Journal of Pharmaceutical Research International

33(44B): 103-113, 2021; Article no.JPRI.74573
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Compounds from *Vernonia arborea* Buch.-Ham. Inhibit Microbes that Impair Wound Healing

Lalitha Vaidyanathan[1*] and T. Sivaswamy Lokeswari[2]

[1]Department of Biomedical Sciences, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
[2]Department of Biotechnology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i44B32655
Editor(s):
(1) Dr. Sawadogo Wamtinga Richard, Ministry of Higher Education, Scientific Research and Innovation, Burkina Faso.
Reviewers:
(1) Mohammed Gamal Mahmoud Mohammed, Beni-Suef University, Egypt.
(2) Manuel Alejandro Melendez Jimenenz, Universidad Central de Venezuela, Venezuela.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74573

Original Research Article

Received 10 July 2021
Accepted 20 September 2021
Published 21 September 2021

ABSTRACT

Aims: To identify the antimicrobial potency of the leaf fractions of *Vernonia arborea* against selected wound microbes viz., *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Stenotrophomonas maltophilia*.
Background: Wound healing is often delayed due to the presence of polymicrobial load, that have to be abolished to facilitate the healing process. A major class of antimicrobial phytocompound reported to occur in *Vernonia arborea* species include sesquiterpenes. Reports on the wound healing potency of *V. arborea* in wound models of Wistar rats however did not report antimicrobial activity of the aqueous or methanolic extracts.
Methodology: The column fractions of the hexane leaf extract were tested against the selected strains by agar well diffusion assay and the zone of inhibition confirmed with TLC bioautography at specific Rf. The minimum inhibitory concentration (MIC) of the bioactive fractions was identified using resazurin microtiter assay (REMA) and the minimum bactericidal concentration (MBC) was determined. HPTLC quantification was also performed.
Results: Out of the 30 pooled fractions, six showed antimicrobial potency against all the five tested wound microbes. The minimum inhibitory concentrations of these fractions were determined,

**Corresponding author: E-mail: lalithav@sriramachandra.edu.in;*

ranging from 15.62 µg/mL to 500 µg/mL for the different microbes. Quantitative High-Performance Thin Layer Chromatography (HPTLC) revealed two compounds (a and b) in the bioactive fraction10 with yields of 633 mg (63%) and 97 mg (9.7%) per gram of the extract.
Conclusion: The findings suggest the potential use of the bioactive compound in chronic infectious wound management therapy.

Keywords: Vernonia arborea; Wound microbes; Minimum inhibitory concentration; HPTLC.

1. INTRODUCTION

Vernonia arborea, the tree species of the family Asteraceae is found in Western Ghats of India and Sri Lanka [1]. Apart from the traditional decoctions used for wound healing, the methanol and aqueous leaf extracts of this species showed wound healing activity in excision, incision and dead space wound models of Wistar rats [1-5].

Wounds require a complex repertoire of cellular events to heal. The major reason for impairment in wound healing in most cases has been wound infection caused by a poly-microbial community [6]. Some of these opportunistic strains maybe resistant to regular antibiotics while the others impact the wound microenvironment by producing digestive enzymes like proteases and hyaluronidase, contributing to the increasing surface area and depth of the wound [7].

In the ordered series of events, the early onset and resolution of wound inflammation is a kick start for the actual healing process, apparently delayed by the microbial load at the wound site. Notwithstanding a wide range of the microbes in the polymicrobial community, the most commonly found microbes in clinical cases were chosen for this study, *viz*., *Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Klebsiella pneumoniae (ATCC 27736)* and *Stenotrophomonas maltophilia (ATCC 13637)* [8].

Among the various classes of phytocompounds, sesquiterpenes showed promising antimicrobial potency and happens to be a major phytocompound reported to occur in *V. arborea* species [1,2]. Sesquiterpenes from other plant species like, Cnicin, Cynaropicrin, Ferusinol, Secodesma showed activity against *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae* respectively [9,10].

Guaianolide and Germacranolide class of sesquiterpene lactones were found active against *S. aureus* [11]. Isabelin, a sesquiterpene lactone was reported to be active against *S. maltophilia* [12].

Reports on the wound healing potency of *V. arborea* in wound models of Wistar rats, prompted us to investigate the antimicrobial activity of hexane leaf extracts [1,3,5]. Zaluzanin D, a sesquiterpene from the hexane leaf extract of *V. arborea* was reported to possess anti-fungal activity [10,12]. Considering the polymicrobial community colonising a wound and impairing the healing process, novel compounds active against a wide range of wound microbes are vital in wound management [7-10,13,14].

In this study we report fractionation of V. *arborea* leaf extracts, the HPTLC profiles and the bioactivity of selected fractions against the five wound microbes using agar well plate assays, the minimum inhibitory and bactericidal concentrations.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The *Vernonia arborea* leaves were collected from Anaimalai Hills, Pollachi District (10°22′N 77°07.5′E) in the month of October. The collected material was identified and authenticated and a voucher specimen maintained in the department (PARC/2012/1392).

