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Studies on essential oil compounds from wild *Cymbopogon giganteus* (Hochst.) Chiov

Manjula S[1], Ashwini M[2], Vinutha M[3], KJ Thara Saraswathi[4*]

[1] Student, 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, Bangalore, Karnataka, India

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

Abstract
Cymbopogon giganteus belonging to Poaceae is an aromatic grass and medicinal plant used to treat various diseases in traditional medicine. The genus Cymbopogon consist of perennial grasses distributed world wide and used for their essential oil. The commercial uses of the various species of cymbopogon are well documented. The ethanopharmacological evidence showed that cymbopogons Possess array of properties and are used for pest control, in cosmetics and as anti-inflammatory agents, besides holding promise as potent antitumor and chemopreventive drugs. In the present study, *Cymbopogon giganteus* collected from Gnanabharathi campus, Bangalore university, Bangalore. was studied for the essential oil content and composition. The essential oil was extracted by Hydro-distillation method and the oil was subjected to GC/MS analysis to get fingerprint compounds. Dihydrocarveol was found as major compound (28.52%), followed by D-limonene (10.93%), Cis-carveol(7.38%), Cis-p-mentha-2,8- diene-1-ol(7.57%), Trans-p-mentha-2,8-dienol(13.81%), Carvotanacetone(3.47%), 3-octadecyne(1.65%).

Keywords: wild *Cymbopogon giganteus*, essential oil, GC-Ms analysis, terpenoid biosynthesis

Introduction
Cymbopogon giganteus also called as "Citronelle de Madagascar", grass which can grow up to 2-3m height and found extensively growing in regions tropical africa (Toukourou *et al.,* 2020). It is used in traditional medicine to treat various skin diseases (Pale *et al.*, 2020). Mental illness, broncho-pulmonary affections, bilharzia, jaundice, cold, conjuctivites, migraine, dermatoses, rheumatic pains, childhood coughs and hepatitis (Alitonou 2006, Popielas *et al.*,1991) [2, 20]. The plant possess antibacterial and anti-inflammatory properties (Alitonou *et al.*, 2012, B. Bayala *et al.,* 2018). and are dominated by the presence of compounds such as Carveol, Limonene, Cis-p- mentha derivatives in the essential oil (Kpoviessi *et al.,* 2014) [26]. *Cymbopogon giganteus* is an aromatic perennial grass belonging to Poaceae, It is a sweet smelling grass that grows spontaneously in the savannahs of Asian and African tropical regions, It possess a rhizome- bearing stem and can grow up to 3m high (Letouzey 1972) [25]. The solvent extracts of C. giganteus contain flavanoids (Vale *et al.*, 1966) [17] and amino acids (Keita *et al.*, 1986). The decoction of the leaves are used to treat Headaches, common cold, conjuctivitis, sickling, cellular diseases and for tranquilizing epileptic seizures (Adjanohoun EJ 1989) [5].

Materials and Methods
Plant collection and ecological details
The wild Cymbopogon Species was collected from Jnanabharathi campus, Bangalore University, Bangalore. The area of plant collection consisted of 1100 acres, altitude 874 m above sea level, with latitude of 12.949357 and longitude of 77.505389. The annual temperature recorded was 28.40C, with humidity of 56%, wind flow of 7.2mt/sec, and precipitation 10%. The average annual rainfall was 22.6mm.

Plant identification
Barcoding studies
Isolation of total cellular DNA and primer designing for Barcode loci amplification
Fresh and young leaves of the plant was collected and subjected to extraction of total cellular DNA using CTAB method (Murray and Thompson, 1980). Specific primers (*rbcL, matK* and ITS1 and 2 spacers) were used for DNA barcoding sequence (Bishoyi *et al.,* 2017) [7].

Barcode amplification, sequencing, validation and data analysis
The isolated DNA from the fresh young leaves were amplified using the primers two chloroplast loci (rbcL, matK) and one nuclear DNA locus (ITS region)). The PCR reaction mixture contained the template DNA, buffer, MgCl2, dNTPS, designed primer and DNA polymerase.

