Mysore J. Agric. Sci., 55 (4) : 162-174 (2021)

Studies on Carotol Rich Essential Oil from Wild *Cymbopogon martini* (Roxb.) Watson

J. Krupa, J. Hemalatha, M. Vinutha and K. J. Thara Saraswathi
Department of Microbiology and Biotechnology, Bangalore Universtiy, Bengaluru - 560 056
e-Mail : krupagowda100@gmail.com

Abstract

Essential oils are volatile liquids having aromatic fragrance. There are secondary metabolites that plants produce for protection from pest & predators, attraction of pollinators & seed dispersal. The essential oils are made up of mixture of compounds that give a characteristic flavor and odor. *C.martinii* belonging to poaceace commonly known as Palmarosa, which one of the important essential oil-bearing plant having rich geraniol content, the oil is extensively used in perfumes, soaps, cosmetics, tobacco and medicine. During the present study, the essential oil of *Cymbopogon martinii* (Roxb.) Wats. was collected from Akkur village, Ramanagara Taluk and District was analyzed using GC-MS to reveal the fingerprint compounds. Gas Chromatography-Mass Spectrometric analysis of the oil provided 59 constituents of compounds dominated by terpenoid compounds like carotol (12.34%), alpha-pinene (3.06%), camphene (9.32%), D-limonene (8.22%), borneol (6.03%), geraniol (2.60%), camphor (7.51%), bornyl acetate (3.65%), linalool (2.56%), alpha-terpeniol (1.18%), geranyl acetate (8.44%) and caryophyllene oxide (5.45%) along with the presence of other components in traces. The study showed the presence of new compounds in high percentages, rather than the standard geraniol. The essential oil compounds are known to possess huge utility in biopesticides, pharmaceutical and food industries. The variation in composition of the essential oil in wild *C. martinii* is due to the ecological conditions for the plant growth.

Keywords : Wild *Cymbopogon martinii*, DNA Bar-coding, Essential oil, GCMS, Terpenoids, Carotol

Cymbopogon martinii (Roxb.) belonging to Ruseae series of genus Cymbopogon possess two varieties such as motia (2n=20) and sofia (2n=40) (Google Wikipedia). It is commonly known as Rosha grass which yield essential oil rich in geraniol and at a commercial scale utilized for manufacture of soaps, perfumery, cosmetics, medicine and aromatic products (Verma *et al.,* 2010). Majority of these *C. martinii* contain essential oil with several biological activities such as insecticidal, anti-protozoan, anticancer, anti-HIV, anti-inflammatory and anti-diabetes effects (Avoseh *et al.,* 2015). India's overall export value of Palmarosa oil has increased by 61.75 since 2018, with the shipments worth 2.969 USD million (Google Wikipedia). Chemically these oils are diverse mixtures of terpenes or phenylpropenes (Sangwan *et al.,* 2000). *C. martinii* is widely distributed in India under diverse adverse climatic conditions covering the planar and the hill stressed regions (Sangwan *et al.,* 2000). This crop native to India is largely cultivated in Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Uttar Pradesh, besides cultivating in Brazil, Madagascar, Indonesia (Verma *et al.,* 2010). *C. martinii* is a highly cross-pollinated crop and survives by with standing environmental stress conditions and hence a lot of variations are observed with yield of essential oils in the species (Smitha *et al.,* 2008). These cultivars of *C. martinii* differ in oil content and quality at the intra and inter-species levels (Neelam *et al.,* 2001). Genotype was identified and assisgned to its classification by utilization of taxonomic literature (Nirmala *et al*., 2017). The variations are attributed to genetic makeup of the genotypes and its interaction with environmental variations (Vinutha and Hegde, 2014).

The plant derived essential oils form the basis of many large chemical, pharmaceutical and perfumery industries and make up a significant proportion of the agro chemical trade worldwide (Wissal *et al.,* 2016).

Chemically the oils are diverse mixtures of terpenes, alkaloids and phenylpropenes (Verma *et al.,* 2010, Meenakshi *et al.,*2018). Impact of environmental factors and geographical conditions like temperature, relative humidity, irradiance, photoperiod, wind, soil properties, harvest time along with genetic variations such as fertilization, cross pollination etc., influence the composition and quality of essential oils (Meenakshi *et al.*, 2018). The essential oils are biosynthesized via Shikimic acid pathway for phenolics and terpenes from Mevalonate and Methyl Erythritol Phosphate (MEP) pathway (Bourgaud *et al.,* 2001). The emergence of the specialized secondary metabolic pathways improved the adaptive ability of plants during their evolution (Waters, 2003). Monoterpenes are synthesized in plastids and sesquiterpenes in cytosol (Vranova *et al.,* 2013). Close observation of the essential oil obtained revealed the presence of different aroma than in the cultivars. Hence, the present study was conducted to examine the essential oil profile and composition with respect to cultivars.

MATERIAL AND METHODS

Ecological Details and Plant Collection

The wild plant of *Cymbopogon martinii* were collected from Akkur village, situated 20 km away from Ramanagara taluk and district, Karnataka, India. It is located within the latitude 12.8266 °N and longitude 77.1951 °E covering an area of 289.37 hectares. The annual temperature ranges between 25.4°C - 30.4°C with an average rain fall of 931.58 mm annually. The plant was found growing in rocky hills (under water stressed condition) with limited soil availability and was authenticated as *Cymbopogon martinii* based on the morphological and essential oil details. 1.4 ml of oil was obtained from 280 grams of the herbage. The essential oil was collected by hydro distillation method using Clevenger apparatus and the oil was yellowish in color with turpentine aroma.

DNA barcoding and Phylogenetic Analysis

The 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). 50 ul of PCR reaction mixture contained 50 ng of gDNA, 100 ng of each forward and reverse primers, 2 ul of 10 mM dNTPs mix, 5 ul 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 ^{0}C for 5 min, followed by 35 cycles at 94 ^{0}C for 1 min, annealing temperature standardized at 60 ^{0}C, extension temperature at 72 ^{0}C for 2 min and final extension was at 72 ^{0}C for 10min. PCR product was run on one per cent 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 two spacers. PCR primer pairs were mapped out from the conserved regions using software primer 3.0 (version 0.4.0).