2.2 Extraction and Column Fractionation of the Crude Extract

The dried, ground leaf material (1 kg) was extracted by exhaustive percolation for three days in *n*-hexane with intermittent stirring for every 18 hours at room temperature. The extract was concentrated in rotary vacuum evaporator (IKA Werke ® HB4 Basic, Germany) and yielded 29.68 g of the crude hexane leaf extract (HLE). Five grams of the HLE was chromatographed over a silica gel column in an eluotropic series

with hexane and ethylacetate [9]. The pooled elutions were concentrated on a rotary vacuum evaporator and stored in a cool dry place in a glass container for further analysis [1,2,15-18].

2.3 Antimicrobial Activity of the Column Fractions

The column fractions were tested against five wound microbes to screen for their antimicrobial activity using agar well diffusion assay. The wound microbes tested include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* [19,20].

Thin layers of (1% Muller Hinton) agar seeded with the test strains (0.5 MacFarland Standard concentrations) were added along with the indicator, tetrazolium chloride (0.1%) [18]. The column fractions were added to the agar wells at 1000 µg concentration in triplicates. Ampicillin at the inhibitory concentrations for the respective bacteria was used as positive control. Ampicillin has been studied to inhibit the growth of the test strains by mechanisms including induction of cell wall damage by action on autolysin inhibitor, inhibition of cell wall penicillin-binding proteins and β-lactamase inhibition [19-22]. After incubation for 18 h at 37 ºC, the mean diameter of inhibition zones as (mm) ± SD were determined. Reproducibility of these assays was tested at least five times. The fractions that had antimicrobial activity against all of the five bacterial strains were selected for further analysis.

2.3.1 TLC bioautography

TLC of the bioactive fractions were developed and bioautography was performed by overlaying agar containing the test organism on the developed chromatogram. The zone of inhibitions at the specific Rf were observed.

2.3.2 MIC microbroth dilution method

The minimum inhibitory concentration (MIC) of the selected bioactive fractions were determined using resazurin microtiter assay (REMA) plate method individually against all the five bacterial strains as per the standard reference method (CLSI 2000). The selected bioactive fractions were serially diluted from 1mg/mL concentration with 100 µL of uninoculated broth in the microtiter plate. Ten microlitres of the standard inoculum (1:100 dilution of the McFarland standard) was added and incubated overnight. After overnight incubation, 30 µL of 0.01% resazurin was added to all the wells and observed for color change after one hour of incubation. Uninoculated broth control, inoculated broth control and with ampicillin drug (at inhibitory concentration for the respective strain) were controls used for comparison. The MIC was identified to be the least concentration of the fraction that showed absence of growth (i.e., inhibition) indicated by no change in color of the dye. Growth was seen as change from purple to pink color due to reduction of the indicator dye [15,16]. The assay was independently performed twice for reproducibility and duplicates for each concentration were maintained.

2.3.3 Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) of the fractions were determined by plating the sample from the REMA plates after determining the MIC through visual observation. A loop full of the suspension from the wells at which MIC was noted and with no apparent color change was streaked onto Muller Hinton agar plates and incubated overnight. The concentration of each fraction inhibiting 99.9% of bacterial growth (yielding no bacterial colonies) on the solid medium was taken as MBC.

2.4 HPTLC Quantification

Quantification of the F10 fraction using the photo-densitometric HPTLC method with a stock solution of the column purified compound (5 mg/mL) dissolved in chloroform was done. A CAMAG Automatic TLC Sampler 4 (ATS4) was used to spot (2 µl, 4 µl, 6 µl and 8 µl) and developed using hexane: ethylacetate (95:5, v/v) and derivatized with methanol: sulphuric acid (90:10, v/v) reagent and chromatograms scanned at 254 nm. The hexane crude leaf extract (5 mg/mL) was (4 µl and 6 µl) spotted on the HPTLC plates precoated with Si-gel Si60F254 (E. Merck). The calibration curve for linearity was obtained by plotting peak area vs. concentration of compound (ng/spot). The data analysed by the linear least square regression method was used to estimate the percentage of the compound (F10) in the HLE of *V. arborea* sample. Limit of detection and limit of quantification were estimated statistically for three determinations of the sample [19,23-27].

3. RESULTS AND DISCUSSION

Wound microbes contribute to major impairment in healing process, altering the wound

microenvironment by prolonging inflammation, inducing excessive exudate formation, digesting wound tissue with extracellular enzymes, exacerbating devascularization caused by the wound, impeding re-epithelialization [28,29]. Among the listed mechanisms, prolonged inflammation is the primary stumbling block that expedites the repertoire of the other challenging events. Chronic inflammation due to polymicrobial wound infection, is characterised by increased expression of proinflammatory mediators, altering the infiltration of inflammatory cells like neutrophils and macrophages, secretion of tissue degrading enzymes like protease and hyaluronidase. The early resolution of inflammation is an indispensable factor to progress with the actual healing phase, including reepithelialisation and tissue remodelling [30,31].

