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# Seasonal Variations In Essential Oil Composition And Antimicrobial Activity Of *Erythroxylummonogynum*roxb. Leaves And Stems

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**Abstract**

The aim of this study is to assess the seasonal variation in the chemical composition and antimicrobial activity of essential oils extracted from leaves and stems of *Erythroxylummonogynum*.The essential oil from leaves and stems of *Erythroxylummonogynum* during summer, rainy, winter and spring seasons were obtained by hydrodistillation and the chemical composition was determined by GC and GC-MS. The main components identified in leaf essential oil in summer and rainy seasons were Limonene, sabinene, β-pinene, isofenchol, dihydrocarveol, isobornyl acetate, germacrene D and farnesol. While winter and spring seasons essentially contained geijerene, pregeijerene, germacrene D, isobornyl acetate, nerolidol and farnesol. The major compounds identified in stem essential oil in summer and rainy seasons were limonene, trans-β-ocimene, methyl eugenol, isobornyl acetate, decanal and germacrene D whereas winter and spring samples contained limonene, methyl eugenol, geijerene, pregeijerene, spathulenol and farnesol. The essential oil extracted from leaves and stems during spring season was evaluated for antimicrobial activity against two gram-positive and two gram-negative bacteria and four pathogenic fungi using agar disc diffusion technique. Further the minimum inhibitory concentration (MIC) was also determined.Among all the bacteria tested, *B. subtilis* was most susceptible at 100µg/ml with zone of inhibition of 23.3mm and 19.8 respectively for leaf and stem oils. While, among all the fungal species tested, *A. niger* inhibited more effectively at 100µg/mlwith a zone of inhibition of 18.2mm and 13.9mm respectively for leaf and stem oils. The results obtained were remarkable suggesting that the different seasons profoundly influences the chemical composition of plants and essential oil of this plant possess excellent antimicrobial

 

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properties where the studies can be extended for further field trials to explore this plant as an alternative bio-friendly source for various pharmaceutical industries.

**Keywords:** Essential oil composition, seasonal variations, sesquiterpenes, *Erythroxylummonogynum*and antimicrobial activity

**Introduction**

*Erythroxylummonogynum* (*E. monogynum*) is commonly known as Red Cedar belonging to the family Erythroxylaceae. It is a tropical tree native to India and Sri Lanka and is well known to possess rich medicinal value and has been implicated in curing many diseases such as Stomachic, Dyspepsia, Fever, and Dropsy in Ayurveda medicine (Alagesaboopathi, 2013).Various antimicrobial preparations have been developed for use as household antiseptic sprays and antimicrobial bandages (Andrade *et al.*, 2013; Rizzello, L, Pompa, 2014). With the continuous bombardment by synthetic compounds, microorganisms have developed resistance and posing lot of problem across the world and therefore, this problem can now be recognized as a promising global challenge to all the researchers. Hence, there is a need for new and novel alternative antimicrobial agents derived from plant products which are safe and offer good efficacy as that of synthetic compounds. Essential oils derived from medicinal and aromatic plants are complex mixture of natural compounds consisting volatilecompounds with characteristic aroma and are being employed in various perfumery and pharmaceutical industries. These essential oils have shown promising pharmacological effects and this attracted many researchers and scientists throughout the world to use as natural antimicrobial agents leading to the development of novel lead products for the treatment (Demo etal., 2005; Mickiene*et al.*, 2011; Swamy*et al.*, 2016).

However, the effect of environmental factors on the essential oil composition was observed to be typical and specific to each and every species (Lawrence *et al.*, 1998). To our knowledge, there are no published reports on the seasonal variation of the essential oil composition of *E. monogynum*. Further, for the first time, geijerene, pregeijerene and germacrene D in appreciable amounts has been identified in the volatile extracts of this species. Therefore, the aim of the present study is to analyze the composition of oil from leaves and stems in various seasons and also to assess the antimicrobial properties and we present herein, the results of our investigative study

 

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## Materials and methods

### Chemicals and Standard Compounds

The chemicals and solvents required for the study were of analytical grade and procured from Merck, India.