Sequencing, Validation and Data Analysis

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 90 ^{0}C for 5 minutes, followed by annealing stage at 60 ^{0}C for 1 minute, extension stage at 70 ^{0}C for 2 minutes and final extension at 72 ^{0}C for 10 minutes. The PCR products were purified and sequenced. Sanger sequencing of amplicons were carried out using BDT v3.1 Cycle sequencing kit on Abi 3730xl Genetic Analyzer. Annotation software were used to annotate the sequenced data. Validation of the designed primers and sequenced data 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 sample (100g) was collected from the experimental site and whole plant was used for the oil extraction. Leaves, stem, roots and inflorescence were separated and were washed under tap water followed by distilled water to remove dust particle and dry at ambient temperature for two days in the laboratory to remove the moisture content. The dried plants were cut into small pieces. The dried plant was weighed and used for the 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 sulfate & was stored vials under a refrigerator until analysis.

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

Gas Chromatography and Mass Spectrometry (GC MS) : Gas Chromatography Mass Spectroscopic 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, 30 m length × 0.25 µm film thickness. ID:0.25 mm and Injector of 250 °C. Sample injection:0.1 µl. Temperature programming was done initial of 40 °C for 2 mins 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 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).

Identification of constituents were done on the basis of retention time, Retention Index (RI, determined with reference to homologous series of n-alkanes (C9-C26, Polyscience Corp., Niles IL) under identical experimental condition), coinjection with standards (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data [27-28]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Results and Discussion

Identification of Wild Cymbopogon

The plant was identified as *Cymbopogon martini* (Roxb.) Wats. based on the morphology characterization (Fig. 1), DNA bar-coding and essential oil studies.

DNA Bar-coding

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.

Details of the Primers Developed for the Barcode Amplification in Cymbopogon Species

Out of twelve primer pairs screened for three barcode loci, eight primer pair (two pair from each loci) were validated for the study as they successfully amplified specific bar code region of interest. From the validated two primer pairs for each locus, the primer pair which had the higher annealing temperature (considering its comparatively higher reproducibility) was taken for amplification and sequencing of the concerned barcodes (Table 1).

Fig. 1: Habit and habitat of wild *Cymbopogon martinii* growing in hilly region of Akkur village, Ramanagara

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 species. 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 OK094433

TABLE 1

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

Primer name	Designed sequence (52 to 32)	Annealing temp (°C)	Product size (bp)	Remarks
Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene				
CNRBCLF1 CNRBCLR1	TGTTGGATTTAAAGCTGGTGTT CATTTGCAAGCTGCTTTGAT	53.9	1323	Primer pair was validated
CNRBCLF2 CNRBCLR2	GCAAGTGTTGGATTTAAAGCTG CAGCACTCCATTTGCAAGC	60.0	1336	Primer pair was taken for this study
Maturase K (matK) gene				
CNMTKF1 CNMTKR1	TTTGATAAACCGAGAAATGCTT GCCTTTCCTTGATATCGAACAT	60.0	909	Primer pair was taken for this study
CNMTKF2 CNMTKR2	ATGTATCATCATTTGATAAACCGAGAA TGCCTTTCCTTGATATCGAACAT	58.5	910	Primer pair was validated
Internal transcribed spacer 1, 5.8 S ribosomal RNA gene and internal transcribed spacer 2				
CFITSF1 CFITSR1	CAAAACAGACCGCGAACG GGTGCTCGATGGGTCCTTAG	60.0	555	Primer pair was taken for this study
CFITSF2 CFITSR2	GTAGGTGAACCTGCGGAAG GGTGCTTGATGGGTCCTTAG	59.0	595	Primer pair was validated

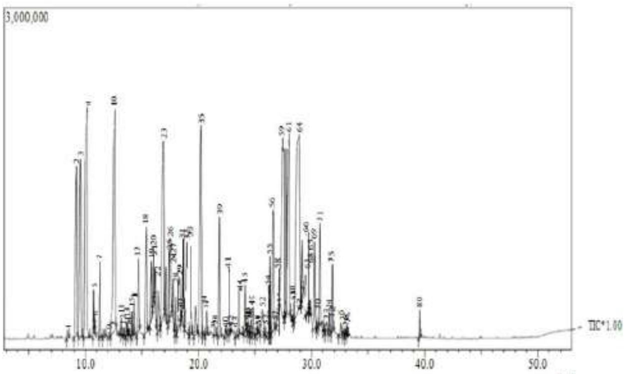

Fig. 2: GC-MS chromatogram of wild *Cymbopogon martini*

Essential Oil Profiling

The GC-MS analysis of the essential oil from wild *C. martinii* showed entirely different spectra of chemical compounds when compare to their cultivar counterpart. The essential oil, showed total of 66 compounds (Table 2, Fig. 2) were analyzed.

TABLE 2

Composition of essential oil and categorization of compounds found in wild *Cymbopogon martinii*