Essential oil sudies
Extraction
The fresh herbage consisting of root, stem, leaf, inflorescence were colected from the experimental sites. The herbage was washed under tap water followed by washed with distilled water, to remove dust particles and dried at ambient temperature for two days under shade. The shade dried leaves were cut into small pieces and used for the extraction of essential oil. The oil was extracted using hydro-distillation method using a clevenger apparatus for 3

hours (joseph Franklin Clevenger, 1928). The oil was collected and dried over anhydrous sodium sulfate and stored in sealed vials under refrigerator untill analysis.

Gas chromatography and mass spectroscopy

GC-MS analysis of the essential oil was carried out on an Acquistion-general, Shimadzu GC- MS, Model number: QP2010S Equipped with Electron Ionization using a column Rxi-5SilMS, 60m length x 250 µm thickness. Temperature programming was done Initial 40℃ hold for 2 minutes Ramp at 5℃ to 280℃ at 20 ℃ to 300 ℃ holds for 2 mins; 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 identical conditions. Interpretation of the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST 11) and WILEY 18 library.

Result and Discussion

Identification of the plant

The plant was identified as *Cymbopogon giganteus* (Hochst.) Chiov. based on the morphological and essential oil studies.

DNA barcoding studies

Out of three loci (rbcL, matK, and ITS spacers 1and 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 length in the same units as those of the evolutionary distances used to infer the phylogenetic tree. 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 tha the studied plant sample was very closely grouped under clad of *Cymbopogon sp*. and the result supports study of NCBI BLAST leading to confirmation of the species as *Cymbopogon giganteus* and the sequence was submitted in NCBI GenBank under the accession number of OK094427.

Essential oil studies

The essential oil obtained from hydro distillation method yielded 0.5 ml of oil from 100g of the herbage. The colour of the essential oil was pale yellow. GCMS analysis revealed the presence of chemical compounds from the essential oil. A number of 32 compounds were identified from the essential oil.(Fig 1) Dihydrocarveol was found at highest percentage (28.52%), followed by D-limonene (10.93%), Trans-p-mentha-2,8-dienol (13.81%), Cis-p-mentha-2,8-diene-1-ol (7.57%), Cis-carveol (7.38%) and found as major compounds. The compounds were categorized into different chemical classes (Table 1). The bioactivity studies of the essential oil compounds as reported by earlier workers are tabulated (Table2).

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 monoterpenoid precussor. Which undergo isomerization, acetylation, diacylation, cyclization and dehydrogenation to form other monoterpene and terpenoid compounds. The enzymes: Limonene 1, 2 monooxygenase, Limonene oxidoreductase, Trans-carveol oxidoreductase, Carveol dehydrogenase, Perillyl Alcohol dehydrogenase, dihydrocarveol dehydrogenase, Myrtenol dehydrogenase were utilized in the monoterpenoid pathway.

GPP get converted into D-limonene along with diphosphate molecule in the presence of limonene synthase and further get converted into 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. D- Limonene also converted into Trans-carveol in the presence of Limonene oxidoreductase and it is further get converted into carvone in the presence of Trans-carveol oxidoreductase. Carvone further get converted into Cis-carveol in the presence of Carveol dehydrogenase. Carvone also converted into Dihydrocarveol in the presence of dyhydrocarveol dehydrogenase. GPP get converted into Myrtenal in the presence of Myrtenal dehydrogenase. (Fig 2) 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 3)

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.

