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# GC-MS PROFILING FOR THE DETECTION OF BIO-ACTIVE COMPOUNDS IN *EUGENIA ROXBURGHII* AND THEIR ACTIVITY AGAINST DIFFERENT MICROBES

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## Abstract

*Eugenia roxburghii* DC. is an underutilized plant with immense medicinal benefits under the family Myrtaceae. The plant is distributed in tropical coastal areas of India and Srilanka. Most of this plant species is used in curing of diabetes, piles, diarrhoea, and dysentery and has anticancer and antibacterial activity. Like other *Eugenia* species, the plant has several phytochemicals and secondary metabolites, which attribute for the medicinal property of the plant. In order to find the active principles present in the plant, GC-MS analysis was carried out by taking the methanolic extract of *Eugenia roxburghii* fresh leaves. Twenty eight compounds were identified from the analysis out of which the major compounds were Caryophyllene, trans-α-Bergamotene, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-, "Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-, α-Cubebene, 13-Docosenamide, (Z)-,"2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- etc. However, Caryophyllene was one of the most abundant compounds with highest percentage (45.93%) of peak area. The methanolic leaf extract of *E. roxburghii* was further tested against strains of multidrug resistant bacteria and found exhibition of antimicrobial activity. So the present investigation about the identification and chemical fingerprinting of *Eugenia* speciescan help in proper utilization of *Eugenia roxburghii* in food, nutraceutical and pharmaceutical industries.

***Key words*** **:** Caryophyllene· Medicinal Plant· GC-MS· Minimum Inhibitory Concentration· Staphylococcus aureus.

## Introduction

Myrtaceae family consists of about 1200 species and more than 75 species are found from India (Anand *et al.,* 1999). *Eugenia roxburghii* DC. is an underutilized plant under the family Myrtaceae. Distribution of this plant species is found mostly in tropical and coastal areas of India and Srilanka. It also known as Roxburgh's cherry for the delicious nature of its fruit. It has been reported that plant of *Eugenia* species possess antidiabetic, anti-inflammatory, antifungal, antibacterial and antioxidant activity (Chaudhuri*et al.,* 1990; Nonaka *et al.,* 1992; Djipa *et al.,* 2000; Nassar *et al.,* 2007; Park *et al.,* 2007; Ayoola *et al.,* 2008; Kumar *et al.,* 2008). As it is known medicinal plants are rich source of phytochemicals and secondary metabolites which attributes the medicinal properties of the plant.

Previously, fingerprinting of metabolites presents in *Eugenia jambolana* Lam. fruit extract was carried out using Gas Chromatography-Mass spectrophotometer (GC-MS) (Sharma *et al.,* 2015). Another report has been given about the GC-MS analysis and antimicrobial study from the leaves of *Syzygium calophyllifolium Walp* (Vignesh *et al.,* 2013). But till now there has been no such report about the biological activities of leaves of *E. roxburghii*.

Hence, in this study GC-MS analysis was carried out by taking *E. roxburghii* fresh leaves in order to find out the active principle present in this plant.

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## Materials and Methods

### Sample collection

Fresh leaf samples of *E. roxburghii* (Fig. 1) were collected from Konark, Odisha, India (19.878,86.101). Then the leaves were washed with tap water and dried with the help of tissue paper**.**

### Sample preparation and analysis by GC-MS

Approximately 25g fresh leaves of *E. roxburghii* were pulverized in a mortar and pestle with the help of liquid $N_2$. Then the powder form was subjected to put into a Soxhlet apparatus followed by addition of ~300ml of methanol (MeOH) which operates at a constant temperature of $50^0$C. The process continued till the colour of the solution becomes transparent. Finally, the suspended sticky extract was taken out from the apparatus with the help of a spatula and stored in an eppendrof tube.

The Gas Chromatography-Mass Spectroscopy analysis of sample was done by the GC (HP 6890) (Hewlett-Packard, USA) which was associated with an MSD (mass selective detector) (HP 5973). Here Helium was taken as stationary phase and injection of sample was made in split less mode in a column HP5 Phenyl methyl siloxane [25µm (film thickness) × 320µm (internal diameter) × 30m (length of column)]. Mass spectra were acquired over a 50-650 atomic mass unit range. The parameter for temperature were $60^0$C, $3^0$C/min and $243^0$C for initial temperature, ramping rate and eventual temperature respectively. The total run was for 24mins. Identification of major compounds in the analyte was done by comparative study of the mass spectra with NIST library through appropriate software and previously available data.

