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Research Article

Profile of Bioactive Phyto-compounds in Essential Oil of *Cymbopogon martinii* from Palani Hills, Western Ghats, INDIA

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Abstract

Worldwide interest in use of plants based natural products (PBNPs) has been growing, and its beneficial effects being rediscovered for the development of novel drugs. Literature survey on indigenous traditional knowledge bestows ethnopharmacological potentials of PBNPs that has inspired current research in drug design and discovery; PBNPs provide baseline for the development of novel drug leads against various pharmacological targets. Studies indicate that *Cymbopogon martini* Essential Oil (CMEO) exhibit wide range of biological activities such as hepatoprotective, antifungal, insecticide, antioxidant and antibacterial. Pharmacological properties in Palmarosa Essential Oil (PEO) may be due to the presence of compounds like 4-Decen-6-yne, (Z), 2-Ethylimino-4-methyl-pent-3-enenitrile, Dihydrocarvyl acetate, 2-Methylbenzaldehyde, Geranyl butyrate, 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene. However, its application is limited because of the odor, color and taste. In the present study, GCMS based profile of bioactive phyto-compounds in essential oil of *Cymbopogon martinii* along with its physiochemical, biological, molecular, pharmacological and drugable properties has been envisaged.

Keywords: *Cymbopogon martinii* Essential Oil (CMEO); Pharmacological Activity; ADMET Properties; Bioactive Compounds; Plant Based Natural Products (PBNPs);

INTRODUCTION

Genus *Cymbopogon* is widely distributed in the tropical and subtropical regions of Africa, Asia and America. The genus *Cymbopogon* comprises of more than 144 species, and is well known for its high content of essential oils[1,2]. Studies have led to the isolation of alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids and tannins from every part of *Cymbopogon* species. *Cymbopogon martinii* (lemongrasses) is native to India and Indochina, but widely cultivated in many places for its aromatic essential oil. Known as Palmarosa, the plant has other names: Indian geranium, ginger grass, rosha, and rosha grass. Besides, therapeutic application, it is commonly used as a condiment and food preservative. PEO contains bioactive molecules, phyto-compounds, endowed with pharmacological activities[3]. PEO contains geraniol, used as scent and in a number of traditional medicinal. PEO is of commercial importance, being extensively used in perfumes, soaps, cosmetics, toiletry and tobacco products[4]. PEO has effective insect repellent property when applied to stored grain and beans[5], antihelmintic against nematodes[5], antifungal[6,7] and mosquito repellent[8] activity. CMEO is used in aromatherapy due to its antimicrobial properties. It is used in Ayurvedic medicine to treat skin problems and relieve nerve pain. Immunomodulatory action of CMEO was evaluated towards production of pro- and anti-inflammatory cytokines (TNF-α and IL-10) by human monocytes *in vitro*[9].

Essential Oils (EOs) a major group of Phytogenic Bio-Active Compounds (PBAC) have been used for variety of purposes. Due to their physiochemical properties and bioactive nature, EOs has been used in aromatherapy, as flavor and fragrances in cosmetics, foods, and more recently as pharmaceuticals, natural preservatives, additives, and biopesticides[10,11]. EOs are concentrated form of liquid mixtures of volatile compounds of plant origin with unique structural chemistry including terpenoid and non-terpenoid hydrocarbons and their oxygenated derivatives, with natural color, odor and flavor, or “essence” of their source - volatile/ odoriferous oil. EOs are extracted from various plant parts such as leaves, fruit, bark, root, wood, heartwood, gum, balsam, berries, seeds, flowers, twigs, and buds[12].

