Chettinad Health City Medical Journal
Volume 13, Issue 2 - 2024, Pg. No. 63-68
Peer Reviewed & Open Access Journal

Research Article

Screening of *Justicia simplex* for Skin Lesions: An *In Vitro* Study

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DOI: https://doi.org/10.24321/2278.2044.202430

I N F O

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How to cite this article:
Kumar R P, Chithra S K J. Screening of *Justicia simplex* for Skin Lesions: An *In Vitro* Study. Chettinad Health City Med J. 2024;13(2):63-68.

Date of Submission: 2023-09-06
Date of Acceptance: 2024-04-16

A B S T R A C T

Introduction: Skin diseases demand excessive and continuous use of antibiotics and skin ointments that are expensive for the general public. Treatment in the later stages makes it more complicated and incurable. This would also result in the emergence of resistant strains of microbes, among other side effects. The present study evaluates the wound healing, antibacterial, and antifungal efficacies of the hexane extract of leaves of the medicinal herb *Justicia simplex* and detects the potent compounds.

Materials and Methods: The wound healing efficacy of the extract was evaluated by *in vitro* scratch assay. Antimicrobial activities were studied by the agar well diffusion method. The potent compounds present in the extracts were identified by FTIR analysis. Drug-likeness of the compounds was predicted by employing the SWISSADME web tool and pharmacological activities by the PASS online software.

Results: The study proved the effectiveness of *J. simplex* against pathogenic bacteria and fungi, and its wound healing properties. The bioactive compounds identified were ethyl 6,9,12-hexadecatrienoate, 17-octadecynoic acid, and n-hexadecanoic acid. All the compounds were very likely to exhibit anti-eczematic activity and obey the Lipinski rule of drug-likeness.

Conclusions: The study reveals the synergistic action of various compounds present in *J. simplex* which prompted the use of the plant as a curative agent in folk medicine. The plant can be used as a source for novel drug discovery but requires further investigation.

Keywords: *Justicia simplex*, Antimicrobial, FTIR, n-hexadecanoic Acid

Introduction

Microbes, including bacteria and fungi, are the main agents of skin infections, which are one of the major diseases in humans. The conditions necessitate the immoderate use of antibiotics and other ointments. Many times, people do not seek medical treatment in time due to social stigma. This makes treatment unaffordable, besides the side effects. Additionally, this results in the development of resistant strains of microorganisms. The preparation of natural bioactive products for the treatment of skin diseases would be more affordable and make the treatment easier for the common people.

It has long been believed that plants have therapeutic properties. Traditional healers have applied poultices and ingested infusions made from native plants to prevent or cure infectious conditions. Medicinal plants are rich in potent bioactive compounds and many of them are used as sources of pharmaceutical drugs. Medicinal plants have been reported to have antibacterial, anti-inflammatory, and antioxidant potencies, which are contributed by secondary metabolites.[1] Compound groups, including alkaloids, flavonoids, terpenoids, and tannins, are abundant in plants and have been proven to have activities against many microbes. Such bioactive chemicals can be isolated from plants employing modern techniques and used against antibiotic-resistant and pathogenic microbes. The antimicrobial chemicals from medicinal plants may limit the growth of bacteria, fungi, viruses, and protozoa by processes other than those employed by currently used antimicrobials.[2] Some of these active substances lack the potency of antibiotics on their own, but when combined with antibiotics, they can break the resistance to antibiotics that bacteria have developed. Chemically complex substances are found to have higher therapeutic potential and are likely to cause resistance.[3] Extracts of the plants *Myrtus communis, Cinnamomum tamala, Verbena officinalis, Oxalis corniculata, Artemisia vulgaris,* and *Ageratina adenophora* exhibit antimicrobial activities against various strains of bacteria.[4] Likewise, essential oils were proven to have antimicrobial activity against *Helicobacter pylori* and *Mycoplasma pneumoniae*.[5]

The wound-healing process involves many cellular and biochemical changes. Several synthetic medications are used to speed up the wound healing process, which can speed up the time needed for healing and improve wound healing. Due to their adverse effects, several synthetic medications are subject to restrictions. This also necessitates the discovery and formulation of plant-derived drugs. Medicinal herbs can speed up wound healing through a variety of processes, including promoting blood clotting, preventing infection, accelerating the healing process, etc.[6] *Aloe vera* extract is reported to have the potency to decrease inflammation and to accelerate wound healing process.[7] Eucalyptus is a traditionally used plant for skin care including herpes, wounds, skin infections and insect bites.[8] But it is unlikely that many traditional medicinal plants are still unexplored or sometimes undisclosed by the healers, with the intention of protecting the knowledge and preventing its commercialisation.