### Study of antibacterial activity

### Disc diffusion method

Four different bacterial strains *viz., Staphylococcus aureus* (Rosenbach, 1884) (ATCC-96), *Escherichia coli* (Migula, 1895; Castellani and Chalmers,1919) (ATCC-443), *Vibrio cholera* (Pacini, 1854) (ATCC-3906) and *Pseudomonas aeruginosa* (Schroeter, 1872; Migula, 1900) (Clinically isolated from SCB medical college, Microbiology department, Cuttack, Odisha, India) were used for analysis of antibacterial activity of *E. roxburghii* extract. Active cultures were revived from the stock cultures maintained at $4^0$C by taking a loop full of bacteria and inoculated in nutrient broth followed by incubation at $37^0$C for 24hrs. Disc diffusion method was performed for this experiment where the discs were infused with four different concentrations *i.e.* 120µg $ml^{-1}$, 160µg $ml^{-1}$, 200µg $ml^{-1}$ and 240µg $ml^{-1}$. The discs were placed over the bacteria spreaded nutrient agar media plates and were incubated at $37^0$C for 24hrs.

### Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration against *Staphylococcus aureus* was measured by adding four concentrations (120µg $ml^{-1}$, 160µg $ml^{-1}$, 200µg $ml^{-1}$and 240µg $ml^{-1}$) of *E. roxburghii* leaf extract in each of the four conical flasks having 25 ml of nutrient broth each. Then to these flasks 100µl of *Staphylococcus aureus* bacterial culture was added and absorbance was taken in a UH 5300 Hitachi UV-Vis Double Beam Spectrophotometer (Japan) as $0^{th}$ hour reading. Then the flasks contain media bacterial culture and extract were incubated in a shaker incubator at $37^0$C at 800rpm. Then the absorbances were taken at 600nm in an interval of 2hrs from initial reading to 12hrs of incubation *i.e.* $0^{th}$, $2^{nd}$, $4^{th}$, $6^{th}$, $8^{th}$, $10^{th}$ and $12^{th}$ hrs.

## Results and Discussion

### Analysis and Evaluation of GC-MS data

Twenty-eight compounds were detected from the leaf extract of E. roxburghii through GC-MS analysis. These compounds may be attributed for the medicinal property of the plant. Details of the active compounds and their mass spectral characters are presented in Table1 and Fig. 2. The compound with lowest retention time *i.e.* 5.22 was found to be 2-Pentanone,4-hydroxy-4-methyl- and the one which has highest retention time *i.e.* 27 was 2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)-. Among the detected components the major compounds were Caryophyllene, trans-a-Bergamotene, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-, "Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-, α-Cubebene, 13-Docosenamide, (Z)-,"2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- etc. having retention time 14.19, 14.28, 14.64, 15.42, 15.57, 25.35 and 27 respectively. These were found to be major compounds as they attribute the maximum area of percentage from other compounds *viz.,*Caryophyllene attained 45.93%, trans-a-Bergamotene attained 4.09%, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- attained 10.79%, "Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- attained 2.85%, a-Cubebene attained 5.15%, 13-Docosenamide, (Z)-attained 3.05% and "2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- attained 27%. However, the most abundant compound with highest percentage (45.93%) of peak area was found to be Caryophyllene.

In order to find out the active principle and antibacterial activity present in the *Eugenia* species, a