### Sample collection

Leaves and stems of *E. monogynum* were collected from the forests of Nallamalaregion, near Tirupathi, Andhra Pradesh, India. A voucher specimen was deposited at herbarium of Department of Botany, Osmania University, Hyderabad (No. OUBOT 9784).

### Essential oil extraction

The air dried leaves and stems of *E. monogynum* were subjected to hydrodistillation in a Clevenger apparatus for 4 hr. The oil thus obtained was dried over anhydrous sodium sulphate and stored at 4 °C under nitrogen until further use. The oil content was expressed as ml/100 g of dried leaves. The oil was analyzed for chemical composition by Gas chromatography (GC) and Gas chromatography Mass spectrometry (GC-MS).

### GC Analysis

GC analysis was carried out, on a Varian-gas chromatograph fitted BP-1 capillary column (30m X 0.2mm i.d., film thickness 0.25µm). Helium was used as a carrier gas at a flow rate of 1.0 ml/min with 8 p.s.i inlet pressure. The temperature was programmed from 60°C to 220°C at a ramp rate of 5°C/min and a final hold time of 6 min. The injector and detector temperatures were maintained at 250°C and 300°C respectively. Sample of 0.2µl was injected with a split ratio of 1:100

### GC-MS Analysis

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GC-MS analysis was performed on an Agilent 6890 GC fitted with HP-5 capillary column (30m X 0.25mm X 0.25µ) and a 5973 N mass selective detector. The temperature of the oven was programmed from 50°C to 280°C at a ramp rate of 4°C/min with a final hold time of 5 min. The Inlet and interface temperatures were maintained at 250°C and 280°C respectively. Helium at a flow rate of 1.0 ml/min (constant flow) was employed as a carrier gas. Sample of 0.2µl was injected with a split ratio of 20:1. EIMS: electron energy, 70ev. The Ion source and quadrupole temperatures were maintained respectively at 230°C and 150°C.

**Identification of Compounds**

GLC facilitated the identification of individual components by comparing the retention indices (RI) of the peaks determined on a BP-1 column with reference to a saturated mixture of $C_8$-$C_{22}$ n-alkanes with linear interpolation of those of literature (Adams, 1989; Jennings andShibamoto, 1980). Further identification was accomplished by GC-MS by comparing their mass spectra with mass spectral databases such as Wiley and NIST which are resident in the system and of standard compounds reported in literature (Jennings andShibamoto, 1980; Davies, 1990).

**Antimicrobial activity**

Two gram +ve bacteria, *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 10707), two gram –ve bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and fungi such as *Aspergillusniger* (ATCC 10553), *Fusariumoxysporum* (ATCC 10960), *Sclerotiumrolfsii* (ATCC 15206), *Rhizoctoniasolani* (ATCC 16120) were obtained from Department of Microbiology, and Department of Botany, Osmania University, Hyderabad and cultured on nutrient agar and potato dextrose agar media respectively. The antimicrobial activity of essential oil obtained in spring season was investigated by disc diffusion method (NCCLS 2007a, b). To each Petri plate containing 20ml of sterilized culture medium, 2 ml of diluted bacterial strains were added and uniformly spread with a sterilized spreader over the surface. Sterile paper discs (6mm diameter) were impregnated with different concentrations of extracts (10-100µg) in dimethyl sulphoxide (DMSO, 0.5%). The paper discs treated with essential oils were placed in the plates and incubated at 37°C for

 

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24h and 30°C for 48h respectively for bacteria and fungi and the zones of inhibition were determined. Streptomycin (dissolved in DMSO to give a concentration of 1-10µg) and Bifonazole (dissolved in DMSO to give a concentration of 1-10µg) were used as positive controls and acetone served as negative control in the present study. All the experiments were performed in quintuplicate.

**Minimum inhibitory concentration (MIC)**

The MIC values were determined by broth microdilutionmethod (NCCLS 2007a, b). All the experiments were performed in quintuplicate in Mueller Hinton Broth and Potato Dextrose Broth for bacteria and fungi respectively supplemented with Tween 80 (0.5% v/v). All the bacterial and fungal strains were suspended in the broth to obtain a final density of 108 c.f.u./ml. Various concentrations of essential oil obtained during spring season (2-250 µg/ml) were prepared in a 96 well microtitre plate and incubated at 37°C for 24 h and 30°C for 48 h respectively for bacteria and fungi. Control tests were also performed in parallel using DMSO and reference compounds for comparison.