The Acanthaceae family includes the genus *Justicia,* and many of its members are said to offer therapeutic benefits. Many species of *Justicia* were reported to have therapeutic properties for the treatment of inflammatory diseases like eczema, rheumatism, lumber pain, and swelling.[9] *J. simplex* D. Don is reported to have many ethnopharmacological uses.[10] We report the use of *Justicia simplex* D. Don as an effective oral folk medicinal plant for the treatment of skin lesions by folk medicine practitioners in Thiruvananthapuram district, Kerala state, India. The preparations were made by mixing the ground slurry of the plant with milk. However, there are no scientific studies regarding its therapeutic application to skin diseases.

The initial steps for the development of new natural pharmaceuticals are scientific evaluation and the identification of the potent compounds in plant extracts. The preliminary identification of powerful chemicals in bioactive plant extracts could be accomplished using spectral analysis techniques. Hexane is a suitable solvent for the extraction of phytocompounds as it has a high evaporation rate and a low boiling point. Phytochemical analysis of the hexane extract of *J. simplex* is reported to have the presence of alkaloids, flavonoids, terpenoids and tannins.[11]

The current study aimed at evaluating the wound healing, antibacterial, and antifungal potencies of hexane extract of the leaves of *J. simplex* and identifying the bioactive compounds.

Materials and Methods

Preparation of the Plant Extract

The leaves of *J. simplex* collected from local areas in Thiruvananthapuram district, Kerala, were shade-dried and ground to powder. The dried leaf powder was extracted with hexane, filtered and concentrated to dryness. Identification of plant species was done by Dr V Viji, Department of Botany, Government College for Women, Thiruvananthapuram. This study was conducted during January–June 2022.

Scratch Wound Healing Assay

The *in vitro* scratch assay was done as per the procedure of Liang et al. with slight modifications, using a RAW cell line (monocyte/ macrophage-like cells) procured from the National Centre for Cell Sciences (NCCS), Pune, India.[12] The

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202430

cells were cultured in DMEM with supplements. The test samples were prepared in cell culture grade DMSO and diluted with DMEM medium at concentrations of 15, 20, and 25 µg/mL, filtered using a 0.2 µm Millipore syringe filter. A 200 µL pipette tip was used to scrape a straight line through the cell monolayer. The control and treated wells were incubated at 37 °C and photographed at intervals.

In Vitro Study of Antibacterial and Antifungal Activities

Antimicrobial properties were studied by the agar-well diffusion method. The microbial inocula were procured from Microbial Type Culture Collection and Gene Bank, (MTCC) Chandigarh, India. Gram-positive *Streptococcus pyogenes* (MTCC 442) and gram-negative *Escherichia coli* (MTCC 443) bacterial strains were used. The fungal strains used were *Aspergillus niger* (MTCC 872) and *Candida albicans* (MTCC 227). The test samples (25, 50 and 100 µL) were prepared from 10 mg/mL sample stock. Gentamycin and clotrimazole were used as positive controls for antibacterial and antifungal activities, respectively. The solvent used for sample dilution was the negative control. Bacterial plates were incubated for 24 h at 36 °C ± 1 °C, under aerobic conditions. The fungal plates were incubated for 24 h at 27 °C ± 1 °C, under aerobic conditions. The diameter of the clear zone of inhibition was measured against the test culture.

Identification of Bioactive Compounds

Identification of bioactive compounds present in the extract was done by FTIR analysis followed by a literature review.

Drug-Likeness and Prediction of Pharmacological Activities of the Compounds

Physicochemical properties and drug-likeness of the identified compounds were predicted using SWISSADME.[13] ChemDraw MDL Molfile (MOL) formats of the compounds were used to predict the pharmacological activities using PASS online software.[14]

Results

Wound Healing Activity

The wound healing efficiency was found to be concentration and time-dependent. At a concentration of 25 µg/mL, the wound closure was completed at 36 h of treatment (Figure 1).

In Vitro Study of Antimicrobial Assay

The hexane extract of leaves of *J. simplex* exhibited growth inhibitory activity against the two strains of pathogenic bacteria and the pathogenic fungus *C. albicans* tested based on their concentration gradient, MIC value of 100 µL (Table 1 and Figure 2).