**Table 1:** Analysis of active principles present in fresh leaves of *E. roxburghii*by GC-MS.

| S.No. | Name of thecompound | RT | Mol.Formula | MW | PeakArea |
|---|---|---|---|---|---|
| 1 | 2-Pentanone,4-hydroxy-4-methyl- | 5.22 | $C_6H_{12}O_2$ | 116.1583 | 0.18 |
| 2 | Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R) | 8.25 | $C_{10}H_{16}$ | 136.234 | 0.28 |
| 3 | 4HPyran4one,2,3dihydro3,5dihydroxy6methyl | 10.02 | $C_6H_8O_4$ | 144.1253 | 0.26 |
| 4 | α-Cubebene | 13.15 | $C_{15}H_{24}$ | 204.3511 | 0.98 |
| 5 | α-Copaene | 13.57 | $C_{15}H_{24}$ | 204.3511 | 0.43 |
| 6 | Caryophyllene | 14.02 | $C_{15}H_{24}$ | 204.3511 | 2.33 |
| 7 | Caryophyllene | 14.19 | $C_{15}H_{24}$ | 204.3512 | 45.93 |
| 8 | trans-α-Bergamotene | 14.28 | $C_{15}H_{24}$ | 204.3511 | 4.09 |
| 9 | cis-α-Bisabolene | 14.44 | $C_{15}H_{24}$ | 204.3511 | 0.46 |
| 10 | 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- | 14.64 | $C_{15}H_{24}$ | 204.3511 | 10.79 |
| 11 | Isoledene | 14.82 | $C_{15}H_{24}$ | 204.3511 | 0.48 |
| 12 | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aα)]- | 15.07 | $C_{15}H_{24}$ | 204.3511 | 1.94 |
| 13 | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aâ)]- | 15.15 | $C_{15}H_{24}$ | 204.3511 | 1.12 |
| 14 | Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- | 15.42 | $C_{15}H_{22}$ | 202.3352 | 2.85 |
| 15 | α-Cubebene | 15.57 | $C_{15}H_{24}$ | 204.3511 | 5.15 |
| 16 | Caryophyllene oxide | 16.23 | $C_{15}H_{24}O$ | 220.3505 | 0.47 |
| 17 | Cubenol | 16.91 | $C_{15}H_{26}O$ | 222.3663 | 0.39 |
| 18 | 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- | 17.6 | $C_{15}H_{26}O$ | 222.3663 | 0.34 |
| 19 | 3,3,5,5-Tetramethylcyclopentene | 18.81 | $C_9H_{16}$ | 124.2233 | 2.42 |
| 20 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 18.92 | $C_{20}H_{40}O$ | 296.531 | 0.3 |
| 21 | 1,3Cyclopentadiene,5,5dimethyl1ethyl- | 19.26 | $C_9H_{14}$ | 122.2075 | 3.61 |
| 22 | n-Hexadecanoic acid | 20.1 | $C_{16}H_{32}O_2$ | 256.4241 | 0.79 |
| 23 | Phytol | 21.6 | $C_{20}H_{40}O$ | 296.531 | 0.52 |
| 24 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 21.81 | $C^{18}H_{30}O_2$ | 278.4296 | 0.79 |
| 25 | Epistephamiersine | 23.05 | $C_{21}H_{27}NO_6$ | - | 1.33 |
| 26 | 13-Docosenamide, (Z)- | 25.35 | $C_{22}H_{43}NO$ | 337.5829 | 3.05 |
| 27 | 2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- | 26.82 | $C_{16}H_{14}O_4$ | 270.28 | 4.95 |
| 28 | 2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- | 27 | $C_{16}H_{14}O_4$ | 270.28 | 2.37 |

**Table 2:** Antibacterial study of *E. roxburghii* leaf extract by disc diffusion method.

| Bacteria | Zone of inhibition(mm) | | | | |
|---|---|---|---|---|---|
| | Control | 120µg/ml | 160µg/ml | 200µg/ml | 240µg/ml |
| *Escherichia coli* | 0 | 4±0.02 | 4.6±0.03 | 5.2±0.02 | 5.9±0.01 |
| *Pseudomonas aeruginosa* | 0 | 4.2±0.01 | 4.8±0.01 | 5±0.02 | 5.6±0.03 |
| *Vibrio cholerae* | 0 | 4.1±0.03 | 4.7±0.01 | 5.3±0.01 | 6±0.02 |
| *Staphylococcus aureus* | 0 | 6±0.01 | 6.5±0.02 | 7.2±0.01 | 8±0.01 |

**Table 3:** MIC test of *E. roxburghii* leaf extract against *Staphylococcus aureus.*

| Concentration (µg/ml) | Optical density (OD) at different time Interval (Hour) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0th | 2nd | 4th | 6th | 8th | 10th | 12th |
| 120 | 0.110 | 0.119 | 0.132 | 0.159 | 0.303 | 0.53 | 0.764 |
| 160 | 0.117 | 0.116 | 0.113 | 0.107 | 0.100 | 0.09 | 0.086 |
| 200 | 0.116 | 0.113 | 0.111 | 0.100 | 0.097 | 0.085 | 0.080 |
| 240 | 0.115 | 0.100 | 0.091 | 0.084 | 0.072 | 0.065 | 0.057 |

