Chemical Diversity in the Essential Oil of Wild *Cymbopogon giganteus* (Chiov.)

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Abstract

The wild *Cymbopogongi ganteus* (Chiov.) collected from Jnanabharathi campus, Bangalore University, Bangalore was subjected to hydro-distillation for extraction of its essential oil. GC-MS analysis was carried out to study the chemical composition of the essential oil. The yield of the essential oil obtained from hydro-distillation method was found to be 0.2 per cent v/w and a total of 50 compounds were identified. The dominant compounds were found to be Isocarveol (25.89%), trans-p-mentha-2,8-dienol (13.39 %), Limonene (9.66 %), 1,4-Methano phthalazine, 1, 4, 4a, 5, 6, 7, 8, 8 a-octahydro-9, 9-dimethyl-, (1α, 4α, 4aα, 8aα) (8.30%), cis-p-mentha-2, 8-dien-1-ol (6.62 %), Carvone (4.40%) and Carveol (3.19%). These secondary metabolite compounds are known to possess potential bioactivities of medical significance.

Keywords : Cymbopogon giganteus, Essential oil, GC-MS analysis, Monoterpenoids, Sesquiterpenoids

The genus Cymbopogon comprises about 140 species (Kumari *et al.,* 2007) of which *Cymbopogon giganteus* (Chiov.) is one of the species that is known to be economically important for the production of essential oils. Essential oils are complex mixture of volatile secondary metabolites. The variation in the composition of essential oil depends upon environmental conditions and the methods used for extraction. *Cymbopogongi ganteus* (Chiov.) is a perennial grass that belongs to the polytypic genus Cymbopogon in the family Poaceae (Graminae) and the species is widely spread in the tropic and subtropic regions of Asia, Africa and America with a distribution ranging from hilly and savanna regions to deserts and xeric shrub lands (Rao, 1997). The plant has a rhizome-bearing stem that enables it to perennate (survive an annual unfavourable season) underground and can grow up to 6 to 9 feet (Letouzey, 1972). The Essential oil of *Cymbopogon giganteus* has vast commercial values in flavors, fragrances, cosmetics, perfumery, soaps, detergents, toiletry, tobacco products and pharmaceuticals (Ganjewala *et al.,* 2009).

GC-MS analysis of the essential oil showed that, out of the different compounds formed, terpenoid sare most abundant and are present as monoterpenes, sesquiterpenes and their derivatives like alcohols, esters, ketones and others. Terpenoids form a unique group in the sense of the range and diversity of compounds they represent. The structural type and their derivatives comprise thousands of compounds and form the vast groups in nature (Connolly and Hill, 1992). The presently studied essential oil of wild *Cymbopogongi ganteus* constituted variouster penoids that are produced from isoprene units through Mevalonate pathway. The characteristic odour of the essential oil of *Cymbopogongi ganteus* is due to its high content of monoterpenoid alcohols *i.e.,* Isocarveol, trans-p-mentha-2, 8-dienol, cis-p-mentha-2, 8-dien-1-ol, Carveol etc. The trace constituents present in the oil are responsible for the characteristic olfactory note of the oil (Raina *et al.,* 2003).

Investigation of the chemical composition of the essential oil from leaves, inflorescence and stem have been carried out. (Raina *et al.,* 2003). During the present investigation, the essential oil from wild *Cymbopogongi ganteus* was analysed by GC-MS and the finger print compounds present in the oil were recorded.

MATERIAL AND METHODS

Plant Collection

The plant sample for study was collected from Jnanabharathi campus, Bangalore University, Bengaluru, Karnataka. The site for plant collection was located at an elevation of 829 m and a latitude and longitude of 12°50'58.1342" N and 77°30'31.8008" E, respectively with an area of 4.452 sq. kilometres. Due to its elevation, it receives about 1200 mm of rain annually, with the wettest months being August, September and October. The temperature recorded on the day the sample was collected (*i.e.,* 11th January 2021) was 28 °C. The collected plant sample was subjected to Essential oil extraction and analysis.