Hence, clinical wound management predominantly entails destruction of the microbial load. Though wound management strategies including mechanical debridement and regular dressing of the wound microenvironment attempts to clear the necrotized, avascular tissue with exudates and bacterial load, wound palliation in immunocompromised patients warrants the need for a topical exogenous drug that can keep a check on the wound microbes and accelerate the healing process as well. Drugs which are claimed to possess wound healing activity fail to do so either due to the lack of inherent antimicrobial property or due to surging resistance to antibiotic agents among the wound microbes [32-35].

3.1 Antimicrobial Activity of the Column Fractions

The 790 elutions obtained were grouped based on their TLC characteristics. They were pooled into 30 fractions and tested for antimicrobial activity in agar well assays.

3.1.1 Agar well diffusion assay

The 30 fractions tested (at 1mg/mL) against the wound microbes, showed varying inhibition zones as compared to the standard ampicillin drug at inhibitory concentrations for the respective microbe. Six of them showed activity against all five or four bacteria with over 10 mm diameter of zones [Table 1, Fig. 1].

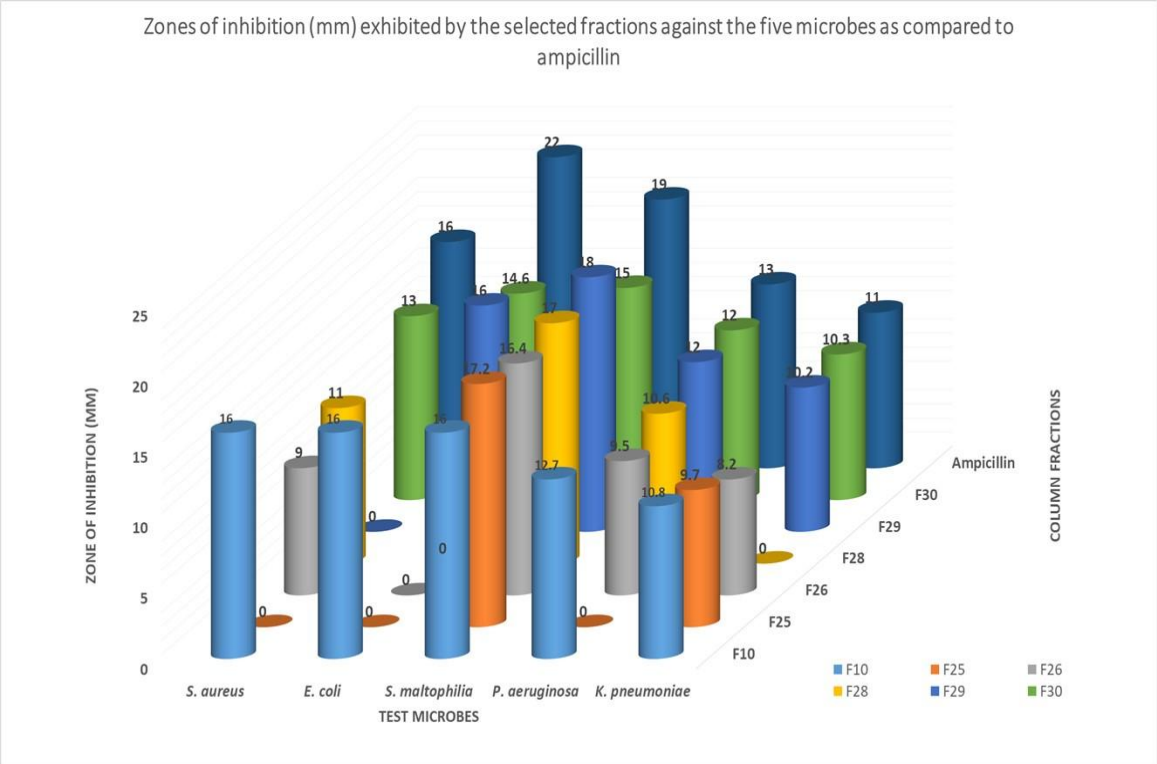

Fig. 1. Zones of inhibition seen in agar well bioassay of the bacteria tested with 5 fractions of *V. arborea* leaf extracts and ampicillin. Fractions 10 and 30 inhibited all the tested bacteria

Table 1. Antibiosis of the six selected fractions tested at 1mg/ml concentration recorded in the agar well plate assay against wound microbes

Selected column fractions	Zone of inhibition (mm) of selected bacterial strains				
	S. aureus	***E. coli***	***S. maltophilia***	***P. aeruginosa***	***K. pneumoniae***
Ampicillin tested at 50µg or 75µg	16	22	19	13	11
F10	16	16	16	12.7	10.8
F25	0	0	17.2	0	9.7
F26	9	0	16.4	9.5	8.2
F28	11	0	17	10.6	0
F29	0	16	18	12	10.2
F30	13	14.6	15	12	10.3

Triplicates for each fraction and organisms were tested and the values are mean readings. Over 34 fractions were assayed for antibacterial activity against the five bacteria in agar well plate assays. Six of the pooled fractions chosen for agar well plate assay were active against two or more bacteria with zones equal to that obtained with ampicillin.

