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Original Article

# GC-MS Analysis of Bioactive Phytocompounds in Polyherbal Formulation Extracted Ointment Used in the Management of Wound Healing

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## ABSTRACT

**Background:** The scientific validation of traditional as well as proprietary Ayurvedic formulations is crucial for the purpose of healing of wound in chronic non healing wounds. Polyherbal Formulation Extracted Ointment, a proprietary formulation made up of Water-soluble extracts of Nirgundi patra, Amalaki Bark and Bhringraja whole plant with simple ointment base. All three drugs are having Wound healing activity which mentioned in ayurveda classical texts as Vranaropana, Vrana-shodhana, Krimighna activities. **Aim and Objectives:** Gas Chromatography-Mass Spectrometry (GC-MS) applied in this study to analyze chemical composition of Polyherbal Formulation Extracted Ointment, targeting to signify correlations between bioactive chemical compounds and therapeutic effects in wound healing. **Materials and Methods:** A 10 g sample Polyherbal Formulation Extracted Ointment was prepared in KLE's GMP certified Ayurvedic Pharmacy, Belagavi and submitted to GC-MS analysis utilizing conventional analytical techniques in order to determine bioactive chemical components. **Results and Discussion:** Bioactive substances were identified by the analysis, including Linoleic acid, Oleic acid, Palmitic acid and derivatives, Erucic acid, Stearic acid, Tetradecanol, Octacosane, Limonene derivative, which are known for their ability to heal wounds. These results are consistent with the conventional application of Polyherbal Formulation Extracted Ointment in reducing pain, inflammation, supporting its role in chronic non healing wounds where wound healing potential is necessary. **Conclusion:** The presence of bioactive compounds confirms medicinal use of Polyherbal Formulation Extracted Ointment as an effective wound healing formulation in chronic non healing wounds. Additional research on-going to investigate its clinical effectiveness and wound healing potential to chronic non healing wounds.

**Keywords** Chronic non healing wounds, GC-MS, Polyherbal Formulation Extracted Ointment, Wound healing activity.

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## INTRODUCTION

Wound Healing is the primary focus in surgical discipline. It is becoming a challenge in day today practice due to comorbidities of the patient, unhealthy wound environment or Resistance etc. (Sen, 2021). It's a complex physiological process. Chronic non healing wounds characterized by microbial colonization, chronic inflammation and poor angiogenesis, all of which contribute to the delayed for nonexistent healing process (Schilrreff *et al.*, 2022).

The frequency of chronic wounds is increasing worldwide. About 15-25% of diabetes patients will acquire diabetic foot ulcers at some time in their lifespan, and 1-2% of population in affluent nations are considered to have chronic and non-healing wounds (Sen, 2023). Up to 1% of adults have venous leg ulcers, 5-15% of hospitalized patients develop pressure ulcers (Maheshwari, 2024). Chronic wounds: A rising public health concern. Currently, more than 40 million people worldwide are impacted, and as the population ages and the frequency of hypertension, diabetes, obesity rises and so does this percentage (Rasero *et al.*, 2015). The economic burden is immense, the United States alone spends over USD 25 billion a year on managing chronic wounds, while European nations indicate that this condition accounts for 2-4% of all healthcare expenses (Sharma *et al.*, 2024). Such injuries significantly reduce mobility, independence and general quality of life in addition to expense.

The current therapies include the use of antibiotics, wound therapy of negative pressure, surgical debridement, dressings and hyperbaric oxygen. Yet, these methods are having drawbacks such high cost, limited availability, recurrence, drug resistance and improper wound closure. Significantly, they frequently focus

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on symptoms rather than the complex factors that contribute to delayed wound healing.

Herbal formulations are showing potential as alternatives in this context. Antimicrobial, antioxidant, anti-inflammatory and angiogenesis-stimulating qualities are all provided by medicinal plants, which together target several different healing pathways. They are useful in both modern and traditional medicine since they are typically safe, affordable and acceptable. Therefore, researching herbal remedies may offer a long-term and efficient way to treat chronic non healing wounds (Dubey *et al*., 2023).

Among these, Polyherbal Formulation Extracted Ointment, a proprietary Ayurvedic ointment, enriched with potent anti-inflammatory, anti-microbial, analgesic, antioxidant compounds (Talekar *et al*., 2017). Discovering the therapeutic advantages of Ayurvedic therapies might offer a different and supplementary method of treating chronic non healing wounds. In order to determine interactions between Polyherbal Formulation Extracted Ointment's bioactive compounds and its therapeutic effects in healing of wound, evaluation of GC-MS of the medicinal plants chemical composition was conducted.

## MATERIALS AND METHODS

Polyherbal Formulation Extracted Ointment prepared from a GMP certified KLE's Ayurvedic pharmacy, Belagavi and GC-MS analysis was performed using conventional protocols. Polyherbal Formulation Extracted Ointment is a proprietary formulation, made up of Water-soluble extracts of Nirgundi-leaves (*Vitex negundo-Verbenaceae*), Amalaki- bark (*Emblica officinalis-Euphorbiaceae*) and Bhringraja-whole plant (*Tridex Procumbens-Asteraceae*) with simple ointment base. Supplementary material for the British Pharmacopoeia method of preparing ointments is available.

### Study conduction

The GC-MS analysis was performed at Amrith Pvt. Lab., Nisargam Pvt. Ltd., in Shivamogga, Karnataka.

### Instrument

The instrument for GC-MS, model Shimadzu QP 2010SE, which provides High sensitivity and dependability, is frequently utilized for accurate qualitative and quantitative analysis in a variety of applications.

### Sample Preparation

Polyherbal Formulation Extracted Ointment was prepared in GMP certified KLE's Ayurveda Pharmacy, a reliable provider of authentic Ayurvedic products. The preparation for GC-MS analysis involved multiple steps are as follows:

### Preparation

The Ayurvedic formulation was carefully examined and homogenized to assure consistency before extraction for analysis.

### Acetone Extraction

A sample of formulation 1 mL was diluted in acetone of quantity 9 mL.

### Analysis

The acetone extract which is filtered (1 µL) was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

### Chromatography and Mass Spectrometry Method

The analysis of the Polyherbal Formulation Extracted Ointment on a Shimadzu GCMS-QP-2010SE instrument was used for the analysis, helium was used as carrier gas. To inject 1 µL of the extract, a 1 mL sample was diluted with 10 mL acetone. The instrument settings were as follows- The temperature for injection was set to 280.00ºC, the column oven temperature was kept at 80.0ºC. The split ratio for the injection mode was 10.0. At a pressure of 24.2 kPa, the flow control mode was linear velocity. With a linear velocity of 45.1 cm/sec and a column flow of 1.50 mL/min, the overall flow rate was 19.5 mL/min. 3.0 mL/min was remained as the purge flow rate. The splitter retained, carrier gas saver and high-pressure injection were key characteristics also shut down. The program of column temperature was as follows- The temperature was first regulated at 80.0ºC for duration of 2 min. After that, it was raised to 200.0ºC at a rate of 10.00ºC per minute and kept there for 5 min. It was then elevated to 280.0ºC at a rate of 5.00ºC per minute and maintained for a period of 3 min. With an interface temperature of 280.0ºC, the ion source temperature was set at 200.0ºC. 1.40 min was decided to be the solvent cut time. The detector gain mode was set to 0.95kV + 0.00 kV with threshold of 0 for detection, taking into consideration the adjusted result. The MS Table sets were as follows- The analysis started at 2.00 min and completed at 33.00 min. The collection method was set to scan, with 0.30 sec for event time. The scan speed was 1666, covering a mass range of 35.00 to 500.00 m/z. The GC-MS system was used in the sample intake instrument.