Compounds	Area %	R. Time	Mol. Weight	Mol. formula
Monoterpenoid hydrocarbons				
Tricyclene	3.17	9.117	136	$C_{10}H_{16}$
Alpha-pinene	3.06	9.5	136	$C_{10}H_{16}$
Camphene	***9.32***	***10.032***	***136***	$\mathbf{C_{10}H_{16}}$
Sebinene	0.27	10.704	136	$C_{10}H_{16}$
Myrcene	0.66	11.263	136	$C_{10}H_{16}$
M-Cymene	0.22	12.31	134	$C_{10}H_{14}$
D-Limonene	**8.22**	**12.549**	**136**	$\mathbf{C_{10}H_{16}}$
Beta-Ocimene	0.34	12.732	136	$C_{10}H_{16}$
2-methyl cyclooctanone	0.17	13.15	140	$C_9H_{16}O$
Bicyclo (2.2.1) heptane-2-one, 3, 3-dimethyl	0.43	14.099	138	$C_9H_{14}O$
Trans-P-mentha-2, 8-dienol	0.5	15.293	152	$C_{10}H_{16}O$
Alpha-pineneoxide	0.17	15.593	152	$C_{10}H_{16}O$
Citronellal	0.51	18.462	156	$C_{10}H_{18}O$
Borneol	**6.03**	**16.765**	**154**	$\mathbf{C_{10}H_{18}O}$
Alpha- curcumene	0.17	25.884	202	$C_{15}H_{22}$
Oxygenated monoterpenes				
Geranoil	2.60	19.245	152	$C_{10}H_{18}O$
Cis-carveol	0.73	19.633	152	$C_{10}H_{16}O$
Alpha-terpineol	1.18	17.023	154	$C_{10}H_{18}O$
Camphor	**7.51**	**15.997**	**152**	$\mathbf{C_{10}H_{16}O}$
Bornyl acetate	3.65	20.146	196	$C_{12}H_{20}O_2$
Limonene oxide	0.4	13.805	170	$C_{10}H_{16}O$
Menthe-1, 8-dien-7-ol				$C_{10}H_{16}O$
Linalool	2.56	14.682	154	$C_{10}H_{18}O$
4-methen-8-ol	0.86	14.287	154	$C_{10}H_9NO$
P-cymene-8-ol	0.22	17.223	150	$C_{10}H_{14}O$
Carvotanacetone	2.21	18.914	150	$C_{10}H_{16}O$
Geranyl acetate	**8.44**	**22.751**	**196**	$\mathbf{C_{12}H_{12}O_2}$
Nerol-E	2.6	19.245	154	$C_{10}H_{18}O$
Perillyl acetate	0.39	20.723	194	$C_{12}H_{18}O_2$
Alpha-citral	0.73	19.633	194	$C_{12}H_{16}O$
Sesquiterpene hydrocarbons				
Alpha-elemene	0.16	24.405	204	$C_{15}H_{24}$
Caryophyllene	0.49	23.756	204	$C_{15}H_{24}$
Beta-sesquip hellandrene	0.43	29.117	204	$C_{15}H_{24}$
Globulol	0.87	29.545	222	$C_{15}H_{26}O$
Calarene	0.3	24.088	204	$C_{15}H_{24}O$
Carotol	**12.34**	**28.679**	**222**	$\mathbf{C_{15}H_{26}O}$
Nerolidol-E	3.48	27.033	222	$C_{15}H_{26}O$
Selina-6-en-4-ol	3.51	26.145	222	$C_{15}H_{26}O$
Alpha-Cadinol	0.7	29.418	222	$C_{15}H_{26}O$
3-oxo-beta ionone	1.56	30.179	206	$C_{13}H_{18}O$
Alpha-cadrene epoxide	0.13	31.222	220	$C_{15}H_{24}O$
Oxygenated sesquiterpene				
Caryophyllene oxide	**5.45**	**27.857**	**220**	$\mathbf{C_{15}H_{24}O}$
Verbenone				$C_{10}H_{14}O$
Sesquiterpenoid alcohol				
Humulane-1, 6-dien-3-ol	0.24	25.31	222	$C_{15}H_{26}O$
Guaiol	0.12	28.992	222	$C_{15}H_{26}O$
Aromatic hydrocarbons				
M-cymene	0.22	12.31	134	$C_{10}H_{14}$
Terpene ketone				
Farnesylacetone	0.2	32.529	262	$C_{18}H_{30}O$
tricyclo (6.2.1.0)1,6 undec-4en-3-one 2,2,7,7-tetramethyl	0.3	31.739	218	$C_{14}H_{22}O_3$
Acetic acid 1- (2-(2,2,6trimethyl-bicyclo(4,1,0) hept-1-yl-vinyl ester	0.22	31.51	250	$C_{15}H_{22}O$
(1S,2E,4S,5R,7E,11E) -cembra-2,7, 11-trien-4,5-diol	1.8	30.648	306	$C_{20}H340_2$
(6,8-bis-hydroxy methyl-4-isopropy l-7-methylene-bicyclo	0.38	29.771	254	$C_{15}H26O_3$

CAROTOL showed highest percentage with (12.34 %) which is key compound followed by Camphene (9.34 %), Geranyl acetate (8.44 %) Limonene (8.22 %), Camphor, Borneol, Caryophyllene oxide, Nerolidol, Seline-6-en-4-ol, Tricyclene and Alpha-pinene.

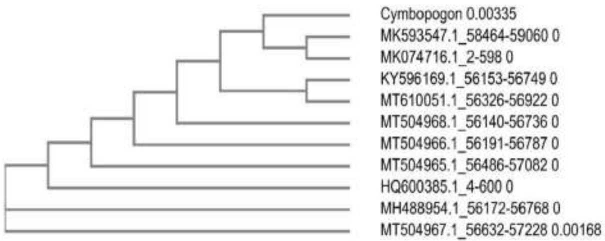

Fig. 3: Phylogenetic tree constructed based on rbcL gene nucleotide sequences of *Cymbopogon species*

CAROTOL is a major source of sesquiterpene alcohol. It is used as a fragrance component in cosmetics & perfumes and a flavor ingredient in different categories of food products (Elwira *et al.,* 2016). . The biological activity of the essential oil compounds of *C. martinii* as reported by earlier studies has been listed (Table 3).

Biosynthesis of Terpenoid Compounds

In present work, biosynthesis of terpenoid compounds via the essential oil pathway was determined based on structural details of essential oil compounds. The monoterpenoid pathway consisted of a C10 carbon backbone including two isoprene units and divided into acyclic, monocyclic and bicyclic groups (Fig 4**)**. The

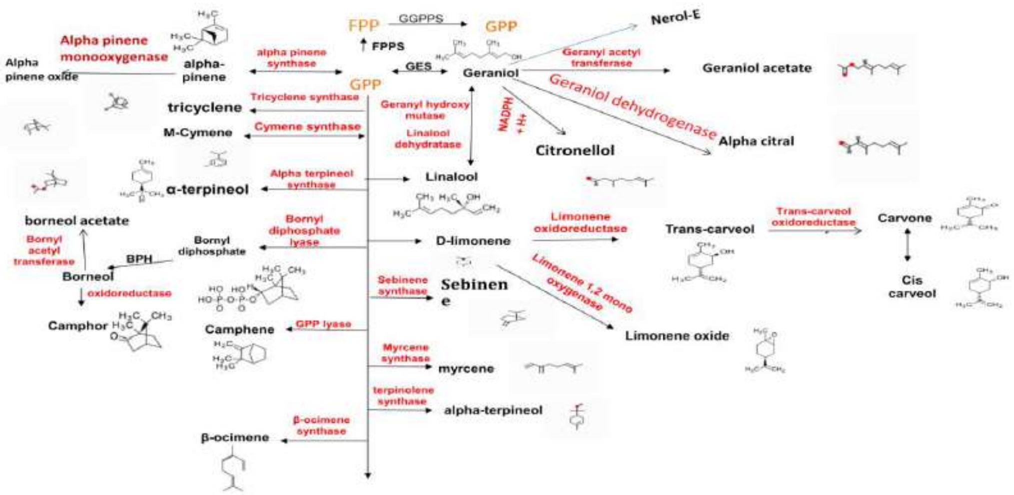

Fig. 4: Overview of monoterpenoid biosynthesis pathway in wild *Cymbopogon martini*

