



Studies on biosynthesis of terpenoid compound from the essential oil of wild *Cymbopogon giganteus* (Hochst.) Chiov

Harshitha A R¹, Hemalatha J², Vinutha M³, K J Thara Saraswati⁴

¹ Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University, Bengaluru, Karnataka, India

² Research scholar, Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University, Bengaluru, Karnataka, India

³ Assistant Professor, MS Ramaiah College of Arts Science and Commerce, MSRIT, Bengaluru, Karnataka, India

⁴ Professor, Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University, Bengaluru, Karnataka, India

Abstract

The wild species of *Cymbopogon* collected from hilly region of Allimaranahalli (Village) Kanakapura (Taluk), Ramanagara (Dist.) Karnataka was taken up for the present study. The species was identified as *Cymbopogon giganteus* based on morphological characters and DNA Barcoding studies and the sequence was deposited in NCBI GenBank under the accession no. OK094431. GC- MS analysis of the essential oil led to the identification of 64 fingerprint compounds belonging to monoterpenoids, diterpenoid and sesquiterpenoids. Dihydrocarveol (15.30%), Trans-p-mentha- 2,8-dienol (14.32%), Limonene (12.19%), 1,4-methanophthalazine,1,4,4a,5,6,7,8a-octohydro- 9,9dimethyl- (10.17%), trans-pinocarveol (9.48%) were found in higher percentage in the essential oil.

Keywords: wild *Cymbopogon giganteus*, DNA barcoding, essential oil, GC-MS analysis, terpenoid biosynthesis

Introduction

The plants produce abundant secondary metabolites and these secondary metabolites show potential providing resistance against biotic and abiotic stress. (Opeyemi Avoseh *et al.*, 2015) [34]. The defence mechanism of plant differ depending on their environment and climatic conditions. (Opeyemi Avoseh *et al.*, 2015) [34] The most common type of secondary metabolites produced by plants are alkaloids, phenols and terpenoids. (Pavan kumar gupta *et al.*,2009) The biological utilizes of these secondary metabolites as therapeutic agents for a wide range of disease and microbial infections has proven to be beneficial. (Opeyemi Avoseh *et al.*, 2015) [34]. The genus *Cymbopogon* comprises of approx. 156 species and belong to poaceae are aromatic and medicinal species. (Lydia Popielas *et al.*, 2006) *Cymbopogon giganteus* Chiov., is a perennial grass, sweet-smelling grass that grows wild in Asian and African tropical savannahs. (Lydia Popielas *et al.*, 2006) It posses a rhizome-bearing stem and the plant reaches upto height of 3 metres. (Jean Brice Boti *et al.*,2005) *C. giganteus* (Hochst) Chiov., having aromatic medicinal properties (J. P Noudogbessi *et al.*, 2012) [23] The *C.giganteus* is also called as "Citronelle de Madagascar" and this plant is used in folk medicine against various diseases such as skin disorder, mental illness, bronchopulmonary affections, bilharzia, jaundice, cold, conjunctivities, migraine, dermatoses, rheumatic pains, childhood coughs and hepatitis. (Toukourou *et al.*, 2020) [21] *C. giganteus* show activity against chloroquine resistant plasmodium. (Bedi Sahouo *et al.*,2003) [17] The essential oil obtained from leaf, stem and inflorescence of *C. giganteus* showed good antibacterial and Antiinflammatory properties. (Bedi Sahouo *et al.*,2003) [17] The essential oil also showed antiseptic and antibacterial

against sore throat caused by bacteria and viruses and used extensively in cosmetics, pharmaceuticals, and perfumery applications. (D Ganjewala 2009 and Toukourou *et al.*, 2020) [12, 21]. Most of the essential oils contains the natural monoterpenes and sesquiterpenes with several functional groups (Adorjan *et al.*, 2020) [2]. The essential oil of cymbopogons are rich in monoterpenes. Monoterpenes are the most important constituents of flavors and fragrances and it serve as therapeutic agents. (Ganjewala *et al.*,2009) [12]. After several secondary transformations such as isomerization, acetylation, deacetylation, cyclization, and dehydrogenation, the monoterpenes are generated from geranyl diphosphate (GPP). Geranyl diphosphate is a monoterpene precursor found in secondary metabolites producing plants. (Ganjewala *et al.*,2009) [12]. During the present study, wild *C. giganteus* explored from Allimaranahalli village, kanakapura taluk was subjected to essential oil analysis extraction and to reveal fingerprint compounds. The essential oil showed rich monoterpene compound followed by sesquiterpenes and diterpenes.

Material and Methods

Plant collection and ecological details

The plant sample for study was collected from Allimaranahalli (V), Kanakapura (T), Ramanagara (D). The place Allimaranahalli is located in Ramanagara district with an area of 4.42km². The latitude and longitude of village is reported as 12.3638 and 77.2316 respectively. The village showed a temperature of 22.6 C to 30.6 C, humidity of 53% with wind flow of 6.52m/sec. with an average rainfall is 822mm.

