Research Article

# Comparative Phytochemical Analysis of Two Wound Healing Flora

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## ABSTRACT

*Chromolaena odorata* (L.) R.M. King & H. Rob., shrub of Family Asteraceae and *Hemigraphis alternata* (Burm.f.) T.Anderson, herb of Family Acanthaceae are widely used as wound healers in local medicine. The leaf extract of both plants is used in local medicine which immediately prevent bleeding from wounds. The two plants possess several phytocompounds which can be cited as responsible for the wound healing process, but the presence of common phytocompounds in these two plants have not been worked out. Leaf extract studied using Gas Chromatography- Mass spectrometry method and phytocompounds identified by mass spectra. The comparative study of the components revealed the presence of common compounds including steroids, phthalates and sesquiterpenes - Diazoprogesterone, Diisooctyl phthalate and Caryophyllene derivatives causing blood clotting, wound healing, preventing inflammations and pathogenic attack. The presence of terpenes can be concluded as reason for the wound healing property as they have antioxidant and anti-inflammatory action; presence of steroids may be accounted for the blood clotting process and the presence of pthalates considered as preventing infection in the wound areas. Isolation and optimization of components can help in modern medical practices instead of chemical combinations.

**Keywords:** *Chromolaena odorata, Hemigraphis alternata*, GC-MS, Sesquiterpenes, Phthalates.

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## INTRODUCTION

Unveiling of ethnomedicinal efficacies of flora has always been a boon to modern medicine. Human civilizations had utilised the healing powers of plants throughout the evolutionary period in different cultures. Majority drugs used in modern medicine claim their sources from plants. But along with this the inclusion of synthetic drugs is creating life-long complications in human life. Understanding this potential danger, retrospective outlook to nature has commenced. Drug development from plants can be factualised by intriguing to the traditional use of plant, identifying the chemical content, conducting toxicity analysis of the components, randomised selection of one or several properties. Ethnopharmacology has been a corner stone for the entire procedure. In conjuncture with this basic idea, two plants *Chromolaena odorata* and *Hemigraphis alternata,* two wound healing ethnic plants were used for phytocomponent studies for the constituents helpful in wound healing property. *Chromolaena odorata* (L.) R.M. King and H. Rob., a perennial shrub belonging to family Asteraceae, identified much for its wound healing as well as invasive properties is native to America.[1] The plant is used for treating cuts, infections and various ailments. *Chromolaena odorata* is reported to possess several medically relevant properties.[2] Reports are there on the detection of tannins, steroids, phenolics, flavonoids, saponins and terpenoids in the plant leaves.[2] *Hemigraphis alternata* (Burm.f.) T. Anderson, a herbaceous perennial with a creeping growth habit is widely used in Kerala for its incredible potency to heal wounds. Apart from wound healing, the screening of various bioactive compounds from plant has led to the discovery of

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anti-inflammatory, antidiuretic, anti-diabetic, antiulcer activity.[3] The common wound healing property of the plant can be attributed to some bioactive compounds of the plants. Since the plant leaves are used in crude form for traditional wound healing, the leaves are subjected to phytochemical analysis. Biological relevance of the plant can be identified by basic pharmacological and chemical properties of plant by phytochemical screening methods like spectrometry and chromatography.[4] Bioactive compounds and functional groups in plants are usually tracked by Fourier-Transform Infrared (FTIR) and gas chromatography-mass spectrometry (GC-MS).[5] In this study, therefore a comparative analysis of the phytocomponents observed in both plants are done to elucidate the wound healing property.

