



## Studies on chemical composition of essential oil from wild *Cymbopogon giganteus*

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### Abstract

*Cymbopogon giganteus* belong to aromatic grasses of the family Poaceae. During the present study wild *Cymbopogon giganteus* collected from Gangondanahalli, Bangalore was investigated for essential oil content comparison, morphological characterization and DNA barcoding of the species carried out confirmed the species investigated as *C.giganteus*. The essential oil of the plant constituted rich amount of monoterpenoids compounds such as Dihydrocarveol (25.02%), Menthane (14.71%), Citral (13.26%), Limonene (7.85%) and Limonene 1,2 epoxide(3.65%). Exploration of wild cymbopogon species revealed the diverse compounds present in essential oil. *C. giganteus* is widely used in traditional medicine against several diseases.

**Keywords:** wild *Cymbopogon giganteus*, DNA barcoding, essential oil, terpenoids, carveol

### Introduction

*Cymbopogon* genus belongs to family Poaceae consist of perennial, aromatic grasses, grows up to 2-3 m height and posses potential essential oil. The oil is used to treat boils, stomach pain, toothache and the decoction of leaf was used to treat headaches, common cold, conjunctivitis, sickling, cellular diseases, or for tranquilizing epileptic seizures. The essential oil is also called "Citronelle de Madagascar" is widely commercialized. The plant posses antiseptic and antifungal properties. (Habib Toukourou *et al.*,2020) [29]. The essential oil is a complex odoriferous mixtures of volatile and scent-laden compounds consisting of monoterpenes, sesquiterpenes, and their derivatives such as aldehydes and phenols. The composition of the essentil oil differs between species differs between the seasons of the year. The essential oils known for their antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties. (Imaël Henri Nestor Bassolé *et al.*,2012). During the present study, the wild cymbopogon essential oil shows antimicrobial activities were found against Gram-(+)- and Gram-(-)-bacteria as well as the yeast *Candida albicans*. (Leopold Jirovetz *et al.*,2007) The species of *Cymbopogon giganteus* is widely used in traditional medicine against several diseases.(G.A. Alitonou *et al.*,2006) [10].

### Materials and Methods

#### Plant collection and ecological details

Fresh plant of *Cymbopogon* including leaves, roots, inflorescence from Wild *Cymbopogon sp.*, were collected for the study, It was collected from the place called Gangodanahalli, It is located in Bangalore South-560039. Elevation, Altitude-874 meters (Above sea level), Temperature-29°C, Humidity-45%, Wind-7.2mt/sec towards

West, Station name- Kanjia Bhavan, Average rainfall-186.26mm.

#### Essential oil studies

##### Essential oil extraction

The fresh plant was collected from the experimented sites, leaves and inflorescence were separated and are washed under tap water followed by distilled water to remove dust particles and dry at ambient temperature for two days in the laboratory.

The dried leaves were cut into small pieces. The dried plant leaves are used for the extraction of essential oils. The plant material were subjected to hydro-distillation using a Clevenger-tube apparatus for 3hours. The oil was dried over anhydrous sodium sulfate and was stored in sealed vials under a refrigerator until analysis.

##### Gas chromatography and mass spectroscopy

GC-MS analysis of the essential oil was carried out on an Acquisition-General, Shimadzu GC-MS, Model number: QP2010S Equipped with Electron Ionisation using a column Rxi-5sil MS, 60nm length×250 micro metre thickness. Temperature programming was done Initial 40°C hold for 2mins Ramp at 5°C to 280°C Ramp at 20°C to 300°C holds for 2min, Flow rate:1 ml/min; carrier gas helium.

##### Identification of compounds

Essential oil constituents were identified by comparing retention times of the chromatogram peaks with those of reference compounds run under identified conditions. Interpretation of the Mass Spectrum was conducted using the database of National Institute Standard and Technology (NIST2) And WILEY18 Library.