Plant identification

DNA barcoding and Phylogenetic analysis

Total Genomic DNA was isolated from the plant sample using Plant Genomic DNA Mini-spin kit. DNA was amplified using the plant specific selective universal region oligo primers (rbcL and matK) (Ashok *et al.*, 2017). 50ul of PCR reaction mixture contained 50mg of gDNA, 100ng of each forward and reverse primers, 2ul of 10mM dNTPs mix, 5ul of 10X Taq Polymerase buffer, 3U of Taq polymerase enzyme and made up with PCR grade water. The PCR program was as follows: an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1min, annealing temperature standardized at 60°C, extension temperature at 72°C for 2 min and final extension was at 72°C for 10min. PCR product was run on 1% agarose gel in 1X TAE buffer and the products were purified using Nucleo-pore, Genetix Biotech PCR clean up kit and purified fragments were sequenced. The sequenced data was edited using Bio edit tool. The experiment was repeated thrice for validation of reproducibility of the barcode sequence.

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). (Bishoyi *et al.*, 2017)^[9]

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, MgCl₂, 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. (Bishoyi *et al.*, 2017)^[9]. 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 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 XR+ (Biorad).

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 CLUSTALW in OMEGA 6.0 and constructed NJ derived phylogenetic tree.

Essential oil studies

Extraction

The fresh herbage consisting of root, stem, culm and inflorescence were collected from the experimental sites. The herbage was washed under tap water followed by distilled water to remove dust particles and dried at ambient temperature for two days under shade. The dried leaves were cut into small pieces and used for extraction of the essential oil by hydro-distillation method using a Clevenger type apparatus for 3 hours. The oil obtained was dried over anhydrous sodium sulphate and stored in sealed vials under refrigeration until further analysis.

Analysis (GC-MS)

Analysis of the essential oil was carried out on an acquisition general, Shimadzu GCMS model number; QP2010S equipped with electron ionization using a column Rtx- 5m, 30m length×0.25µm film, thickness, ID: 0.25mm and injector of 250°C. The carrier gas flow rate: 0.7ml/min with carrier gas helium with split ratio: 1:100 sample injection: 0.1µl. 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 2 mins

Identification of compounds

Essential oil constituents were identified by comparing retention times of chromatogram peaks with those of reference compounds run under identical conditions. Interpretation of the mass spectrum was conducted using the database of National Institute Standard and Technology

Result

Identification of the plant

The plant was identified as *Cymbopogon giganteus* based on the morphological characters and essential oil studies. (Fig.1)

DNA barcoding and phylogenetic analysis

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 Neighbour-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 used to infer the phylogenetic tree. (Fig 3) 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 the study of NCBI BLAST leading to confirmation of the species as *Cymbopogon giganteus* and the sequence was submitted to in NCBI GenBank under the accession number of OK094431.

Essential oil studies

GC-MS analysis revealed the presence of various chemical compounds in the essential oil of *C. giganteus*. (Fig 2) Totally 64 fingerprint compounds were identified from the essential oil. (Table1) Dihydrocarveol (15.30%) shows the highest percentage followed by Trans-p-mentha-2, 8-dienol, Limonene, Trans-pinocarveol, cis-p-mentha-2,8-dien-1-ol,

Isopentyl hexanoate. The compounds were categorised into different chemical classes (Table 1).

The composition of the essential oil consisted of monoterpenoids (63.57%), sesquiterpenoid (0.65%),

diterpenoid (0.63%), steroids (2.87%), hydrocarbon (0.33%), phenol (0.06%) and alcohol (0.17%) The bioactivity studies of the essential oil compounds as reported by earlier workers are tabulated (Table 2).

