International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 25-10-2021, Accepted: 11-11-2021, Published: 26-11-2021
Volume 6, Issue 6, 2021, Page No. 525-533

Studies on terpenoid compounds in the essential oil of wild *Cymbopogon martinii* (Roxb.) watson

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Abstract
Cymbopogon belongs to the aromatic grasses and possess high medicinal properties. These aromatic grasses produce essential oil of commercial values and offer protection from pests and predators, attracts pollinators, and helps in seed dispersal. During the present study, wild *Cymbopogon martini* collected from Balluru near Attibele, Anekal Taluk was subjected for GC-MS analysis to study the chemical composition present in the essential oil. The whole plant (leaf, stem, root and inflorescence) was used to extract essential oil by hydro-distillation method using Clevenger type apparatus. GC-MS analysis revealed the presence of 80 compounds which were identified. Cubenol a sesquiterpene compound was found as major compound (11.40%) in the essential oil followed by Camphene (10.24%), alpha-Pinene (7.94%), Limonene (7.91%), Caryophyllene oxide (7.52%), Borneol (6.64%), Iso-bornyl acetate (5.36%) and Nerolidol (5.09%). Plant was subjected to DNA barcoding studies for confirmation of the species.

Keywords: wild *Cymbopogon martini*, DNA barcode, essential oil, GC-MS, terpenoid pathway

Introduction

The genus *Cymbopogon* belonging to Poaceae comprise of aromatic grasses having significant medicinal property. It is derived from the Greek word, Cymbo-Hallow boat like vessel, pogon-beard (Milica Acimovic *et al*., 2019) [2]. *Cymbopogon* are widely distributed in different parts of the world such as Asia, Africa, America, Tropic and subtropic regions of India. In India, they are distributed in Kashmir, Punjab, Rajasthan, Mumbai and southern states. The genus *Cymbopogon* belongs to Poaceae Family (Milica Acimovic *et al*., 2019) [2]. *Cymbopogon martini* commonly called as Rosha grass and is utilised commercially for different purpose. There are two varieties of *C. martinii* (Sofia and Motia) (Dagar *et al*., 2007) [14]. Sofia gives Ginger grass oil and Motia gives Palma Rosa oil which are extensively used in skin care and aromatherapy (Anupama 2017) [6]. *C. martinii* is a sweet-scented grass of 1.5 to 3.5m hight. It provides aromatic essential oil having large applications in fragrance, perfumery, cosmetics and pharmaceutical industries (Jeliazkov (Zheljazkov) *et al*., 2016).

The essential oil from this genus has two dominating monoterpenes such as geranyl acetate and geraniol (Gurminder Kaur *et al*., 2019) [29]. Having a huge application of the essential oil from this genus studying the terpenoid biosynthetic pathway is highly desirable to improve its quality and yield of the oil (Gurminder Kaur *et al*., 2019) [29]. *Cymbopogons* provide phytochemicals such as alkaloids, ketones, alcohol, phenolics, terpenoids, glycosides (Gupta *et al*., 2019) [18]. The quality and quantity of the essential oil produced by the plant depends on the climate, soil type, age and vegetable cycle stage, preparation method, chemotypes, as well as on the ontogeny of the plant (Fongang Fotsing Yannick Stephane *et al*., 2020). Environment plays a very important role on chemical composition of the essential oil. The essential oils are volatile substances extracted from plants belongs to secondary metabolites and show various bioactivities (BUTNARIU *et al*., 2018) [12]. Essential oils are composed of complex mixture of compounds. Oxygenated monoterpenes are the major contributor to the taste and aroma of the essential oils (Lydia *et al*., 2020). Most of the essential oils contains the natural monoterpenes and sesquiterpenes with various functional groups (Adorjan *et al*., 2020) [3].

Terpenes are synthetized in the cytoplasm of plant cells through the mevalonic acid pathway (Nashwa Fathy Sayed Morsy 2017) [39]. Terpenoids are produced by the biochemical modifications such as oxidation and rearrangements of terpenes. Terpenoids are oxygenated derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, acids, ethers, and esters. Monoterpenes are made from the combination of two isoprene units (Abuzar Kabir *et al*., 2020) [27]. Monoterpenes are produced in nearly all the essential oil and Sesquiterpenes are produced as other major group. Sesquiterpenes are derived from three isoprene units and exist in a wide variety of forms, including linear, monocyclic, bicyclic, and tricyclic and form the most diverse group of terpenoids (Ludwiczuk *et al*., 2017) [1]. During the present study, wild *Cymbopogon martinii* explored from Balluru near Attibele, Anekal taluk

was identified by DNA barcoding studies and studied for the fingerprint compounds present in the essential oil.