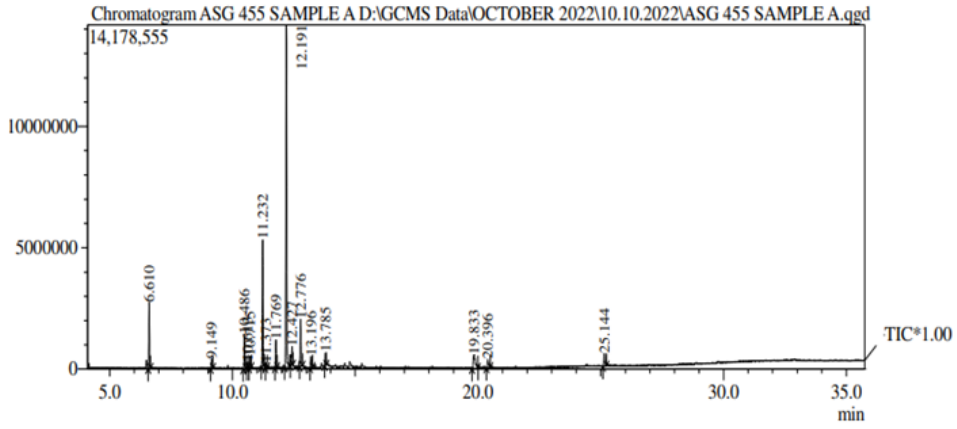
## MATERIALS AND METHODS

The leaves of *Chromolaena odorata* and *Hemigraphis alternata* was collected, cleaned, shade dried and powdered. The powder obtained was weighed out and 75 g of each of this was mixed with 150 mL of ethyl acetate and placed in the rotary shaker for 24 hr to separate the components. After 24 hr, the extract was filtered and the filtrate was allowed to dry out at room temperature for 2 days in dark. The extract (2 mL) was taken and phytochemical components were identified using GC-MS method at KFRI, Peechi. The specifications of the instrument used was Shimadzu GC-MS; Model Number: QP2010S; Column ELITE-5MS; 30 meter length; 0.25 mm ID; 0.25 µm thickness. GCMS Software: GCMS Solutions and Libraries used: NIST 11 and WILEY 8. A comparison of the GC-MS profile was done for the similar components.

## RESULTS

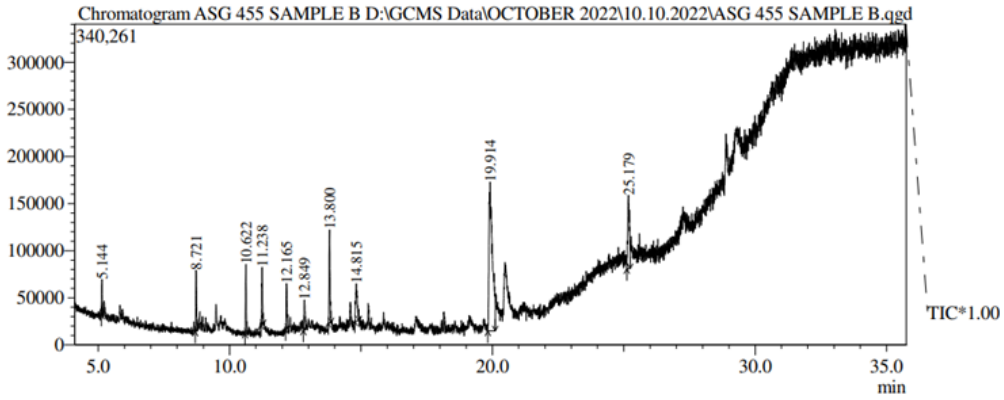
2 mL extract of each plant was used for phytochemical analysis. The compounds identified in *Chromolaena odorata* are Cyclohexene, 5,6-diethenyl-1-methyl-, Cyclohexene, 3,4-diethenyl-3-methyl-, Copaene, (-)-1,2,2.alpha.,3,3,4,6,7,8,8.alpha.-decahydro-2.alpha.,7,8-trimethylacenaphthylene, beta.-Elemene, (Z)-.beta.-Caryophyllene, alpha.-ylangene, Humulene, beta.-copaene, Germacrene B, delta.-Cadinene, Elemol, Caryophyllene oxide, 5.alpha.,17.alpha.-Pregnan-12-one, Phytol, and Diisooctyl phthalate starting from a retention time of 6.6 min up to a retention time of 25.144 min. Beta-copaene was the major compound identified at a retention time of 12.91 min.