Table 1: Classification of the compound into chemical group

Sl.no	Compounds	Area%	R.Time	Mol.weight
Monoterpenoid hydrocarbons				
1.	Alpha pinene	0.09	9.452	136.23
2.	Camphene	0.16	9.902	136.23
3.	D-limonene	12.19	12.731	136.23
4.	Myrcene	0.21	11.259	136.23
5.	Dihydrocarveol	15.30	19.110	152.23
6.	Perillaldehyde	0.36	19.932	150.21
7.	Trans-carane	0.23	20.262	138.25
8.	Trans-pinocarveol	9.48	17.802	152.23
9.	P-cymene	0.26	14.306	132.20
10.	Trans-p-Mentha-2,8-dienol	14.32	15.778	152.23
11.	Cis-p-Mentha-2,8-dien-1-ol	2.51	16.169	152.23
12.	1-p-Mentha-9-al	0.21	18.128	152.23
13.	p-Menth-1(7)-en-9-ol	1.87	22.678	154.25
14.	(2R,4R)-p-Mentha-[1(7),8]-diene, 2- hydroperoxide	0.10	22.091	168.23
15.	(1S,4R)-p-Mentha-2,8-diene, 1- hydroperoxide	0.08	32.953	168.23
16.	5-Isopropenyl-2-methylcyclopent-1- enecarboxaldehyde	0.38	18.296	150.22
Oxygenated monoterpenoid				
17.	Limonene oxide	4.93	15.989	152.23
18.	Citral	0.89	152.23	152.23
Sesquiterpenoid hydrocarbon				
19.	Caryophyllene	0.31	23.784	204.36
20.	Delta.-neoclovene	0.14	40.763	204.35
Oxygenated sesquiterpenoid				
21.	Carryophyllene oxide	0.20	27.786	220.35
Esters				
22.	Isoamyl butyrate	0.27	13.301	158.24
23.	Isopentyl hexanoate	3.56	19.290	186.29
24.	Phenethyl octanoate	0.29	33.358	248.36
25.	Isopentyl octanoate	1.02	24.322	214.34
26.	2-phenylethyl hexanoate	0.63		220.32
Steroids				
27.	Androst-1-en-3-one,4,4-dimethyl-,(5 alpha.)-	2.63	39.710	300.5
28.	11.alpha.-hydroxy-17.alpha.-methyl testosterone	0.24	40.004	318.45
Unsaturated aliphatic hydrocarbon				
29.	Pentacosane	0.10	44.775	352.69
30.	Dotriacontane	0.08	47.932	450.9
31.	Nonanal	0.08	14.741	142.24
32.	1,7-octadine,3-methylene	0.07	9.689	122.21
Phenol				
33.	Phenol, 2-ethyl-4,5-dimethyl	0.06	13.520	150.24
Diterpenoid				
34.	Steviol	0.63	41.465	317.4
Alcohol				
35.	Isoamyl alcohol	0.09	3.979	88.15
36.	Stearyl alcohol	0.08	37.930	270.49
Ungrouped compound				
37.	Trans-hydrindane	0.23	9.358	124.22
38.	Ethanone,1-(1,4-dimethyl-3- cyclohexen-1-yl)-	0.30	13.841	152.23
39.	1,3-Benzodioxole, 3a,7a-dihydro- 2,2,4-trimethyl-	0.21	14.588	166.21
40.	Tetrahydrofuran-2-ol,3,4-di[1-butenyl]-	0.22	15.079	196.29
41.	Bicyclo[3.3.0]oct-2-en-7-one,6- methyl-	1.44	16.904	136.19
42.	Cyclohexene,2-ethenyl-1,3,3- trimethyl	1.75	17.325	150.26
43.	Tricyclo[4.2.1.1(2,5)]decan-9-ol, stereoisomer	1.51	17.936	152.23
44.	3,6,6-Trimethyl-cyclohex-2-enol	0.75	18.030	140.22
45.	2-cyclopentylcyclopentanone	3.21	18.498	152.23
46.	5H-Inden-5-one,1,2,3,3a,4,7a- hexahydro-7a-methyl-, trans-	1.19	18.611	150.22
47.	Grandlure II	0.07	19.484	154.25
48.	Bicyclo[4.1.0]heptane,-3- cyclopropyl,-7-hydroxymethyl, (cis)	0.08	20.141	166.26
49.	Naphthalene,decahydro-1,6- dimethyl-	0.31	20.446	166.3

50.	1,4-Methanophthalazine, 1,4,4a,5,6,7,8,8a-octahydro-9,9- dimethyl-	12.06	21.030	178.27
51.	1,4-Methanophthalazine, 1,4,4a,5,6,7,8,8a-octahydro-9,9-dimethyl-,(1.alpha.,4.alpha.,4a.	1.89	21.301	178.27
52.	Bicyclo[2.2.2]oct-2-ene,1,2,3,6- tetramethyl-	0.83	22.284	164.29
53.	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-,[1R-(1.	0.09	26.915	222.36
54.	Cycloheptane,4-methylene-1- methyl-2-(2-methyl-1-propen-1yl)- 1-vinyl-	0.13	27.580	204.35
55.	Androst-1-en-3-one,3-ethyl-3- hydroxy-,(5.alpha.)-	2.63	39.710	318.5
56.	Diepi-alpha.-cedrene epoxide	0.08	40.980	220.35
57.	4-isopropyl-7,11-dimethyl-3,7,11- cyclotetradecatrienone	0.46	41.622	274.4
58.	6.beta.bicyclo[4.3.0]nonane,5.beta.- iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl	0.34	42.478	332.26
59.	6-Isopropenyl-4,8a-dimethyl- 1,2,3,5,6,7,8,8a-octahydronaphthalene	0.11	43.126	236.35
60.	Tricycle[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	0.18	45.586	444.7
61.	Cosmene	0.17	11.680	134.22
62.	4-isopropenyl cyclohexanone	0.37	16.448	140.22