Materials and Methods

Ecological details and plant collection

The wild species of Cymbopogon for study was collected from Balluru village near Attibele, Anekal Taluk, Bangalore district, Karnataka, in the month of January 2021. The ecological details of the plant growth were studied. The place of the plant growth had an area of 431.21 hectares and possessed red sandy soil. The collection site is situated 3.4 kms (East) away from Attibele with an Altitude of 888 meters above the sea level, Latitude of 12.78°N and Longitude of 77.363° E. It receives the average rain fall of 750 mm. The place showed a temperature of max-36°C, min-23°C, Humidity-52° with the wind flow of 26km/hour towards West.

Plant identification

DNA barcoding studies and Phylogenetic analysis was carried out to identify the species.

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 ITSI 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) [8].

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) [8]. 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 form 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 plant (root, leaf, stem, inflorescence) was collected from the experimental site was used for essential oil extraction. Whole plant is taken for the oil extraction. Leaves, stem, roots and inflorescence were separated and are washed under tap water followed by distilled water to remove dust particle. The plant was dried at ambient temperature for 2 days in the laboratory to remove the moisture content and the dried plants were cut into small pieces, weighed and used for extraction of essential oil. The plant materials were subjected to hydro-distillation using Clevenger type apparatus for 3 hours. The oil was dried over anhydrous sodium sulphate and was stored in sealed vials in the refrigerator (4°C) until analysis.

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

Gas chromatography and mass spectrometry (GC-MS)

GC-MS analysis of the essential oil was carried out on an acquisition- Shimadzu GC-MS, Model number-QP-2010 plus equipped with electron ionization using a column Rtx-5MS, 30m length ×0.25µm film thickness. ID: 0. 25mm and Injector of 250°C. Sample injection:0.1µl. Temperature programming was done initial of 40°C for 2mins Ramp at 5°C to 280°C Ramp at 20°C to 300°C holds for 2mins.

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 data base of National Institute Standard and Technology (NIST5) library.

Result

Plant identification

Based on the morphological characterization, DNA bar-coding and essential oil studies. The species of Cymbopogon undertaken for the study belonged to the Cymbopogon martini (Fig 1).

Fig 1: Habit and Habitat of wild *Cymbopogon martinii*

DNA bar-coding studies

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 2). 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 spp*. This result supports the study of NCBI BLAST leading to confirmation of the species as *Cymbopogon martinii* and was submitted the same in NCBI GenBank under the accession number of OK094430.

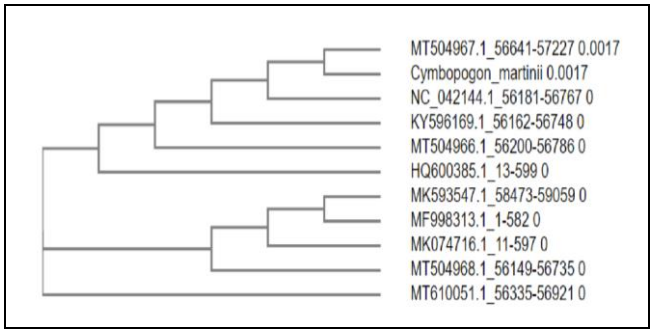

Fig 2: Phylogenetic tree constructed based on *rbcL* gene nucleotide sequences of *Cymbopogon species*

Essential oil studies

180g of the dried herbage sample yield approximately 0.5ml of essential oil. The essential oil was found to be pale green colour and was characterized by fresh, sweet-smelling lemon aroma. GC-MS analysis of the essential oil revealed the presence of various compounds present in wild *C.martinii*. (Fig 3). The essential oil gave 73 fingerprint compounds which were identified and recorded. Cubenol expressed in highest percentage of 11.40% followed by Camphene (10.24%), Alpha-Pinene (7.94%), Limonene (7.91%), Caryophyllene oxide (7.52%), Borneol (6.64%), Iso-bornyl acetate (5.36%), Nerolidol (5.09%) are the major compound analysed by GC-MS. All the chemical compounds were categorised into different chemical classes (Table 1). The composition of the oils was dominated by monoterpenoids (51.48%), sesquiterpenoid (31.1%), diterpenoid (0.32%), ketones (3.62%), hydrocarbons (2.47%), esters (0.09%), cyclic hydrocarbon (0.11%), isomeric hydrocarbon (0.60%). Bioactivity studies of the compounds as reported by earlier workers (Table 2).