**Results and Discussion**

The essential oil obtained by hydrodistillation of leaves and stems in various seasons were analyzed by GC and GC-MS where the yields of oils were approximately constant i.e., 0.21 – 0.3% from leaves and 0.12 – 0.2% from stems in different seasons, but there was significant variation in the relative composition of chemical constituents.Monoterpene hydrocarbons content varied significantly over time with a low level during spring and winter seasons but was found to be maximum in rainy and summer seasons in leaves (table-1). While low levels of monoterpene hydrocarbons was observed in summer and spring seasons with maximum in rainy and winter seasons in stem oil (table-2). Nine monoterpene hydrocarbons were identified with limonene as a major constituent in both leaf and stem oils. As evident from the table 1& 2, the limonene content varied between 4.18-9.02% in both the leaves and stems. The variation in other monoterpenes was not very significant. Major oxygeneatedmonoterpene components identified are Isofenchol, Isopulegol, isothujanol, borneol and Dihydrocarveol. Methyl eugenol was found to be in higher amounts in stems where it varied between 3.98-4.49% in various

 

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seasons. The maximum content of oxygenated monoterpenes accounting for 19.09% during summer in leaf oil and 23.53% during winter in stem oil, whereas it was 16.18% in spring and 21.91 in summer respectively for leaf and stem essential oils.

**Table-1: Chemical composition of essential oil from leaves of *E. monogynum* in various seasons**

| S. No. | Compound | RI | % Composition in various seasons | | | | Method of Identification |
|---|---|---|---|---|---|---|---|
| | | | Summer | Rainy | Winter | Spring | |
| 1. | α-pinene | 937 | 2.09 | 1.86 | 1.54 | 1.27 | a, b, c |
| 2. | Sabinene | 977 | 2.72 | 2.92 | 3.12 | 3.01 | a, b, c |
| 3. | β-pinene | 981 | 2.79 | 2.99 | 3.43 | 3.18 | a, b, c |
| 4. | Myrcene | 984 | 1.12 | 1.22 | 1.52 | 1.88 | a, b, c |
| 5. | α-phellendrene | 1009 | 1.35 | 1.04 | 1.19 | 1.13 | a, b, c |
| 6. | α-terpinene | 1020 | 0.97 | 0.63 | 0.47 | 0.42 | a, b, c |
| 7. | Limonene | 1024 | 8.78 | 9.02 | 5.13 | 4.95 | a, b, c |
| 8. | Cis-β-ocimene | 1035 | 1.03 | 1.12 | 1.16 | 1.01 | a, b, c |
| 9. | Trans-β-ocimene | 1040 | 2.34 | 2.11 | 2.11 | 1.78 | a, b, c |
| 10. | Linalool | 1085 | 0.98 | 0.68 | 0.53 | 0.56 | a, b, c |
| 11. | Isofenchol | 1101 | 3.21 | 2.77 | 2.33 | 2.02 | a, b, c |
| 12. | Sabinene hydrate | 1116 | 0.95 | 0.76 | 0.70 | 0.68 | a, b, c |
| 13. | Geijerene | 1143 | - | - | 4.26 | 6.62 | a, b, c |
| 14. | Isopulegol | 1146 | 2.57 | 2.88 | 2.52 | 2.42 | a, b, c |
| 15. | Isothujanol | 1157 | 2.48 | 2.69 | 2.58 | 2.19 | a, b, c |
| 16. | Terpinene-4-ol | 1166 | 0.15 | 0.19 | 0.15 | 0.18 | a, b, c |
| 17. | Borneol | 1180 | 2.21 | 2.49 | 2.44 | 2.28 | a, b, c |
| 17. | Decanal | 1192 | Tr | - | - | - | a, b, c |
| 18. | Dihydrocarveol | 1195 | 3.78 | 3.38 | 2.99 | 2.73 | a, b, c |
| 18. | Geraniol | 1240 | 0.21 | 0.27 | 0.32 | 0.30 | a, b, c |
| 19. | Isobornyl acetate | 1271 | 5.32 | 5.42 | 5.30 | 4.69 | a, b, c |