Plant Identification

Isolation of Total Cellular DNA and Primer Designing for Barcode Loci Amplication : Fresh and young leaves of the wild plant were taken and subjected to total extraction of the cellular DNA using CTAB method. The corresponding gene sequences of the genus Cymbopogon were retrieved from NCBI Gen-Bank data domain for precisely designing the specific primers for the amplification of three barcoding loci, *i.e.*, rbcL, matK and ITS one and two spacers. PCR primer pairs were mapped out from the conserved regions using software primer 3.0 (version 0.4.0) (Bishoyi *et al.,* 2017).

Barcode Amplification, Sequencing and Validation and Data Analysis : Two chloroplast loci (rbcL, matK) and one nuclear DNA locus (ITS region) of the isolated DNA from the fresh young leaves were amplified using the primers that were designed. The PCR reaction mixture contained the template DNA, buffer, $MgCl_2$, 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 94 ºC for 5 minutes followed by an annealing stage at 60 ºC for one minute, extension stage at 72 ºC for two minutes and final extension at 72 ºC for 10 minutes. The PCR products were purified and sequenced (Bishoyi *et al.,* 2017). Sanger sequencing of amplicons were carried out using BDT v 3.1 Cycle sequencing kit on Abi 370xl Genetic Analyzer. Annotation softwares 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 per cent agarose gel for the visualization of the amplified products. The gel was pictured with a Gel Doc XR+(Biorad). Annotated counting barcode sequences were subjected to BLASTn (NCBI domain) for verification and were finally submitted to Gen Bank of NCBI. The DNA sequences were aligned automatically using the program CLUSTALW in MEGA 6.0 and constructed NJ derived phylogenetic tree.

Essential Oil Studies

Extraction of Essential Oil : The fresh plant materials collected from the experimental sites were washed under tap water followed by distilled water to remove the dust particles and dried at ambient temperature for two days in the laboratory. The dried materials were minced and subjected to hydro-distillation for three hours using Clevenger-type apparatus for the extraction of essential oil. The oil was dried over anhydrous sodium sulphate and stored in sealed vials under a refrigerator until analysis. The amount of essential oil concentration was calculated by using the formula given below.

$$\text{Essential oil extration (\%)} = \frac{\text{Amount of essential oil recovered (ml)}}{\text{Amount of crop biomass distilled (g)}} \times 100$$

Analysis of Essential Oil (GC-MS) : GC-MS analysis of the essential oil was carried out on an Acquisition - General, Shimadzu GCMS, Model Number: QP2010Plus equipped with Electron Ionization using a capillary column Rtx-5MS 30 m length × 0.25 µm thickness with an internal diameter of 0.25 mm. 0.1 µl sample was injected at the head of the column. The column temperature was initially programmed at 40 °C with a hold time of two minutes and then at a Ramp rate of 5 °C to 280 °C followed by 20 °C to 300 °C for two minutes. The injector and the interface temperature was 250 °C and 280 °C, respectively. Helium was used as a carrier gas at a flow rate of 0.7 ml / min with a split ratio of 1:100.

Identification of Components : The identification of the constituent was assigned on the basis of comparison of the retention indices and their mass spectra with those given in the literature (Adams, 2001; Joulain and Konig,1998). The interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST5).

Results and Discussion

Plant Identification

The DNA barcoding studies of wild *Cymbopogongi ganteus* showed that out of three locus (rbcL, matK, and ITS spacers 1 and 2), only rbcL 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. 1) (Table 1). 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 supported the study of NCBI BLAST leading to confirmation of the species as *Cymbopogongi ganteus* and the same was submitted in the NCBI Gen Bank under the accession number OK094429.