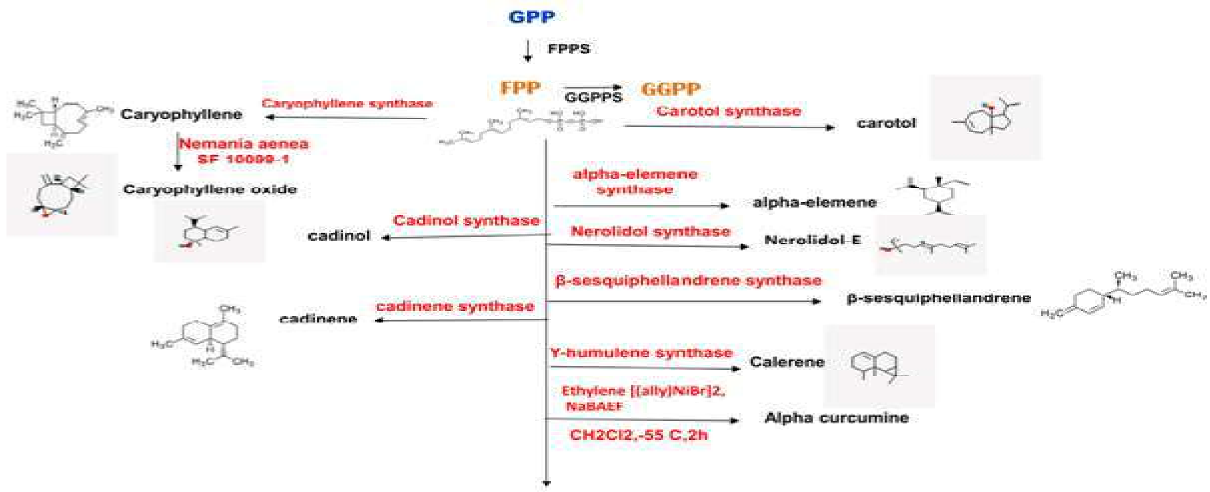

Fig. 5: Overview of sesquiterpenoid biosynthesis pathway in wild *Cymbopogon martini*

TABLE 3

Biological activity of essential oil compounds from wild *Cymbopogon martinii*

Compound	Bioactivity	Reference
Sebinene	anti-diabetic and anti-hyperlipidemic effects	Subramani *et al.* (2015)
Myrcene	Anti-angiogenic, antioxidant,antiinflammatory and anticatabolic effects	Gay *et al,* (2008)
M-Cymene	Antioxidant, anti-inflammatory, antinociceptive, anxiolytic,	Anna Marchese *et al.* (2017) anticancer and antimicrobial effects
D-Limonene	antibacterial activity against food-borne pathogensbut it has an ambiguous antimicrobial susceptibility and mechanism against Listeria monocytogenes	Yingjie Han *et al.* (2020)
Beta-Ocimene	anticonvulsant activity, antifungal activity, antitumor activity and pest resistance	Ethan and Jahan (2017)
Linalool oxide	Produce fragrances and aromatic chemicals with various applications in modern society. It also shows antimicrobial, anti-inflammatory, anticancer, anti-oxidant properties.	Guy *et al.* (2008)
Bicyclo(2.2.1) heptane-2-one, 3,3-dimethyl	prevention and treatment of chronic diseases such as cancer, cardiovascular disease, diarrhoea diseases and infections. Play important roles as a source of antimicrobial and antioxidant	Olubunmi and Anthony *et al.* (2017)
Linalool	Antimicrobial, anti-inflammatory, anticancer, anti-oxidant properties and several in vivo studies have confirmed various effects of linalool on the central nervous system.	Guy *et al.* (2008)
Alpha-pineneoxide	production of various fragrances, cosmetics, and pharmaceuticals and also exibits anti inflammatory and Anti oxidant properties	Eelco *et al.* (2015)
Camphor	Camphor exhibits a number of biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, anti-nociceptive, anticancer and antitussive activities, in addition to its use as a skin penetration enhancer.	Weiyang *et al.* (2013)
Citronellal	Antimicrobial properties	Ravi *et al.* (2015)
Borneol	Anti-inflammatory and antimicrobial Agents, antioedematogenic activity	Maria *et al.* (2020)
Alpha-terpineol	antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, anti-nociceptive compound	Christina *et al.* (2018)
Verbenone	antifungal and herbicidal activities	Qiong Hu *et al.* (2017)
Cis-carveol	use of (-)-cis-carveol may be suitable for decreasing AD-related symptoms. Alzheimer's disease (AD) could be considered a multifactorial neurodegenerative disorder characterized by the accumulation of the ß-amyloid-peptide (Aß) within the brain leading to cognitive deficits, oxidative stress and neuroinflammation	Lucian and Razvan (2020)
Citronellal	Anti-fungal properties	Lee seong and Iran (2007)
E-nerol	antibacterial activity,antioxidant properties	Chung-Yi Wang *et al.* (2019)
Alpha-citral (or) Geraniol	presents many pharmacological properties including antifungal and antibacterial activity	Maria *et al.* (2020)