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|---|---|---|---|---|---|---|---|
| 20. | Pregeijerene | 1285 | - | - | 5.33 | 5.64 | a, b, c |
| 21. | Delta elemene | 1337 | 0.97 | 1.05 | 0.89 | 0.77 | a, b, c |
| 22. | Methyl cinnamte | 1342 | 0.46 | 0.86 | 0.76 | 0.62 | a, b, c |
| 23. | Geranyl acetate | 1370 | 0.98 | 1.13 | 1.21 | 1.18 | a, b, c |

| **S. No.** | **Compound** | **RI** | **% Composition in various seasons** | | | | **Method of Identification** |
|---|---|---|---|---|---|---|---|
| | | | **Summer** | **Rainy** | **Winter** | **Spring** | |
| 24. | β-Bourbonene | 1386 | 2.14 | 2.33 | 1.97 | 1.78 | a, b, c |
| 25. | β-Elemene | 1389 | 0.98 | 1.10 | 1.01 | 0.78 | a, b, c |
| 26. | Methyl eugenol | 1403 | 1.01 | 1.14 | 1.08 | 1.02 | a, b, c |
| 27. | β-caryophyllene | 1421 | 2.36 | 2.29 | 2.02 | 1.86 | a, b, c |
| 28. | α-humulene | 1446 | 1.17 | 1.46 | 1.38 | 1.24 | a, b, c |
| 29. | Germacrene D | 1480 | 11.82 | 5.87 | 7.36 | 9.18 | a, b, c |
| 30. | D-cadinene | 1536 | 0.49 | 1.49 | 1.34 | 0.99 | a, b, c |
| 31. | Nerolidol | 1544 | 3.17 | 3.27 | 3.29 | 3.03 | a, b, c |
| 32. | Spathulenol | 1564 | - | 3.61 | 3.32 | 2.98 | a, b, c |
| 33. | Caryophyllene oxide | 1574 | 2.94 | 0.99 | 0.84 | 0.65 | a, b, c |
| 34. | τ-cadinol | 1642 | 2.18 | 1.02 | 1.08 | 1.12 | a, b, c |
| 35. | β-bisabolol | 1672 | 1.88 | 1.88 | 1.77 | 1.43 | a, b, c |
| 36. | Farnesol | 1699 | 4.63 | 4.17 | 4.08 | 3.01 | a, b, c |
| 37. | Farnesyl acetate | 1790 | 0.73 | 0.88 | 0.67 | 0.71 | a, b, c |

a – Retention time; b – Retention indices; c – Mass spectra

Tr – trace amounts

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**Table-2: Chemical composition of essential oil from stems of *E. monogynum* in various seasons**