## Plant Identification

### Isolation of total cellular DNA and primer designing for barcode loci amplification

Fresh and young leaves of the wild plant were taken and subjected to total extraction of cellular DNA using CTAB method. The corresponding gene sequences of the genus *Cymbopogon* were retrieved from NCBI Gene-Bank data domain for precisely designing the specific primers for the amplification of three barcoding loci and ITS1 and 2 spacers. PCR primer pairs were mapped out from the conserved regions using software primer 3.0 (version 0.4.0)

### Barcode amplification, sequencing, validation and data analysis

Two chloroplast loci and one nuclear DNA locus (ITS region) of the isolated DNA from the fresh young leaves were amplified using primers that were designed. The PCR reaction mixture contained the template DNA, Buffer, MgCl<sub>2</sub>, dNTPS, designed primer and DNA polymerase. The PCR program that was set involved 35 cycles, each cycle

starting from an initial stage of denaturation at 90°C for 5 minutes, followed by annealing stage at 60°C for 1 minute, extension stage at 70°C for 2 minutes and final extension at 72°C for 10 minutes. The PCR products were purified and sequenced. Sanger sequencing of amplicons were carried out using BDT v3.1 cycle sequencing kit on Abi 3730xl Genetic Analyzer. Annotation software were used to annotate the sequenced data. Validation of the designed primers and sequenced data. Validation of the designed primers and sequenced data was done by repeating the experiment twice from the starting DNA isolation step to the sequencing step. The PCR Products were also subjected to 1.6% agarose gel for the visualization of the amplified products. The gel was pictured with a Gel Doc X+.

Annotated contig barcode sequences were subjected to BLASTA (NCBI domain) for the verification and were finally submitted to GenBank of NCBI. The DNA sequences were aligned automatically using the program CLUSTAL in OMEGA 6.0 and constructed NJ derived Phylogenetic tree.