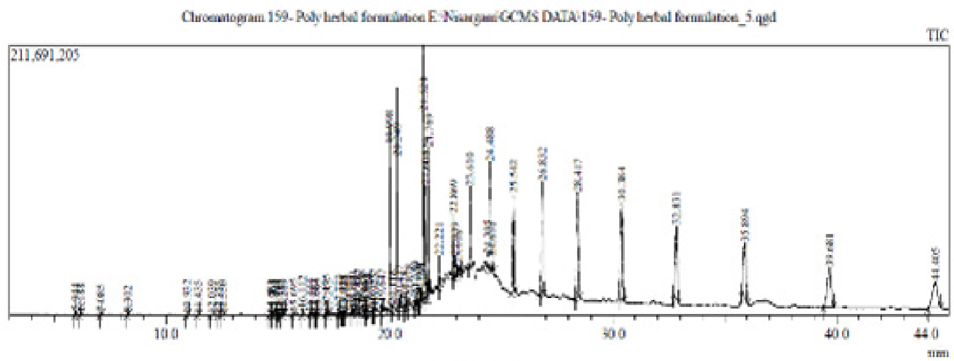
## RESULTS

The Evaluation of Gas Chromatography-Mass Spectrometry of Polyherbal Formulation Extracted Ointment identified several bioactive compounds which is beneficial for chronic non healing wounds Tables 1-4 and Figure 1.

The main elements detected were- 1- Tetradecanol (Ret. Time:19.998 min, 5.26%), Tetradecanoic acid, 12-methyl-, methyl ester (Ret. Time:20.349 min, 5.49%), Oxirane, decyl- (Ret. Time:21.528 min, 7.73%), Carbonic acid, decyl nonyl ester (Ret. Time: 24.488 min, 5.00%), 2-Ethyl-1-dodecanol (Ret. Time:

26.832 min, 6.58%), Octadecane, 5-methyl- (Ret. Time:25.542 min, 4.69%),

Nonyl tetradecyl ether (Ret. Time: 28.417 min, 7.70%), Nonyl tetradecyl ether (isomer)- (Ret. Time: 30.384 min, 8.79%), Octacosane (Ret. Time: 32.831 min, 7.26%), Octacosane (isomer)- (Ret. Time: 35.894 min, 6.44%), Tetratetracontane (Ret. Time:39.681 min, 6.57%), Tetratetracontane (isomer)- (Ret. Time: 44.405 min, 5.76%). Nonyl tetradecyl ether plays an important part in the formulation, enhancing solubility and penetration of contents and balances moisture. Octacosane suggests antimicrobial activity ( ). Tetracontane maintains moist environment in wounds ( ). 1- Tetradecanol aids re-epithelization, maintains moisture. Oxirane, decyl- potentially contributing Antimicrobial, Antioxidant, and Infection control properties. 9-Octadecenoic acid signifying Fibroblast proliferation, collagen synthesis and Angiogenesis in wound healing. Oxineundecanoic acid, 3-pentyl-, methyl ester(cis-) signifying Antioxidant, Antimicrobial, reduces oxidative stress during wound healing. 2-Ethyl-1-dodecanol signifying Antimicrobial, enhances penetration of other active compounds. These compounds collectively supporting use of Polyherbal Formulation Extracted




Peak Report TIC

| Peak# | R.Time | Area | Area% | Height | CAS# | Name |
|---|---|---|---|---|---|---|
| 1 | 5.944 | 3626574 | 0.07 | 1888147 | 627-82-7 | Diglycerol |
| 2 | 6.188 | 4344207 | 0.08 | 2243805 | 0-0-0 | 1-[(Trimethylsilyl)oxy]propan-2-ol |
| 3 | 7.085 | 3458470 | 0.06 | 1983483 | 5989-54-8 | Cyclohexene, 1-methyl-4-(1-methylethenyl)-, ( |
| 4 | 8.402 | 1805759 | 0.03 | 1185952 | 111-11-5 | Octanoic acid, methyl ester |
| 5 | 10.942 | 1581744 | 0.03 | 676041 | 629-50-5 | Tridecane |
| 6 | 11.435 | 1154709 | 0.02 | 381638 | 110-42-9 | Decanoic acid, methyl ester |
| 7 | 12.039 | 2503838 | 0.04 | 461168 | 0-0-0 | Carbonic acid, prop-1-en-2-yl tridecyl ester |
| 8 | 12.353 | 1276614 | 0.02 | 433931 | 1560-96-9 | Tridecane, 2-methyl- |
| 9 | 12.550 | 1241520 | 0.02 | 330553 | 6418-41-3 | Tridecane, 3-methyl- |
| 10 | 14.681 | 3007750 | 0.05 | 716692 | 6165-40-8 | Pentadecane, 7-methyl- |
| 11 | 14.768 | 1764078 | 0.02 | 456147 | 17312-80-0 | Undecane, 2,4-dimethyl- |
| 12 | 14.894 | 1299591 | 0.02 | 502959 | 6117-97-1 | Dodecane, 4-methyl- |
| 13 | 14.998 | 2508535 | 0.04 | 834511 | 1560-95-8 | Tetradecane, 2-methyl- |
| 14 | 15.152 | 1961557 | 0.04 | 760729 | 18435-22-8 | Tetradecane, 3-methyl- |
| 15 | 15.269 | 2480513 | 0.04 | 875343 | 28303-42-6 | Formic acid, dodecyl ester |
| 16 | 15.695 | 4804862 | 0.09 | 1990810 | 544-76-3 | Hexadecane |
| 17 | 16.112 | 9136940 | 0.16 | 4175849 | 111-82-0 | Dodecanoic acid, methyl ester |
| 18 | 16.444 | 4930749 | 0.09 | 1610172 | 6165-40-8 | Pentadecane, 7-methyl- |
| 19 | 16.684 | 3199092 | 0.06 | 1778482 | 1560-96-9 | Tridecane, 2-methyl- |
| 20 | 16.795 | 3709972 | 0.07 | 1834328 | 6418-44-6 | Heptadecane, 3-methyl- |
| 21 | 17.175 | 8886243 | 0.16 | 4374558 | 112-95-8 | Eicosane |
| 22 | 17.275 | 3165379 | 0.06 | 1208979 | 593-45-3 | Octadecane |
| 23 | 17.723 | 14308507 | 0.26 | 4488790 | 6165-40-8 | Pentadecane, 7-methyl- |
| 24 | 17.928 | 5946976 | 0.11 | 2606991 | 1560-95-8 | Tetradecane, 2-methyl- |
| 25 | 17.928 | 4636023 | 0.08 | 2872339 | 1560-95-8 | Tetradecane, 2-methyl- |
| 26 | 18.020 | 5283696 | 0.09 | 3093308 | 18435-22-8 | Tetradecane, 3-methyl- |
| 27 | 18.320 | 9269743 | 0.17 | 5294553 | 112-95-8 | Eicosane |
| 28 | 18.388 | 8339725 | 0.15 | 2950486 | 112-95-8 | Eicosane |
| 29 | 18.587 | 11284643 | 0.20 | 7222835 | 124-10-7 | Methyl tetradecanoate |
| 30 | 18.758 | 17031281 | 0.31 | 6141447 | 6165-40-8 | Pentadecane, 7-methyl- |
| 31 | 18.894 | 2685263 | 0.05 | 2469404 | 26429-11-8 | Heptadecane, 4-methyl- |
| 32 | 18.943 | 4490254 | 0.08 | 3239699 | 1560-86-7 | Nonadecane, 2-methyl- |
| 33 | 19.024 | 6159414 | 0.11 | 3798458 | 6418-44-6 | Heptadecane, 3-methyl- |
| 34 | 19.277 | 10475062 | 0.19 | 6203975 | 112-95-8 | Eicosane |
| 35 | 19.325 | 14187404 | 0.25 | 3541552 | 112-95-8 | Eicosane |
| 36 | 19.647 | 19457188 | 0.35 | 6677327 | 544-76-3 | Hexadecane |
| 37 | 19.998 | 293306677 | 5.26 | 128318441 | 112-72-1 | 1-Tetradecanol |
| 38 | 20.118 | 6603453 | 0.12 | 3291717 | 4292-19-7 | Dodecane, 1-iodo- |
| 39 | 20.349 | 305964829 | 5.49 | 118635998 | 5129-66-8 | Tetradecanoic acid, 12-methyl-, methyl ester |
| 40 | 20.437 | 16186931 | 0.29 | 6293338 | 630-1-3 | Hexacosane |
| 41 | 20.675 | 2981493 | 0.05 | 2170242 | 6418-44-6 | Heptadecane, 3-methyl- |
| 42 | 20.878 | 18752576 | 0.34 | 6437113 | 112-95-8 | Eicosane |
| 43 | 21.159 | 27927489 | 0.50 | 7868388 | 630-1-3 | Hexacosane |
| 44 | 21.318 | 9798502 | 0.18 | 2846534 | 1560-84-5 | Eicosane, 2-methyl- |
| 45 | 21.391 | 12671709 | 0.23 | 4651032 | 638-36-8 | Hexadecane, 2,6,10,14-tetramethyl- |
| 46 | 21.528 | 430893944 | 7.73 | 120421806 | 2855-19-8 | Oxirane, decyl- |
| 47 | 21.605 | 147480652 | 2.64 | 88268663 | 56134-3-3 | 1,8,11-Heptadecatriene, (Z,Z)- |