Identification of Bioactive Molecules

FTIR peaks from 1665 to 1675 cm^{-1} indicate C=C stretching of alkene, the peak at 2848 cm^{-1} indicates OH stretching of carboxylic acids, and the peak at 1440 cm^{-1} indicates OH bending of carboxylic acids (Figure 3). The FTIR peaks and their comparison with previous literature[15] indicate the presence of the compounds ethyl 6,9,12-hexadecatrienoate ($C_{18}H_{30}O_2$, mass 278.22), 17-octadecynoic acid ($C_{18}H_{32}O_2$, mass 280.24) and n-hexadecanoic acid ($C_{16}H_{32}O_2$, mass 256.24).

Physicochemical Properties and Drug Likeness of the Identified Compounds

The SWISSADME analysis showed the molecular weight of ethyl 6,9,12-hexadecatrienoate as 278.43 g/mol, molar refractivity 88.50 and TPSA 26.30 Å. The molecular weight of 17-octadecynoic acid was seen to be 280.45 g/mol, molar refractivity 88.57 and TPSA 37.30 Å. The molecular weight of n-hexadecanoic acid was 256.42 g/mol, molar refractivity was 80.80 and TPSA was 37.30 Å. All the compounds obey the Lipinski rule of drug-likeness. All the compounds were predicted to have anti-inflammatory anti-eczematic activities as their probability of being active (Pa) is higher than the probability of being inactive (Pi) (Table 2).

Table 1.Antibacterial and Antifungal Activities of Hexane Extract of *J. simplex* Leaves

Microbial Strains		Inhibition Zone (mm)				
		Negative Control	Positive Control	Concentration		
				25 µL	50 µL	100 µL
Bacteria	*Streptococcus pyogenes*	-	29.00 ± 0.81	-	-	9.00 ± 0.81
	Escherichia coli	-	25.00 ± 0.81	-	-	15.33 ± 1.24
Fungi	*Aspergillus niger*	-	17.67 ± 1.24	-	-	-
	Candida albicans	-	23.67 ± 1.24	-	-	10.67 ± 0.58

- No activity, Values are mean ± SD of three replicates.

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202430

Table 2.Pass Analysis of the Phytocompounds Present in the Hexane Extract of *J. simplex*

Compounds	Anti-inflammatory		Anti-eczematic	
	Pa	Pi	Pa	Pi
Ethyl 6,9,12-hexadecatrienoate	0.764	0.009	0.963	0.002
17-octadecynoic acid	0.522	0.051	0.872	0.007
n-hexadecanoic acid	0.515	0.052	0.920	0.004

Pa: Pharmacological Activity, Pi: Pharmacological Inactivity

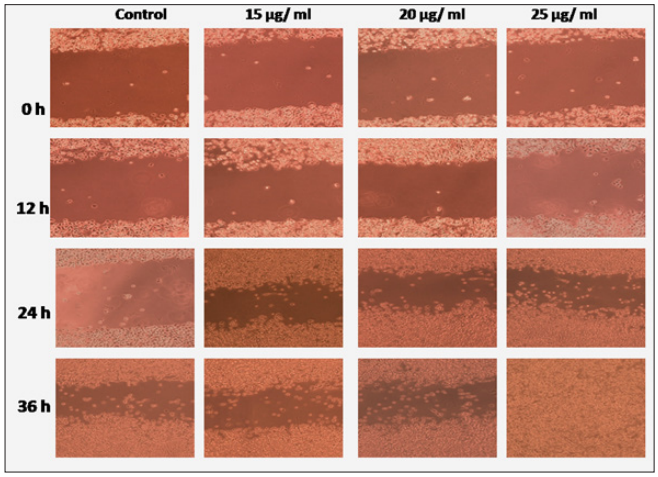

Figure 1.Scratch Wound Healing Assay Showing the Effect of Hexane Extract of Leaves of *J. simplex* on RAW Cells at Various Concentrations