Geranyl diphosphate (GPP) belongs to monoterpene group and it undergo isomerization, acetylation, diacylation, cyclization and dehydrogenation to form various monoterpenes. The enzymes involved in the process include, alcohol O-acetyltransferase, Geraniol synthase, Sabinene-hydrate synthase, Geraniol isomerase, Linalool dehydrogenase, Alpha pinene synthase, Alpha pinene monooxygenase, Linalool hydrolyse, Myrcene synthase, Beta- phellandrene, Alpha phellandrene, Camphene synthase, Limonene-6-hydroxylase, Bornyl pyrophosphate hydrolase, Carveol dehydrogenase (Fig 4). Geranyl diphosphate (GPP) is reacts with water and get converted to Sabinene hydrate in the presence of Sabinene hydrate synthase. GPP is converted α-pinene in the presence of α-pinene synthase and α-pinene is further converted into trans pinocarveol in the presence of α-pinene monooxygenase. GPP get converted into β-Phellandrene in the presence of β-Phellandrene synthase. GPP also get converted into Geraniol in the presence of Geraniol synthase and Geraniol further get converted to Geraniol acetate in the presence of alcohol O-acetyltransferase. GPP with water get converted into Linalool in the presence of Linalool dehydratase which further converts to form Geraniol in the presence of Geraniol isomerase. GPP upon conversion to Myrcene in the presence of Myrcene synthase get converted further to Linalool in the presence of Linalool hydro-lyase. GPP is converted to α-Phellandrene in the presence of α-Phellandrene synthase. Limonene gets converted from GPP in the presence of Limonene synthase and Limonene is further converted into Perillyl alcohol in the presence of Limonene hydroxylase. Limonene is converted into Trans-carveol in the presence of Limonene-6-hydroxylase and Trans-carveol further converts to Carvone in the presence of Carveol dehydrogenase. Carvone is converted to Carveol and the enzyme involved is unclear. GPP is converted to Camphene in the presence of Camphene synthase and Camphene is converted into Camphene hydrate and the enzyme involved is unclear. GPP is converted into Borneol diphosphate (BDP) in the presence of borneol diphosphate

lyase. BDP with water is converted to Borneol in the presence of Borneol pyrophosphate hydrolase. Borneol is converted to Beta camphor in the presence of Borneol dehydrogenase (conversion of NAD+ to NADH with the removal H+). Borneol is converted to Borneol acetate in the presence of Menthol transacetylase.

Farnesyl diphosphate (FPP) is formed by the initiation of Geranyl diphosphate (GPP) in the presence of farnesyl diphosphate synthase leading to the formation of sesquiterpenoid pathway. The enzymes such as farnesyl diphosphate synthase, cubenene synthase, cubenol synthase, nerolidol synthase, valencene synthase, alpha bisabolene synthase, caryophyllene synthase, calarene synthase, gamma muurolene synthase, beta-cubenene synthase are involved for the conversion. (Fig 5). FPP is converted to Cubenene in the presence of Cubenene synthase. FPP get converted into Cubenol in the presence of Cubenol synthase. FPP converts Nerolidol in the presence of Nerolidol synthase and also converts Valencene in the presence of Valencene synthase. FPP is converted into Alpha-bisabolene in the presence of Alpha-bisabolene synthase and it converts β -Cubenene in the presence of β-Cubenene synthase. FPP is converted to γ-Muurolene in the presence of γ-Muurolene synthase and FPP converts Calerene in the presence of Calerene synthase. FPP is converted to Caryophyllene in the presence of Caryophyllene synthase and further get converted into Caryophyllene oxide. Mevalonate pathway is involved in the production of Steviol, diterpene derivative which is synthesised from kaurene. The precursor of Steviol is synthesised by plastid localised in methylerythritol-4-phosphate (MEP) pathway results in formation of isopentenyl diphosphate (IPP) and dimethyl diphosphate (DMAPP) which get converted into Geranyl geranyl diphosphate (GGDP) by plastid phenyl transferase.