| Peak# | R.Time | Area | Area% | Height | CAS# | Name |
|---|---|---|---|---|---|---|
| 48 | 21.629 | 156027336 | 2.80 | 98175623 | 112-62-9 | 9-Octadecenoic acid (Z)-, methyl ester |
| 49 | 21.769 | 194990680 | 3.50 | 115029927 | 5129-66-8 | Tetradecanoic acid, 12-methyl-, methyl ester |
| 50 | 22.221 | 38043285 | 0.68 | 23523765 | 112-95-8 | Eicosane |
| 51 | 22.869 | 97746975 | 1.75 | 48261249 | 629-92-5 | Nonadecane |
| 52 | 22.939 | 50617000 | 0.91 | 15884224 | 1560-78-7 | 2-Methyltetracosane |
| 53 | 23.116 | 41487862 | 0.74 | 8222245 | 593-49-7 | Heptacosane |
| 54 | 23.610 | 146084292 | 2.62 | 62602095 | 25117-35-5 | Octadecane, 5-methyl- |
| 55 | 24.395 | 35792014 | 0.64 | 9416755 | 7459-33-8 | 9,12-Octadecadienoyl chloride, (Z,Z)- |
| 56 | 24.488 | 278727756 | 5.00 | 83257976 | 0-0-0 | Carbonic acid, decyl nonyl ester |
| 57 | 24.610 | 60050877 | 1.08 | 11706468 | 38520-30-8 | Oxiraneundecanoic acid, 3-pentyl-, methyl este |
| 58 | 25.542 | 261709109 | 4.69 | 76489884 | 25117-35-5 | Octadecane, 5-methyl- |
| 59 | 26.832 | 367109931 | 6.58 | 88222130 | 19780-33-7 | 2-Ethyl-1-dodecanol |
| 60 | 28.417 | 429495244 | 7.70 | 82164954 | 0-0-0 | Nonyl tetradecyl ether |
| 61 | 30.384 | 490155602 | 8.79 | 76648009 | 0-0-0 | Nonyl tetradecyl ether |
| 62 | 32.831 | 404835023 | 7.26 | 57259804 | 630-2-4 | Octacosane |
| 63 | 35.894 | 359077098 | 6.44 | 43210113 | 630-2-4 | Octacosane |
| 64 | 39.681 | 366612549 | 6.57 | 31369045 | 7098-22-8 | Tetratetracontane |
| 65 | 44.405 | 321520115 | 5.76 | 20450517 | 7098-22-8 | Tetratetracontane |
| | | 5577543822 | 100.00 | 1532278564 | | |

**Figure 1:** GC-MS Analysis report of Polyherbal Formulation Extracted Ointment.

**Table 1: Composition of Polyherbal Formulation Extracted Ointment.**

| Sanskrit Name | Latin Name | Family | Part Used | Proportion |
|---|---|---|---|---|
| Nirgundi | *Vitex negundo* Linn. | Verbenaceae | Leaves | 1 |
| Amalaki | *Emblica officinalis* Gaertn | Euphorbiaceae | Bark | 1 |
| Bhringaraj | *Tridex procumbens* Linn. | Asteraceae | Whole plant | 1 |
| Simple Ointment Base | 1. White Soft Paraffin- 85 g<br>2. Hard Paraffin - 05 g<br>3. Wool Fat - 05 g<br>4. Cetostearyl Alcohol - 05 g | | | |

**Table 2: Phytocomponents identified in the Polyherbal Formulation Extracted Ointment extracted by Acetone using GC-MS.**

| Peak# | R. Time | Area | Area% | Height | CAS# | Name |
|---|---|---|---|---|---|---|
| 1 | 5.944 | 3626574 | 0.07 | 1888147 | 627-82-7 | Diglycerol |
| 2 | 6.188 | 4344207 | 0.08 | 2243805 | 0-0-0 | 1-[(Trimethylsilyl)oxy]propan-2-ol |
| 3 | 7.085 | 3458470 | 0.06 | 1983483 | 5989-54-8 | Cyclohexene, 1-methyl-4-(1-methylethenyl)-, ( |
| 4 | 8.302 | 1805759 | 0.03 | 1185952 | 0111-11-5 | Octanoic acid, methyl ester |
| 5 | 10.932 | 1581744 | 0.03 | 626041 | 629-50-5 | Tridecane |
| 6 | 11.435 | 1154703 | 0.02 | 381638 | 110-42-9 | Decanoic acid, methyl ester |
| 7 | 12.039 | 2503838 | 00:04 | 461168 | 0-0-0 | Carbonic acid, prop-1-en-2-yl tridecyl ester |
| 8 | 12.355 | 1276614 | 0.02 | 433931 | 1560-96-9 | Tridecane, 2-methyl- |
| 9 | 12.55 | 1241520 | 0.02 | 350553 | 6418-41-3 | Tridecane, 3-methyl- |
| 10 | 14.681 | 3007750 | 0.05 | 716697 | 6165-40-8 | Pentadecane, 7-methyl- |
| 11 | 14.768 | 1263028 | 0.02 | 456142 | 17312-80-0 | Undecane, 2,4dimethyl-- |
| 12 | 14.893 | 1299591 | 0.02 | 507959 | 6117-97-1 | Dodecane, 4-methyl- |
| 13 | 14.998 | 2508535 | 0.04 | 834544 | 1560-95-8 | Tetradecane, 2-methyl- |
| 14 | 15.152 | 1961557 | 0.04 | 760729 | 18435-22-8 | Tetradecane, 3-methyl- |
| 15 | 15.269 | 2480513 | 0.04 | 875343 | 28303-42-6 | Formic acid, dodecyl ester |
| 16 | 15.695 | 4804862 | 0.09 | 1990840 | 544-76-3 | Hexadecane |
| 17 | 16.112 | 9136940 | 0.16 | 4125849 | 111-82-0 | Dodecanoic acid, methyl ester |
| 18 | 16.444 | 4920749 | 0.09 | 1610177 | 6165-40-8 | Pentadecane, 7-methyl- |
| 19 | 16.684 | 3199092 | 0.06 | 1728482 | 1560-96-9 | Tridecane, 2-methyl- |
| 20 | 16.795 | 3709972 | 0.07 | 1834328 | 6418-44-6 | Heptadecane, 3-methyl- |
| 21 | 17.175 | 8886243 | 0.16 | 4374558 | 112-95-8 | Eicosane |
| 22 | 17.275 | 3165379 | 0.06 | 1208979 | 593-45-3 | Octadecane |
| 23 | 17.723 | 14308507 | 0.26 | 4488790 | 6165-40-8 | Pentadecane, 7-methyl- |
| 24 | 17.928 | 5946976 | 0.11 | 2606991 | 1560-95-8 | Tetradecane, 2-methyl- |
| 25 | 17.928 | 4636023 | 0.08 | 2872339 | 1560-95-8 | Tetradecane, 2-methyl- |
| 26 | 18.02 | 5283696 | 0.09 | 3093308 | 18435-22-8 | Tetradecane, 3-methyl- |
| 27 | 18.32 | 9269743 | 0.17 | 5294553 | 112-95-8 | Eicosane |
| 28 | 18.388 | 8339725 | 0.15 | 2950486 | 112-95-8 | Eicosane |
| 29 | 18.587 | 11284643 | 0.2 | 7222835 | 0124-10-7 | Methyl tetradecanoate |
| 30 | 18.758 | 17031281 | 0.31 | 6141447 | 6165-40-8 | Pentadecane, 7-methyl- |
| 31 | 18.894 | 2685263 | 0.05 | 2469404 | 26429-11-8 | Heptadecane, 4-methyl- |
| 32 | 18.943 | 4490254 | 0.08 | 3239699 | 1560-86-7 | Nonadecane, 2-methyl- |
| 33 | 19.024 | 6159414 | 0.11 | 3798458 | 6418-44-6 | Heptadecane, 3-methyl- |
| 34 | 19.277 | 10475062 | 0.19 | 6203975 | 112-95-8 | Eicosane |
| 35 | 19.325 | 14187404 | 0.25 | 3541552 | 112-95-8 | Eicosane |
| 36 | 19.647 | 19457188 | 0.35 | 6677327 | 54476-3 | Hexadecane |
| 37 | 19.998 | 293306677 | 5.26 | 128318441 | 112-72-1 | 1-Tetradecanol |

