Puthanpura Sasidharan (2025). Phytochemical, antioxidant and antibacterial effects of *Jatropha multifida* L.. Ann. Phytomed., 14(2):430-439. http://dx.doi.org/10.54085/ap.2025.14.2.41.**