Among them, fractions F10 and F30 inhibited all the five bacterial species with zones of inhibition > 10 mm. F25 inhibited two of the test strains while F26 inhibited all bacteria except *E. coli* with moderate inhibition zones. F29 fraction showed two compounds, while one did not inhibit any of the bacteria, the other showed inhibition against four of the bacteria. We therefore selected F10 and F30 fractions which showed promising ability to inhibit all the tested bacterial species. A TLC bioautography overlay assay was also used to identify the Rf of these two fractions that were active against all five microbes [Fig. 2].

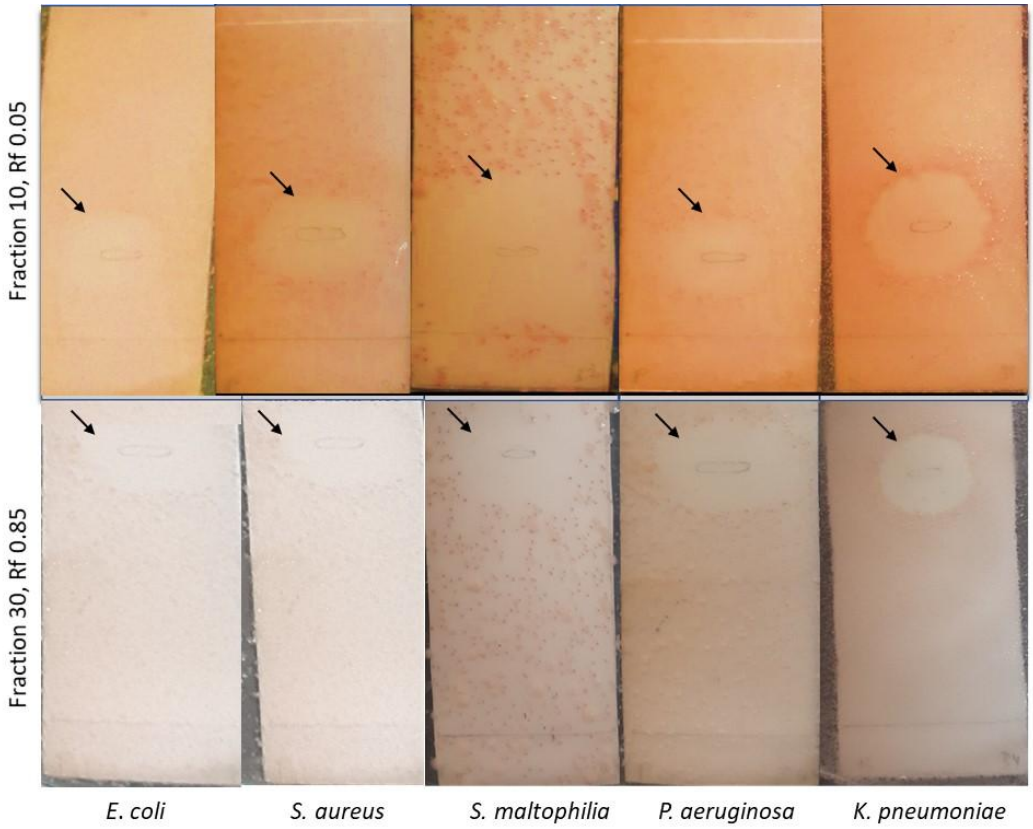

Fig. 2. Bioautography of fraction 10 and 30 overlayed with five wound microbes showing zones of inhibition at Rf 0.05 and 0.85 respectively

3.1.2 Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentrations of the six fractions were determined using resazurin dye reduction method and recorded as shown in Table 2.

As shown in Fig. 3, F10 showed an MIC ranging from 31.5 µg/mL against *S. aureus* and *K. pneumoniae* to 125 µg ug/ mL against *P. aeruginosa*. Fraction 30 was also effective with MICs ranging from 15.62 µg/ mL against *S. maltophila* to 125 µg/mL against *P. aeruginosa*. Other fractions showed higher MICs against the five bacteria tested. Among the bacteria, the most resistant was *P. aeruginosa*. The MBC also supports the same conclusions. Fraction 10 showed MBC at 31.5 µg/mL against *S. aureus* and *K. pneumoniae*. The other fractions showed a slightly higher MBC.