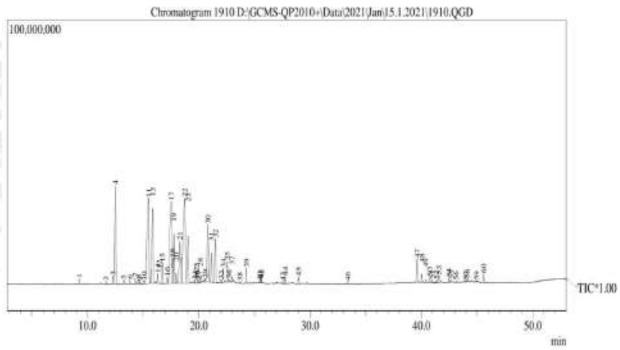

Fig. 1: Peaks observed in GC-MS analysis

Table 1

Details of the primers developed for the barcode amplification in *Cymbopogon* species

Primer name	Designed sequence (52 to 32)	Annealing temp (°C)	Annealing temp (°C)	Remarks
Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene				
CNRBCLF1CNRBCLR1	TGTTGGATTTAAAGCTGGTGTTCA TTTGCAAGCTGCTTTGAT	53.9	1323	Primer pair was validated
CNRBCLF2CNRBCLR2	GCAAGTGTTGGATTTAAAGCTGCA GCACTCCATTTGCAAGC	60.0	1336	Primer pair was taken for this study
Maturase K (matK) gene				
CNMTKF1CNMTKR1	TTTGATAAACCGAGAAATGCTTGC CTTTCCTTGATATCGAACAT	60.0	909	Primer pair was taken for this study
CNMTKF2CNMTKR2	ATGTATCATCATTTGATAAACCGA GAATGCCTTTCCTTGATATCGAACAT	58.5	910	Primer pair was validated
Internal transcribed spacer 1, 5.8 S ribosomal RNA gene and internal transcribed spacer 2				
CFITSF1CFITSR1	CAAAACAGACCGCGAACGGGTGCTC GATGGGTCCTTAG	60.0	555	Primer pair was taken for this study
CFITSF2CFITSR2	GTAGGTGAACCTGCGGAAGGGTGCTT GATGGGTCCTTAG	59.0	595	Primer pair was validated

Characterization of Essential Oil

The essential oil from wild *Cymbopogongi ganteus* was qualitatively and quantitatively analysed. 0.2 ml of essential oil was obtained from 100 g of herbage with an yield of 0.2 per cent v/w. The colour of the essential oil was pale yellow. The essential oil was dominated by monoterpenoids with the presence of few sesquiterpenoids. The essential oil showed balanced content of hydrocarbons and oxygenated terpenes. A total of 52 compounds were identified from the essential oil by GC-MS analysis (Fig. 2) (Table 2).

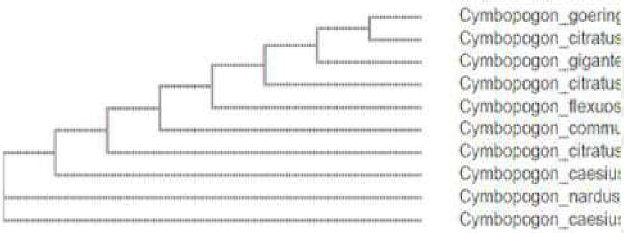

Fig. 2: Phylogenetic tree constructed based on rbcL gene nucleotide sequences of *Cymbopogon* species

The major compounds present in the oil were limonene (9.66%), 1,4-methano phthalazine, 1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-9, 9-dimethyl-, (1α, 4α, 4aα, 8aα), (8.30 %), carvone (4.40 %) and a set of monoterpene alcohols : isocarveol (25.89 %), trans-p-mentha-2, 8-dienol (13.39%), cis-p-mentha-2, 8-dien-1-ol (6.62 %) and carveol (3.19 %). There were quantitative differences present in the essential oil suggesting that the environmental factors strongly influenced its chemical composition. It was noted that Isocarveol, a monoterpenoid alcohol in the essential oil contributed strongly to the aroma of the oil. The components of the essential oil due to which the plant gets its biological activity has vast commercial and medicinal values. The major constituent Isocarveol possesses antimicrobial (Janaina B. Seibert, 2019), trypanocidal and antiplasmodial activities, trans-p-mentha-2, 8-dienol possesses anti microbial activity (Bassole *et al.*, 2014) and cis-p-mentha-2, 8-dien-1-ol possesses antimicrobial (Bassole *et al.*, 2014), antibacterial and antioxidant activities (Carmen *et al.*, 2021). Further more, Limonene is known to possess chemopreventive and