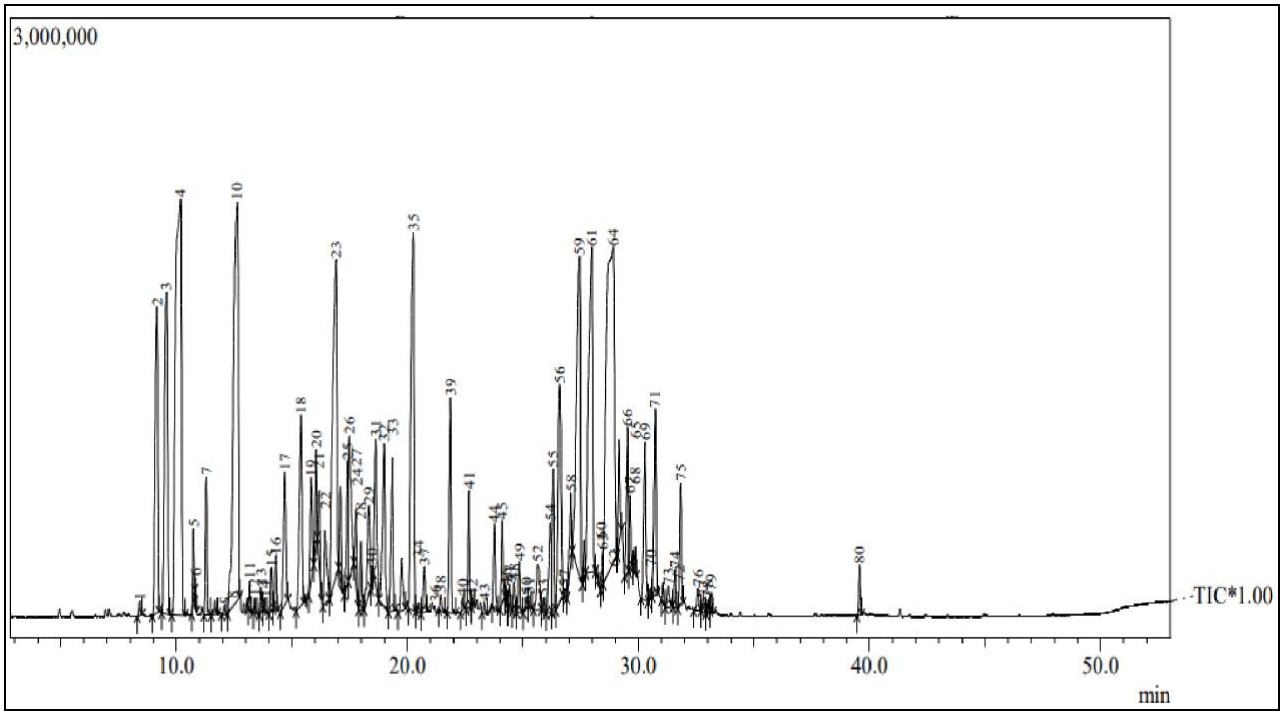

Fig 3: GC-MS chromatogram of wild *Cymbopogon martini*

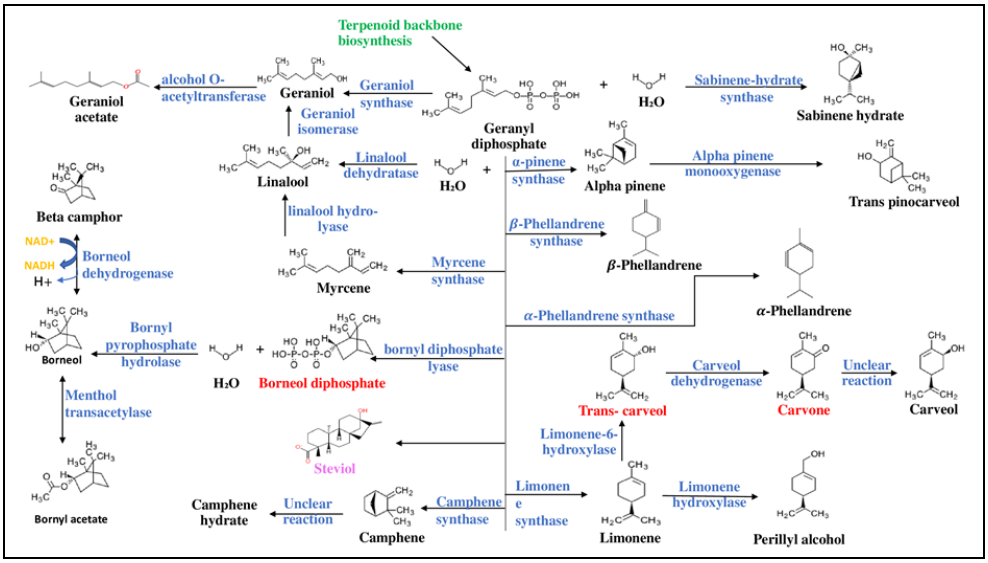

Fig 4: Overview of monoterpenoid