Table 2: Bioactivity of compound with reference

Sl.no	Compound name	Bioactivity of compound	Reference
1	Isoamyl alcohol	Isoamyl alcohol were effective in inactivating various micro- organisms, and antimicrobial mechanism of volatile isoamyl acetate against E. coli was clarified based on proteome analysis.	H Ando <i>et al.</i> , (2015)
2	Alpha-pinene	The antimicrobial activities of the isomers and enantiomers of pinene were evaluated against bacterial and fungal cells.	Rivas da Silva <i>et al.</i> , (2012)
3	Camphene	Antioxidant activity Antiradical activities	Lijuan Yang <i>et al.</i> , (2020)
4	Myrcene	The aim of this study was to investigate the anti-ulcer effects of β -myrcene on experimental models of ulcers that are induced by ethanol, NSAIDs (non-steroidal anti-inflammatory drugs), stress, <i>Helicobacter pylori</i> , ischaemia–reperfusion injury (I/R)	Flavia Bonamina <i>et al.</i> , (2014)
5	Limonene	The therapeutic effects of limonene have been extensively studied, proving anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic,	Vieira <i>et al.</i> , (2018)
		antihyperalgesic, antiviral, and gastroprotective effects,	
6	p-cymene	p-Cymene [1-methyl-4-(1- methylethyl)-benzene] is a monoterpene. used for medicine and food purposes. It shows a range of biological activity including antioxidant, anti-inflammatory, antinociceptive, anxiolytic, anticancer and antimicrobial effects.	Anna Marchese <i>et al.</i> , (2017)
7	Nonanal	Nonanal has reported to exhibit antimicrobial activity against gram positive and gram negative bacteria.	Ji-hong Zhang <i>et al.</i> , (2017)
8	Cis-p-mentha- 2,8-dien-1ol	Antioxidant and antibacterial, antimicrobial activity against the pathogenic bacteria	Ambrosio <i>et al.</i> , (2021)
9	Trans-limonene oxide	Antimicrobial activity	Ambrosio <i>et al.</i> , (2021)
10	Trans-pinocarveol	The antimicrobial activity against <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Candida albicans</i> , and <i>Aspergillus niger</i> .	Anita Bansal <i>et al.</i> , (2006)
11	Dihydrocarveol	The essential oil exhibited strong antimicrobial activity against strains the bacteria, <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> , <i>Escherichia coli</i> , <i>Shigella dysenteriae</i> , and a strain of the fungus <i>Candida albicans</i> .	Qing Zhu <i>et al.</i> , (2020)
12	Citral	Citral have been demonstrated to show antimicrobial, antifungal, and antiparasitic characteristics.	Canan Ece Tamer <i>et al.</i> , (2019)
13	L-perillaldehyde	Perillaldehyde was found to preserve fruits and promote the antioxidant activity of blueberries and Chinese bayberries. perillaldehyde elicited antidepressant-like effects on the olfactory nervous system in mice	Miho Igarashi <i>et al.</i> , (2013)
14	Trans-carane	Antifeedant activity against the lesser mealworm,	Agata Koziol <i>et al.</i> , (2018)

		Alphitobius diaperinus Panzer, and peach potato aphid (<i>Myzus persicae</i>). In addition,	
		its moderate antibacterial activity was observed against the <i>Bacillus subtilis</i> strain	
15	caryophyllene	Several biological activities are attributed to β -caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities.	Jean Legault <i>et al.</i> , (2007)
16	caryophyllene oxide	Caryophyllene oxide was found to exhibit anti-inflammatory, antioxidant, antiviral, anticarcinogenic, and analgesic properties	Klaudyna Fidyt <i>et al.</i> , (2016)
17	[-]-caryophyllene- [II]	caryophyllene exerts anti- inflammatory action via inhibiting the main inflammatory mediators, such as inducible nitric oxide synthase (iNOS), Interleukin 1 beta (IL-1 β), Interleukin-6 (IL-6),	Fabrizio Francomano <i>et al.</i> , (2019)
18	Steviol	steviol induces the antioxidant system and stimulates the steviol glycosides biosynthesis in stevia leaves.	Mojtaba karimi <i>et al.</i> , (2014)
19	Dotriacontane	Antibacterial, Antifungal, antioxidant effect	Asong <i>et al.</i> , (2019)

Biosynthesis of terpenoid compounds

Monoterpenoids (C₁₀ terpenoids) are a group of terpenoids consisting of two isoprene units. They are derived from geranyl diphosphate (GPP). Geranyl diphosphate (GPP) is a monoterpene precursor. Which undergo isomerization, acetylation, diacylation, cyclization and dehydrogenation to form other monoterpene and terpenoid compounds. The enzymes: geraniol dehydrogenase, dihydrocarveol dehydrogenase, Limonene synthase, Limonene 1,2 monooxygenase, Perillyl alcohol dehydrogenase, 3-carene synthase, alpha-pinene synthase, Myrcene synthase, camphene synthase, alpha-pinene monooxygenase were utilized in the monoterpene pathway

The GPP get converted into alpha-pinene in the presence of alpha-pinene synthase with release of diphosphate and further converted into trans pinocarveol in the presence of alpha-pinene monooxygenase with release of water. In the presence of geraniol dehydrogenase, the GPP is converted into cis-citral. GPP get converted into D-limonene along with diphosphate molecule in the presence of limonene synthase and further get converted into limonene oxide and perillyl alcohol in the presence of Limonene 1, 2 monooxygenase and perillyl alcohol combine with NAD⁺ get converted into the perillaldehyde and NADH in the presence of perillyl alcohol dehydrogenase respectively. GPP is converted into 3-Carene in the presence of 3-Carene synthase. GPP get converted into myrcene and diphosphate in the presence of myrcene synthase. GPP also get converted into camphene and diphosphate in the presence of camphene synthase enzyme. (Fig 4)

Sesquiterpenoids (C₁₅ terpenoids) are a group of terpenoids consisting of three isoprene units. In sesquiterpenoid pathway, Geranyl diphosphate (GPP) get initiated to form farnesyl diphosphate (FPP) in the presence of farnesyl diphosphate synthase. The enzymes used in this pathway are farnesyl diphosphate synthase and caryophyllene synthase. (Fig 5)

GPP get converted into FPP in the presence of farnesyl diphosphate synthase. FPP is converted to caryophyllene and release diphosphate in the presence of caryophyllene synthase enzyme and it further converted into caryophyllene oxide.