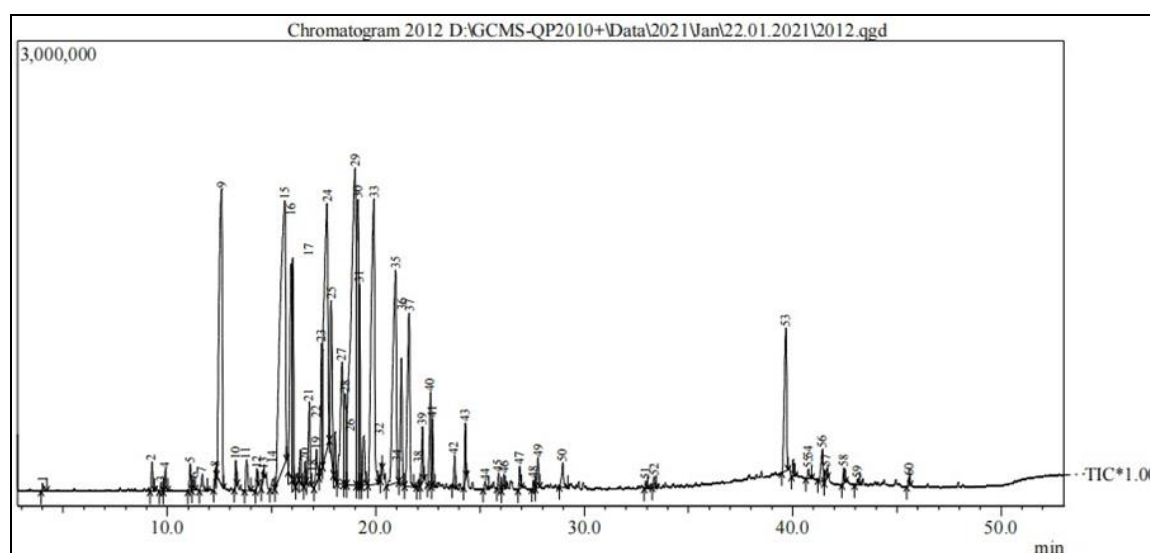


Fig 1: GCMS-Chromatogram of wild *Cymbopogon giganteus*

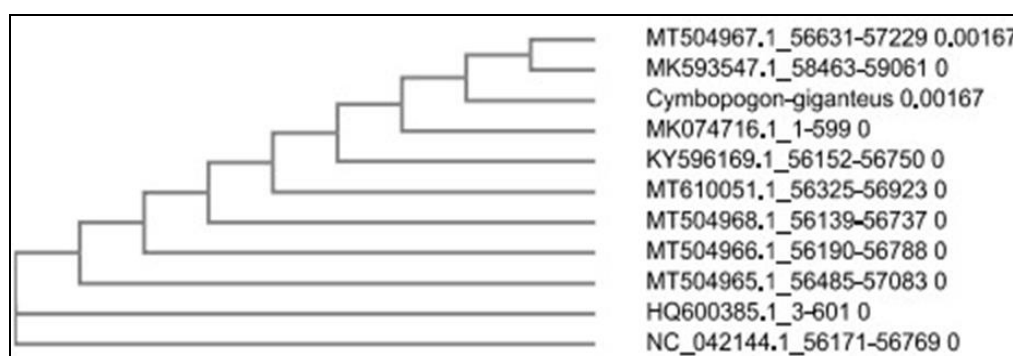


Fig 2: Phylogenetic tree constructed based on rbcL gene nucleotide sequences of *Cymbopogon species*

## Result and Discussion

The essential oil extracted from *Cymbopogon giganteus*, an aromatic herb that is commonly used in Medicinal purposes, Commercial purposes and also in Food Industry. The essential oil has a pale yellow colour, a slightly Lemonyodour has been observed. GC-MS analysis revealed the presence of more than 50 compounds of the total oil. The Major compounds were Carveol, Menthane, Citral, Limonene, Geraniol, Nerol and Beta-Chamigrene, Geranyl

acetate were minor but significant components. The quality of lemon grass is generally determined by its Major compounds. The oxygenated compounds such as p-menthane derivatives, carveone, and carveol possessing antimicrobial activities (Toukourou, *et al.*,2020) <sup>[29]</sup>

### Essential oil characteristics

In the present study, 150g of the dried herbage sample yielded approximately 0.07ml of essential oil. The essential

oil has a pale yellow colour, a slightly Lemony odour has been observed.