biosynthesis pathway in wild *Cymbopogon martinii*

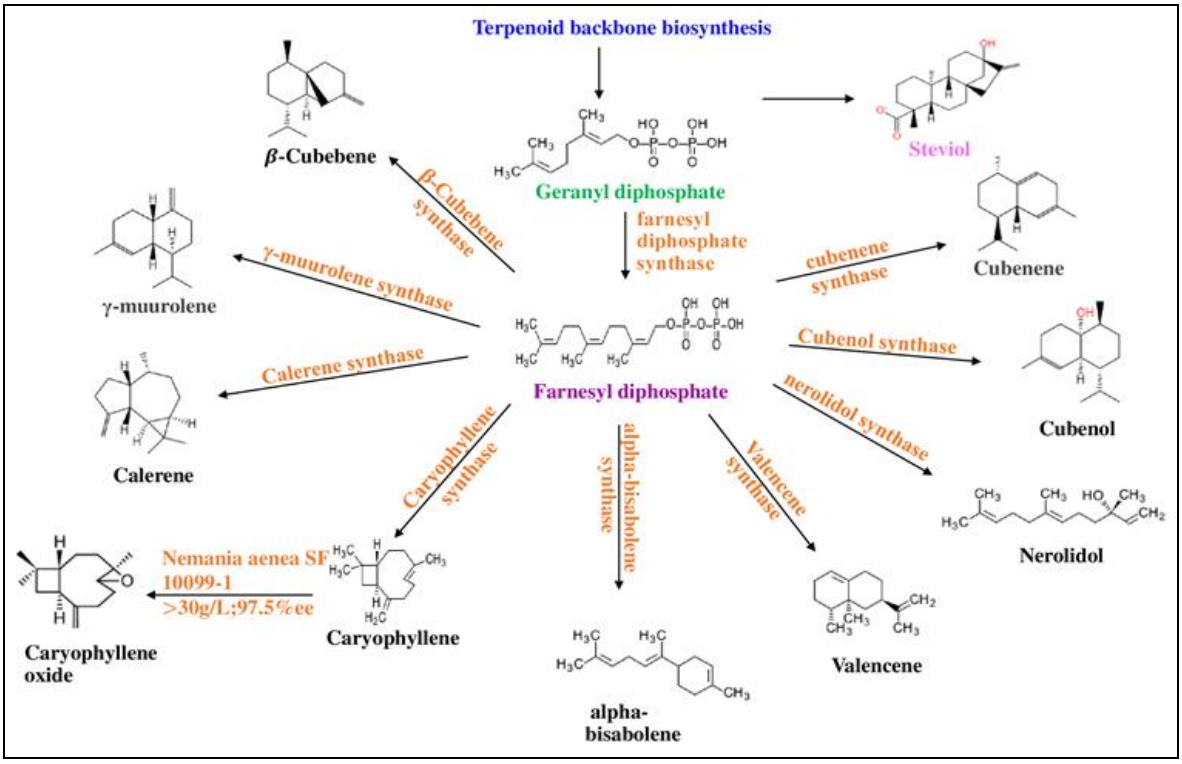

Fig 5: Overview of sesquiterpenoid biosynthesis pathway in wild *Cymbopogon martinii*