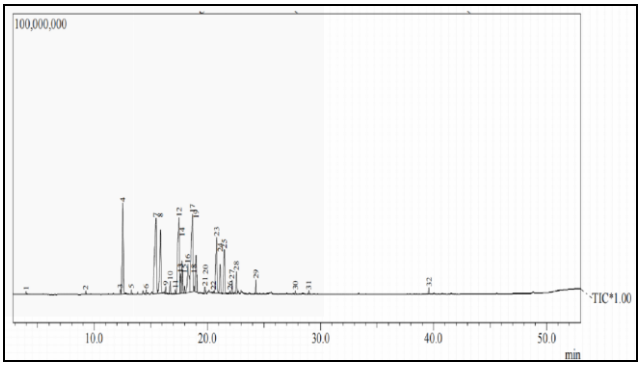

Fig 1: GC-MS chromatogram of wild *Cymbopogon giganteus*

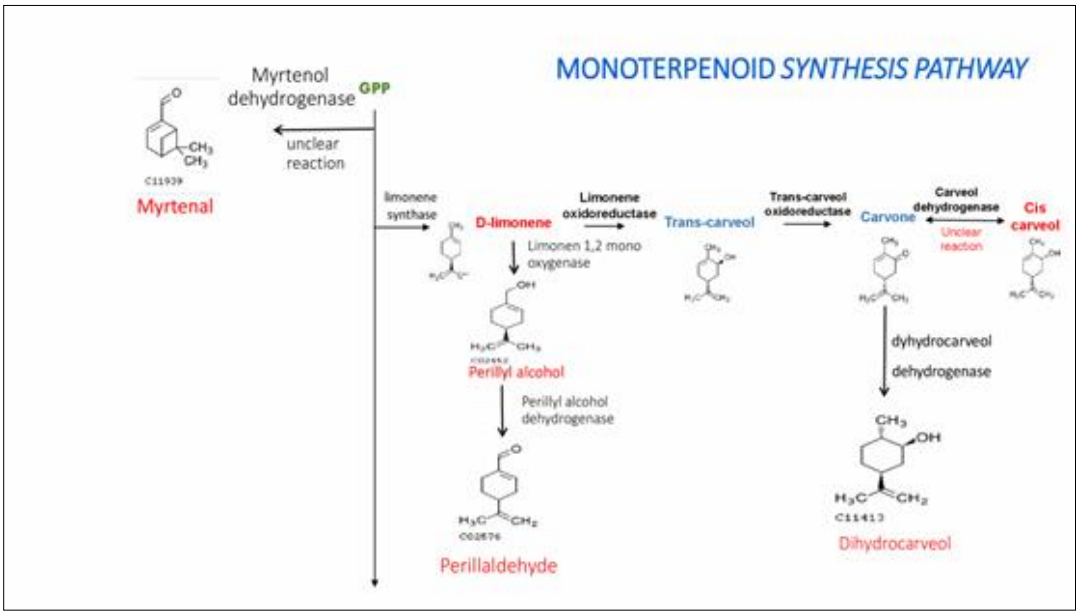

Fig 2: Pathway for monoterpenoid biosynthesis in wild *Cymbopogon giganteus.*

Monoterpenoids are synthesized by by MEP pathway from geranyl diphosphtae (GPP) as their precursor. GPP get converted into D-limonene and further get converted into Trans-carveol, Carvone, cis-carveol. D-limonene get converted into perillyl alcohol and converted to perillylaldehyde. Carvone get converted into Dihydrocarveol. GPP get converted into Myrtenal. The enzymes identified via KEGG and META CYC include: Limonene 1, 2 mono oxygenase, limonene oxidoreductase, trans-carveol oxidoreductase, carveol dehydrogenase, Perillyl alcohol dehydrogenase, dihydrocarveol dehydrogenase, myrtenol dehydrogenase.