Steviol, a diterpenoid is synthesized from kaurene, via Mevalonate pathway (MEP). The isopentenyl diphosphate (IPP) and dimethyl diphosphate (DMAPP) produced at the

end of the MEP pathway get transformed into Geranyl geranyl diphosphate (GGDP) by plastid phenyl transferase.



Fig 1: Habit and habitat of wild *Cymbopogon giganteus*

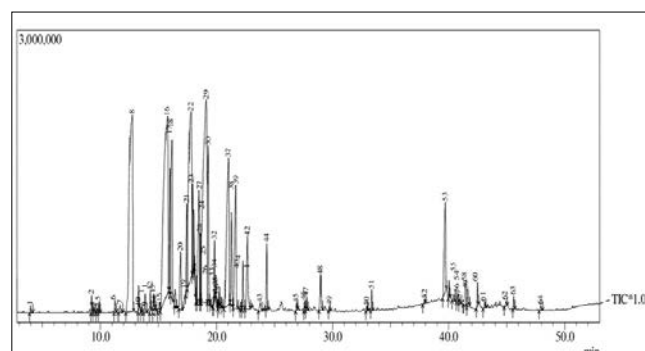


Fig 2: GC-MS Chromatogram of wild *Cymbopogon giganteus*

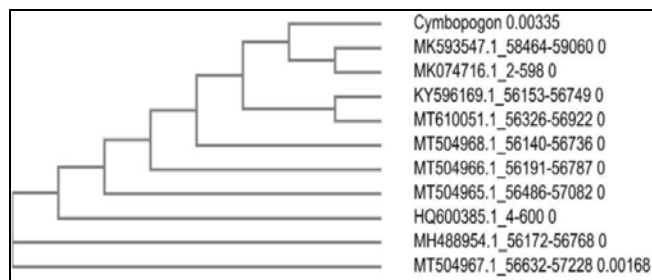


Fig 3: Phylogenetic tree constructed based on rbcL gene nucleotide sequence of *Cymbopogon* species

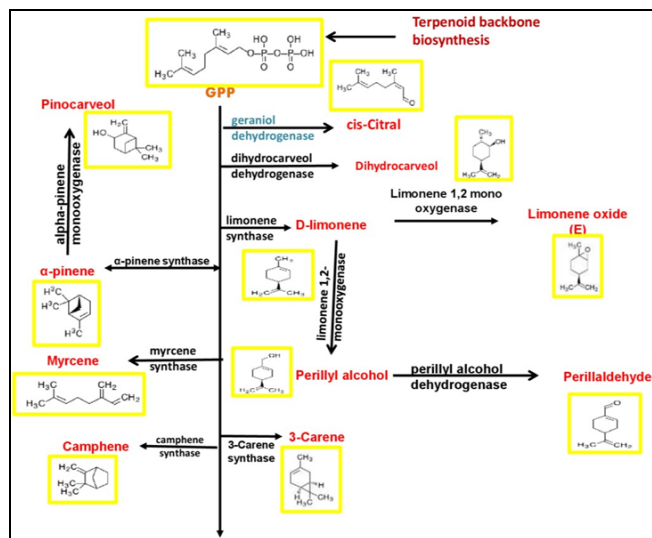


Fig 4: overview of monoterpene biosynthesis pathway in wild *Cymbopogon giganteus*

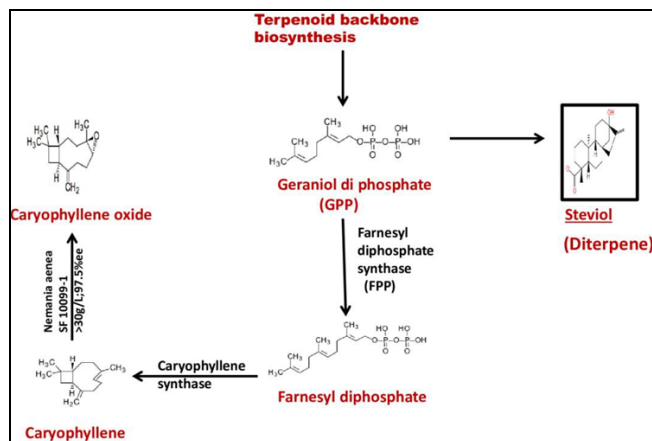


Fig 5: Overview of sesquiterpene biosynthesis pathway in wild *Cymbopogon giganteus*

Discussion

During the present study the essential oil from wild *Cymbopogon giganteus* showed highest production of dihydrocarveol compound which is reported as stereoisomer. (jiyang Guo *et al.*, 2018) [27]. Dihydrocarveol is found as the major compound in higher percentages present in wild *C. giganteus*. It is a p-menthane monoterpene and a dihydro derivative of carveol. (Małgorzata *et al.*, 2020) Dihydrocarveol is a secondary alcohol and derived from a carveol. (Graham Carr, *et al.*, 1999) [19] Dihydrocarveol tastes herbal, menthol, and minty utilized in a number of food items. This make dihydrocarveol a potential biomarker for the consumption of food products and used as additives

in the flavor industry. In the present study the compounds with mentha group were also found in higher percentages.