Leaf extracts from *Vernonia cinerea* have been reported to show approximately 10, 13, 12, 12 mm zone of inhibition in agar well diffusion assay and MICs ranging from 2-6, 3-6, 3.5-6, 3-12 mg/mL against *S. aureus, E. coli, K. pneumoniae and P. aeruginosa*, respectively [36,37]. The compound vernolide from *Vernonia amydalina* showed a maximum activity of 19 mm inhibition zone against *S. aureus* in agar well diffusion assay [38-40]. Hexane leaf extract from *Vernonia colorata* showed 13 mm inhibition zone and 3.12 mg/mL MIC against *S. aureus* [41]. The extract inhibited *P. aeruginosa* at 25 mg/mL. Hexane leaf extracts of *Vernonia tenoreana* showed 11, 12.3, 16.3, 18.3 mm zone of inhibition and 15, 20, 10, 20 mg/mL MIC against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, respectively. In this study the results obtained from Fraction 10 are better than most of the other species studied earlier [42-45].

Several phytocompounds from the Genus *Vernonia* have been demonstrated to be antimicrobial in earlier studies. Vernolide from *Vernonia galamensis* was found to be potent against *S. aureus*, *E. coli*, *B. subtilis*, *K. pneumoniae* and *Proteus mirabilis*, with the maximum inhibition zone being 17 mm against *S. aureus*. This is comparable to the action of the phytocompound isolated from *Vernonia arborea* (16 mm) against *S. aureus* in the present study. Chondrillasterol isolated from acetone leaf extract of *Vernonia adoensis* is reported to be very effective against *P. aeruginosa*, with an MIC of 16 mg/mL, while this study reports a several fold lower MIC of 125 µg/mL. The compound Vernolide from the species *V. galamensis* was reported to be effective against *Shigella boydii* and *Salmonella typhi* with maximum inhibition zones of about 25-28 mm; studies against wound microbes are not reported though. There are no distinct reports on antimicrobial activity reported from the species of Vernonia against the burn wound microbe, *S. maltophilia* (16 mm inhibition and 62.2 µg/ml MIC) considered in this study. The crude extracts of the material from the tree *Vernonia* have also been reported to be active against a number of pathogens, the most predominant being the chloroform stem extract of *Vernonia schimperi* showing 38 mm zone of inhibition against *E. coli* [46-48].

Six selected samples of the 30 column fractions of the leaf extract showed activity at their minimum inhibitory and bactericidal concentrations as comparable with the standard antibiotic, ampicillin [49,50]. A few of these samples were found to be mixture of two phytocompounds thus leaving behind a possibility of synergistic role in inhibiting or killing the bacteria.

3.2 Quantification of the Partially Purified Bioactive Fraction in HLE by HPTLC Analysis

The partially purified column fraction, F10 which showed good activity (in terms of MIC and MBC) was selected as the standard for HPTLC analysis and the quantity of the same in the HLE was deduced. The densitogram in Fig. 4 showed two prominent peaks in the putative standard and the HLE with the linear regression correlation shown for the two peaks (a and b) [38]. From the regression equation, the compounds a and b were quantified to be 633mg/g (63%) and 97mg/g (9.7%) of the HLE respectively [Table 3] [26-27,51].

The quantification of the bioactive compounds in HLE revealed good yield from the leaf material. Further structural elucidation would open way to QSAR studies and understanding the class of phytoconstituent(s) that could be a lead compound to develop an effective drug. The fraction with potent anti-wound microbial activity could be an effective constituent of medications used in topical management of infectious wounds and is the future objective of this study.

Table 2. Minimum inhibitory concentration of the column fractions tested against wound microbes

Wound microbe	MIC (µg/mL) of fractions					
	F10	F25	F26	F28	F29	F30
Staphylococcus aureus	31.5	250	250	31.5	31.5	31.5
Escherichia coli	62.2	250	250	125	125	62.2
Stenotrophomonas maltophilia	62.2	250	250	125	125	15.62
Pseudomonas aeruginosa	125	500	500	125	250	125
Klebsiella pneumoniae	31.5	125	125	125	250	31.5