**Fig. 1:** Eugeniaroxburghii DC. (in its natural habitat)

comparative study was carried out between *E. roxburghii* with *E. jambolana* and *E. caryophyllata.*

GC-MS analysis was carried out by taking volatile oil of *E. jambolana* (Sharma *et al.,* 2015) to know the active principle present in fruits and it was found out that there were 8 major compounds present *i.e.* Myrtenal (21.4%), α-Pinene (18.6%), β-Pinene (14.0%), γ-Terpinene (9.2%), δ-Carene (6.2%), α-Phellandrene (5.8%), Terpinolene (3.9%) and Bornyl acetate (1.5%). Also the result for GC-MS analysis of *E. caryophyllata* (Sifniades *et al.,* 2000) revealed about presence of numerous compounds among which Eugenol (19.46 %), 1,2,3-Benzenetriol (5.48 %) and Caryophyllene (4.48%) were the major compounds. Caryophyllene is used as a non-steroidal anti-inflammatory drug. So *E. roxburghii* leaf extract may be used for the treatment of pain and inflammation. Though Caryophyllene is present in *E. caryophyllata* and *E. roxburghii* the concentration of the compound is more in the latter species. So, *E. roxburghii* may be the better option as a source of Caryophyllene than *E. caryophyllata.* From this present study, 28 compounds were found among which Caryophyllene (45.93%), 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (10.79%), α-Cubebene (5.15%),"2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- (4.95%), trans-a-Bergamotene (4.09%), 13-Docosenamide, (Z)- (3.05%) and "Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- (1.94%) are the major compounds. Some of the compounds has been found with their biological activities such as 2-Pentanone, 4-hydroxy-4-methyl- (Diacetone alcohol) is used in cellulose ester lacquers (Sifniades *et al.,* 2000), α-Copaene is used as a potential rendezvous cue for Mediterranean fruit fly (Nishida *et al.,* 2000), caryophyllene is used for the treatment of inflammation and pain (https://monq.com/eo/terpenes/beta-caryophyllene/), trans-α-Bergamotene a precursor in the biosynthesis of fumagillin, ovalicin and