| Peak# | R. Time | Area | Area% | Height | CAS# | Name |
|---|---|---|---|---|---|---|
| 38 | 20.118 | 6603453 | 0.12 | 3291717 | 4292-19-7 | Dodecane, 1-iodo- |
| 39 | 20.349 | 305964829 | 5.49 | 118635998 | 5129-66-8 | Tetradecanoic acid, 12-methyl-, methyl ester |
| 40 | 20.437 | 16186931 | 0.29 | 6293338 | 0630-1-3 | Hexacosane |
| 41 | 20.675 | 2981493 | 0.05 | 2170242 | 6418-44-6 | Heptadecane, 3-methyl- |
| 42 | 20.878 | 18752576 | 0.34 | 6437113 | 112-95-8 | Eicosane |
| 43 | 21.159 | 27927489 | 0.5 | 7868388 | 0630-1-3 | Hexacosane |
| 44 | 21.318 | 9798502 | 0.18 | 2846534 | 1560-84-5 | Eicosane, 2methyl-- |
| 45 | 21.391 | 12671709 | 0.23 | 4651032 | 638-36-8 | Hexadecane, 2,6,10,14-tetramethyl- |
| 46 | 21.528 | 430893944 | 7.73 | 120421806 | 2855-19-8 | Oxirane, decyl- |
| 47 | 21.605 | 147480652 | 2.64 | 88268663 | 56134-3-3 | 1.8.11-Heptadecatriene, (Z Z)- |
| 48 | 21.629 | 156027336 | 2.8 | 98175623 | 112-62-9 | 9-Octadecenoic acid (Z)-, methyl ester |
| 49 | 21.769 | 194990680 | 3.5 | 115029927 | 5129-66-8 | Tetradecanoic acid, 12-methyl-, methyl ester |
| 50 | 22.221 | 38043285 | 0.68 | 23523765 | 112-95-8 | Eicosane |
| 51 | 22.869 | 97746975 | 1.75 | 48261249 | 629-92-5 | Nonadecane |
| 52 | 22.939 | 50617000 | 0.91 | 15884224 | 1560-78-7 | 2-Methyltetracosane |
| 53 | 23.116 | 41487862 | 0.74 | 8222245 | 593-49-7 | Heptacosane |
| 54 | 23.61 | 146084292 | 2.62 | 62602095 | 25117-35-5 | Octadecane, 5methyl-- |
| 55 | 24.395 | 35792014 | 0.64 | 9416755 | 7459-33-8 | 9,12-Octadecadienoyl chloride, (ZZ)- |
| 56 | 24.488 | 278727756 | 5 | 83257976 | 0-0-0 | Carbonic acid, decyl nonyl ester |
| 57 | 24.61 | 60050877 | 1.08 | 11706468 | 38520-30-8 | Oxiraneundecanoic acid, 3-pentyl-, methyl este |
| 58 | 25.542 | 261709109 | 4.69 | 76489884 | 25117-35-5 | Octadecane, 5-methyl- |
| 59 | 26.832 | 367109931 | 6.58 | 88222130 | 19780-33-7 | 2-Ethyl-1-dodecanol |
| 60 | 28.417 | 429495244 | 7.7 | 82164954 | 0-0-0 | Nonyl tetradecyl ether |
| 61 | 30.384 | 490155602 | 8.79 | 76648009 | 0-0-0 | Nonyl tetradecyl ether |
| 62 | 32.831 | 404835023 | 7.26 | 57259804 | 0630-2-4 | Octacosane |
| 63 | 35.894 | 359077098 | 6.44 | 43210113 | 0630-2-4 | Octacosane |
| 64 | 39.681 | 366612549 | 6.57 | 31369045 | 7098-22-8 | Tetratetracontane |
| 65 | 44.405 | 321520115 | 5.76 | 20450517 | 7098-22-8 | Tetratetracontane |
| | | 5577543822 | 100 | 1532278564 | | |

Ointment in Chronic Non healing wounds, supporting its role as an effective formulation for wound healing.

## DISCUSSION

The GC-MS analysis of Polyherbal Formulation Extracted Ointment disseminating a complex chemical composition and contains essential bioactive components that may promote wound healing, Antimicrobial, Anti-inflammatory, Angiogenesis, Collagen synthesis, Fibroblast Proliferation.

Linoleic acid promotes healing of wound as enhancing the phase of inflammation of the healing, accelerating migration of immune cells like neutrophils and keratinocytes, improving skin barrier function and hydration (Figure 2). Oleic acid can accelerate wound healing by positively influencing the initial inflammatory phase. It increases wound tissue mass, promotes angiogenesis and stimulates production of cytokines of pro-inflammatory which helps to clear debris which prepare the wound for repair (Figure 3).