Compound	Bioactivity	Reference
Borneol acetate	Anti-Inflammatory Functions	HeYang, *et al.* (2014)
Thioflavin	It is cytotoxic on several tumor cell line	Yamini *et al.* (2017)
Geranyl acetate	Anti-Inflammatory, Anxiolytic, Antimicrobial, Diuretic, Antiseptic, Anti-cancerous	Chahal (2017)
Alpha-elemene	antioxidant activity	Natasha *et al.* (2019)
Humulane-1, 6-dien-3-ol	antioxidant activity effect of essential oils and supercritical carbon dioxide extracts from Cinnamomum spp. barks and fruits against food bacterial pathogens in liquid and vapor phase	Zhao *et al.* (2014)
Cyclocopa camphenol	Antimicrobial effect	Katerina *et al.* (2021)
Alpha-curcumene	activity against bacteria, yeasts and an alga was inspected by the applying the microdilution method	Silva *et.al.* (2015)
Selina-6-en-4-ol	anti-proliferative, antibacterial and antioxidant activity	Sakina *et al.* (2016)
E-Nerolidol	various pharmacological and biological activities of nerolidol have been reported such as anti-microbial, anti-biofilm, anti-oxidant, anti-parasitic, skin-penetration enhancer, skin-repellent, anti-nociceptive, anti-inflammatory and anti-cancer	Weng-Keong *et al.* (2016)
Carotol	Antifungal. Phytopathogenic fungi are the major problems causing harmful damage to the rice crop. Only available control of these diseases is synthetic fungicides but their repeated use led to serious environmental issues, residual toxicity, and development of resistanc	Mukta Sharma *et al.* (2019)
Guaiol	Antiviral, anti-inflammatory, anticancer and antibacterial activities	Tao Liua *et.al.* (2013)
Sesquip-hellandrene	exhibits anticancer activity.	Amit Kumar *et al.* (2015)
Alpha-cadinol	a-Cadinol was said to act as anti-fungal and as hepatoprotective and was proposed as a possible remedy for drug-resistant tuberculosis.	Chen-Lung *et al.* (2011)
(-)-Globulol	Antimicrobia activity	Manliang *et al.* (2008)
(6,8-bis-hydroxy hydroxymethyl-4-isopropyl-7-methylene-bicyclo	anti-efflux activity	Marjan *et al.* (2020)
(1S,2E,4S,5R, 7E,11E)-cembra-2,7,11-trien-4, 5-diol	Used to treat fevers, malaria, and sore throat.	Prabodh *et al.* (2018)
2,2,7,7-trtra methyl tricyclo 6.2.1.0(1,6) undec-4-en-3-one	Antioxidant and antimicrobial agents.	Olubunmi and Antony *et al.* (2018)
Farnesy lacetone	antioxidant activities	Yavuz *et al.* (2021)

sesquiterpenoids pathway consisted of three isoprene units with the molecular formula $C_{15}H_{24}$ and may be acyclic or contain rings, including many unique combinations (Fig 5).

Monoterpenes are derived biosynthetically from units of isopentenyl pyrophosphate, which is formed from acetyl-CoA via the intermediacy of Mevalonic acid in the HMG-CoA reductase pathway. An alternative, unrelated biosynthesis pathway of IPP is known in the plastids. MEP-(2-methyl-Derythritol-4-phosphate) pathway get initiated from C_5 sugars. Geranyl pyrophosphate serve as the precursor for monoterpenes. Elimination of the pyrophosphate group from geranyl pyrophosphate leads to the formation of acyclic monoterpenes such as ocimene and myrcene. Hydrolysis of phosphate groups leads to the prototypical acyclic monoterpenoid geraniol. Additional rearrangements and oxidations provide compounds such as citral, citronellal, citronellol and linalool and many others. Geranyl diprosphate(GPP) is the monoterpene and is synthesized by condensation of IPP and its isomer dimethylallyl diphosphate (DMAPP) by geranyl diphosphate synthase (GPPS). GPP under goes further isomerazation, acetylation, deacetylation, cyclization and dehydrogenation to form varies monoterpene and terpenoid compounds in the presense of Terpene synthase (GES) and terpene cyclase (TEC) enzymes. GPP initiates the formation of Farnesyl diphosphate (FPP) a precursor molecule leading to sesquiterpenoid compounds by addition of IPP in the presense of enzyme Farneyl diphosphate synthase (FPPS). A diterpine precursor molecule Geranylgeranyl diphosphate (GGPP) is obtained by the addition of IPP for FPP with the enzyme Geranylgeranyl diphosphate synthase (GGPPS). The reaction of geranyl pyrophosphate with isopentenyl pyrophosphate results in the 15-carbon fernesyl pyrophosphate (FPP), which is an intermediate in the biosynthesis of sesquiterpenes. GPP initiates the formation of Farnesyl diphosphate (FPP) a precursor molecule leading to sesquiterpenoid compounds by addition of IPP in the presense of enzyme Farneyl diphosphate synthase (FPPS). A diterpine precursor molecule Geranylgeranyl diphosphate (GGPP) is obtained by the addition of IPP for FPP with the enzyme Geranylgeranyl diphosphate synthase (GGPPS).

Ethno-medical Properties of the Essential Oil Compounds

C. martinii shows medicinal properties and used for the treatment of joint pain, respiratory diseases, anorexia, intestinal worms, skin diseases, diarrhea, fever, disorders of spleen, liver disorders, bleeding disorders, cough, epilepsy, heart disease. *C. martinii* was used in Ayurveda since ancient times. Charak gave the decoration of whole plant in the treatment of abdominal disorders, liver disorders of spleen (Avicenna, 2019).

Pale greenish-yellow scented palmarosa oil obtained from the plant used in different formulations and applied topically for alopecia, lumbago, skin diseases, dryness, wrinkles, dermatitis & stiff joints (Lodhia *et al.,* 2009). It is also used in Aromatherapy to alleviate stress, tendion, anxiety and calm the emotions and it stimulates cell rejuvenation (Kandhasamy and Kim, 2016). The oil is given internally for bilious complains (Lodhia *et al.,* 2009). The oil is in high demand in perfumery, soap, cosmetics and blending tobacco products industries (Asja and Iris, 2016). Oil is applied topically to minimise appearance of stretch marks and scars (Ane Orchard and Sandy, 2017). The leaf in fusion is applied to treat joint pain (Sonali *et al.,* 2014). The plant shows antifungal, antiseptic, antiviral activities. It has bactericidal, Cicatrizant, Cytrophylactic, Febrifuge, Hydrating, Nervine properties (Sangeeta and Soni, 2014). Palmorosa essential oil is non-toxic, completely safe for topical use and shows fertility reducing & abortifacient activities on oral use (Lodhia *et al.,* 2009).