The compounds identified for Hemigraphis alternata are Undecane, 5,7-dimethyl-, 3-Ethyl-3-methylheptane, Cedrene-V6, Caryophyllene, Undecane, 3,8-dimethyl-, Hexane, 3,3-dimethyl-, Caryophyllene oxide, beta-Eudesmol, Diazoprogesterone, Diisooctyl phthalate. A total of 10 phytoconstituents was identified in the present study. Retention time taken for analysis was from 5.144 min to 25.179 min. The maximum height obtained was for peak 9 in the spectra representing diazoprogesterone at a retention time of 19.914 min. The prominent fractions identified in the present analysis are 3-Ethyl-3-methylheptane, Cedrene-V6, Caryophyllene, Caryophyllene oxide, Diazoprogesterone, Diisooctyl phthalate.




**Chromatogram of *Chromolaena odorata***

| Peak Report TIC | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Peak#** | **R.Time** | **Area** | **Area %** | **Height** | **Height %** | **Name** | **Base m/z** |
| 1 | 6.610 | 4881577 | 7.50 | 2709456 | 8.73 | Cyclohexene, 5,6-diethenyl-1-methyl- | 79.10 |
| 2 | 9.149 | 802073 | 1.23 | 394798 | 1.27 | Cyclohexene, 3,4-diethenyl-3-methyl- | 79.10 |
| 3 | 10.486 | 2744713 | 4.22 | 1433623 | 4.62 | Copaene | 105.20 |
| 4 | 10.613 | 417064 | 0.64 | 231917 | 0.75 | (-)-1,2,2.alpha.,3,3,4,6,7,8,8.alpha.-decahydro-2. alpha.,7,8-trimethylacenaphthylene | 189.20 |
| 5 | 10.715 | 927248 | 1.42 | 478471 | 1.54 | .beta-Elemene | 93.15 |
| 6 | 11.232 | 10017124 | 15.39 | 5242829 | 16.90 | (Z)-.beta-Caryophyllene | 93.10 |
| 7 | 11.373 | 376714 | 0.58 | 182378 | 0.59 | .alpha.-ylangene | 161.20 |
| 8 | 11.769 | 2213146 | 3.40 | 1152529 | 3.71 | Humulene | 93.15 |
| 9 | 12.191 | 27816313 | 42.74 | 14093841 | 45.42 | .beta.-copaene | 161.20 |
| 10 | 12.427 | 2433471 | 3.74 | 789017 | 2.54 | Germacrene B | 121.20 |
| 11 | 12.776 | 3999032 | 6.14 | 1932770 | 6.23 | .delta.-Cadinene | 161.20 |
| 12 | 13.196 | 1140621 | 1.75 | 459978 | 1.48 | Elemol | 59.05 |
| 13 | 13.785 | 903053 | 1.39 | 508291 | 1.64 | Caryophyllene oxide | 79.10 |
| 14 | 19.833 | 3359649 | 5.16 | 552403 | 1.78 | 5.alpha. 17.alpha. -Pregnan-12-one | 233.15 |
| 15 | 20.396 | 1156278 | 1.78 | 336070 | 1.08 | Phytol | 71.10 |
| 16 | 25.144 | 1891730 | 2.91 | 529073 | 1.71 | Diisooctyl phthalate | 149.10 |
| | | 65079806 | 100.00 | 31027444 | 100.00 | | |