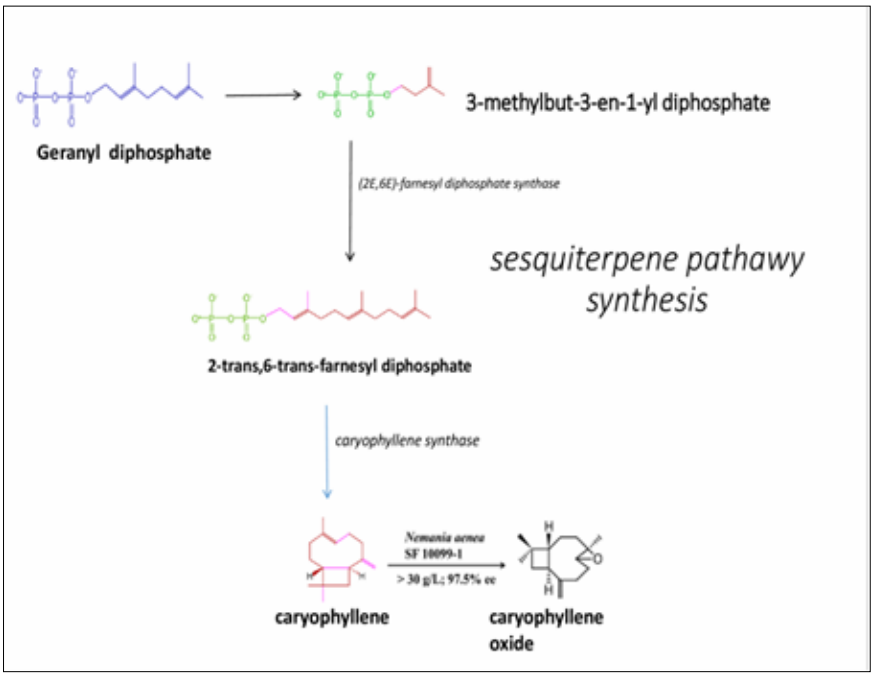

Fig 3: Sesquiterpene biosynthesis pathway in wild *Cymbopogon giganteus.*

Table 1: Finger print compounds from wild *C. giganteus*

SL No	Compounds	Area%	Retention time	Mol. weight
Monoterpenoid hydrocarbons				
1	D-Limonene	10.93%	12.524	88.15
2	Trans-p- mentha-2,8-dienol	13.81%	15.456	152.23
3	Cis-p-mentha-2,8-diene-1-ol	7.57%	15.852	152.23
	L-perillaldehyde	0.57%	19.775	150.22
Menthane Monoterpenoids				
4	Carvotanacetone	3.47%	19.001	179.26
5	(2R-4R)-p-Mentha-6,8-diene,2-hydroperoxide	1.02%	22.187	168.23
P-menthane Monoterpenoids				

6	Gamma-terpineol	0.29%	18.821	154.25
Monoterpenoids				
7	Di-hydrocarveol	28.52%	17.489	152.23
8	L-perillyl alcohol	0.24%	20.555	152.23
Oxygenated Monoterpenes				
9	Cis-carveol	7.38%	17.762	152.23
10	1,3-Bis-(2-cyclopropyl,2- methylcyclopropyl)-but-2-en-1-one	4.98%	21.503	258.4
Primary alcohols				
11	Isoamyl alcohol	0.25%	3.961	88.15
Esters				
12	Isoamyl butyrate	0.33%	13.282	158.24
Bicyclic Monoterpenoids				
13	Myrtenal	0.36%	14.583	150.22
Saturated Monocyclic hydrocarbons				
14	Hexahydrocumene	0.48%	16.134	138.21
Cyclic ketones				
15	Bicyclo[3.3.0] oct-2-en-7-one,6-methyl-	1.01%	16.717	136.19

Sesquiterpenoids are synthesized by MVA pathway in Cytosol from Farnesyl diphosphate as precursor. The enzymes identified via METACYC include:farnesyl diphosphate synthase and caryophyllene synthase.

Conclusion

The genus Cymbopogon contain varied compounds showing varied biological activities. The essential oil obtained from the wild *C. giganteus* analyzed by gas chromatography-mass spectrometry (GC-MS) showed total number of 32 compounds. The essential oil rich in terpenoid compounds contained Monoterpenoids, oxygenated Monoterpenes, Sesquiterpene hydrocarbons, Oxygenated sesquiterpene hydrocarbons which can be tested further for various bioactivities.

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