Role of EOs in drug development has been well documented since antiquity nevertheless, they are directly used as therapeutic agents due to fact that they have proven record in traditional indigenous systems of medicine such as Ayurveda, Siddha, Unani and Homeopathy and in modern medicine, EOs contain bioactive compounds of GRAS nature. Furthermore, concern about the negative effect of synthetic chemicals as food additives warrants “GO” products with no or lesser side effects. Therefore, growing interest in natural

extracts as alternatives for synthetic additives is attributed to (a) their synergy with other preservation methods (b) generally regarded as safe, and (c) PBNPs are endowed with antioxidant, antidiabetic, antimutagenic, antitoxigenic and antibacterial properties. Apart from effective antioxidants of CMEO viz., cyclic diterpene diphenols, carnosolic acid and carnosol CMEO contains carnosic acid, epirosmanol, rosmanol, methylcarnosate and isorosmanol[13] however, needs scientific validation[14].

Cymbopogon martinii (Palmarosa) has been traced for its origin from the Mediterranean region. It is an aromatic plant, a unique spice commercially available for use as an antioxidant. CMEO extracts have been used in the treatment of diseases, due to its phytotherapeutic potential[14]. On the other hand, it is used in food preservation, PEO could even decrease the use of synthetic antioxidants in foods. EFSA (European Food Safety Authority) recently, reviewed the safety of CMEO extracts and concluded that there are high-intake estimates ranging from 0.09 (elderly) to 0.81 (children) mg/kg per day.

Cymbopogon martini (PALMAROSA)

Botanical Description: Perennial from a short woody rootstock. Culms tufted, up to 3 m tall, lower nodes often swollen, mealy. Leaf sheaths glabrous; leaf blades lanceolate, usually glaucous below, dark green above, up to 50 × 2–3 cm, glabrous, base cordate, often amplexicaul, apex filiform; ligule 2–4 mm. Spathate panicle narrow, dense, erect, 20–30 cm; spatheoles green becoming reddish, 2–4 cm; racemes 1.5–2 cm; rachis internodes and pedicels ciliate on margins, back sometimes pubescent; pedicel of homogamous pair swollen, barrel-shaped, shiny, fused to internode at base. Sessile spikelet oblong, 3.5–4.5 mm; lower glume flat, deeply grooved below middle, keels winged above middle, vein less or 2-veined between keels; upper lemma 2-lobed; awn 1.4–1.8 cm. Pedicelled spikelet 3.5–4 mm. Fl. and fr. Jul–Oct. This grass is native to India, but is cultivated elsewhere in tropical region of the world for its essential oils.

In traditional medicine both the plant and its oils are used to treat rheumatism, hair loss, arthritis, lumbago and spasms. The essential oil is a strong fungicide. In laboratory tests it was more effective than several synthetic fungicides against pathogenic fungi and yeasts, including *Aspergillus* spp., *Candida albicans*, *Monilia sitophila* and *Trichophyton tonsurae*[14,15]. In Ayurvedic medicine - Charak gave the decoction of whole plant in the treatment of abdominal disorders, the liver disorders, jaundice, fever and disorders of the spleen. In Sushruta, decoction of whole plant is prescribed in inflammation of throat, chest pain, indigestion, bronchitis, cough and asthma.

MATERIALS AND METHODS

Collection, Preparation and Extraction of Oil from the sample

The leaf samples were collected from wild in the Perumalmalai Region (Perumalmalai is a hillock in the Palani Hills, Dindigul District, TamilNadu) Western Ghats, INDIA during December 2020. The leaf sample were well preserved, taken to laboratory, identified by using flora[16,17] shade dried and processed as per the protocol for preparation of sample according to the methods previously described by Eleyinmi[18], however, with modifications in the temperature and duration of processing of the sample. As much as 100 g leaf was weighed and dried in an oven at 60°C. Dried sample was ground into powder using Thomas-Willey milling machine and sieved on a wire mesh screen (3 × 3 mm^2). Sample was stored at 4°C in air-tight container with screw caps. Sample was prepared according to the methods previously described by Rašković et al.[13]. 25 g of sample was suspended in 250 mL of distilled water in stoppered flasks and allowed to stand for 24 h, filtered with Whatman No 24 filter paper, concentrated in a rotary evaporator for 12 h at 50°C and dried in vacuum desiccator. Yield was calculated to be 6.06% w/w. Extract was suspended in ethyl acetate and subjected to GC-MS analysis.