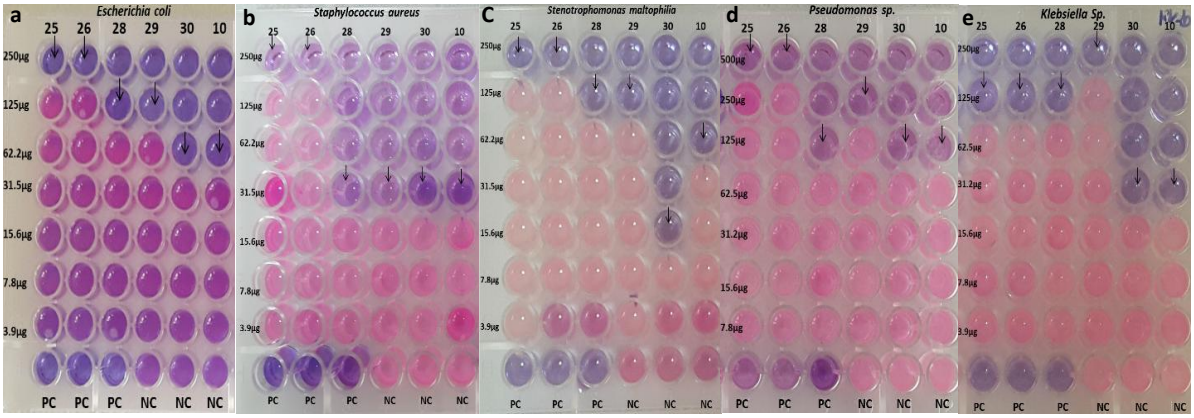

Fig. 3. Resazurin microtiter assay plates showing MICs of the fractions 10 and 30 tested against the wound microbes. a, *E. coli* (62.2 µg/mL) ; b, *S. aureus* (31.5 µg/mL); c, *S. maltophilia* (62.2 and 15.6 µg/mL); d, *P. aeruginosa* (125 µg/mL) and e, *K. pneunoniae* (31.2 µg/mL)

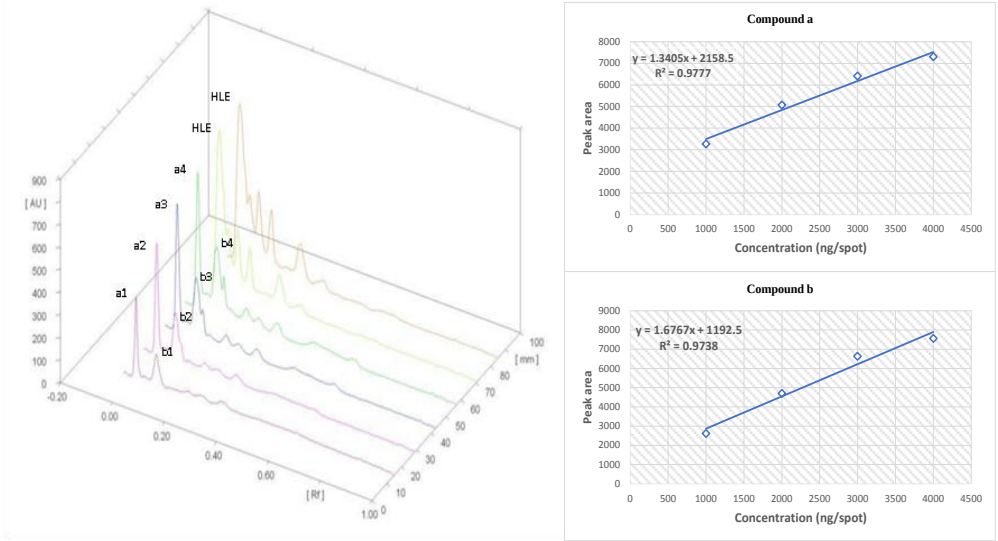

Fig. 4. HPTLC densitogram of the standard (F10, Left) at various concentration ranges and the hexane leaf extract (HLE) scanned under optimum chromatographic conditions at 254 nm. Right, calibration plots of concentration (ng/spot) vs peak area for compound a and b

Table 3. Statistical parameters from the calibration curve for two compounds in F10 and the interpolated amount of bioactive compounds a and b in hexane leaf extract (HLE) using data from HPTLC densitograms

Calibration parameters	Compound a	Compound b
Number of standard, n (F10)	4	4
Linearity range	1000-4000 ng/spot	1000-4000 ng/spot
Peak area range	3000-8000	2000-8000
Mean peak area	5509.75	5384.25
Regression equation	Y=1.3405x + 2158.5	Y=1.6767x + 1192.5
Correlation coefficient	0.9777	0.9738
LOD	1.92µg	1.51µg
LOQ	5.84µg	4.58µg
Concentration of HLE/spot	20µg/µl	20µg/µl
Amount/g HLE	633mg	97mg
% recovery	63%	9.7%

4. CONCLUSION

The hexane leaf extracts were fractionated with at least two fractions being antimicrobial against the five wound microbes tested in this study. Fractions 10 showed MICs of 31.5-125 µg/mL while fraction 30 showed a low MIC of 15.62 µg/mL against *S. maltophilia.* Wound healing by methanol and aqueous leaf extracts of this species in wound models of Wistar rats were reported. The antibiosis against the poly-microbial community impairing wound healing was however not reported and this study demonstrated this in the hexane leaf extracts for the first time.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors acknowledge the support from "VELS INSTITUTE OF SCIENCE, TECHNOLOGY & ADVANCED STUDIES (VISTAS), CHENNAI" for HPTLC analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eloy R, Towers GHN, Mitchell JC. Biological activities of sesquiterpene lactones. Phytochem. 1976;15:1573-80.
2. Krishna Kumari GN, Masilamani S, Ganesh MR, Aravind S, Sridhar SR, Zaluzanin D. A fungistatic sesquiterpene from *Vernonia arborea*. Fitoterapia. 2003a;74:479-82.
3. Krishna Kumari GN, Masilamani S, Ganesh MR, Aravind S. Microbial transformation of Zaluzanin D. Phytochem. 2003b;62:1101-04.
4. Pradhan D, Panda PK, Tripathy G. Wound healing activity of aqueous and methanolic bark extracts of Vernonia arborea Buch. - Ham. In Wistar rats. Nat Pro Rad. 2009;8(1):6-11.
5. Tom JM, Zeinab AB, William GP, Samuel BJ. Systemic implications of flavonoids and sesquiterpene lactones in species of Vernonia. Biochem Syst Ecol. 1975;2:185-192.
6. Bowler PG, Duerden BI and Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev. 2001;14(2):244-69.
7. Sara McCarty M, Cochrane CA, Clegg PD and Percival SL. The role of endogenous and exogenous enzymes in common