**Table 1:** Compounds present in the essential oil report

Sl. no	Compounds	Area of %	RT value	Mol. weight	Mol. formula
1. Mono Terpenoid Compounds					
1	Camphene	0.23	9.911	136.23	C10H16
2	Limonene	7.85	12.596	136.24	C10H16
3	P-Cymene	0.18	14.297	134.21	C10H14
4	Menthane	14.71	31.646	152.237	C10H16O
5	Carvone	3.65	15.934	150.22	C10H14O
6	Dihydrocarveol	25.02	109.658	154.25	C10H18O
7	Nerol	4.82	19.140	154.25	C10H18O
8	Neryl Acetate	0.63	22.715	196.29	C12H20O2
9	Geraniol	0.78	19.421	154.253	C10H18O
10	Carveol acetate	2.38	17.855	194.27	C12H18O2
11	P-Mentha-1-en-9-ol	0.48	18.045	154.25	C10H18O
2. Bicyclic Monoterpene					
12	Verbenol	0.25	16.611	152.24	C10H16O
3. Oxygenated Monoterpene					
13	1,3 Bis-(2-cyclopropyl,2- methyl cyclopropyl)-but-2-en-1- one	3.52	21.597	258.4	C18H26O
4. Diterpene					
14	Steviol	3.39	81.101	318.45	C20H30O3
5. Sesquiterpenoids					
15	Caryophyllene	0.45	51.347	204.36	C15H24
16	Chamigrene	0.10	25.235	204.35	C15H24
17	Toluene	0.18	25.892	92.14	C7H8
18	Caryophyllene oxide	0.37	27.777	220.35	C15H24O
19	Elemol	0.23	26.907	222.37	C15H26O
20	Neoclovene	0.12	40.749	204.35	C15H24
6. Alcohols (Organic Compound)					
21	Isoamyl alcohol	0.08	4.014	88.148	C5H12O
7. Ketones					
22	Sulcatone	0.27	11.093	126.20	C8H14O
23	Propyl ketone amyl	0.49	13.795	142.24	C9H18O
8. Pyrans					
24	Dehydrocineole	0.13	11.249	152.2334	C10H16O
Alkatetrane					
25	Cosmene	0.40	25.973	134.2182	C10H14
9. Esters					
26	Isoamyl Butyrate	0.24	13.284	158.24	C9H18O2
10. Fatty Acid Esters					
27	Hexanoic acid	1.55	19.214	116.1583	C6H12O2
28	Octanoic acid	0.55	24.295	144.21	C8H16O2
29	Phenethyl Octanoate	0.12	33.349	248.36	C16H24O2
11. Steroids					
30	Cholest-22-ene-21-ol,3,5-dehydro-6- methoxy, Pivalate	0.18	40.019	498.8	C33H54O3
12. Acyclic Terpenoid					
31	Lemonal	8.44	19.907	152.24	C10H16O
13. Other Compounds					
32	Trans Hydrindane	0.29	9.263	124.22	C9H16
33	1,7-Octadiene,3-methylene-	0.08	9.697	136.238	C9H16
34	1,3-Bendioxole,3a,7a,-dihydro-2,2,4 trimethyl-	0.11	14.581	166.22	C9H12O2
35	Tetrahydrofuran-2-ol,3,4-di(1-butenyl-)	0.19	15.064	196.29	C12H20O2
36	Cyclopentane,1-methyl-1-(2-methyl-2-propenyl)-	0.15	16.224	138.25	C10H18
37	4-Isopropenyl Cyclohexanone	0.37	16.387	140.23	C9H16O
38	Bicyclo(3.3.0)oct-2-en-7-one,6-Methyl-	1.23	16.819	136.19	C9H12O
39	4,5-Epoxycarene	0.50	17.152	152.2334	C10H16O
40	Durenol	1.63	17.404	150.220	C10H14O
41	3,6,6-Trimethyl -Cyclohex-2-enol	3.14	18.379	140.22	C9H16O
42	2-Methyl5-Isopropenyl (1-Cyclopenten- 1-carboxyaldehyde)	1.65	18.530	150.22	C10H14O
43	(4R*,5R*,9S*)-5,9-Dimethylspiro(3.5)nonan-1-one	0.09	20.274	166.26	C11H18O
44	1,4-Methanophthalazine,1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-9, 9-dimethyl (1.alpha)	7.98	41.504	178.27	C11H18N2
45	Bicyclo(2.2.2)oct-2-ene,1,2,3,6- tetramethyl-	0.66	22.238	164.29	C12H20
46	3-Oxabicyclo(6.3.1)dodec-8-en-2-one	1.54	22.629	-	-
47	Selina -6-en-4-ol	0.16	44	222.3663	C15H26O
48	Tetracyclo(6.3.2.0(2,5).0(1,8) tridecan-9-ol,4,4-dimethyl-	0.40	28.969	220.35	C15H24O
49	Tricyclo(3.1.0.0(2,4)hexane,3,6-diethyl-3,6-dimethyl-trans-,	0.08	32.95246	164.29	C12H20
50	6-Beta Bicyclo(4.3.0)nonane,5beta-iodomethyl)-1.beta isopropenyl-4.alpha	0.16	42.452	-	-
51	6-Isopropenyl-4-8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H- naphthalen-2-one	0.08	43.109	220.35	C15H24O
52	Tricyclo(20.8.0.0(7,16)tri-aconate,1(22),7(16)-diepoxy-	0.09	45.568	444.7	C30H52O2