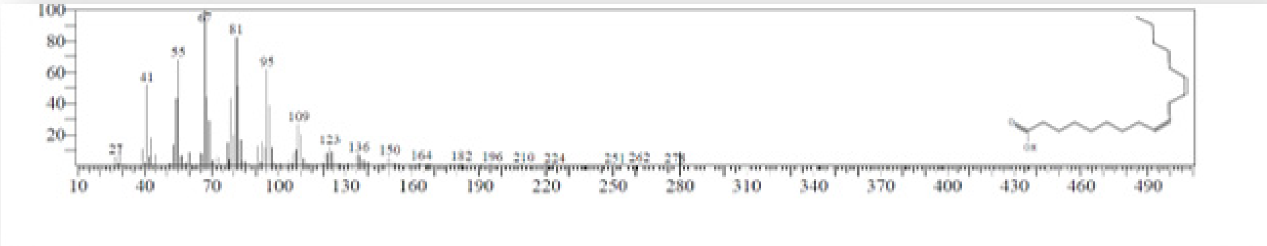



**Figure 2:** Retention time of compound Linoleic acid ($C_{18}H_{32}O_2$).

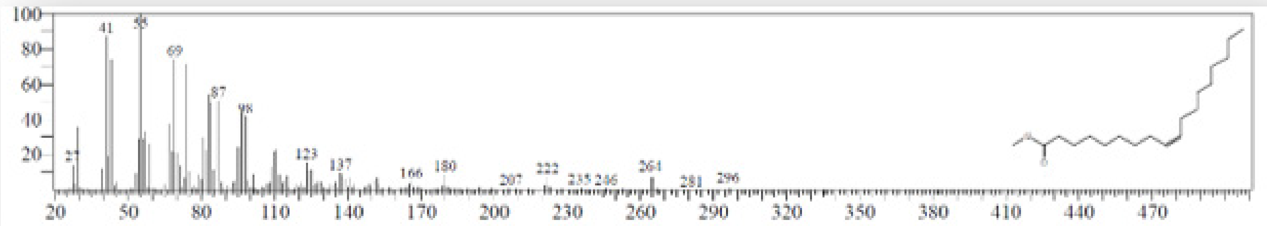



**Figure 3:** Retention time of compound Oleic acid ($C_{18}H_{34}O_2$).

**Table 3: Pharmacological Activity of compounds found in Polyherbal Formulation Extracted Ointment which has potential in wound healing.**

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 1. | Linoleic acid | $C_{18}H_{32}O_2$ | Inflammation → Proliferation (re-epithelialization, barrier repair)<br>Omega-6 essential fatty acid that modulates inflammation, supports keratinocyte migration and membrane/skin-barrier repair; promotes early resolution of inflammation and epithelial proliferation (Prado *et al.*, 2025; Pereira *et al.*, 2008). |
| 2. | Oleic acid | $C_{18}H_{34}O_2$ | Inflammation → Proliferation<br>Monounsaturated fatty acid that modulates the immune infiltrate, reduces excessive inflammation and can accelerate early reparative responses and keratinocyte migration (Pereira *et al.*, 2008). |
| 3. | Palmitic acid and derivatives (methyl esters) | Palmitic acid $C_{16}H_{32}O_2$; methyl palmitate $C_{17}H_{34}O_2$ | Inflammation (anti-inflammatory)<br>Methyl palmitate exhibits anti-inflammatory activity in experimental models (reduces pro-inflammatory mediators), which can help move chronic wounds out of a persistent inflammatory state. Also occurs in oils that support skin repair (Chuah *et al.*, 2018). |
| 4. | Erucic acid (methyl ester) | Erucic acid $C_{22}H_{42}O_2$; methyl erucate $C_{23}H_{44}O_2$ | (Wound-healing evidence)-likely barrier / lipid matrix roles<br>Long-chain mono-unsaturated fatty acid found in some plant oils; plausible effects on membrane/skin lipid environment and formulation permeability, but direct, specific wound-healing pharmacology is not well documented in textbooks-evidence mainly safety/chemical profiles (Poljšak *et al.*, 2020). |
| 5. | Stearic acid | $C_{18}H_{36}O_2$ | Barrier repair / Proliferation (formulation excipient: supports topical delivery)<br>Saturated long-chain fatty acid important for stratum corneum lipid matrix; used in topical formulations as emollient and emulsifier-supports barrier function and vehicle properties that facilitate healing. Direct biological pro-healing actions are less robust than its formulation role (Chen *et al.*, 2019). |

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 6. | Tetradecanol | $C_{14}H_{30}O$ | Formulation/excipient → supports delivery; may aid barrier/occlusion<br>Fatty alcohol used in topical formulations (emollient, occlusive layer) and in advanced delivery systems (e.g., waxy matrix or dressing coatings). Its contribution is often physicochemical (moisture retention, vehicle stability) rather than direct cellular signaling (Braťka *et al*., 2022). |
| 7. | Octacosane | $C_{28}H_{58}$ | Antioxidant / antimicrobial (reported in extracts)-may assist proliferation / remodeling indirectly<br>Long-chain hydrocarbon detected in plant extracts that showed wound-healing activity in animal studies; proposed roles include antimicrobial, antioxidant effects and promotion of collagen/epithelialization in extract-based preparations-evidence largely from phytochemical/extract studies rather than isolated-compound clinical data (Balachandran, *et al*., 2023). |
| 8. | Limonene derivative | $C_{10}H_{16}$ | Inflammation → Remodeling (modulates cytokines, MMPs)<br>Monoterpene with anti-inflammatory and immunomodulatory effects; topical limonene reduced wound size and inflammatory cytokines in animal models and has been linked to up-/down-regulation of remodeling enzymes (MMPs) and promotion of tissue remodeling (Keskin *et al*., 2017; Ahmad *et al*., 2014). |

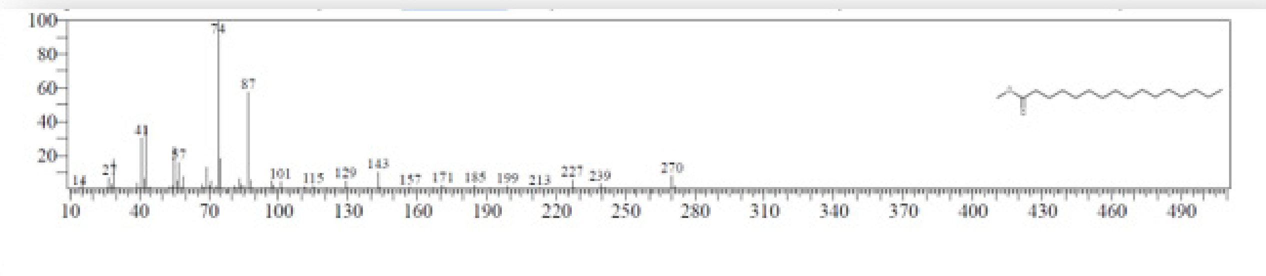



**Figure 4:** Retention time of compound Palmitic acid ($C_{17}H_{34}O_2$).

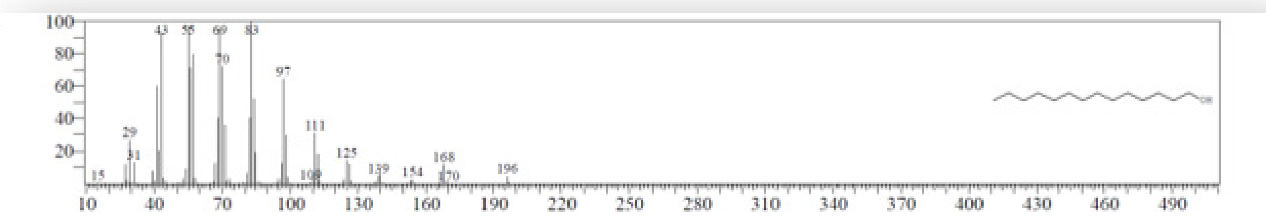



**Figure 5:** Retention time of compound Tetradecanol ($C_{14}H_{30}O$).

Palmitic acid and its derivatives having anti-inflammatory properties and potential roles in tissue regeneration (Figure 4). Erucic acid shown anti-inflammatory and antioxidant agent. Stearic acid aids wound healing by strengthening the skin barrier, preventing moisture loss and potentially enhancing migration of stem cells implicates in tissue repair. Tetradecanol used as a carrier or component in wound healing formulations due to its moisturizing and lipid-based properties (Figure 5). Octacosane having antimicrobial, barrier-enhancing, antioxidant properties, which help prevent infection, support cell membrane function and reduce oxidative stress (Figure 6). Limonene derivatives show healing of wound as a result of their antibacterial, anti-inflammatory and antimicrobial effects (Figure 7).