wounds: a focus on the implications of aberrant levels of both host and bacterial proteases in wound healing. Wound Repair Regen. 2007;20(2):125-36.

8. Narendra N, Gaurav P, Lokesh D, Naveen K. Wound healing activity of latex of *Calotropis gigantea*. Int J Pharm Pharm Sci. 2009;1(1):34-35.
9. Bachelier A, Mayer R and Klein CD. Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA. Bioorg Med Chem Lett. 2006;16(21):5605-09.
10. Awouafack MD, Tane P, Kuete V and Eloff JN. Sesquiterpenes from the medicinal plants of Africa. Med Plant Res. 2013; Available:101016/B978-0-12-405927-6.00002-3
11. Cartagena E, Montanaro S and Bardon A. Improvement of antibacterial activity of sesquiterpene lactones. Revista Latinoamericana de Quimica. 2013;36(1):43-51.
12. Molinaro F, Tyc O, Beekwilder J, Cankar K, Bertea CM, Negre M, et al. The effect of isabelin, a sesquiterpene lactone from *Ambrosia artemisiifolia* on soil microorganisms and human pathogens. FEMS Microbiol Lett. 2018;365(4):1-4.
13. Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ, Jagadeesh Singh SD. Evaluation of wound-healing potency of *Vernonia arborea*. Indian J Pharmacol. 2005;37(4):223-26.
14. Velnar, T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37:1528-42.
15. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *In vitro* antibacterial screening of phytochemicals. Methods. 2007;42:321-24.
16. Sephra NR. Multiple applications of alamar blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors. 2012;12(9):12347-60.
17. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plant extracts. Afr J Tradit Complement Altern Med. 2010;8(1):1-10.
18. Elaine Souza-Fagundes M, Ana Queiroz BR, Olindo Assis Martins Filho, Giovanni Gazzinelli, Rodrigo Correa-Oliveria, Tania Alves MA, et al. Screening and Fractionation of plant extracts with antiproliferative activity on human peripheral blood mononuclear cells, Memorias do Instituto Oswaldo Cruz. 2002;97(8):1207-12.
19. Lin WP, Wang JT, Chang SC, et al. The antimicrobial susceptibility of Klebsiella pneumoniae from community settings in Taiwan, a Trend Analysis. Sci Rep. 2016;6:1-11.
20. Guliĭ OI, Markina LN, Ignatov OV, et al. The effect of ampicillin on the electrophysical properties of Escherichia coli cells. Mikrobiologiia. 2005;74(1):126-31.
21. Brooke JS. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clin Microbiol Rev. 2012;25(1):2-41.
22. Das RR, Kabra SK and Singh M. Treatment of pseudomonas and staphylococcus bronchopulmonary infection in patients with cystic fibrosis. 2013;1-13.
23. Mulugeta KA, Bayeh AB. Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. Tanzan J Health Res. 2011;13(4):31-40.
24. Solanki R, Nagori BP. A review on microorganisms causing wound infections on skin. Asian J Pharm Technol. 2013;3(3):121-124.
25. Isabelle Kagan A, Michael Flythe D. Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. J Vis Exp. 2014;85:51411.
26. Dolowy M, Kulpinska-Kucia K and Pyka A. Validation of thin-layer chromatography for the determination of hydrocortisone acetate and lidocaine in a pharmaceutical preparation. Scientific World Journal. 2014;Article ID 107879.
27. Varkey IC and Kasthuri GM. HPTLC analysis of stem bark extracts of Terminalia chebula Retz. For alkaloid profile. Asian J Biochem. 2016;11(2):97-103.
28. Saraf A, Sankhla S. HPTLC fingerprint profile of extracts from leaf, bark and flower of Tecomella undulata (Seem) in different solvents. Int J Pharmacog Phytochem Res. 2015;7(6):1208-1216.