| S. No. | Compound | RI | % Composition in various seasons | | | | Method of Identification |
|---|---|---|---|---|---|---|---|
| | | | Summer | Rainy | Winter | Spring | |
| 1. | α-pinene | 937 | 1.86 | 1.74 | 1.28 | 1.02 | a, b, c |
| 2. | Sabinene | 977 | 1.08 | 1.27 | 2.28 | 2.04 | a, b, c |
| 3. | β-pinene | 981 | 0.98 | 0.79 | 2.71 | 2.57 | a, b, c |
| 4. | Myrcene | 984 | 0.94 | 1.24 | 1.38 | 1.63 | a, b, c |
| 5. | α-phellendrene | 1009 | 1.32 | 1.45 | 1.63 | 1.72 | a, b, c |
| 6. | α-terpinene | 1020 | 1.98 | 2.79 | 2.44 | 2.33 | a, b, c |
| 7. | Limonene | 1024 | 6.47 | 7.43 | 4.18 | 4.26 | a, b, c |
| 8. | Cis-β-ocimene | 1035 | 0.91 | 1.18 | 1.19 | 1.12 | a, b, c |
| 9. | Trans-β-ocimene | 1040 | 3.38 | 3.49 | 3.21 | 2.89 | a, b, c |
| 10. | Linalool | 1085 | 0.87 | 0.89 | 0.69 | 0.64 | a, b, c |
| 11. | Isofenchol | 1101 | 1.74 | 2.19 | 2.06 | 2.01 | a, b, c |
| 12. | Sabinene hydrate | 1116 | 0.82 | 0.99 | 0.90 | 0.81 | a, b, c |
| 13. | Geijerene | 1143 | - | - | 3.11 | 4.98 | a, b, c |
| 14. | Isopulegol | 1146 | 1.22 | 1.19 | 2.07 | 2.21 | a, b, c |
| 15. | Isothujanol | 1157 | 1.67 | 1.78 | 2.11 | 2.15 | a, b, c |
| 16. | Terpinene-4-ol | 1166 | 0.88 | 0.76 | 0.54 | 0.38 | a, b, c |
| 17. | Borneol | 1180 | 1.24 | 1.36 | 1.57 | 1.47 | a, b, c |
| 17. | Decanal | 1192 | 2.61 | 2.17 | 2.10 | 1.99 | a, b, c |
| 18. | Dihydrocarveol | 1195 | 1.94 | 2.33 | 2.16 | 2.41 | a, b, c |

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|---|---|---|---|---|---|---|---|
| 18. | Geraniol | 1240 | 0.99 | 1.04 | 1.22 | 0.97 | a, b, c |
| 19. | Isobornyl acetate | 1271 | 3.98 | 4.23 | 4.08 | 4.55 | a, b, c |
| 20. | Pregeijerene | 1285 | - | - | 3.26 | 4.62 | a, b, c |
| 21. | Delta elemene | 1337 | 0.78 | 0.93 | 0.91 | 0.90 | a, b, c |
| 22. | Methyl cinnamte | 1342 | 1.69 | 1.72 | 1.82 | 1.27 | a, b, c |
| 23. | Geranyl acetate | 1370 | 1.75 | 2.11 | 2.08 | 1.89 | a, b, c |

| **S. No.** | **Compound** | **RI** | **% Composition in various seasons** | | | | **Method of Identification** |
|---|---|---|---|---|---|---|---|
| | | | **Summer** | **Rainy** | **Winter** | **Spring** | |
| 24. | β-Bourbonene | 1386 | 0.56 | 1.09 | 1.14 | 1.34 | a, b, c |
| 25. | β-Elemene | 1389 | 1.82 | 1.82 | 1.77 | 1.46 | a, b, c |
| 26. | Methyl eugenol | 1403 | 4.49 | 4.21 | 4.21 | 3.98 | a, b, c |
| 27. | β-caryophyllene | 1421 | 1.54 | 1.72 | 1.49 | 1.31 | a, b, c |
| 28. | α-humulene | 1446 | 1.19 | 1.28 | 1.23 | 1.30 | a, b, c |
| 29. | Germacrene D | 1480 | 8.97 | 3.62 | 4.55 | 6.67 | a, b, c |
| 30. | D-cadinene | 1536 | 2.02 | 2.32 | 2.27 | 2.14 | a, b, c |
| 31. | Nerolidol | 1544 | 1.93 | 1.88 | 1.28 | 1.08 | a, b, c |
| 32. | Spathulenol | 1564 | - | 2.76 | 2.62 | 2.34 | a, b, c |
| 33. | Caryophyllene oxide | 1574 | 1.97 | 1.44 | 1.26 | 0.97 | a, b, c |
| 34. | τ-cadinol | 1642 | 1.38 | 0.98 | - | - | a, b, c |
| 35. | β-bisabolol | 1672 | 1.57 | 1.75 | 1.66 | 1.79 | a, b, c |
| 36. | Farnesol | 1699 | 3.85 | 3.79 | 3.83 | 3.11 | a, b, c |
| 37. | Farnesyl acetate | 1790 | - | 0.59 | - | - | a, b, c |