C. giganteus is used as folk medicine to treat various types of cough (Alitonou *et al.*, 2006) [16], and used as prophylactic and curative power against fever, yellow fever and jaundice. It is also are known to reported for treating strokes and mental disorder and relieve stomachache and epilepsy. (Toukourou *et al.*, 2020 and Bagora bayala *et al.*, 2018 and Annick Flore 2020) [21, 8, 7] *C. giganteus* is treat several diseases reported to including cancer. (Bagora bayala *et al.*, 2018) [8] The decoction from leaf and flower are used to treat skin disorders, conjunctiva and hepatitis. (Bassole *et al.*, 2011) [22]

The present study on *C.giganteus* essential oil revealed various compounds belonging to monoterpene, diterpene, sesquiterpenoids which can be explored further utilize them for various bioactivities.

Conclusion

Various terpene compounds are isolated and identified from wild *Cymbopogon giganteus* essential oil. The plant is well known folk medicine treating in several diseases. The essential oil possess several biological activities such as insecticidal, anti-protozoan, anticancer, anti-HIV and anti-inflammatory. Exploring wild *C. giganteus* require further probing to characterize the compounds for various biological activities.

Acknowledgement

We, all authors are thankful to the Department of Microbiology and Biotechnology, Bangalore University and IADFAC Laboratory and Barcode Biosciences, Bangalore for providing support to complete this work.

Conflict of Interest

The authors declare no conflict of interest.

References

- Vieira AJ, Beserra FP, Souza MC, Totti BM, Rozza AL. Limonene: Aroma of innovation in health and disease, *Chemico-Biological Interactions*, 2018, 283, <https://doi.org/10.1016/j.cbi.2018.02.007>.
- Abuzar Kabir, Francesco Cacciagran, Angela Tartaglia, Marica Lipsi, Halil Ibrahim Ulusoy, Marcello Locatelli. Analysis of monoterpenes and monoterpene, *Recent Advances in Natural Products Analysis*, Pages 274-286. <https://doi.org/10.1016/B978-0-12-816455-6.00007-X>. Adorjan B and Buchbauer G (2010). Biological properties of essential oils: an updated review. *Flavour and Fragrance Journal*, 2020;25(6):407-426. <https://doi.org/10.1002/ffj.2024>.
- Agata Koziol, Jakub Fraczak, Ewa Grela, Maryla Szczepanik, Beata Gabrys, Katarzyna Danciewicz *et al.* Synthesis and biological activity of new derivatives with the preserved carane system, *Natural Product Research*, 2018, 34. DOI: 10.1080/14786419.2018.1512992.
- Aleksandra Zielinska, Carlos Martins-Gomes, Nuno R.Ferreira, Amelia M Silva, Izabela Nowak, Eliana B Souto. Anti-inflammatory and anti-cancer activity of citral: Optimization of citral- loaded solid lipid nanoparticles (SLN) using experimental factorial design