GC-MS Analysis

Cymbopogon martini (Palmarosa) Essential Oil was extracted, from the leaf samples collected from the Perumalmalai Region, Palani, Dindigul District, Tamil Nadu, India. Phyto-components were identified using GC–MS detection system as described previously, however with modification, whereby portion of the extract was analysed directly by headspace sampling. GC–MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, and USA). Capillary column used was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 µm; J&W Scientific, CA, USA). Temperature program was set as follows: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/ min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively. Identification of phyto-components was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST 08.L and Wiley 7n.l libraries.

ADMET prediction

Selected phytocompounds were subjected to ADMET prediction using QikProp (version 4.3, Suite 2015-1; Schrödinger, LLC: New York, NY) and toxicity prediction using TOPKAT (Accelrys, Inc., USA). QikProp develops and employs QSAR/QSPR models using partial least squares, principal component analysis and multiple linear regression to predict physic-chemically significant descriptors[19].

RESULTS AND DISCUSSION

GCMS analysis

The chemical composition of EOs depends on plant genetics, growth conditions, development stage at harvest, and processes of extracting active compounds. Different parts of the plant (bark, leaf, fruit and seed) have been extensively investigated for their bioactive phytochemical constituents in various plants[20]. GC-MS analysis revealed that the extract of *Cymbopogon martini* contained different volatile oils (Jummes et al., 2020). 4-Decen-6-yne, (Z)- ($C_{10}H_{16}$), 3.568 min, 10 hits; 2-Ethylimino-4-methyl-pent-3-enenitrile ($C_8H_{12}N_2$), 3.913 min, 10 hits; Cyanogen bromide (CBrN), 4.024, 1 hits; Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alphA.,2.betA. ,5.alphA.) - ($C_{10}H_{18}O$), 4.503 min, 10 hits; Cyclohexa-1,3-diene, 5,6-diethyl- ($C_{10}H_{16}$), 4.915 min, 10 hits; Benzaldehyde, 2-methyl- (C_8H_8O), 8.154 min, 10 hits; Pyrazine ($C_4H_4N_2$), 9.32, 5 hits; 2-Norbornaneacetic acid ($C_9H_{14}O_2$), 9.378, 8 hits; cis-syn-trans-Tricyclo[7.3.0.0 (2,6)]dodec-7-ene ($C_{12}H_{18}$), 9.509 min, 10 hits; 1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl -5-(1-methylethyl)-, [1S (1.alphA. ,2.alphA. ,3A. betA. ,4.alphA. ,5.alphA. ,7A. be tA. ,8S*)]- ($C_{15}H_{24}$), 9.913 min, 10 hits; 1,4,7, Cycloun-decatriene, 1,5,9,9-tetramethyl-Z,Z,Z- ($C_{15}H_{24}$), 10.343 min, 10 hits; Naphthalene, decahydro-4a-methyl-1-methylene- 7-(1-methylethylidene)-, (4aR-trans)- ($C_{15}H_{24}$), 10.738 min, 10 hits; Butanoic acid, 3,7-dimethyl-2,6-

octadienyl ester,(E)- ($C_{14}H_{24}O_2$), 11.772 min, 10 hits; Nerolidol 2 ($C_{15}H_{26}O$), 11.948 min, 10 hits; Caryophyllene oxide ($C_{15}H_{24}O$), 12.525 min, 10 hits; 2-Azidomethyl-1,3,3-trimethyl-cyclohexene ($C_{10}H_{17}N_3$), 15.152 min, 10 hits; Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)- ($C_{16}H_{28}O_2$), 15.423 min, 10 hits; Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)- ($C_{16}H_{28}O_2$), 15.701 min, 10 hits; Farnesol, acetate ($C_{17}H_{28}O_2$), 17.258 min, 10 hits; 2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (Z)- ($C_{13}H_{22}O_2$), 20.158, 10 hits respectively (Table 1).