**Table 4: Pharmacological Activity of other compounds found in Polyherbal Formulation Extracted Ointment**

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 1. | Diglycerol | $C_6H_{14}O_5$ | It is a superior, long-lasting humectant and moisturizer which is crucial for managing wound dehydration, supporting tissue repair through enhanced hydration of the stratum corneum. Its having long lasting action due to its higher molecular weight and improved formulation stability ensuring the dressing remains moist and effective (Verčimáková *et al*., 2024; ALR Pires *et al*., 2018). |
| 2. | 1-[(Trimethylsilyl)oxy] propan-2-ol | $C_6H_{16}O_2SI$ | It acts primarily as a protected alcohol in organic synthesis and enhanced penetration of the compounds in formulation with Antibacterial and Antioxidant properties (Fatwati *et al*., 2025). |
| 3. | Octanoic acid, methyl ester | $C_9H_{18}O_2$ | It acts as a bioactive, ester of fatty acid that supports wound healing primarily through Antimicrobial action, Biofilm eradication, Anti-inflammatory and Antioxidant effects with Stimulation of tissue repair through improved fibroblast activity (WC Lin *et al*., 2023; Balachandran *et al*., 2023; Mostofa *et al*., 2024). |
| 4. | Tridecane | $C_{13}H_{28}$ | It is a volatile component of essential oils, showing Increased tensile strength, Fibroblast proliferation and Anti-inflammatory action. (Amur *et al*., 2025; Venkatesan *et al*., 2025). Benefits of frankincense oil on healing of wounds: Caspase- 3 expression is reduced to help the transition from the initial phase inflammatory till proliferative phase Pharmaceuticals. |
| 5. | Decanoic acid, methyl ester | $C_{11}H_{22}O_2$ | It plays an essential role in healing of wounds, from its chemical actions as a Biodegradable structural component, Enhanced fibroblast proliferation and Migration, Antibacterial and Anti-inflammatory action, Maintains optimal wound microenvironment and having low cytotoxicity and high biocompatibility which leads to faster and effective healing (Shen *et al*., 2021; Dang *et al*., 2019). |
| 6. | Carbonic acid, prop-1-en-2-yl tridecyl ester | $C_{17}H_{32}O_3$ | It acts in wound healing primarily as a degradable, protective component or vehicle for delivering active agents. Its Chemical actions are as Biodegradable film forming, Hydrolysis and compatibility which providing a moist environment crucial for accelerated tissue repair and re-epithelialization (Zamani *et al*., 2026; Manalu *et al*., 2025). |
| 7. | Tridecane, 2-methyl- | $C_{14}H_{30}$ | Its acting through Antibacterial activity, Synergistic effect in essential oils by accelerating the healing process, suppressing inflammation and minimizing infection, Film forming with skin protection which helps maintain moisture (Vijayaraj *et al*., 2023; Kalaskar *et al*., 2024). |
| 8. | Tridecane, 3-methyl- | $C_{14}H_{30}$ | It may act in wound healing through Contribution to Antimicrobial activity, volatile component, Binding affinity in tissue dysfunction with activity of Anti-inflammatory (Aboaba *et al*., 2013; Torrijos *et al*., 2023). |
| 9. | Pentadecane, 7-methyl- | $C_{16}H_{34}$ | It demonstrates significant *in vitro* wound healing properties including Anti-inflammatory, Antimicrobial, Fibroblast stimulating activity, Deposition of Collagen which is essential for repairing the structure of tissues (Chuah *et al*., 2018; Balachandran *et al*., 2023; Okechukwu, 2020; Chuah *et al*., 2018). |
| 10. | Undecane, 2,4-dimethyl- | $C_{13}H_{28}$ | It having activities as Anti-allergic and Anti-inflammatory in skin cells (Choi, *et al*., 2020; Mousa *et al*., 2024; Akayleh *et al*., 2022). |
| 11. | Dodecane, 4-methyl- | $C_{13}H_{28}$ | Its stimulating Neurotrophins, Activating signaling pathways, enhancing regeneration and accelerates wound closure (Abdel *et al*., 2022; Majidi Ghatar *et al*., 2023). |
| 12. | Tetradecane, 2-methyl- | $C_{15}H_{32}$ | it is a branched alkane hydrocarbon contributes in wound healing, exhibiting Antibacterial, Antioxidant and Synergistic effects which are essential for wound contraction and closure (Abdel *et al*., 2022). |

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 13. | Tetradecane, 3-methyl- | $C_{15}H_{32}$ | It is a bioactive constituent found in natural plant extracts for promoting wound healing by its Antimicrobial, Antifungal properties, cleansing and preservative agent on the skin, contributing to a reduced inflammatory reaction, accelerates wound healing by reducing wound surface area and promoting wound contraction, Interaction with biological pathways improves epithelialization, fibroblast proliferation and increase vascular endothelial growth factor expression (Abdel *et al*., 2022; Subramanian *et al*., 2020; Sallam *et al*., 2022). |
| 14. | Formic acid, dodecyl ester | $C_{13}H_{26}O_2$ | It acts as a Decellularization agent which having Antimicrobial properties, wound healing effect, promotes fibroblast proliferation, enhancing cell migration, accelerating angiogenesis and stimulating collagen deposition (Manakkoth *et al*., 2025). |
| 15. | Hexadecane | $C_{16}H_{34}$ | It acts as a bioactive component in natural extracts, promoting skin regeneration through metabolic and cellular actions. It having Antimicrobial, Antioxidant and Anti-inflammatory actions (Owusu *et al*., 1989; Thajuddin, 2021). |
| 16. | Dodecanoic acid, methyl ester | $C_{13}H_{26}O_2$ | Its showing healing of wounds primarily through activity of Anti-inflammatory, Antimicrobial action and Emollient action that protect skin barrier and acts as an Enhancer of drug delivery to facilitate the penetration of other active components into the skin to improve treatment outcomes (Salem *et al*., 2022; Jiménez-Nevárez *et al*., 2023; M Zaveri, 2021; Ben *et al*., 2025). |
| 17. | Pentadecane, 7-Methyl- | $C_{16}H_{34}$ | It plays a role in healing of wounds through Anti-inflammatory and tissue regenerative properties by migration of Fibroblast with Enhanced collagen deposition (Siyumbwa *et al*., 2019; Okechukwu, 2020; Chuah *et al*., 2018). |
| 18. | Heptadecane, 3-methyl- | $C_{18}H_{38}$ | It is a long-chain alkane found in plant extracts which includes Anti-inflammatory action, Antioxidant activity, ell migration acceleration, Modulation of signaling pathways and Potential Antimicrobial properties (Abdel *et al*., 2022). |
| 19. | Eicosane | $C_{20}H_{42}$ | It is a long chain saturated hydrocarbon which has potential as an alternative for wounds, in accelerating the closure of prolonged and diabetic wounds. it promotes wound repair through Antioxidant activity, Anti-inflammatory actions and fibroblast migration stimulation (Balachandran *et al*., 2023; Chuah *et al*., 2018). |
| 20. | Octadecane | $C_{18}H_{38}$ | It is a long-chain of alkane hydrocarbon identified as a bioactive component in various plant extracts, showing potential in promoting wound healing by Antioxidant activity, Collagen synthesis and Promoting cell proliferation. It promotes wound closure and shows antimicrobial activity (Serra *et al*., 2020; Ekweogu *et al*., 2024; Balachandran *et al*., 2023). |
| 21. | Pentadecane, 7-methyl- | $C_{16}H_{34}$ | It is a saturated alkane hydrocarbon with facilitating healing of wounds in the phases of Inflammatory and Proliferative stages (Chuah *et al*., 2018). |
| 22. | Tetradecane, 2-methyl- | $C_{15}H_{32}$ | Its showing Anti-inflammatory, Antimicrobial, Antioxidant activities, Promotion of cell proliferation and collagen deposition (Benmeddour *et al*., 2015; Samavati *et al*., 2025; Abdel *et al*., 2022; Esad *et al*., 2025). |
| 23. | Methyl tetradecanoate | $C_{15}H_{30}O_2$ | It's a methyl ester of saturated fatty acid that contributes to wound healing through its role as a Skin-conditioning agent, Emollient and potential penetration enhancer for other active compounds (S Vitale *et al*., 2022; YR Boshra *et al*., 2023). |