TABLE 2

Classification of the constituents of the essential oil from wild *Cymbopogongi ganteus*

Compound	Area %	RT (sec)	Molwt (g/mol)
Monoterpenoids	69.34		
Monoterpenoids without functional groups			
Limonene	9.66	12.532	136.23
Monoterpenoid alcohols			
trans-p-Mentha-2,8-dienol	13.39	15.487	152.23
cis-p-Mentha-2,8-dien-1-ol	6.62	15.876	152.23
Isocarveol	25.89	17.522	152.23
Carveol	3.19	17.786	152.23
L-Perillyl alcohol	0.36	20.570	152.23
Monoterpenoid aldehydes			
L-Perillaldehyde	0.73	19.788	150.22
α-Citral	0.16	19.653	152.23
α-Campholenal	0.29	22.986	152.23
p-Menth-1-en-9-al	0.56	17.991	152.23
Monoterpenoid ketones			
1,4-Dimethyl-3-tetra hydroacetophenone	0.27	13.828	152.23
Carvone	4.40	19.034	150.22
Monoterpenoid epoxides			
Limonene epoxide	0.68	15.717	150.23
cis-Carvone oxide	0.12	19.865	166.21
Monoterpenoid peroxides			
(2R,4R)-p-Mentha-[1(7),8] -diene, 2-hydroperoxide	0.26	22.004	168.23
(2S,4R)-p-Mentha-[1(7),8] -diene 2-hydroperoxide	1.61	22.574	168.23
Acyclic monoterpenoids			
Cosmene	0.19	11.672	134.22
α-Citral	0.16	19.653	152.23
Bicyclic Monoterpenoid			
6.β.Bicyclo[4.3.0]nonane, 5 β-iodomethyl-1β-iso propenyl-4α,5α-dimethyl-,	0.39	42.453	332.26
Irregular monoterpenoids			
2-Methylisoborneol	0.17	22.729	168.28
Methyl carveol	0.24	25.641	166.26
Sesquiterpenoids	3.32		

Compound	Area %	RT (sec)	Molwt (g/mol)
Sesquiterpenoids without functional groups			
Caryophyllene	0.89	27.565	204.36
δ-Guaiene	0.19	41.349	204.36
Sesquiterpenoid ketone			
Longiverbenone	1.55	39.606	218.33
Sesquiterpenoid alcohol			
Widdrol	0.28	42.656	222.37
Sesquiterpenoid epoxide			
Caryophyllene oxide	0.41	27.774	220.35
Steroid	0.49		
Steroid with ketone and alcohol			
11α-Hydroxy-17α-methyl testosterone	0.49	40.012	318.45
Hydrocarbons	16.65		
Saturated Hydrocarbons			
Tetracontane	0.11	44.948	563.1
Unsaturated Hydrocarbons			
Cosmene	0.19	11.672	134.22
3-Octadecyne	1.50	17.648	250.5
Nonanal	0.16	14.741	142.23
2-Isopropylidene-3-methylhexa-3, 5-dienal	0.11	25.486	150.22
Cyclic Hydrocarbons			
4-Isopropenylcyclohexanone	0.54	16.325	138.21
Melilotal	0.41	17.171	134.17
1,3-Bis-(2-cyclopropyl, 2-methylcyclopropyl) -but-2-en-1-one	3.80	21.509	258.4
Bicyclo [2.2.2] oct-2-ene, 1,2,3,6-tetramethyl	0.82	22.192	164.29
1,4-Cyclohexadiene, -3-ethenyl-1,2-dimethyl	0.54	12.301	134.22
β, β-Dimethylstyrene	0.35	14.295	132.2
Cyclohexanemethanol, 2-(2-propenyl)-, trans	0.36	20.143	154.25
[1,1-Bicyclopentyl]-2-one	4.28	18.301	152.23
Cyclic Hydrocarbons with Fused Rings			
trans-Hydrindane	0.29	9.248	124.22
1,3-Benzodioxole, 3a,7a-dihydro-2,2,4-trimethyl	0.29	14.579	152.19
Bicyclo[3.3.0] oct-2-en-7-one, 6-methyl	1.37	16.739	136.19