**Table 2:** Bioactivity of the compounds

Sl. no	Name of the compounds	Bioactivity	References
1	Isoamyl alcohol	Cyanobacteriolytic activity	S.J.L. Wright <i>et al.</i> ,(2008)
2	1,7-Octadiene,3-methylene-	Antibacterial activity	Imad Hadi Hameed <i>et al.</i> ,(2015)
3	Camphene	Bactericidal, Anti viral, Anti fungal, expectorant, Anti septic, Antibiotic, Antioxidant, Anti-inflammatory,Lipid- lowering gel,Bronchial stimulant,Plant metabolite, fragrance.	Ethan B. Russo <i>et al.</i> ,(2017)
4	Sulcanote	Mosquito attractant,Endogenous metabolite.	Neha Karl <i>et al.</i> ,(2014)
5	Dehydrocineole	Antimicrobial activity	Mahmoud Ben Othman <i>et al.</i> ,(2017)
6	Cosmene	Antibacterial activity	M.Z.M. Salem <i>et al.</i> ,(2018)
7	Limonene	Antibacterial and Antifungal	H-H Sherry Chow <i>et al.</i> , (2013)
8	Isoamyl butyrate	Flavouring agent	Macedo, G. A <i>et al.</i> ,(2004)
9	Propyl amyl ketone	Flavouring agent	Yannai, Shmuel. <i>et al.</i> , (2004)
10	P-cymene	Antioxidant, anti-inflammatory, antinociceptive, anxiolytic, anti-cancer and anti-microbial effects.	Anna Marchese <i>et al.</i> ,(2017)
11	Menthane	Antifungal activity, Antimicrobial activity	Félicien Avlessi <i>et al.</i> ,(2012)
12	Carvone	Anti bacterial, Antifungal and Cytotoxic to cancer cell lines, Insecticidal blood lipid lowering activity, Ca <sup>2+</sup> channel blocker. It has a role as an allergen.	Arun K Tripathi <i>et al.</i> ,(2003)
13	Verbenol	Effect on tumour cells	Roman Paduch <i>et al.</i> ,(2017)
14	Dihydrocarveol	Antimicrobial activity,Acaricide, a volatile compound and a plant metabolite	Yoshiaki Noma <i>et al.</i> ,(2010)
15	Carveol acetate	Antibacterial Activity and Antioxidant Capacity	Carmen MS. Ambrosio <i>et al.</i> ,(2021)
16	P-Mentha-1-en-9-ol	Potential activity, Anti microbial activity.	Yin Yanga <i>et al.</i> ,(2013)
17	Nerol	Volatile component, and a plant metabolite and a fragrance	Rachel A. Kahn <i>et al.</i> , (2000)
18	Geraniol	Antioxidant, Anti-inflammatory properties exhibit Insecticidal, pest control agent, Exhibiting low toxicity.	Maria Helena Pereira de Lira <i>et al.</i> ,(2020)
19	Lemonal	Antimicrobial, Anti-inflammatory, Anti parasitic, Allelopathic and Mosquito repellent. Used as flavours, fragrances, cosmetics, perfumery and pharmaceuticals. Insecticide, Inhibitor, Plant metabolite.	Fazal Ur Rehman <i>et al.</i> , (2020)
20	Nerol acetate	Volatile oil component and a fragrances and a plant metabolite	Robert Tisserand <i>et al.</i> ,(2014)
21	Caryophyllene	Anti microbial activity, Bactericidal, Fungicidal.	Saad S. Dahham, <i>et al.</i> ,(2015) <sup>[26]</sup>
22	Chamigren	Beta chamigrene synthase(E.C 4.2.3.78) is an enzyme	Shaza M Al-Massarani <i>et al.</i> ,(2014)
23	Toluene	Anti-inflammatory	Inés García-Peña <i>et al.</i> ,(2005)
24	Elemol	Fragrance and a plant metabolite	Chahrazed Bekhechi <i>et al.</i> ,(2019)
25	Caryophyllene oxide	Anti-inflammatory ,Local anesthetic, Anti-oxidant and perhaps in cancer treatment, Non-toxic,Non-sensitizing, Antifungal, insecticidal/antifordant, Genoprotective, Chemosensitizing, Anti proliferative	Ethan B. Russoe <i>et al.</i> ,(2017)
26	Steviol	Bioactivities similar to Gibberallin hormone, Anti oxidant.	Mojtaba karimi <i>et al.</i> ,(2014)
27	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy,pivalate	Anti microbial, Anti Antifungal activity. inflammatory,	Abdul Waheed <i>et al.</i> ,(2019)