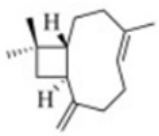

**Component identification in *Chromolaena odorata***

| Chromatogram of *Hemigraphis alternata* and component identification table | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Peak#** | **R.Time** | **Area** | **Area %** | **Height** | **Height %** | **Name** | **Base m/z** |
| 1 | 5.144 | 61094 | 2.15 | 37238 | 5.38 | Undecane, 5,7-dimethyl- | 57.10 |
| 2 | 8.721 | 139524 | 4.91 | 65000 | 9.39 | 3-Ethyl-3-methylheptane | 57.10 |
| 3 | 10.622 | 136537 | 4.81 | 73174 | 10.57 | Cedrene-V6 | 189.15 |
| 4 | 11.238 | 125156 | 4.41 | 61074 | 8.83 | Caryophyllene | 93.10 |
| 5 | 12.165 | 109270 | 3.85 | 48529 | 7.01 | Undecane, 3,8-dimethyl- | 57.10 |
| 6 | 12.849 | 91468 | 3.22 | 32186 | 4.65 | Hexane, 3,3-dimethyl- | 57.05 |
| 7 | 13.800 | 243185 | 8.56 | 98696 | 14.26 | Caryophyllene oxide | 79.15 |
| 8 | 14.815 | 176151 | 6.20 | 39854 | 5.76 | beta.-Eudesmol | 59.15 |
| 9 | 19.914 | 1408141 | 49.58 | 158303 | 22.88 | Diazoprogesterone | 233.10 |
| 10 | 25.179 | 349525 | 12.31 | 77906 | 11.26 | Diisooctyl phthalate | 149.10 |
| | | 2840051 | 100.00 | 691960 | 100.00 | | |

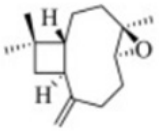
## DISCUSSION

The GC-MS analysis conducted with the leaf samples of two plants revealed some compounds in common. The compounds identified in *Chromolaena odorata* are Cyclohexene, 5,6-diethenyl-1-methyl-,Cyclohexene,3,4-diethenyl-3-methyl-,Copaene,(-)-1,2,2.alpha., 3,3,4,6,7,8,8.alpha.-decahydro-2.alpha.,7,8-trimethylacenaphthylene, beta.-Elemene, (Z)-.beta.-Caryophyllene, alpha.-ylangene, Humulene, beta.-copaene, Germacrene B, delta.-Cadinene, Elemol, Caryophyllene oxide, 5.alpha.,17.alpha.-Pregnan-12-one, Phytol, and Diisooctyl phthalate. The compounds identified for *Hemigraphis alternata* are Undecane, 5,7-dimethyl-, 3-Ethyl-3-methylheptane, Cedrene-V6, Caryophyllene, Undecane, 3,8-dimethyl-, Hexane, 3,3-dimethyl-, Caryophyllene oxide, beta-Eudesmol, Diazoprogesterone, Diisooctyl phthalate.

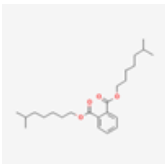
Reports earlier released,[6-8] show the presence of monoterpenes, sesquiterpenes, hydrocarbons, triterpenes/ steroids, alkaloids and flavonoids in *Eupatorium odoarata*. Similarly, phenols, steroids and coumarins were detected in studies,[9] conducted in *Hemigraphis alternata*. In concordance with the previous reports, both samples contain β-Caryophyllene/Caryophyllene, Caryophyllene oxide, Diisooctyl phthalate and class of steroid (Diazoprogesterone/5-α, 17-α- Pregnan-12-one). Critical study of these compounds reveals the reason behind the wound healing property of the plants. β-caryophyllene, Caryophyllene and caryophyllene oxide belong to the sesquiterpene class of terpenoids.[10]



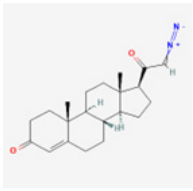
β-caryophyllene[11]



caryophyllene oxide[11]



Diisooctyl phthalate[12]



Diazoprogesterone[13]

5-α, 17-α- Pregnan-12-one[14]

(Credit goes to online websites for molecular structures)

β-caryophyllene possess anti-inflammatory, antioxidant property. It is a bicyclic sesquiterpene, and according to studies can be used as alternative as aircraft fuel, therapeutic compound because of its anti-inflammatory and antioxidant nature.[15] Diisooctyl phthalate belongs to phthalates (esters of o-phthalic acid) that has been detected in higher plants.[16] Xenobiotic and pollutant aspects of phthalates were reported earlier.[17] Work on the endogenous phthalates in plants has led to the view that phthalates has some physiological and biochemical role in plant protection.[18] Diazoprogesterone and 5-α, 17-α-Pregnan-12-one belongs to class of steroids. Steroids are reported as basic constituents of cell membranes they are said to function as signalling molecules in biological systems.[19]