Compound	Area %	RT (sec)	Molwt (g/mol)
Bicyclo [4.1.0] heptane, -3-cyclopropyl, -7-hydroxy methyl, (cis)	0.14	20.026	166.26
5, 9, 9-Trimethyl-spiro [3.5] non-5-en-1-one	0.22	25.575	178.27
Fatty acid esters			
Isoamyl butyrate	0.27	13.278	158.24
Isoamyloctanoate	0.77	24.282	214.34
Phenylethyloctanoate	0.13	33.356	248.36
Tricyclic compound	9.91		
1, 4-Methanophthalazine, 1, 4, 4a, 5, 6, 7, 8, 8a-octahydro -9, 9-dimethyl-, (1α,4α,4α,8α)	8.30	20.839	178.27
Tricyclo [20.8.0.0 (7,16)] triacontane, 1 (22), 7(16) -diepoxy	0.84	43.950	444.7
Tricyclo [7.2.0.0 (2,6)] undecan-5-ol, 2,6,10,10 -tetramethyl-	0.77	41.538	204.36
Tetracyclic compound	0.48		
Tetracyclo [6.3.2.0 (2,5).0 (1,8)] tridecan-9- ol 4, 4-dimethyl	0.48	28.972	220.35

antitumour activity (Jessica A Miller *et al*., 2013). Bioactivity of rest of the compounds present in the essential oil are reported in (Table 3).

The volatile constituents (monoterpenoids and sesquiterpenoids) present in the wild *C. giganteus* interconvert through biosynthetic pathways by the action of certain enzymes (Fig. 3). These volatile constituents are produced from isoprene units which are synthesised as Isopentenyl pyrophosphate (IPP) from Acetyl-CoA through Mevalonate pathway. IPP is isomerized to the other isoprene unit DMAPP, by an isomerase enzyme. These isomers condense together in a head to tail manner to form Geranyl pyrophosphate (GPP). Successive addition of IPP to GPP produces Farnesyl pyrophosphate (FPP) and Geranylgeranyl pyrophosphate (GGPP).

Geranyl pyrophosphate (GPP) serves as the precursor for monoterpenoids (Fig. 4). Geranial is produced from geraniol by the action of the enzyme geraniol

TABLE 3

Bioactivity profile for the components of the essential oil from wild *Cymbopogongiganteus*