Essential oils are widely used in aromatherapy procedures (Camila and Jiri, 2018). The essential oil due to their therapeutic properties are also used in food product, in dermatology, and in the fragrance and cosmetic industries. When compared to the cultivar varieties of CSIR-CIMAP (Palace *et al.,* 2020), the wild *C. martinii* studied showed reduced plant height (1.5 m) with reduced leaf width and erect inflorescence as modification for withstanding drought stress. Environmental stress leading for reduced leaf area and enhanced glandular trichome formation leading to enhanced essential oil production and deporsition in the glands has been reported (Delfine *et al.,* 2005).

The essential oil of wild *C. martinii* showed 66 compounds dominated by oxyginated sesquiterpenes and monoterpenes. The production of higher percentages of essential oil compounds in the wide genotype when compared to cultivar variety is influenced by the ontogeny, developmental stage and environmental condition (Padalia *et al.,* 2011, Smitha *et al.,* 2008). Drought stress reduces transpiration rate and CO_2 intake and increases the supply of $NADPH+H^+$ thus enhancing the secondary metabolic pathways for essential oil production (Khalid, 2006). The environmental stress has led to upregulation of the genes involved in essential oil biosynthesis (Mahajan *et al.,* 2020; Palace *et al.,* 2020).

During the present study, a new carotol-rich chemotype of Palmarosa is identified. The newly identified chemotype is widly distributed in the Aravali range of Akkur, which can be commercially utilized for its essential oil, composition which can be explored further for different bioactivities.

Acknowledgement : Authors are thankful to the Department of Microbiology and Biotechnology, Bangalore University and IADFAC Laboratory and Barcode Biosciences, Bangalore for providing support to complete the work.

REFERENCES

AMIT KUMAR TYAGI, SAHDEO PRASAD, WEI YUAN, SHIYOU LI AND BHARAT B. AGGARWAL, 2015, Identification of a novel compound (-sesquiphellandrene) from turmeric (Cur uma longa) with anticancer potential : Comparison with curcumm. *Investigational new druds journal,* Vol. **33** : 1175-86, DOI:10.1007/sl 0637-015-0296-5.

ANE ORCHARD AND SANDY VAN VUUREN, 2017, Commercial essential oils as potential antimicrobials to treat skin diseases, *Evid Based Complemen Altemat Med. journal,* Doi: 10.1155/2017/4517971

ANNA MARCHESE, CARLA RENATA ARCIOLA, RAMONA BARBIERI, ANA SANCHES SILVA, SEYED FAZEL NABAVI, AROLD OREL TSETEGHO SOKENG, MORTEZA IZADI, LPEK SUNTAR, MARIA DAGLIA AND SEYED MOHAMMAD NABAVI, 2017, Update on monoterpenes as antimicrobial agents : A particular focus on p-Cymene. *Materials (Basel) journal,* Doi:10.3390/mal 0080947.

ASHOK, A. H., MARQUES, T. R., JAUHAR, S., NOUR, M. M., GOODWIN, G. M., YOUNG, A. AND HOWES, O. D., 2017, The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment, *Mol Psychiatry Journal,* Vol. **22** (5) : 666-679, Doi: 10.1038/ mp.2017.16

ASJA SARKIC AND IRIS STAPPEN, 2016, Essential oils and their single compounds in cosmetics - A critical review. *Cosmetics journal,* Vol. **5** : Doi:10.3390/ cosmetics5010011.

AVICENNA J. PHYTOMED, 2019, Treatment of liver and spleen illnesses by herbs : Recommendations of Avicenna's heritage 'Canon of Medicine'. *Avicenna Journal of Phytomed.*

AVOSEH, O., OYEDEJI, O., RUNGQU, P., NKEH-CHUNGAG, B. AND OYEDEJI, A., CYMBOPOGON SPECIES, 2015, *Ethnopharmacology, phytochemistry and the pharmacological importance, molecules journal,* Vol. **23** : 38-53, Doi: 10.3390/molecules20057438.

BOURGAUD, F., ORAVOT, A., MILESI, S. AND GONTIER, E., 2001, Production of plant secondary metabolites: a historical perspective. *Elsevier plant science journal,* Vol. **161**, Doi:10.1016 /SO168-9452(01)00490–3

CAMILA PEREIRA BRAGA AND JIRI ADAMEC, 2018, Encyclopedia of bioinformatics and computational biology. *Elsevier journal,* Vol. 1-3.

CHAHAL, K. K., SINGH, RAVINDER, KUMAR, AMIT, BHARDWAJ AND URVASHI, 2017, Chemical composition andbiological activity of *Coriandrum sativum. Indian Journal of Natural Products and Resources* (IJNPR) [Formerly Natural Product Radiance (NPR)]. Vol 8.

CHEN-LUNG HO, PEI-CHUN LIAO, EUGENE I-CHEN WANG AND YU-CHANG SU NAT PROD COMMUN, 2011, Composition and antimicrobial activity of the leaf and twig oils of Litsea acutivena from Taiwan. *Nat Prod Commun journal,* **6** (11) : 1755 - 8.