The antioxidant property shown by the terpenoids in both plants can be said to have significant function in wound healing process. Antioxidants reported in wound healing process,[20] is in conformity with the finding. Fibroblast conservation and proliferation of keratinocyte in the wounded area due to the presence of antioxidants can be attributed to the wound healing efficiency of the plants.[21] The phthalates, possess antimicrobial[22] property, excretion of phthalates under stress by cells,[23] ability of phthalates to reduce biofilm formation in bacterial phytopathogens[24] all can be helpful in wound healing process exhibited in *Chromolaena odorata* and *Hemigraphis alternata*. The leaves of these plants may contain recommendable amount of phthalates which can be accounted for the reported results. The anti-inflammatory property of terpenoids is also a supporting action for wound healing. Infection, trauma, chemicals, heat, or unrecognized particles can induce inflammation of tissues.[25] The influx of neutrophils and the resulting surge in free radicals can be alleviated by the antioxidant activity.[26] Inflammatory disease pathology involves excessive Reactive Oxygen Species (ROS) generation by Polymorphonuclear Leukocytes (PMNLs). Natural compounds with antioxidant activity may be useful in reducing or regulating the oxidative damage caused by ROS produced from PMNLs. *C. odorata* may also contribute to wound healing by stimulating the production of antioxidants at the wound site, therefore protecting tissues from oxidative damage and providing a favourable environment for tissue healing.[20] The same combination of terpenoid compounds detected in *Hemigraphis alternata* can be attributed to similar activity. Steroids are responsible for diverse physiological activities namely energy metabolism, circadian rhythms, inflammation, growth and reproduction, immunosup-

pression etc.,[27] This property of steroids reported in the plants can be accounted for the blood clotting capability of the extracts. The infections coming in the wounded area may be prevented by the presence of phthalates as these compounds are playing major role in supressing the growth and biofilm formation of bacterial phytopathogens.[18] Based on the present study, the phytocomponents responsible for blood clotting and wound healing are identified. This study presents a preliminary comparative analysis of the two plants based on the phytocomponents and more precise quantitative determination of pharmacogenetic properties especially phthalates are to be conducted as these compounds are considered to be xenobiotic in scientific literature before utilization.

## CONCLUSION

Present study aims to elucidate the common phytocomponents present in two plants *Chromolaena odorata* and *Hemigraphis alternata* that can be accounted for the wound healing properties. The presence of terpenes can be concluded as reason for the wound healing property as they have antioxidant and anti-inflammatory action; presence of steroids may be accounted for the blood clotting process and the presence of pthalates considered as preventing infection in the infected wound areas. Therefore, a proper method of isolation of these compounds and their optimal combination can help in instantaneous wound healing processes rather than moving on to the chemical synthesised combinations.

## ETHICS APPROVAL

The submitted work is original and not placed for publication in any journal. It has not utilised any animal for study or pose threat to nature. Also, no external fund was utilised for the study and no additional contributors have been involved in the study.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GC-MS:** Gas Chromatography-Mass Spectrometry; **FTIR:** Fourier- Transform Infrared Spectroscopy; **ROS:** Reactive oxygen species; **PMNLs:** Polymorphonuclear leukocytes

## SUMMARY

Two plants *Chromolaena odorata* and *Hemigraphis alternata* of family Asteraceae and Acanthaceae respectively were subjected to GC-MS analysis to analyse the common phytocomponents present that can be the responsible factor for the wound healing property of the plants that is commonly used in the local medicine in Kerala. The plant extracts of both were subjected to GC-MS analysis and the study revealed the presence of common components that can be accounted for the wound healing properties. The presence of terpenes can be concluded as reason for the wound healing property as they have antioxidant and anti-inflammatory action; presence of steroids may be accounted for the blood clotting process and the presence of pthalates considered as preventing infection in the infected wound areas. Therefore, a proper method of isolation of these compounds and their optimal combination can help in instantaneous wound healing processes rather than moving on to the chemical synthesised combinations.

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