Name of the Compound	Bioactivity	Reference
Cosmene	Pheromone and semiochemical (insect attractant)	A M EI- Sayed *et al*., (2008).
Limonene	Chemopreventive and antitumour	Jessica A Miller *et al.,* (2013).
Isoamylbutanoate	Aggregation pheromone of the brown spiny bug, Clavigrallatomentosicollis	Hilaire Kpongbe *et al.,* (2019).
4-Acetyl-1, 4-dimethyl-1-cyclohexene	Human metabolite and flavouring agent	Yannai (2004).
Nonanal	Antifungal Metabolite observed in cancer metabolism	Ji-Hong Zhang *et al*., (2017).
trans-p-Mentha -2, 8-dienol	Antimicrobial activity (plant metabolite found in spearmint oil)	Bassole I H N *et al.,* (2014).
Limonene epoxide	Anxiolytic and antioxidant	Antonia Amanda Cardoso de Almeida *et al.,* (2014 Mar).
cis-p-Mentha-2, 8- dien-1-ol	Plant metabolite with antimicrobial potentialAntibacterial and antioxidant	Bassole I H N *et al.,* (2014). Carmen M.S. Ambrosio (2021).
4-Isopropenyl cyclohexanone	Acaricidal and potential inhibitor of tumor cells affecting brain	Ji-Yeon Yang (2013).
Melilotal	Antioxidant	Chia-Pei Liang *et al.,* (2014).
Isocarveol	Antimicrobial and acaricide Trypanocidal and antiplasmodial	Janaina B. Seibert (2019). Salome Kpoviessi *et al., (*2014).
3-Octadecyne	Used in perfumery and cosmetics	Yingngam B *et al*., (2015).
Carveol	Human metabolite of limonene, chemopreventive (prevents breast cancer) Vasorelaxant Antibacterial	Renata Evaristo Rodigues da Silva (2020). Aline Cristina Guimaraes *et al*., (2019).
Carvone	Spasmolytic activityAcaricide Antiplasmodic Anticancer, Insecticidal effect	Damiao Pergentino de Sousa *et al.,* (2015) Magna GalvaoPeixoto *et al.,* (2015)
α-Citral	Antioxidant Antimicrobial Anti-inflammatory and anticancer	Hafsia Bouzenna (2017) Aleksandra Zielinska (2018)
L-Perillaldehyde	Antidepressant Fumigant Antioxidant	Ji-Xiao *et al*. (2019) Wei-Bin Ma *et al.* (2014) Hui Tian *et al.,* (2017)
cis- Carvone oxide	Antimicrobial Plant growth regulator	Maria C Pellegrini *et al*., (2017) Jacek Lyczko (2020)
L-Perillyl alcohol	Vasorelaxant Chemopreventive and antiangiogenic	Ana Carolina Cardoso-Teixeira *et al.,* (2018), Chen TC *et al.,* (2015)
Bicyclo[2.2.2] oct-2-ene, 1,2,3,6-tetramethyl	Anticholinesterase and antiradical	Muhammad Ayaz *et al.,* (2015)
(2S,4R)-p-Mentha -[1(7),8]-diene 2-hydroperoxide	Trypanocidal	Fumiyuki Kiuchi *et al.,* (2002)
2-Methylisoborneol	Cyanobacterial metabolite	Izaguirre and Taylor (2004).
α-Campholenal	AntimicrobialAnalgesic	NaouelChaftar*et al.,* (2015).

Name of the Compound	Bioactivity	Reference
Caryophyllene	Antiflammatory and anti-edematogenicLarvicidalAntioxidant	Oliveira-Tintino *et al.,* (2018). HammadUllah*et al.,* (2021)
Caryophyllene oxide	AntioxidantAnticancer and analgesicAntimicrobial Antifungal	Eman H Reda*et al.,* (2021).
Longiverbenone	Antifungal Fumigant and repellent properties	Abbas Khani *et al.,* (2014)
δ-Guaiene	Larvicidal Insecticidal Platelet activating factor inhibitor	Ephantus J Muturi *et al.,* (2019). Elânia L D Albuquerque (2013)
Widdrol	Antifungal	Nunez *et al.,* (2006)
Tetracontane	Antidengue and antiviral	T Pratheeba*et al.,* (2019)