Table 1: Classification of the compounds into chemical groups

Sl.no	Compounds	Area%	RT	Molecular weight	Molecular formula
	Monoterpenoids				
01.	alpha-Pinene	7.94	9.162	136	$C_{10}H_{16}$
02.	Camphene	10.26	10.18	136	$C_{10}H_{16}$
03.	Myrcene	0.88	11.288	136	$C_{10}H_{16}$
04.	2-Carane	0.45	12.072	176	$C_{10}H_{16}$
05.	Limonene	7.91	12.632	136	$C_{10}H_{16}$
06.	Gamma-Terpinene	0.08	13.387	136	$C_{10}H_{16}$
07.	Sabinene hydrate	0.15	13.651	154	$C_{10}H_{18}O$
08.	Linalool	1.55	13.814	154	$C_{10}H_{18}O$
09.	cis-Isolimonenol	0.97	15.826	152	$C_{10}H_{16}O$
10.	Beta-Camphor	0.67	16.042	152	$C_{10}H_{16}O$
11.	Camphene hydrate	0.49	16.178	210	$C_{10}H_{18}O$
12.	Borneol	6.64	16.444	154	$C_{10}H_{18}O$
13.	trans-Pinocarveol	1.59	17.476	152	$C_{10}H_{16}O$
14.	cis-Piperitol	0.45	17.99	154	$C_{10}H_{18}O$
15.	(Z)-Carveol	1.09	18.328	152	$C_{10}H_{16}O$
16.	Geraniol	1.42	19.352	154	$C_{10}H_{18}O$
17.	Iso geranial	0.52	19.737	152	$C_{10}H_{18}O$
18.	Bornyl acetate	5.36	20.26	196	$C_{12}H_{20}O_2$
19.	Geranyl acetate	0.75	22.668	196	$C_{12}H_{20}O_2$
20.	trans-p-Mentha-2,8-dienol	2.23	15.398	152	$C_{10}H_{16}O$
	Oxygenated monoterpenes				
21.	Perillyl alcohol	0.07	20.464	152	$C_{10}H_{16}O$
	Cyclic monoterpenes				
22.	Beta-Phellandrene	0.37	10.738	136	$C_{10}H_{16}$
23.	alpha-Phellandrene	0.11	11.677	136	$C_{10}H_{16}$
	Sesquiterpenoids				
24.	Caryophyllene	0.73	23.768	204	$C_{15}H_{24}$
25.	Calarene	0.57	24.103	204	$C_{15}H_{24}$
26.	alpha-Bisabolene	0.19	24.604	204	$C_{15}H_{24}$
27.	Gamma-Muurolene	0.07	25.147	204	$C_{15}H_{24}$
28.	Valencene	0.09	25.898	204	$C_{15}H_{24}$
29.	Cubenene	1.11	26.306	204	$C_{15}H_{24}$
30.	Cedrelanol	2.73	26.593	222	$C_{15}H_{26}O$
31.	Caryophyllene oxide	6.79	27.069	220	$C_{15}H_{24}O$
32.	Nerolidol	5.09	27.447	222	$C_{15}H_{26}O$
33.	Cubenol	11.40	28.914	222	$C_{15}H_{26}O$
34.	Gaol	0.81	29.149	222	$C_{15}H_{26}O$
35.	alpha-Cadinol	1.22	29.534	222	$C_{15}H_{26}O$