**Fig 3:** Habit and habitat of wild *Cymbopogon giganteus* growing in Gangondanahalli, Bangalore

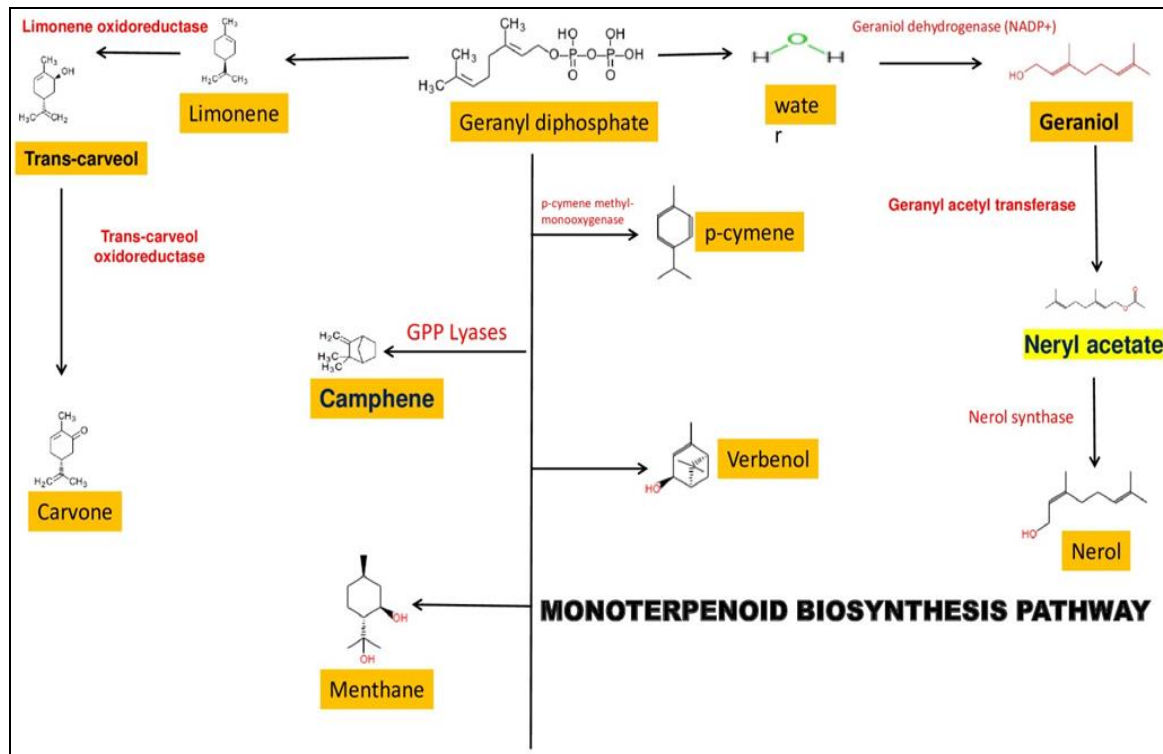


Fig 4: Overview of monoterpenoid biosynthesis pathway in wild *Cymbopogon giganteus*.

Monoterpenoids are synthesized by MEP pathway in chloroplast from Geranyl Diphosphate as their precursor. where Orange colour represents the key compounds found in the wild *C. giganteus*, orange colour for compounds reported in the wild *C. giganteus* and intermediate compounds present in the pathway. The enzymes identified

via KEGG and META CYC include: LO-Limonene oxidoreductases, Trans carveol oxidoreductases, GPP Lyases, Geraniol dehydrogenases, p-cymene methyl monoxygenases, Geranyl acteyl transferases, Nerol synthases.