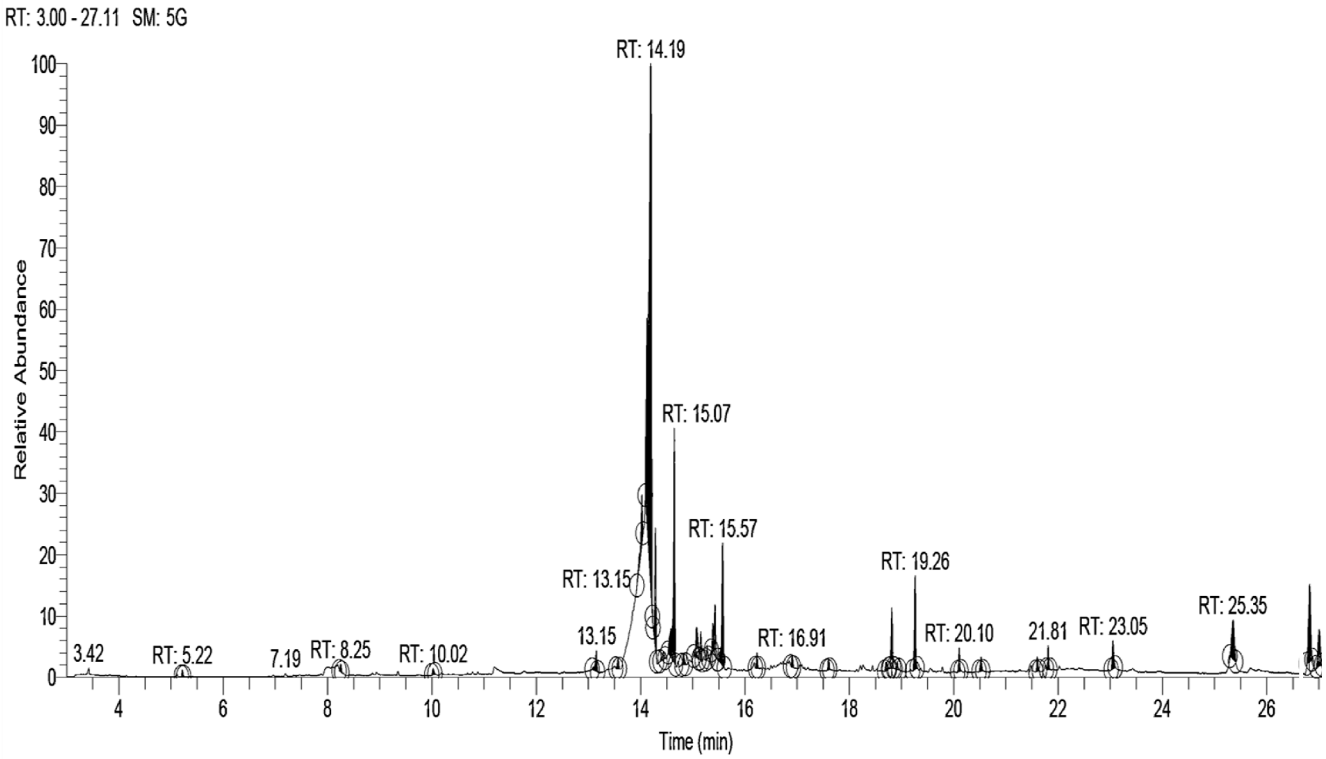


**Fig. 2:** GC-MS analysis of *E.roxburghii* leaf samples.

related antibiotics (Cane *et al.,* 1987; Cane *et al.,* 1989).

**Disc Diffusion method of Antimicrobial test**

Antibacterial activity by both disc diffusion method was represented in table 2. From disc diffusion method, when it comes to controls there was no bacterial inhibition zone was found but further when prepared disks with different concentrations (*i.e.*120µg ml$^{-1}$, 160µg ml$^{-1}$, 200µg ml$^{-1}$ and 240µg ml$^{-1}$) of extracts were infused over the bacterial plates there were formation of zones was observed. From this it was observed that, out of the four bacteria taken for testing it showed highest zone of inhibition against *Staphylococcus aureus* and moderate effect against other three bacteria. It may happen that the other three bacteria were having some more resistant activity than *Staphylococcus aureus* but there also form good zone of inhibition against those three when treated with the extract.

**Examining of MIC test**

For further confirmation, MIC test was carried out by taking bacterial culture of *Staphylococcus aureus* as the extract showed highest zone of inhibition against it table 3. In MIC test spectro readings of the bacterial culture and different concentrations of the extract mixture were taken at different intervals and the result showed that there is continuous increase in absorbance at concentration of 120µgml$^{-1}$ with the increase in time. But in 240µg ml$^{-1}$ concentration there is continuously decrease in the absorbance as time increases. However there was no significant change in other concentration of extract. So at less concentration of extract, *Staphylococcus aureus* may be able to produce some resistant but when concentration of the extract increased, it may be losing its resistant activity and the extract became dominant over the bacteria.

In disc diffusion method, extract showed highest zone of inhibition against *Staphylococcus aureus* than other bacteria and when MIC test was carried out by taking different concentrations of extract there was no inhibition in bacterial growth at 120µg ml$^{-1}$ concentration of leaf extract. But it was observed a significant variation in antimicrobial activity (inhibition of bacterial growth) at 240µg ml$^{-1}$ concentration of the leaf extract. So this result may suggest that due to less concentration of extract it may not be very effective towards bacteria but in higher concentration, the antimicrobial effect of extract getting increased with lapse of time. Antibacterial study of *E. caryophyllata* ethanol extract was exhibited more towards *Klebsiella pneumonia* where as it revealed marginal activity against *Vibrio cholerae, Bacillus subtilis* and *Staphylococcus aureus* (Hemalatha*at al.* 2015). As compared to these reports, present study found

that *E. roxburghii* methanolic leaf extract was exhibited more towards *Staphylococcus aureus*. Although there was moderate activity observed against *Escherichia coli, Vibrio cholera* and *Pseudomonas aeruginosa*. So extract of *E. roxburghii* may be a better antibacterial reagent with respect to *Staphylococcus aureus* than *E. jambolana*. However, to combat *Klebsiella pneumonia* and *Vibrio cholera, E. jambolana* can be a better choice. All this result only reinforced to prove the antibacterial activity of some *Eugenia* species.

The compounds observed through GC-MS analysis from leaf extract can be the alternate source for treatment of antibacterial and anti- inflammatory purposes. But in vivo studies may be carried out to find out the efficacy of the leaf extract for the treatment of bacterial and inflammatory diseases.

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