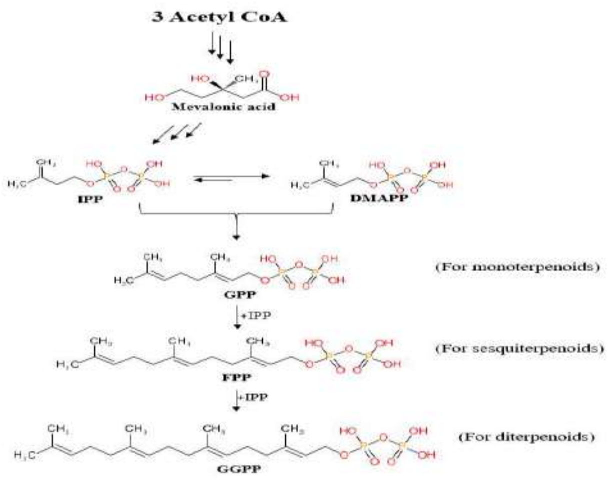

Fig. 3: Mevalonate pathway for biosynthesis of terpenoids

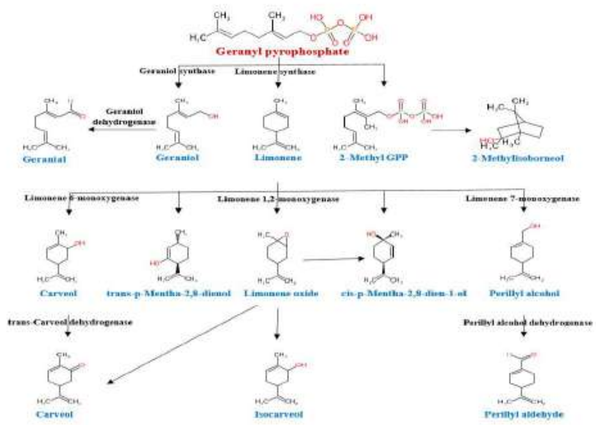

Fig. 4: Biosynthesis of monoterpenoids from the essential oil of wild *Cymbopogongiganteus*

dehydrogenase (Singh Sangwan *et al.,*1993). Geraniol, in turn is produced from geranyl pyrophosphate with the removal of pyrophosphate molecule by the action of geraniol synthase. GPP is cyclized to limonene by the action of limonene synthase. Limonene is then converted by limonene 6-monooxygenase to Carveol, followed by oxidation by carveol dehydrogenase to Carvone. Also, limonene is converted to Perillyl alcohol by limonene 7-monooxygenase, which further gets oxidized to Perillyl aldehyde by perillyl alcohol dehydrogenase. In a similar manner, trans-p-mentha-2, 8-dienol can be biosynthesised from Limonene. Further the biosynthesis of limonene oxide and isocarveol proceeds from limonene *via* a two-step pathway. First, Limonene is converted to Limonene oxide by the action of limonene 1, 2-monooxygenase and later the epoxide ring of the limonene oxide breaks to form Isocarveol, the major compound present in the essential oil of wild *Cymbopogongi ganteus*.

GPP reacts with the nucleophilic IPP unit to yield FPP and served as a precursor to sesquiterpenoids (Fig. 5). FPP undergoes cyclisation *via* carbonium ions to form cyclic sesquiterpenoids. The course of cyclisation depends on the geometry of FPP (IL Finar, 1956). FPP is cyclized to β-Caryophyllene, a humulene type sesquiterpenoid, by the action of (-)-β-Caryophyllene synthase followed by conversion of β-Caryophyllene oxide. The biosynthesis of Widdrol and a-Guaiene proceeds *via* the action of Widdrol synthase and a-guaiene synthase, respectively.

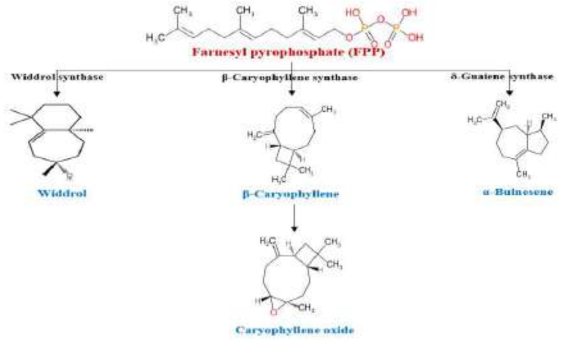

Fig 5: Biosynthesis of sesquiterpenoids from the essential oil of *Cymbopogongiganteus*

The present study showed that the composition of the essential oil of *C. giganteus* collected from Jnanabharathi campus, Bangalore University was dominated by monoterpenoids bearing the menthane skeleton. The essential oil possessed many potential bioactivities of great medical significance. In addition, the essential oil possesses other activities of commercial importance and thus the demand for the wide variety of wild species is increasing with the growth in human needs, numbers and commercial trade (Lambert *et al.*, 1997).

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(*Received :* October 2021 *Accepted :* November 2021)