- and LUMiSizer®. International Journal of Pharmaceutics, 2018; 553(1-2): 428-440. <https://doi.org/10.1016/j.ijpharm.2018.10.065>.
5. Anita Bansal, Amelia K Boehme, Lauren C Eiter, Jennifer M Schmidt, William N Setzer, Michael A Vincent. Chemical Composition and Bioactivity of the Leaf Oil of *Calyptanthus pallens* (Poir.) Griseb. from Abaco Island, Bahamas, Natural Product Communications, 2006. <https://doi.org/10.1177/1934578X0600100407>.
 6. Anna Marchese, Carla Renata Arciola, Ramona Barbieri, Ana Sanches Silva, Seyed Fazel Nabavi, Arold Jorel Tsetegho Sokeng *et al.* Update on Monoterpenes as Antimicrobial Agents: A Particular focus on p-cymene, journal of materials science and engineering, 2017. <https://doi.org/10.3390/ma10080947>.
 7. Annick Flore Arlette Dohoue Bossou, Gbedossou Sophie Reine Bogninou, Cokou P Agbangnan Dossa, Hounnankpon Yedomonhan, Felicien Avlessi, Dominique C K Sohounhloue. Volatile profiles and biological properties of *Cymbopogon citratus*, *Cymbopogon giganteus*, *Cymbopogon schoenanthus* and their isolated compounds: A review. Journal of biomedical and pharmaceutical research, 2020; 9(1): 22-32. doi:10.32553/jbpr.v9i1.711.
 8. Bagora Bayala, Imael H N Bassole, Salwan Maqdasy, Silvere Baron, Jacques Simporé, Jean-Marc A Lobaccaro. *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule, Biochimie, 2018.
 9. Bishoyi AK Kavane, A Sharma A *et al.* A report on identification of sequence polymorphism in barcode region of six commercially important *Cymbopogon* species. Mol Biol Rep, 2017; 44: 19-24 <https://doi.org/10.1007/s11033-017-4097-0>.
 10. Canan EceTamer, SenemSuna, GulsahOzcan-Sinir. 14-Toxicological Aspects of Ingredients Used in Nonalcoholic, Elsevier journals, 2019, 6, <https://doi.org/10.1016/B978-0-12-815270-6.00014-1>.
 11. Carmen MS Ambrosio, Gloria L Diaz-Arenas, Leidy P A Agudelo, Elena Stashenko, Carmen J Contreras-Castillo, Eduardo M da Gloria. Chemical Composition and Antibacterial and Antioxidant Activity of a Citrus Essential Oil and Its Fractions, Molecules, 2021; 26(10): 2888. <https://doi.org/10.3390/molecules26102888>.
 12. Ganjewala D. *Cymbopogon* essential oils: Chemical compositions and bioactivities, International Journal of Essential Oil Therapeutics, 2009; 3: 56-65.
 13. Bourgaud F, Gravot A, Milesi S, Gontier E. Production of plant secondary metabolites: a historical perspective, Plant Science, 2001; 161(2001): 839-851, DOI:10.1016/S0168-9452(01)00490-3.
 14. Fabrizio Francomano, Anna Caruso, Alexia Barbarossa, Alessia Fazio, Chiara La Torre, Jessica Ceramella. β -Caryophyllene: A Sesquiterpene with Countless Biological Properties, applied science, 2019, 9. <https://doi.org/10.3390/app9245420>.
 15. Flavia Bonamin, Thiago M Moraes, Raquel C dos Santos, Helio Kushima, Felipe M Faria, Marcos A Silva *et al.* The effect of a minor constituent of essential oil from *Citrus aurantium*: The role of β -myrcene in preventing peptic ulcer disease, Chemico-Biological Interactions, 2014; 212(5): 11-19. <https://doi.org/10.1016/j.cbi.2014.01.009>
 16. Alitonou GA, Avlessi F, Sohounhloue D, Agnani H, Bessiere J, Menut C. Investigations on the essential oil of *Cymbopogon giganteus* from Benin for its potential use as an anti-inflammatory agent, International Journal of Aromatherapy, 2006; 16(1): 37-41. DOI:10.1016/j.ijat.2006.01.001
 17. Bedi Sahouo G, Tonzibo ZF, Boti B, Chopard C, Mahy JP, Yao T Nguessan. Anti-inflammatory and analgesic activities: Chemical constituents of essential oils of *Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon giganteus* inhibited lipoxygenase L-1 and cyclooxygenase of PGHS, Bulletin of the Chemical Society of Ethiopia, 2003. DOI:10.4314/bcse.v17i2.61681.
 18. Ganjewala D, Luthra R. Essential oil biosynthesis and regulation in the genus *Cymbopogon*. Natural product communications, 2010; 5(1): 163-172.
 19. Graham Carr, Christopher Dean, David Whittaker. Terpenoid ether formation in superacids, Journal of the Chemical Society Perkin Transactions, 1999; 2(12): 2655-2817. DOI:10.1039/p29880000351.
 20. Kimbi K, Fagbenro-Beyioku AF. Efficacy of *Cymbopogon giganteus* and *Enantia chrantha* against chloroquine resistant *Plasmodium yoelii nigeriensis*, East African Medical Journal. Habib Toukourou, Hope Sounouvou, Lucy Catteau, Fatiou Toukourou, Francoise Van Bambeke, Fernand Gbaguidi, Joelle Quetin-Leclercq (2020). *Cymbopogon giganteus* Chiov. essential oil: Direct effects or activity in combination with antibiotics against multi-drug resistant bacteria, Journal of Applied Biology & Biotechnology, 1996, 8(01), DOI:10.7324/JABB.2020.80114.
 21. Habib Toukourou, Francine Uwambayinema, Yousof Yakoub, Birgit Mertens, Anatole Laleye, Dominique Lison *et al.* *In Vitro* and *In Vivo* Toxicity Studies on *Cymbopogon giganteus* Chiov. Leaves Essential Oil from Benin, Journal of Toxicology, 2020. <https://doi.org/10.1155/2020/8261058>.
 22. Bassole HN, LamienMeda A, Bayala B, Obame LC, Ilboudo AJ, Franz C *et al.* Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination, Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 2011; 18(12): 1070-1074. <https://doi.org/10.1016/j.phymed.2011.05.009>
 23. Noudogbessi JP, Alitonou GA, jenontin TD, Avlessi F, Figueredo G, Chalard P *et al.* Chemical compositions and physico-chemical properties of three varieties essential oils of *Cymbopogon giganteus* growing to the spontaneous state in benin, oriental journal of chemistry, 2012; 29(1): 59-67. DOI:10.13005/ojc/290109.
 24. Jean Brice Boti, Alain Muselli, Felix Tomi, Gerard Koukoua, Thomas Yao Nguessan, Jean Costa, Joseph Casanova. Combined analysis of *Cymbopogon giganteus* Chiov. leaf oil from Ivory Coast by GC/RI, GC/MS and ¹³C-NMR, Comptes Rendus Chimie, 2009, 9(1). <https://doi.org/10.1016/j.crci.2005.10.003>.