Biological activities of these secondary metabolites of *Cymbopogon martini* (Palmarosa) have been reported for its antitumor, antioxidant, anti-infectious, anti-inflammatory, and analgesic activities and effects on the central nervous system, endocrine system, disorders such ascardiac remodeling after myocardial infarction, body weight changes, dyslipidemia, cerebral ischemia, hepato-nephrotoxicity, stress, and anxiety. Anti-inflammatory activity of CMEO has been attributed to the presence and synergistic activity of carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids (A). Specifically, anti-inflammatory activity has been attributed to synergic effects of ursolic and micromeric acids present in CMEO[21]. These natural drugs can be proposed for preclinical and clinical studies in different diseases and pathological conditions.

2D, 3D structures of bioactive compounds in *C. martini* essential oil is given in Table 1. Molecular and biological properties (CID, MF, miLogP, TPSA, N atoms, MW (g/mol), Non, n OHNH, N violations, N rotb, volume, GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor) of the bioactive compounds is provided in Table 2. Summary of toxic (mutagenic, toxicology, irritant, reproductive properties) risk assessment towards drugability/ drug score in Table 3 indicates that the compounds were neither mutagenic nor toxic to biological system. All the bioactive compounds studied were drugable candidates and had good score for Druggability Properties - Lipinski's rule of 5 violations, Veber rule, Egan rule, Oral PhysChem score, GSK's 4/400 score, Pfizer's 3/75 score, QEDw score, Solubility, Solubility Index (Table 4).

Similarly, ADMET properties of key molecules in CMEO (Caryophyllene oxide and Geranyl butyrate) towards Human Intestinal Absorption, Blood Brain Barrier, Caco-2 permeable, P-glycoprotein substrate, P-glycoprotein inhibitor I, P-glycoprotein inhibitor II, CYP450 2C9 substrate, CYP450 2D6 substrate, CYP450 3A4 substrate, CYP450 1A2 inhibitor, CYP450 2C9 inhibitor, CYP450 2D6 inhibitor, CYP450 2C19 inhibitor, CYP450 3A4 inhibitor, CYP450 inhibitory promiscuity, Ames test, Carcinogenicity, Biodegradation, Rat acute toxicity, LD50 mol/kg, hERG inhibition (predictor I), hERG inhibition (predictor II) (Table 5) indicate that these molecules can be used for drug formulations.

Table 1: GC-MS profile of compounds s in *C. martini* essential oil

RT	Name of the Compound	Molecular Formula	Hits (DB)
3.568	4-Decen-6-yne, (Z)-	$C_{10}H_{16}$	**10**
3.913	2-Ethylimino-4-methyl-pent-3-enenitrile	$C_8H_{12}N_2$	**10**
4.024	Cyanogen bromide	CBrN	1
4.503	Cyclohexanol, 2-methyl-5-(1-methylethenyl)(1.alpha.,2.beta.,5.alpha.)-	$C_{10}H_{18}O$	**10**
4.915	Cyclohexa-1,3-diene, 5,6-diethyl-	$C_{10}H_{16}$	**10**
8.154	Benzaldehyde, 2-methyl-	C_8H_8O	**10**
9.32	Pyrazine	$C_4H_4N_2$	5
9.378	2-Norbornaneacetic acid	$C_9H_{14}O_2$	8
9.509	cis-syn-trans-Tricyclo[7.3.0.0(2,6)]dodec-7-ene	$C_{12}H_{18}$	**10**
9.913	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S- (1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.be ta.,8S*)]-	$C_{15}H_{24}$	**10**
10.343	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-	$C_{15}H_{24}$	**10**
10.738	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	$C_{15}H_{24}$	**10**
11.772	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester,(E)-	$C_{14}H_{24}O_2$	**10**
11.948	Nerolidol 2	$C_{15}H_{26}O$	**10**
12.525	Caryophyllene oxide	$C_{15}H_{24}O$	**10**
15.152	2-Azidomethyl-1,3,3-trimethyl-cyclohexene	$C_{10}H_{17}N_3$	**10**
15.423	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester,(E)-	$C_{16}H_{28}O_2$	**10**
17.258	Farnesol, acetate	$C_{16}H_{28}O_2$	**10**
20.158	2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (Z)-	$C_{13}H_{22}O_2$	**10**