Christina Khaleel, Nurhayat Tabanca and Gerhard Buchbauer, 2018, A-terpineol, a natural monoterpene, *The journal of Open Chemistry,* Doi:10.1515/chem-2018-0040

Chung-yi Wang, Yu-wei Chen and Chih-yao Hou, 2019, Antioxidant and antibacterial activity of seven predominant terpenoids. *International Journal of Food Properties,* .Vol. **22**, Doi:10.1080/10942912.2019.15825

Delfine. S., Roberto Tognetti, Ersilio Desiderio and Arturo Alvino, 2005, Effect of foliar application of N and humic acids on growth and yield of durum wheat, agronomy for sustainable development journal, Doi:10.1051/agro:2005017

Eelco T. C. Vogt, Gareth T. Whiting, Abhishekdutta Chowdhury and Bert M. Weckhuysen, 2015, Chapter two - zeolites and zeotypes for oil and gas conversion. *Advances in Catalysis journal,* Vol. **58** : 143-314, Doi:10.1016/bs.acat.2015.10.001

Elwira Sieniawska, Lukasz Swiatek, Barbara Rajtar, Ewelinakoziol, Malgorzata Polz- Dacewicz and Krystyna Skalicka, 2016, Wozniak carrot seed essential oil - Source of carotol and cytotoxicity study. *Industrial Crops and Products journal,* Vol. **92 :** 109-115, Doi:10.1016/j .indcrop.2016.08.001

Ethan B. Russo and Jahan Marcu, 2017, Cannabis pharmacology : In advances ology, *Elsevier Journal*, Vol. **80** : 67-134, DOI:10.1016/bs.apha, 2017.03.004

Guy, P., Kamatou, P. and Alvaro M. Viljoen, 2008, Linalool : A review of a biologically active compound of commercial importance research article . *SAGE Journals,* Doi:10.1177/1934578X0800300727

Heyang, Rugang Zhao, Hao Chen, Pu Jia, Li Bao and Hai Tang, 2014, Bornyl acetate has an anti-inflammatory effect in human chondrocytes via induction of IL-11. *IUBMB Life journal,* Doi: 10.1002/iub.1338

Khalid, A., Muhammad Gulbaz Arshad, Baby Shaharoona and Tariq Mahmood, 2006, Plant growth promoting rhizobacteria and sustainable agriculture. *Journal of Microbial Strategies for Crop Improvement,* DOI:10.1007/978-3642019791_7

Kandhasamy Sowndhararaj Anandsongmun and Kim Helmut Viernstein, 2016, Influence of fragrances on human psychophysiological activity : with special reference to human electroencephalographic response, *Scientia Pharmaceutica,* Vol. **84 :** 724 - 752, Doi: 10.3390/scipharm84040724

Katerina Vihanova, Marketa Houdkova, Trinop Promgool, Klara Urbanova, Somdej, Kim, D. S., Lee H. J., Jeon, Y. D., Han, Y. H., Kee, J. Y., Kim, H. J., Shin, H. J., Kang, J., Lee, B. S., Kim, S. H., Kim, S. J., Park, S. H., Choi, B. M., Park, S. J., Um, J. Y. and Hong, S. H. , 2021, Alpha - pinene exhibits anti-inflammatory activity through the suppression of MAPKs and the NF-KB Pathway in mouse peritoneal macrophages. *American Journal of Chinese Medicine*, Vol. **43** : 731 - 742. Doi:10.1142/S0192415X1550045

Lee Seong Wei and Iran J. Microbiol, 2007, Chemical composition and antimicrobial activity of Cymbopogon nardus citronella essential oil against systemic bacteria of aquatic animals. *Iranian journal of microbiology.*

Lodhia, M. H., Bhatt, K. R. and Thaker, V. S., 2009, Antibacterial activity of essential oils from palmarosa, evening primrose, lavender and tuberose. *Indian J. Sci. Journal,*Vol.**71**(2) :134-6. DOI: 10.4103/0250-474X.54278

Lucian Hritcu and Razvan Stefan Boiangiu,2020, (-)- cis – Carveol, a natural compound, improves - Amyloid-Peptide 1-42-Induced memory impairment and oxidative stress in the rat hippocampus. *BioMed Research International journal,* Vol. 2020, Doi:10.1155/2020/8082560

Mahajan, U. V., Vijay R. Varma, Michael E. Griswold, Chad T. Blackshear, Yano An, Anup M. Oommen, Sudhir Varma, Juan C. Troncoso, Olga Pletnikova, Richard O'brien, Timothy J. Hohman, Cristina Legido-quigley, Madhav Thambisetty, 2020, Dysregulation of multiple metabolic networks related to brain transmethylation and polyamine pathways in Alzheimer disease:A targeted metabolomic and transcriptomic study. *Observational study journal,* Doi :10.1371/ journal.pmed.1003439001

MARIA HELENA PEREIRA DE LIRA, FRANCISCO PATRICIO DE ANDRADE JUNIOR, GUSTAVO FERNANDES QUEIROGAMORAES, GIRLENE DA SILVA MACENA, FILLIPE DE OLNEIRA PEREIRA & IGARA 0LNEIRA LIMA ORCID ICON, 2020, Antimicrobial activity of geraniol : An integrative review. *Journal of Essential Oil Research,* Vol. **32**, Doi:10.1080/ 10412905.2020.1745697

MEENAKSHI, POKHRIYAL, PROMILA, KUMARI, SITHARAMAN, UMARAJAMANI AND NAGARAJAN, 2018, Evaluation of solid solution formation between $Th0_2$ and 8- Bii03 by molecular precursor route. Materials Research Bulletin journal, Volume 107, Doi:10.1016/j.materresbull. 2018.07.001

MUKTA SHARMA, KHUSHMINDER KAUR CHAHAL, RAMANDEEP KAUR, RAVINDER SINGH, DALVIR KATARIA, 2019, Antifungal potential and structure activity relationship of carrot seed constituent. *J. Food Biochem journal,* Vol. **43**(9), Doi:10.1111/jfbc.12971

NATASHA TIEMI FABRI AND JOSIANE DE FATIMA GASPARI DIAS, 2019, oxidant properties and biological activities of the essential oil extracted from Ocotea diospyrifolia (Meisn) Mez. *Braz J. Pharm Sci. journal,* Doi: I 0.1590/s2175-97902019000218471

NEELAM S. SANGWAN, AHA FAROOQI, SHABIH FATIMA AND RAJENDER SINGH SANGWAN, 2001, Regulation of essential oil production in aromatic plants. *Journal of plant growth regulation,* Vol. **34** : 3-21.

NIRMALA, K. S., CHAMPA, B. V. AND MAHABALESHWAR HEGDE, 2017, Morphological diversity of jasmin cultivars and wild species in Karnataka. *Mysore J. Agric. Sci.,* **51**(4) : 822 - 821.