a – Retention time; b – Retention indices; c – Mass spectra

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The relative levels of sesquiterpene hydrocarbons during summer and winter were 20-25% but decreased to 16% in spring season in leaf oil. While in stem oils they were found to be maximum of 26.9% in spring season with a low level of 19% in rainy season. The major sesquiterpenes identified were germacrene D, geijerene, pregeijerene, β-elemene and β-caryophyllene, all of which showed significant seasonal differences. Geijerene and pregeijerene were observed only in winter and spring seasons in both the oils.Germacrene D was found to be maximum of 11.8% and 8.97% respectively in leaf and stem oils while the content dropped down to 5.87% and 3.62% respectively in leaf and stem oils during rainy season. The significant observation was that Methyl eugenol, dihydrocarveol, borneol, isothujanol, isopulegol, nerolidol, spathulenol, and farnesol were observed to be present in predominant percentages inboth the essential oil extracts of *E. monogynum*. The chemical class distribution and major compounds of the essential oil was presented in Table 3-4. Interestingly, the group of sesquiterpenes was found to be dominantin both the essential oils. Nevertheless, the oxygenated derivatives of monoterpenes and sesquiterpenes have also been noticed as minor constituents.

The antibacterial activity of essential oils of leaves and stems of *E. monogynum* obtained during spring season was carried out against four organisms by disc diffusion method and the results were presented in Table 5. Both the oils exhibited noticeable antibacterial activity, as evidenced by their zones of inhibition. Moreover, the activity of leaf essential oil at a concentration of 100 µg/mlwas higher when compared to stem oil, showing a strong inhibition against *B. subtilis* and *S. aureus* with inhibition zones of 23.3and 17.6 mm respectively. With increase in the concentration of oil from 20-100 µg/ml, increase in inhibition was noted. The leaf ans stem essentials oil could inhibit the complete visible growth of *B.*

 

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*subtilis* at a minimum concentration of 119.9 and 129.1µg/ml respectively. Streptomycin was used as a reference positive control sample where itsMIC values ranged from 12.6 to 29.7 µg/ml for all the organisms tested. In the present study, *B. subtilis* was found to be more susceptible and *P. aeruginosa* was found to be resistant for the essential oils.

**Table-3: Chemical class distribution and major components in the essential oil from leaves of *E. monogynum***

| Part of the Plant | Season | Compound class | Area percent | No. of compounds | Major compound | Area percent |
|---|---|---|---|---|---|---|
| Leaves | Summer | Monoterpene hydrocarbons | 23.19 | 9 | Limonene | 8.78 |
| | | Oxygenated monoterpenes | 19.09 | 12 | Dihydrocarveol | 3.78 |
| | | Sesquiterpenes | 19.93 | 7 | Germacrene D | 11.82 |
| | | Oxygenated sesquiterpenes | 20.85 | 7 | Isobornyl acetate | 5.32 |
| | Rainy | Monoterpene hydrocarbons | 22.91 | 9 | Limonene | 9.02 |
| | | Oxygenated monoterpenes | 18.19 | 11 | Dihydrocarveol | 3.38 |
| | | Sesquiterpenes | 15.59 | 7 | Germacrene D | 5.87 |
| | | Oxygenated sesquiterpenes | 21.24 | 8 | Isobornyl acetate | 5.42 |
| | Winter | Monoterpene hydrocarbons | 19.67 | 9 | Limonene | 5.13 |
| | | Oxygenated monoterpenes | 17.61 | 12 | Dihydrocarveol | 2.99 |
| | | Sesquiterpenes | 25.56 | 9 | Germacrene D | 7.36 |

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|---|---|---|---|---|---|---|
| | | Oxygenated sesquiterpenes | 20.35 | 8 | Isobornyl acetate | 5.30 |
| | Spring | Monoterpene hydrocarbons | 18.63 | 9 | Limonene | 4.95 |
| | | Oxygenated monoterpenes | 16.18 | 12 | Dihydrocarveol | 2.73 |
| | | Sesquiterpenes | 28.86 | 9 | Germacrene D | 9.18 |
| | | Oxygenated sesquiterpenes | 17.62 | 8 | Isobornyl acetate | 4.69 |