25. Jean Legault, Andre Pichette. Potentiating effect of β -caryophyllene on anticancer activity of α -humulene, isocaryophyllene and paclitaxel, Journal of pharmacy and pharmacology, 2010. <https://doi.org/10.1211/jpp.59.12.0005>.
26. Ji-hong Zhang, He-long Sun, Shao-yang Chen, Li Zeng, Tao-tao Wang. Anti-fungal activity, mechanism studies on α -Phellandrene and Nonanal against *Penicillium cyclopium*, 2017. doi:10.1186/s40529-017-0168-8.
27. Jiyang Guo, Rui Zhang, Jingping Ouyang, Feiting Zhang, Fengyu Qin, Guigao Liu *et al.* Stereodivergent Synthesis of Carveol and Dihydrocarveol through Ketoreductases/Ene-Reductases Catalyzed Asymmetric Reduction, The European society journal for catalysis, 2018. <https://doi.org/10.1002/cctc.201801391>.
28. John A Asong, Stephen O Amoo, Lyndy J McGaw, Sanah M Nkadameng, Adeyemi O Aremu, Wilfred Otang-Mbeng. Antimicrobial Activity, Antioxidant Potential, Cytotoxicity and Phytochemical Profiling of Four Plants Locally Used against Skin Diseases, plants-basel, 2019. DOI:10.3390/plants8090350.
29. Klaudyna Fidy, Anna Fiedorowicz, Leon Strzadala, Antoni Szumny. β -caryophyllene and β -caryophyllene oxide-natural compounds of anticancer and analgesic properties, Cancer Medicine, 2016. <https://doi.org/10.1002/cam4.816>.
30. Lijuan Yang, Haochuang, Dasha Xia, Shifa Wang. Antioxidant Properties of Camphene- Based Thiosemicarbazones: Experimental and Theoretical Evaluation, molecules, 2020. <https://doi.org/10.3390/molecules25051192>
31. Malgorzata Grabarczyk, Wanda M aczka, Anna K Zolnierczyk, Katarzyna Winska. Transformations of Monoterpenes with the p-Menthane Skeleton in the Enzymatic System of Bacteria, Fungi and Insects, *Molecules*, 2020;25(20):4840. <https://doi.org/10.3390/molecules25204840>.
32. Miho Igarashi, Yoshifumi Miyazaki. A Review on Bioactivities of Perilla: Progress in Research on the Functions of Perilla as Medicine and Food, Evidence-Based Complementary and Alternative Medicine (eCAM), 2013. <https://doi.org/10.1155/2013/925342>.
33. Mojtaba Karimi, Javad Hashemi, Ali Ahmadi, Alireza Abbasi, Masoud Esfahani. Study on the bioactivity of steviol and isosteviol in stevia (*Stevia rebaudiana* Bertoni), *Acta Physiol Plant*, 2014;36:3243-3248. DOI 10.1007/s11738-014-1690-x
34. Opeyemi Avoseh, Opeoluwa Oyedeji, Pamela Rungqu, Benedicta Nkeh-Chungag, Adebola Oyedeji. *Cymbopogon* Species; Ethnopharmacology, Phytochemistry and the Pharmacological Importance, *Molecules*, 2015;23:7438-7453. doi:10.3390/molecules20057438
35. Pamela L Crowell, Charles E Elson, Howard H Bailey, Abiodun Elegbede, Jill D Haag, Michael N Gould. Human metabolism of the experimental cancer therapeutic agent d-limonene, *Cancer Chemother Pharmacol*, 1994;35(1):31-7. doi:10.1007/BF00686281.
36. Praveen Kumar Gupta, Rithu BS, Shruthi A, Anushree Vinayak Lokur, Raksha M. Phytochemical screening and qualitative analysis of *Cymbopogon citratus*. *Journal of Pharmacognosy and Phytochemistry*, 2019. DOI:10.13140/RG.2.2.35870.28481.
37. Qing Zhu, Mei-Lin Jiang, Feng Shao, Guang-Qiang Ma, Qiang Shi, Rong-Hua Liu. Chemical Composition and Antimicrobial Activity of the Essential Oil From *Euphorbia helioscopia* L, *Natural Product Communications*, <https://doi.org/10.1177/1934578X20953249>. Rivas da Silva AC, Lopes PM, Barros de Azevedo MM, Costa DC, Alviano CS, Alviano DS (2012). Biological activities of α -pinene and β -pinene enantiomers, Search worldwide, life-sciences literature, 2020. DOI:10.3390/molecules17066305
38. Geetha TS, Geetha N. Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogon citratus* (DC) stapf. leaves from Kodaikanal hills, Tamilnadu, *International Journal of PharmTech Research*, 2014, 6(153). DOI:10.1016/j.biochi.2018.02.013.