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 24. | Heptadecane, 4-methyl- | $C_{18}H_{38}$ | As a saturated hydrocarbon, 4-methylheptadecane is largely unreactive with aqueous solutions of acids, alkalis, and most oxidizing or reducing agents under normal conditions. Its action and uses are generally physical in nature or related to its presence in complex biological and industrial mixtures (Abdel *et al*., 2022; Wu *et al*., 2024; Kandimalla *et al*., 2016). |
| 26. | Nonadecane, 2-methyl- | $C_{20}H_{42}$ | As a saturated hydrocarbon (an alkane), 2-methylnonadecane is generally a stable and unreactive compound, primarily acting through physical properties rather than specific chemical or biological actions (Jiménez-Nevárez *et al*., 2023; Esad *et al*., 2025; Kłósek *et al*., 2023). |
| 27. | Heptadecane, 3-methyl- | $C_{18}H_{38}$ | 3-Methylheptadecane is a branched-chain alkane, which is a type of saturated hydrocarbon. Utilized as Solvent and Industrial Use, Cosmetic Ingredient, Fragrance Ingredient, Biomarker (Abdel *et al*., 2022; Kim *et al*., 2013; Sathiyavimal *et al*., 2026). |
| 28. | 1-Tetradecanol | $C_{14}H_{30}O$ | Its action in the context of healing of wounds is related to the use as a component in novel materials, such as Phase Change Material (PCM) microcapsules. These microcapsules are integrated into textiles (like cotton fabric) to create "climate-responsive textiles" that help regulate temperature and improve comfort, which can be beneficial for managing the microenvironment of a wound dressing. It is a fatty alcohol used in cosmetics and industrial applications as an emollient, emulsifier, and thickening agent to provide a smooth texture and stability to formulations (Park *et al*., 2017; Braťka *et al*., 2022). |
| 29. | Dodecane, 1-iodo- | $C_{12}H_{25}I$ | The pure compound 1-iodododecane is primarily used in organic synthesis and as a chemical intermediate. It having potential of Broad-spectrum antimicrobial activity, Biofilm breakdown, Inflammation modulation, Enhanced tissue formation (Leaper *et al*., 2008; Gupta Jr *et al*., 2022; Ibragimova *et al*., 2025). |
| 30. | Tetradecanoic acid, 12-methyl-, methyl ester | $C_{16}H_{32}O_2$ | There is no direct information found that specifically describes the action in healing of wound. But its having Antimicrobial activity, Anti-inflammatory, antioxidant activity and Promoting cell proliferation for wound healing and collagen deposition (Abdel *et al*., 2022; Das *et al*., 2024; Juárez-Rodríguez *et al*., 2021). |
| 31. | Hexacosane | $C_{26}H_{54}$ | Hexacosane, a long-chain alkane hydrocarbon found in certain plant and marine sponge extracts, appears to promote tissue regeneration through a multifaceted approach which includes: Anti-inflammatory effects, Antioxidant properties, proliferation of cells with Angiogenesis (Marrufo *et al*., 2013; Chin *et al*., 2018). |
| 32. | Eicosane, 2-methyl- | $C_{21}H_{44}$ | 2-methyleicosane (EIC) contributes to wound healing through several key actions as Antioxidant activity, Fibroblast Migration, Epithelialization with Collagen synthesis and Potential Antitumor and Antifungal properties. It supports several phases of complex process of healing of wounds, particularly by oxidative stress management with tissue regeneration and cell migration (Balachandran *et al*., 2023; Chuah *et al*., 2018; Balachandran *et al*., 2023). |
| 33. | Hexadecane, 2,6,10,14-tetramethyl- | $C_{20}H_{42}$ | Phytane is a saturated isoprenoid alkane, a derivative of chlorophyll found in many organisms and geological samples. Its unsaturated alcohol form, phytol, and related compounds like tocopherol (vitamin E) and phylloquinone (vitamin K1), are biologically important molecules. It showing Scavenging free radicals and reducing inflammation, Modulating growth factors and cytokine levels (e.g., IL-6, VEGF, TNF-α) and Increasing deposition of collagen and aiding re-epithelialization (Silva *et al*., 2007; Akwu *et al*., 2019). |
| 34. | Oxirane, decyl- | $C_{12}H_{24}O$ | The primary use of decyloxirane is as a chemical intermediate in industrial applications such as in the production of lubricants and as a catalyst (Savitri *et al*., 2025; Bagheri *et al*., 2022). |

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 35. | 1,8,11-Heptadecatriene, (Z,Z)- | $C_{17}H_{30}$ | Its potential contribution to wound healing is likely as one of many phytochemicals within a complex natural extract, possibly contributing to general antimicrobial or anti-inflammatory activities (Najda *et al*., 2021; Likittrakulwong *et al*., 2023). |
| 36. | 9-Octadecenoic acid (Z)-, methyl ester | $C_{19}H_{36}O_2$ | It aids in wound healing by possessing anti-inflammatory, antibacterial, and skin repair properties, often acting as an emollient to condition skin and promote tissue regeneration (Salem *et al*., 2022; Abdel *et al*., 2022; Saifullah *et al*., 2023). |
| 37. | Nonadecane | $C_{19}H_{40}$ | Nonadecane itself is a relatively unreactive, very hydrophobic lipid molecule. It is largely insoluble in water and is generally considered inert in biological systems compared to more active compounds like flavonoids or phenolic compounds. It is a major component of plant waxes that serve a protective barrier function for the plant (Criollo *et al*., 2023; Nandhini *et al*., 2024). |
| 38. | 2-Methyltetracosane | $C_{25}H_{52}$ | 2-Methyltetracosane is a bioactive compound, often found in plant extracts such as *Sonneratia apetala* and various other natural sources, that exhibits significant wound healing properties primarily through anti-inflammatory activity with antioxidant action. It acts as a free radical scavenger, which helps to reduce oxidative stress at the site, which fastens tissue repair (Bhawani *et al*., 2025). |
| 39. | Heptacosane | $C_{27}H_{56}$ | It is a long-chain saturated hydrocarbon found in various plant essential oils and cuticle waxes that contributes to wound healing by its anti-inflammatory, membrane-stabilizing, potential antimicrobial properties. While specifically recognized for its role in modulating multidrug resistance (P-gp) in cancer studies, its structural characteristics as a long-chain alkane suggest a role in strengthening the lipid barrier in skin regeneration (Abdel *et al*., 2022; Labbozzetta *et al*., 2022; Criollo *et al*., 2023). |
| 40. | Octadecane, 5-methyl- | $C_{19}H_{40}$ | It is a branched alkane hydrocarbon identified in various medicinal plant extracts, which possess wound healing properties. Its chemical action in wound healing based on related long-chain hydrocarbon behaviors as Antibacterial, Antioxidant with Anti-inflammatory activity and helps in Epithelialization (Alhazmi *et al*., 2018; Balachandran *et al*., 2023). |
| 41. | 9,12-Octadecadienoyl chloride, (Z,Z)- | $C_{18}H_{31}C_{l}O$ | It is also known as Linoleoyl chloride. It is a fatty acid derivative which identified in various plant extracts through GC-MS analysis for their medicinal, antimicrobial and healing of wounds. Its role in healing of wound is associated with Anti-inflammatory action, Antimicrobial activity and potential Regeneration of tissue (Marathe *et al*., 2019; Alotaibi *et al*., 2026; Bhattacharya *et al*., 2019). |
| 42. | Carbonic acid, decyl nonyl ester | $C_{20}H_{40}O_3$ | It is a specific carbonate ester, having action of Lipid based barrier and Moisture retention, Controlled degradation and Potential Acidification and Drug delivery vector to enhance the solubility and absorption of other active compounds for facilitating faster wound healing. Essentially, this compound acts as an emollient with potential for enhancing the delivery of therapeutic agents in wound repair (Zhu *et al*., 2007; Kumar *et al*., 2025). |
| 43. | Oxiraneundecanoic acid, 3-pentyl-, methyl ester | $C_{19}H_{36}O_3$ | It is a fatty acid derivative containing an epoxide (oxirane) ring, acting primarily as a bioactive compound in plant extracts. It having antioxidant activity, free radical scavenger and potential pharmacological agent with Antimicrobial, Anti-inflammatory and Anti parasitic actions and creates Environment for moist wound which is good for re-epithelialization (Abdel *et al*., 2022; Pooranachithra *et al*., 2019). |