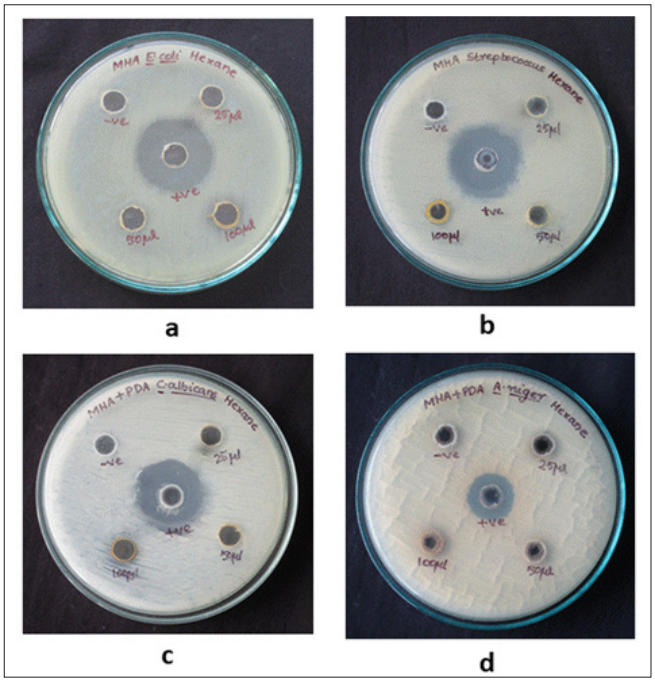

Figure 2.Antimicrobial Activity of Hexane Extract of Leaves of *J. simplex* (a). *E. coli*, (b). *S. pyogenes*, (c). *C. albicans*, (d). *A. niger*

Discussion

The present study proves the effectiveness of *J. simplex* against pathogenic microbes and its wound-healing activity. The study evaluates the use of *J. simplex* as a folk medicine for skin infections. The wound healing assay allows the investigation of cell-to-cell communication and mimics the movement of cells *in vivo*. A thin scratch is placed into a confluent monolayer of cells in the wound assay to detect cell migration *in vitro*. At the wound's edge, cells polarise and move inside the wound. The result of the study indicates the presence of phytocompounds in the hexane extract of *J. simplex* that can induce cell proliferation and migration. Curcumin, N-acetyl cysteine, quercetin and chitosan are antioxidant compounds that have been proven to enhance wound healing activity in *in vitro* and *in vivo* studies.[16]

Fungi and bacteria are the major agents of skin infections and become resistant to antibiotics and antifungal drugs, respectively. In the present study, the hexane extract of *J. simplex* showed antimicrobial activity against bacteria and fungi. The microbes investigated in the study are reported to cause superinfections in COVID-19 patients.[17] The hexane extract was shown to have antifungal activity against the Candida species. It is one of the most prevalent species causing skin infections.[18] These observations substantiate the traditional use of *J. simplex* as a therapeutic agent for skin diseases.

Identification of phytocompounds in the bioactive extract is an important step in herbal therapy. The compounds identified from the hexane extract of *J. simplex* are esterified compounds of the fatty acids decanoic acid and decenoic acid. Antibacterial and antifungal efficacies of water-soluble decanoic acid formulations were proved.[19] Topical application of the fatty acid palmitoleic acid has been found to hasten the wound healing process.[20] Fatty acids are also important in diminishing various inflammatory skin diseases.[21] Plants possess metabolic diversity and can synthesise structural derivatives of a compound, thereby increasing their bioactivities. These studies show that the hexane extract of *J. simplex* is a compound composition of biologically active compounds imparting synergistic effects that form the basis of the plant's usage as folk medicine. The plant has been used as an oral drug by traditional practitioners for a long time, indicating its nontoxicity. For drug-likeness, TPSA should be between 20 and 130 Å.[13] The TPSA of the compounds in the present study ranges between 26 and 38 Å indicating the fitness of individual compounds to be bioactive.

Conclusion

The present study reveals the synergistic action of various compounds present in *J. simplex*, which prompted the use

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202430

of the plant as a curative agent in folk medicine and should have long-lasting effects as a natural product. The study was conducted using hexane extract of *J. simplex*, which is a non-polar solvent. By employing more spectral and other analytic methods, more potent compounds could be characterised from the extract. Also, polar solvents like ethyl acetate, methanol and ethanol would elute more phytocompounds with higher potencies. We conclude the plant can be used as a source for novel drug discovery but requires further investigations, including the isolation of the active molecules, their bioassays, chemical modifications to augment the activities and their synthesis.

Acknowledgements

The authors are very grateful to the Department of Zoology, Government College for Women, Thiruvananthapuram, Kerala, India, for providing laboratory facilities for the completion of the research work. The authors are thankful to the Department of Chemistry, Government College for Women, Thiruvananthapuram and Athmic Biotech Solutions Pvt. Ltd. R&D Laboratory, Thiruvananthapuram, for providing laboratory facilities for the completion of the study.

Source of Funding: None

Conflict of Interest: None

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ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202430