29. Bernardi T, Bortolini O, Massi A, Sacchetti G, Tacchini M and De Risi C. Exploring the synergy between HPTLC and HPLC-DAD for the investigation of wine-making by-products. Molecules. 2019;24:3416.
30. Gupta A, Maheta P, Chauhan R, Pandey S, Yadav JS and Shah S. Simultaneous quantification of bioactive triterpene acids (Ursolic acid and Oleanolic acid) in different extracts of *Eucalyptus globulus* (L) by HPTLC method. Pharmacogn J. 2018;10(1):179-185.
31. Giacometti A, Cirioni O, Schimizzi M, Del Prete MS, Barchiesi F, Derrico MM *et al*. Epidemiology and microbiology of surgical wound infections. J Clin Microbiol. 2000;38(2):918-22.
32. Mustoe TA, O'Shaughnessy K, Kloeters O. Chronic wound pathogenesis and current treatment strategies: A unifying hypothesis. Plast Reconstr Surg. 2006;117 (7):35S-41S.
33. Eming S, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol. 2007;127(3):514-25.
34. Koh GCKW, Peacock SJ, Van der Poll T, Wiersinga WJ. The impact of diabetes on the pathogenesis of sepsis. Eur J Clin Microbiol Infect Dis. 2012;31(4): 379-88.
35. Bishara SA, Michel C, Shady NH, Saad AD. Effect of silver on burn wound infection control and healing: Review of the literature. Burns. 2007;33:139-148.
36. Hanghui C, Zhenbo W, Fan W, Chunmao H. Pathogen distribution and drug resistance in a burn ward: A three-year retrospective analysis of a single center in China. Int J Clin Exp Med. 2015;8(10):19188-19199.
37. Wariso BA, Nwachukwu CO. A survey of common pathogens in wound in patients at the University of Port Harcourt Teaching Hospital (U.P.T.H), Port Harcourt. West Afr J Med. 1998;22(1):50-4.
38. Idowu OF, Muhibat AR, Nasiru AI, Andrew OU, Samuel A. Bacteriology of infected burn wounds in the burn wards of a teaching hospital in Southwest Nigeria. Burns. 2013;39:168-173.
39. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother. 2008;61(6):1295-1301.
40. Gulmez D, Hascelik G. *Stenotrophomonas maltophilia*: antimicrobial resistance and molecular typing of an emerging pathogen in a Turkish university hospital. Clin Microbiol Infect. 2005;11:880-886.
41. Mounyr B, Moulay S, Saad KI. Methods for in vivo evaluating antimicrobial activity a review. J Pharm Anal. 2016;6(2):71-79.
42. Christy S, Niveditha MS. Antimicrobial efficacy of *Azhadirachta indica* against *Streptococcus mutans*-An invitro study. Asian J Pharm Tech. 2019;9(3):149-153.
43. Somasundaram A, Velmurugan V, Senthilkumar GP. *In vitro* antimicrobial activity of *Vernonia cinerea* (L) Less. Pharmacol. 2010;960:957-960.
44. Selem Habtom and Sibhatu Gebrehiwot. *In vitro* antimicrobial activities of crude extracts of *Vernonia amygdalina* and *Croton macrostachyus* against some bacterial and fungal test pathogens. J Phytopharmacol. 2019;8(2):57-62.
45. Ogundare AO, Adetuyi FC and Akinyosoye FA. Antimicrobial activities of *Vernonia tenoreana*. Afr J Biotechnol. 2006;5(18):1663-1668.
46. Julien GK, Sorho S, Nathalie G, Yaya S, Joseph DA, Mireille D. Phytochemical assessment and antimicrobial activity of leaves extract of *Vernonia colorata* (Wild.) Drake on resistant germs of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Chem Pharm Res. 2012;4(5):2490-2494.
47. Mozirandi W, Tagwireyi D, Mukanganyama S. Evaluation of antimicrobial activity of chondrillasterol isolated from *Vernonia adoensis* (Asteraceae). BMC Comp Alt Med. 2019;19:249.
48. Tafesse G, Mekonnen Y, Makonnen E, Majinda RRT, Moleta GB and Yeboah S. Antibacterial activities of crude extracts and pure compounds isolated from *Vernonia galamensis* leaves. Afr J Pharm Pharmacol. 2018;12(11):136-141.
49. Rajasekaran R, Asefaw Y, Gebrekidan Y, Medhanie G and Yamane B. Antimicrobial activity of *Vernonia Shimperi* and *Vernonia amygdalina* against selected clinical pathogens. Int J Pharm Sci Rev Res. 2017;47(1):141-144.
50. Jagtap NS, Khadabadi SS, Ghorpade DS, Banarase NB, Naphade SS. Antimicrobial and antifungal activity of *Centella asiatica* (L.) Urban, Umbelliferae. Res J Pharm Tech. 2009;2(2):328-330.

51. Stepfanie NS, Sareh K, Gabriel AA, Teo SS, Farahnaz A and Patrick NO. The HPTLC validated method development for the quantification of Naringin from the partially purified *Labisia Pumila* dichloromethane extract. J Chromatogr Sep Tech. 2015;6(4): 271.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74573