36.	beta-Cubebene	0.10	25.281	204	$C_{15}H_{24}$
37.	Dehydro-beta-ionone	0.06	28.199	194	$C_{13}H_{22}O$
38.	Allo-aromadendrene	0.14	29.856	204	$C_{15}H_{24}$
	Diterpenes				
39.	Steviol	0.32	39.564	318	$C_{20}H_{30}O_3$
	Ketones				
40.	Camphenilone	0.26	14.108	138	$C_9H_{14}O$
41.	Carvomenthone	1.43	18.634	154	$C_{10}H_{18}O$
42.	Carvotanacetone	1.78	18.988	152	$C_{10}H_{16}O$
43.	Cyclohexanone	0.15	13.168	98.14	
	Esters				
44.	Dodecyl acetate	0.09	23.326	228	$C_6H_{10}O$
	Hydrocarbons				
45.	Alpha Terinyl Acetate	1.76	21.868	196	$C_{12}H_{20}O_2$
46.	Bicyclo[3.1.1]heptane	0.08	10.827	136	C_7H_{12}
47.	trans-beta-lonone	0.63	25.642	192	$C_{13}H_{20}O$
	Cyclic hydrocarbons				
48.	2-Bornene	0.11	8.412	136	$C_{10}H_{16}$
	Isomeric hydrocarbons				
49.	Terpinene	0.60	17.108	136	$C_{10}H_{16}$
	Ungrouped compounds				
50.	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	0.92	17.398	150	$C_{11}H_{18}$
51.	[1,1'-Bicyclopentyl]-2-one	0.57	17.773	152	$C_{10}H_{16}O$
52.	Cyclooctene, 3-(1-methylethenyl)-	0.06	18.473	150	$C_{11}H_{18}$
53.	(1S,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	0.41	20.723	-	-
54.	(2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide	0.14	21.436	168	-
55.	trans-Shisool	0.08	22.383	154	$C_{10}H_{18}O$
56.	Decanoic acid	0.07	22.821	172	$C_{10}H_{20}O_2$
57.	Isopentyl octanoate	0.06	24.26	214	-
58.	(+)-Epi-bicyclosesquiphellandrene	0.50	24.409	204	$C_{15}H_{24}$
59.	Selina-6-en-4-ol	0.97	26.177	222	$C_{15}H_{26}O$
60.	Cadala-1(10),3,8-triene	0.06	26.79	202	$C_{15}H_{22}$
61.	6-Methyl-6-nitroheptan-2-one	0.21	27.639	173	$C_8H_{14}O$
62.	1,3-Hexadiene, 3-ethyl-2,5-dimethyl-	0.20	28.449	138	$C_{10}H_{18}$
63.	Spiro [2.5] octane, 3,3-dimethyl-2-(1-buten-3-on-1-yl)-	0.53	29.644	206	$C_{14}H_{22}O$
64.	2H-Cycloprop[c]indene-2,3(3ah)-dione, hexahydro-3a,7,7-trimethyl-	1.52	30.281	206	-
65.	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-	0.07	30.532	192	$C_{13}H_{20}O$
66.	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	1.59	30.732	306	-
67.	"1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-"	0.12	31.054	238	$C_{15}H_{26}O$
68.	Acetic acid, 1- [2-(2,2,6-trimethyl-bicyclo [4.1.0]hept-1-yl)-ethyl]-vinyl ester	0.23	31.578	238	-
69.	"2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one "	0.93	31.827	218	$C_{15}H_{22}O$
70.	Cycloartenol acetate	0.19	32.563	470	$C_{32}H_{52}O_2$
71.	"6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol"	0.12	32.811	236	$C_{15}H_{24}O_2$
72.	"3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol "	0.07	33.012	238	$C_{15}H_{26}$
73.	"Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- "	0.11	33.147	236.35	$C_{15}H_{24}O_2$

Table 2: Bioactivity of the compounds with references

Sl.no	Compounds	Bioactivity	References
	Alpha- pinene	Exhibits anti- inflammatory activity. Anti-carcinogenic effect.	Kim *et al*.,(2017)
	Camphene	present in many cannabis chemovars in low titer. application in human chronic obstructive pulmonary disease.	Ethan *et al*., (2017)
	Myrcene	used to treat diabetes, diarrhea, dysentery, and hypertension	Joshua *et al*., (2016)
	Alpha -Phellandrene	Antimicrobial agent.	Salas-Oropeza *et al*., (2021)
	Limonene	Antibacterial agent, Antifungal agent, Insectisidal effect.	Lis-Balchin *et. al*., (1996)
	Linalool	Anti-inflamatory property.	Peana *et al*., (2002)
	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-,trans-	Used as adjuncts in probiotic cheese making.	Kourkoutas *et al*., (2006)
	Beta-Camphor	Assessed by evaluation of fumigant activity against C. ferrugineus under laboratory and storage conditions.	Rozman et.al.,(2006)
	Camphene hydrate	Antibacterial agent Anti-fungal	Gebze-Kocaeli *et al*., (2008)
	Borneol	Reaction mechanism on analgesia, putridity elimination and flesh regeneration, and repair of damaged cells.	Zhen-Yu Xiong *et al*., (2013)
	Carvomenthone	Anti-inflammatory and anti-allergic remedy	Revista Brasileira de Farmacognosia. (2019)
	Carvotanacetone	Strongest bactericidal activity, moderate cytotoxic activity and acetylcholinesterase inhibitory effect	Nasser *et al*., (2012)
	Geraniol	Antimicrobial, Anti-oxidant, Anti-inflammatory.	W.Chen *et al*., (2010)
	Bornyl acetate OR Isobornyl acetate	Anti-Inflammatory Functions.	He Yang *et al*., (2014)
	Geranoil acetate	Anti-Inflammatory, Anxiolytic, Antimicrobial, Diuretic, Antiseptic,Anti-cancerous	Yasiel Arteaga Crespoa *et al*., (2019)
	Decanoic acid OR Capric acid	Antibacterial agent Antioxident.	Renugadevi *et al*., (2021)
	Caryophyllene	anticancer activities, affecting growth and proliferation of	Fidyt, *et al*., (2016).