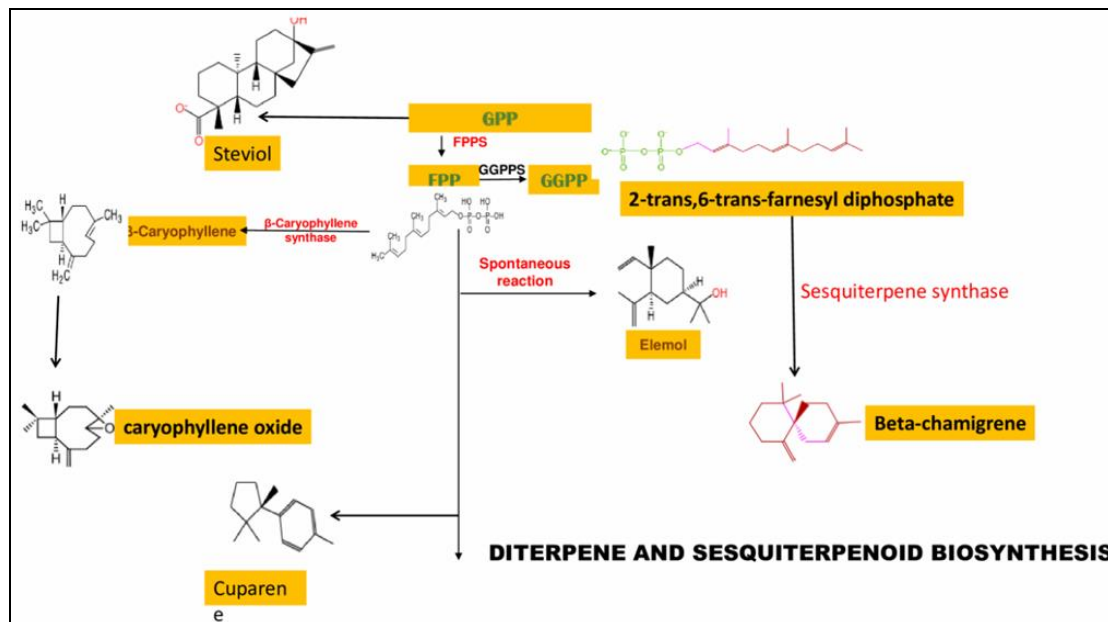


Fig 5: Overview of sesquiterpenoid biosynthesis pathway in wild *Cymbopogon giganteus*.

Sesquiterpenoids are synthesized by MVA pathway in cytosol from Farnesyl Diphosphate as their precursor. The enzymes identified via METACYC include: Sesquiterpene synthase and CS (-)-beta caryophyllene synthase.

**Conclusion**

The present study confirmed that the composition of the essential oil of *C. giganteus* growing wild in

Gangondanahalli, is dominated by Monoterpenoids, Sesquiterpenoids. *C. giganteus* essential oil including oxygenated monoterpenes, sesquiterpene, steroids, fatty acid esters, esters, alcohols, diterpenes, and ketones. (Jean Brice Botiab *et al.*,(2006) Essential oils have to be used with caution because of the potential toxic effects of some of them. therefore, toxi-cological studies should be always

performed to determine the safe doses for each essential oil. Habib Toukourou *et al.*, (2020) [29]

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### Conflict of Interest

The authors declare no conflict of interest.

### DNA Barcoding

Out of three loci (*rbcL*, *matK* and ITS spacers 1 and 2), only *rbcL* loci was amplified successfully and evolutionary analysis was conducted in Clustal Omega using Neighbouring-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Phylogeny indicates that the studied plant sample is very closely grouped under clad of *Cymbopogon sp.* This result supports study of NCBI BLAST leading to confirmation of the species as *cymbopogon giganteus* and was submitted the same in NCBI GenBank under the accession number of OK094432.

### Ethanomedicinal Properties

*Cymbopogon giganteus* plant is used in traditional medicine against various disease such as skin disorders, mental illness, Broncho-pulmonary affections, jaundice, cold, conjunctivitis, Rheumatic pains, Childhood coughs and Hepatitis.

The essential oil of *Cymbopogon giganteus* get by hydro-distillation of its leaves showed good anti-bacterial and anti-inflammatory properties and is sold for these activities.

It was characterized by the presence of limonene, carveol, and p-menthane, citral derivatives.

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