PADALIA, R. C., RAM SWAROOP VERMA, SAH, A. N. AND NEHA KARKI, 2011, Leaf and rhizome oil composition of Zingiber officinale Roscoe and their antibacterial and antioxidant activities. *Asian Journal of Traditional Medicine.*

RAVI KANT AGRAWAL AND BHOJ R SINGH, 2015, Antimicrobial activity of citronella essential oil on antimicrobial drug resistant bacteria from veterinary clinical cases. *Clinical and biochemistry journal,* Doi:10.4172/2471-2663.1000106

SAKINA YAGI, RANDA BABIKER, TZVETOMIRA TZANOVA AND HERVE SCHOHN, 2016, chemical, antiproliferative, antioxidant and antibacterial activities of essentialoils from aromatic plants growing in Sudan. *Asian Pac J. Trop Med. journal,* Vol. **9**(8) : 763-770. Doi: 10.1016/ j.apjtm.2016.06.009

TAO LIUA, CHUN-JUAN WANG, HUI-QIN XIEB AND QING MUA, 2013, Guaiol a naturally occurring insecticidal sesquiterpene. *Nat. prod. commul. journal,* Vol. (10): 1353-4.

PALACE, S. G., YI WANG, DANIEL HF RUBIN, MICHAEL A. WELSH, TATUM D. MORTIMER, KEVIN COLE, DAVID W. EYRE, SUZANNE WALKER AND YONATAN H. GRAD, 2020, RNA polymerase mutations cause cephalosporin resistance in clinical Neisseria gonorrhoeae isolate, *Elife journal,* Doi: 10.7554/eLife.51407

SANGEETA SONI AND SONI, U. N., 2014, *In-vitro* anti-bacterial and anti-fungal activity oils, *International Journal of Pharmacy and Pharmaceutical Sciences.* Vol. 6.

SANGWAN, N. S., FAROOQI, A. H. A., SHABIH, F. AND SANGWAN R. S., 2000, Regulation of essential oil production in plants. *Journal of Plant Growth Regulation,* Vol. **34**, Doi:10.1023/A:1013386921596

SILVA, POZZATTI, G. A., RIGATTI, P., HORNER, F., ALVES, R., MALLMANN, S. H. AND EINZMANN, C. A., 2015, Antimicrobial evaluation of sesquiterpene a-cu:c9 ne and its synergism with imipenem. *Journal of Microbiology, Biotechnology and Food Sciences,* Vol. **04**(05) : 434-436, DOI: 10. f5414/jmbfs.2015.4.5.434-436

SMITHA, B. R., SANJEEVAN, V. N., VIMALKUMAR, K. G. AND REVICHANDRAN, C., 2008, On the upwelling off the southern tip and along the West Coast of India. *Journal of coastal research,* Doi: 10.2112/06-0779.1

SUBRAMANI PARASURAMAN, SUBRAMANI BALAMURUGAN, PARAYIL VARGHESE CHRISTAPHER, RAJENDRAN RAMESH

PETCHI, JEYABALAN SUJITHRA AND CHOCKALINGAM VIJAYA, 2015, Evaluation of antidiabetic and Aantihyperlipidemic effects of hydroalcoholic extract of leaves of ocimum tenuiflorum (Lamiaceae) and prediction of biological activity of its phytoconstituents. *Pharmacognosy Research journal.* Vol. **7**(2) : 156-165. Doi: 10.4103/0974-8490.151457

VERMA, A., BHATTACHARYA, R., REMADEVI, I., LI, K., PRAMANIK, K., SAMANT, G. V., HORSWILL, M., CHUN, C. Z., ZHAO, B., WANG, E., MIAO, R. Q., MUKHOPADHYAY, D., RAMCHANDRAN, R. AND WILKINSON, G. A., 2010, Endothelial cell-specific chemotaxis receptor (ecscr) promotes angioblast, migration during vasculogenesis and enhances VEGF receptor sensitivity. *Blood journal,* DOI: 10.1182/blood-2009-10-248856

VINUTHA, N. S. AND HEGDE, N. K., 2014, Evaluation of citronellaa (*Cymbopogon winterianus* Jowitt) genotypes for growth parameters under Northern dry zone of Karnataka. *Mysore J. Agric. Sci.* **48**(4) : 553 - 559.

VRANOVA EVA , DIANO COMAN AND WILHELM GRUISSEM, 2013, Network analysis of the MVA and MEP pathways for isoprenoid synthesis, annurev-arplant journal, Vol. **64** : 665-700, Doi: 10.1146/050312-120116

WATERS DONALD, 2003, Logistics: An introduction to supply chain management. *Journal of global health.*

WEIYANG CHEN, ILZE VERMAAK AND ALVARO VILJOEN, 2013, Camphor - a fumigant during the black death and a coveted fragrant wood in ancient egypt and babylon-review, *Molecules journal,* Vol. **10**, DOI: 10.3390/molecules18055434

WENG-KEONG CHAN, LOH TENG-HERN TAN, KOK-GAN CHAN, LEARN-HAN LEE, BEY-HING AND NEROLIDOL, 2016, A sesquiterpene alcohol with multi-faceted pharmacologigal and biological activities. *Molecules journal*, Vol. **28**, Doi: 10.3390/molecules21050529

WISSAL DHIFI, SANA BELLILI, SABRINE JAZI, NADA BAHLOUL AND WISSEM MNIF, 2016, Essential oils' chemical characterization and investigation of some biological activities; A critical review. *Medicines (Basel) journal,* Vol. **3**, Doi: 10.3390/medicines3040025

YAMINI MUTREJA AND TRUMAN C. GAMBLIN, 2017, Optimization of *in vitro* conditions to study the arachidonic acid induction of 4R isoforms of the microtubule-associated protein tau. *Methods Cell Biology journal,* Vol.-I(41) : 65-88, Doi: 10.1016/bs.mcb.2017.06.007

YINGJIE HAN, ZHICHANG SUN AND WENXUE CHEN, 2020, Antibacterial mechanism of limonene against : Listeria monocytogenes. *Molecules journal,* Vol. **25**, Doi:10.3390/molecules25010033.

ZHAO CHEN-XING, ZHANG MR, HE JING, DING YA-FANG AND BAO-CAI, L. R., 2014, Chemical composition and antioxidant activity of the essential oil from the flowers austro-yunnanensis. *Journal of Chemical and Pharmaceutical Research*, **6**(7) : 1583 - 1587.

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