**Table-4: Chemical class distribution and major components in the essential oil from stems of *E. monogynum***

| Part of the Plant | Season | Compound class | Area percent | No. of compounds | Major compound | Area percent |
|---|---|---|---|---|---|---|
| **Stems** | Summer | Monoterpene hydrocarbons | 18.92 | 9 | Limonene | 6.47 |
| | | Oxygenated monoterpenes | 21.91 | 13 | Methyl Eugenol | 4.49 |
| | | Sesquiterpenes | 22.15 | 9 | Germacrene D | 8.97 |
| | | Oxygenated sesquiterpenes | 14.68 | 6 | Isobornyl acetate | 3.98 |
| | Rainy | Monoterpene hydrocarbons | 21.38 | 9 | Limonene | 7.43 |
| | | Oxygenated monoterpenes | 22.74 | 13 | Methyl Eugenol | 4.21 |
| | | Sesquiterpenes | 19.92 | 9 | Germacrene D | 3.62 |
| | | Oxygenated sesquiterpenes | 17.42 | 8 | Isobornyl acetate | 4.23 |
| | Winter | Monoterpene hydrocarbons | 20.3 | 9 | Limonene | 4.18 |
| | | Oxygenated monoterpenes | 23.53 | 13 | Methyl Eugenol | 4.21 |

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|---|---|---|---|---|---|---|
| | | Sesquiterpenes | 24.85 | 11 | Germacrene D | 4.55 |
| | | Oxygenated sesquiterpenes | 14.73 | 6 | Isobornyl acetate | 4.08 |
| | Spring | Monoterpene hydrocarbons | 19.58 | 9 | Limonene | 4.26 |
| | | Oxygenated monoterpenes | 22.18 | 13 | Methyl Eugenol | 3.98 |
| | | Sesquiterpenes | 29.6 | 11 | Germacrene D | 6.67 |
| | | Oxygenated sesquiterpenes | 13.84 | 6 | Isobornyl acetate | 4.55 |

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**Table – 5: Antibacterial activity of essential oil from leaves and stems of *E. monogynum***

| Organism | Extract | Concentration (µg/ml) | | | | | | Streptomycin | |
|---|---|---|---|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 | 100 | MIC | DD | MIC |
| *E. coli* | SO | 2.3±0.14 | 3.1±0.13 | 5.5±0.21 [c] | 8.2±0.32 | 10.1±0.21 | 178.3±3.37 | 18.5±0.59 | 23.4±0.32 |
| | LO | 3.6±0.19 | 5.4±0.22 [b] | 9.2±0.18 | 10.6±0.26 | 11.9±0.28 | 166.2±2.78 | | |
| *P. aeruginosa* | SO | 3.5±0.18 [a] | 4.2±0.29 | 6.1±0.22 | 9.2±0.32 [d] | 11.8±0.32 | 198.2±4.27 [f] | 23.6±0.66 | 29.7±0.40 |
| | LO | 4.8±0.21 | 6.6±0.31 | 10.9±0.29 [c] | 11.6±0.29 | 13.8±0.34 | 174.1±3.73 [f] | | |
| *S. aureus* | SO | 5.9±0.27 | 7.3±0.33 | 8.7±0.25 | 11.5±0.39 [d] | 14.6±0.36 [e] | 141.7±2.88 | 30.2±0.89 | 16.6±0.39 |
| | LO | 8.1±0.31 [a] | 9.7±0.35 [b] | 13.6±0.23 | 15.4±0.36 | 17.6±0.39 | 132.8±3.36 | | |
| *B. subtilis* | SO | 7.2±0.23 | 8.8±0.31 | 12.1±0.33 | 15.2±0.33 | 19.8±0.38 | 129.1±2.92 [f] | 36.4±1.07 | 12.6±0.42 |
| | LO | 9.1±0.27 | 12.7±0.28 [b] | 17.3±0.36 [c] | 19.8±0.46 | 23.3±0.48 [e] | 119.9±3.29 | | |

Mean diameter of zone of inhibition in mm (mean ± S.E) (Excluding the diameter of the disc – 6 mm)
Numbers with the same letters in each column means differences are not significant (P > 0.05)