Table 2: IUPAC Name, 2D, 3D structure of bioactive compounds in CMEO

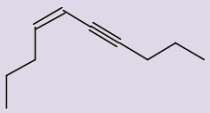
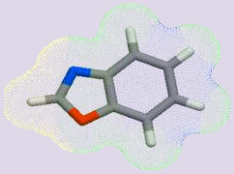
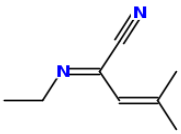
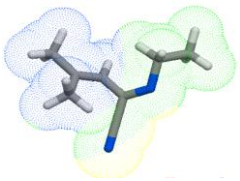
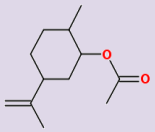
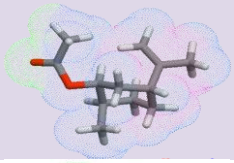
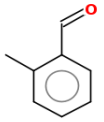
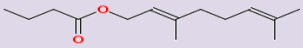
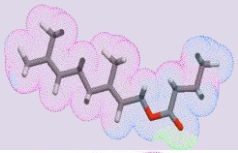
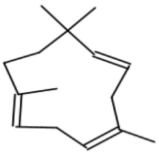
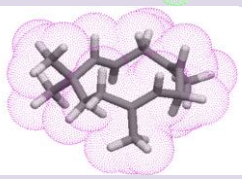
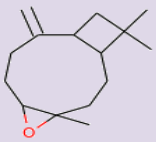
IUPAC Name	2D Chemical Structure	3D Chemical Structure
Cyclodecyne; 4-Decen-6-yne, (Z)		
2-Ethylimino-4-methyl-pent-3-enenitrile		
Dihydrocarvyl acetate		
2-Methylbenzaldehyde		
Geranyl butyrate		
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene		
Caryophyllene oxide		

Table 3: Molecular properties of bioactive compounds in CMEO

PROPERTY	BIOACTIVE COMPOUNDS						
CID	137799	68315	73918	998	5282854	5281522	1742210
MF	$C_{10}H_{16}$	$C_8H_{12}N_2$	$C_{10}H_{18}O$	C_8H_8O	$C_{14}H_{24}O_2$	$C_{15}H_{24}$	$C_{15}H_{24}O$
miLogP	4.54	2.09	3.35	2.13	4.83	5.07	4.14
TPSA	0.00	36.16	26.30	17.07	26.30	0.00	12.53
N atoms	10	10	4	9	16	15	16
MW (g/mol)	136.24	136.20	154.24	120.15	224.34	204.36	220.36
Non	0	2	2	1	2	0	1
n OHNH	0	0	0	0	0	0	0
N violations	0	0	0	0	0	1	0
N rotb	3	2	3	1	8	0	0
volume	162.53	146.66	208.06	119.59	245.69	234.00	234.01

Table 4: Biological properties of compounds in CMEO

PROPERTIES	BIOACTIVE COMPOUNDS						
CID	**137799**	**68315**	**73918**	**998**	**5282854**	**5281522**	**1742210**
GPCR ligand	-0.56	-1.64	- 0.47	- 2.33	- 0.26	**0.03**	**0.08**
Ion channel modulator	**0.57**	-1.04	**0.23**	- 1.80	**0.05**	**0.132**	**0.14**
Kinase inhibitor	-1.05	- 2.08	- 1.25	- 2.40	- 0.86	- 0.95	-0.86
Nuclear receptor ligand	-0.18	- 2.06	- 0.17	- 2.20	**0.03**	**0.40**	**0.62**
Protease inhibitor	-0.76	- 1.92	- 0.44	- 2.91	- 0.56	- 0.63	0.00
Enzyme inhibitor	**0.43**	- 0.84	- 0.12	- 1.91	**0.30**	**0.41**	**0.57**