		numerous cancer cells	
	alpha-Bisabolele	It has been used in cosmetics for hundreds of years because of its perceived skin-healing properties.	Nara *et.al*., (2013).
	beta-Cubebene	Antimicrobial activity.	Mirjana Skočibušić *et al*., (2004).
	trans-beta-lonone	A toxicologic and dermatologic review of trans-beta-Ionone when used as a fragrance ingredient is presented.	Lalko *et.al*., (2007).
	Valencene	Anti-oxident property	Kehai Liu *et.al*., (2012).
	Cubenene	Antimicrobial agent. Anti-oxidant property.	Yuan-Hui Wang ORCID Icon *et.al*., (2019).
	Cadala-1(10),3,8-triene	Antifungal property. Antibacterial property.	Imad Hadi Hameed *et.al*., (2016)
	Caryophyllene oxide	anticancer activities, affecting growth and proliferation of bnumerous cancer cells	Fidyt *et al*., (2016).
	Nerolidol	They exhibit, inter alia, olfactory, germicidal and antimicrobial properties.	Radosław Bonikowski *et.al*, (2015).
	Cubenol	Cytotoxic activity against the retinoblastoma cancer cell line	Nadechanok Jiangseubchatveera *et.al*, (2015).
	Guaiol	Antimicrobial and insecticidal properties	Tao Liua *et.al*, (2013).
	alpha-Cadinol	Anti inflamatory activity	Titilayo OmolaraJohnson *et.al*, (2020).
	Allo-aromadendrene	Antifungal and Antibacterial property.	Zi-qian He *et al*, (2019).
	Steviol	antioxidant capacity	Mojtaba Karimi *et a*., (2014).

Discussion

The present investigation wild *Cymbopogon martinii* was analysed for the characteristic fingerprint essential oil, which contained Cubenol (11.40%) as major constituent. Cubenol is a sesquiterpenoid and it serves as a refreshing agent. Camphene (10.26%) is the second major compound belonging to monoterpenoid group used as fragrance and flavouring. Steviol is synthesised as Diterpene compound and they are derived from the kaurenoid precursor of Gibberllic acid (Brandle *et al*., 2007) [23]. The diversity of steviol glycosides results from elaboration of glycone steviol by various glycosyl transferase (Brandle *et al*., 2007) [23]. The species of *Cymbopogon martinii* has been traditionally used to treat central nervous system (CNS) disorders such as neuralgia, epileptic fits and anorexia (Buch *et al*., 2012) [11]. Biological significance of the *Cymbopogon* essential oils, little is known about their biosynthesis and regulatory mechanisms (Ganjewala *et al*., 2010) [17].

Since it has high medicinal properties, it is used in the treatment of skin, respiratory disorders, and have an application in food industry, cosmetic industry. The essential oil compounds obtained from wild *Cymbopogon martini* require further probing to understand their significance to be adopted in different fields. Unlike other cultivar varieties of *Cymbopogon martini*, the presently studied wild *C. martinii* showed geraniol in trace amount which may be due to the environmental stress condition offered to wild species.

Conclusion

Various compounds have been identified from essential oils. The action of *Cymbopogon martini* essential oil on oxidative stress can prevent the toxicity in liver and prevent growth of bacteria by damaging their membrane. In food industry product can be supplied without any contamination.

Acknowledgement

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

Conflict of Interest

The authors declare no conflict of interest.

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