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**Table – 6: Antifungal activity of essential oil from leaves and stems of *E. monogynum***

| Organism | Extract | Concentration (µg/ml) | | | | | | Bifonazole | |
|---|---|---|---|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 | 100 | MIC | DD | MIC |
| *A. niger* | SO | 5.4±0.16 | 6.1±0.26 | 7.6±0.27 | 10.8±0.33 | 13.9±0.37 | 134.4±3.44$^{f}$ | 34.4±1.36 | 15.2±0.27 |
| | LO | 6.6±0.23 | 7.8±0.37$^{b}$ | 9.7±0.19 | 13.4±0.32 | 18.2±0.39 | 125.6±3.24 | | |
| *F. oxysporum* | SO | 4.7±0.29 | 5.6±0.33 | 6.6±0.29$^{c}$ | 8.9±0.36 | 12.1±0.28 | 146.2±3.19 | 27.9±1.76 | 18.4±0.36 |
| | LO | 5.9±0.31$^{a}$ | 6.1±0.37 | 7.7±0.35$^{c}$ | 11.1±0.39$^{d}$ | 14.7±0.45$^{e}$ | 136.2±3.33 | | |
| *R. solani* | SO | 3.1±0.31$^{a}$ | 4.9±0.37 | 5.7±0.24 | 7.5±0.39$^{d}$ | 10.2±0.41 | 168.1±2.93 | 23.1±1.07 | 24.9±0.44 |
| | LO | 4.5±0.24 | 5.4±0.31$^{b}$ | 7.7±0.38 | 9.3±0.33 | 11.0±0.34 | 145.7±3.55$^{f}$ | | |
| *S. rolfsii* | SO | 2.4±0.22 | 3.9±0.23 | 4.4±0.21 | 6.5±0.35 | 8.1±0.39$^{e}$ | 201.3±4.23$^{f}$ | 18.3±1.23 | 31.1±0.46 |
| | LO | 3.7±0.19 | 4.6±0.28 | 5.8±0.30 | 8.6±0.37$^{d}$ | 10.2±0.33 | 177.7±3.44 | | |

Mean diameter of zone of inhibition in mm (mean ± S.E) (Excluding the diameter of the disc – 6 mm)
Numbers with the same letters in each column means differences are not significant (P > 0.05)

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The essential oils were evaluated for their antifungal activity using the disc diffusion technique and the results were depicted in Table 6. As evident from the table, *A. niger* was inhibited more effectively at 100 µg/ml with a zone of inhibition of 18.2 mm and 13.9 mmrespectively for leaf and stem oil.. Subsequently MICs were also determined and are presented wherein, leaf oil was more potential with a MIC value of 125.6µg/ml and stem oil has a MIC value of 134.4µg/ml for the same *A. niger* species.Bifonazolewas used as reference positive control sample and the its MIC values ranged from 12.8 to 29.1µg/ml for all the organisms tested. The antimicrobial activity of essential oils have been reported in a number of reports of reports (Chouhan*et al.*, 2017; Man *et al.*, 2019; Cazella*et al.*, 2019) where it was showed a very good inhibitory activity against both gram +ve and gram –ve bacteria. In the present study, the essential oils of *E. monogynum*was effective against all the tested bacterial and fungal strains and showed that gram-positive bacteria are more sensitive than gram-negative bacteria. These results are in contrast with those of earlier reports available in the literature (Smith-Palmer *et al.*, 2001; Delaquis 2002). The mild activity of gram-negative bacteria can be attributed due to presence of an outer membrane with which these bacteria are coated (Mann *et al.* 2000). The potential anti-microbial susceptibility of the organisms in the present study might be due to the varying membrane permeability of the essential oilswhich is in agreement with the published reports (Lambert *et al.* 2001).

**Conclusion**

The present study indicates that the seasonal variations have a profound effect on the chemical composition of essential oils obtained from *E. monogynum*. It is also interesting to note germacrene D, geijerene, pregeijerene, isothujanol and isopulegol were observed for the first time in this species and noticed as major compounds. The results obtained with antimicrobial activity are remarkable showing maximum antimicrobial activity of the essential oilsof *E. monogynum*and need to be explored as a viable alternative source of natural antimicrobial agent.

 

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