Table 5: Summary of MTIR/ DL/DS score of bioactive compounds in CMEO

COMPOUND	MP	TP	IP	RE	DL	DS
Cyclodecyne; 4-Decen-6-yne, (Z)-	None	None	High	None	-10.80	0.21
2-Ethylimino-4-methyl-pent-3-enenitrile	None	None	None	None	-4.87	0.48
Dihydrocarvyl acetate	None	None	High	None	-19.56	0.26
2-Methylbenzaldehyde	None	None	Medium	High	-5.59	0.23
Geranyl butyrate	None	None	High	None	-5.84	0.21
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	None	None	None	None	-5.08	0.28
Caryophyllene oxide	None	Medium	None	Medium	-4.77	0.25

Note: MP = Mutagenic property; TP = Toxicology property; IP = Irritant property; RE = Reproductive property; DL = Drug Likeness; DS = Druggable Score

Table 6: Druggability Properties of bioactive compounds in CMEO

Druggability Property	BIOACTIVE COMPOUNDS					
	$C_{10}H_{16}$	$C_8H_{12}N_2$	$C_{10}H_{18}O$	C_8H_8O	$C_{14}H_{24}O_2$	$C_{15}H_{24}$
Lipinski's rule of 5 violations	0	0	0	0	0	0
Veber rule	Good	Good	Good	Good	Good	Good
Egan rule	Good	Good	Good	Good	Good	Good
Oral PhysChem score	0	1	2	2	1	2
GSK's 4/400 score	Good	Good	Good	Good	Good	Good
Pfizer's 3/75 score	Warning	Bad	Bad	Bad	Bad	Bad
QEDw score	0.521	0.506	0.493	0.434	0.433	0.434
Solubility	12379.28	8150.46	4750.64	5166.30	4350.64	5166.30
Solubility Index	Good	Good	Good	Good	Good	Good

Druggability scoring schemes were computed using FAF-Drugs 4(28961788) and FAF-QED (28961788) open-source Chem-informatics platform.

Studies have led to the isolation of alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids and tannins from *Cymbopogon* species. β-Caryophyllene from CMEO has been reported to be directly beneficial for colitis, osteoarthritis, diabetes, cerebral ischemia, anxiety and depression, liver fibrosis. Biological activities of these secondary metabolites of *Cymbopogon martini* (Palmarosa) have been reported for its antitumor, antioxidant, anti-infectious, anti-inflammatory, and analgesic activities and effects on the central nervous system, endocrine system, disorders such as cardiac remodeling after myocardial infarction, body weight changes, dyslipidemia, cerebral ischemia, hepato-nephrotoxicity, stress, and anxiety. Anti-inflammatory activity of CMEO has been attributed to the

presence and synergistic activity of carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids (A). Specifically, anti-inflammatory activity has been attributed to synergic effects of ursolic and micromeric acids present in CMEO. These natural drugs can be proposed for preclinical and clinical studies in different diseases and pathological conditions.

CONCLUSION

Cymbopogon species have been used as traditional medicine in many countries since antiquity. CMEO has been used in traditional and in conventional medicine due to the pharmacological potential of their phytochemicals. *C. martini* (Palmarosa) contains a large variety of bioactive molecules with great therapeutic potential and biological activities such as insecticidal, anti-protozoan, anticancer, anti-HIV, anti-inflammatory and anti-diabetes effects. CMEO has remarkable anti-inflammatory, antimicrobial, and antioxidant properties, which have been extensively reported in several formulations. However, development of new formulations containing other less common CMEO extracts is warranted through trials to establish the credentials of pharmacologically active phyto-compounds towards safety/ efficacy, in treating various pathological conditions including COVID-19 and other viral infections owing to the physiochemical properties and druggable nature of CMEO.

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