| Sl. No. | Compound | Chemical Formula | Pharmacological Activity |
|---|---|---|---|
| 44. | 2-Ethyl-1-dodecanol | $C_{14}H_{30}O$ | It is a branched-chain fatty alcohol identified as a bioactive constituent in various plant extracts, acting as an Emollient, Solvent, Antimicrobial agent that aids in wound healing. Its chemical structure allows it to contribute to the wound healing process through direct inhibition of microbes and by potentially modulating inflammation. Its chemical actions are Antimicrobial, Antifungal, Anti-inflammatory with Moisturizing and Emollient properties and Synergistic action with plant extracts to promote fibroblast proliferation and neovascularization in plant-based creams and ointments (Muhamad *et al.*, 2025; Raza *et al.*, 2024; Satheesan *et al.*, 2024). |
| 45. | Nonyl tetradecyl ether | $C_{23}H_{48}O$ | It is an organic ether compound having potential chemical actions in wound healing are as Emollient or Skin conditioning agent, Formulation component to deliver active agents for treating infections, wounds and physical properties as highly hydrophobic, nonpolar compound which making it suitable for providing a water- resistant barrier to protect the wound environment (Bibi *et al.*, 2025; Rahman *et al.*, 2017). |
| 46. | Tetratetracontane | $C_{44}H_{90}$ | It is a long-chain aliphatic hydrocarbon identified as a bioactive constituent in plant extracts through GC-MS analysis which is used for wound healing. It contributes through Antibacterial, Antimicrobial, Antioxidant activities and having synergistic effect in which it works alongside other compounds to promote wound repair. Its main role is reducing the microbial load which is helpful in chronic, non-healing wounds (Ghiulai *et al.*, 2020; Sharma *et al.*, 2021; Prasathkumar *et al.*, 2022). |

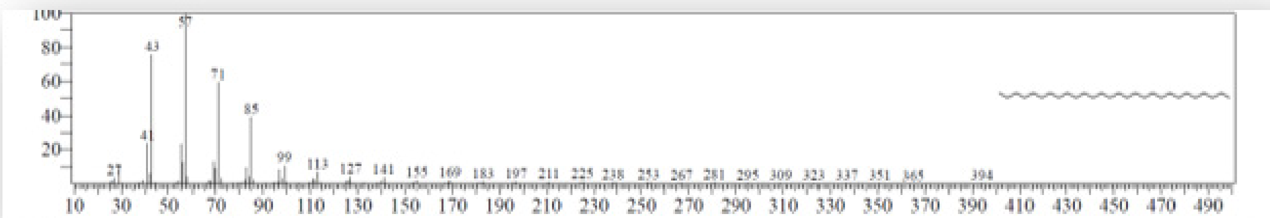


**Figure 6:** Retention time of compound Octacosane ($C_{28}H_{58}$).

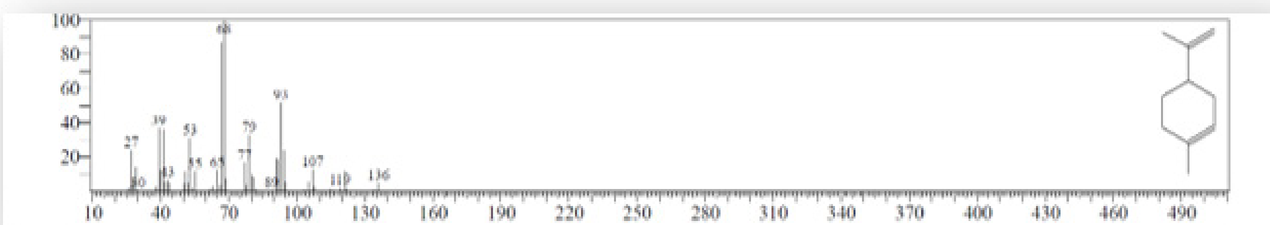


**Figure 7:** Retention time of compound Limonene derivative ($C_{10}H_{16}$).

## CONCLUSION

The ability of Polyherbal Formulation Extracted Ointment to alleviates healing of wound in Chronic Non healing wounds has been recognized. Its potent combination of organic components promotes wound healing, Fibroblast proliferation, Angiogenesis, Antimicrobial, Anti-inflammatory, Collagen Synthesis. It encourages deep absorption and promotes wound healing activity. The GC-MS findings scientifically substantiate its synergistic effect by combining Cleansing, Antimicrobial, wound healing actions consistent with Ayurvedic principles of Vranashodhana, Vranaropana potential in the management of Dusta Vrana. This proprietary formulation reveals promise as a potential complementary approach for the management of Dusta Vrana (Chronic Non healing wounds).

## ABBREVIATIONS

**GC-MS:** Gas Chromatography-Mass Spectrometry; **GMP:** Good Manufacturing Practice; **KLE:** Karnatak Lingayat Education; **CAS:** Chemical Abstracts Service; **R. Time:** Retention Time; **MS:** Mass Spectrometry; **m/z:** Mass-to-Charge Ratio; **PCM:** Phase Change Material; **P-gp:** P-Glycoprotein; **IL-6:** Interleukin-6; **VEGF:** Vascular Endothelial Growth Factor; **TNF-α:** Tumor Necrosis Factor-Alpha; **USD:** United States Dollar; **BP:** British Pharmacopoeia; **GCMS-QP-2010SE:** Gas Chromatography-Mass Spectrometry Quadrupole 2010SE; **cm/sec:** Centimeters per Second; **mL/min:** Milliliters per Minute; **kPa:** Kilopascal; **kV:** Kilovolt; **µL:** Microliter; **mL:** Milliliter; **ºC:** Degrees Celsius.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

Polyherbal Formulation Extracted Ointment has shown various bioactive components have demonstrated potential in the healing of wounds.

The utilization of the formulation in chronic non healing wounds done by its anti-inflammatory, antioxidant, antimicrobial activity. Its ability to heal the chronic wounds by the presence of Anti-inflammatory, antioxidant, antimicrobial components. Its therapeutic utility is further supported by scientific research, which emphasizes its function as an alternative method for Chronic non healing wounds.

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