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Ocimum kilimandscharicum Guerke: Phytochemical and Pharmacological Aspects: A Review.

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ABSTRACT

Ocimum kilimandscharicum is a plant belonging to family Lamiaceae. It is widely distributed in different parts of the world and has a long history of traditional medicinal uses. The plant has been used traditionally in African countries as mosquito repellent and for GIT disorders. The volatile oil from plant is found to be rich in camphor. The phenolics, flavonoids and terpenoids have been isolated from aerial parts of plant. The comprehensive account of detailed biotechnological, phytochemical and pharmacological aspects of *Ocimum kilimandscharicum* along with patented formulations are presented in this review.

INTRODUCTION

Ocimum kilimandscharicum Guerke (*Syn. Ocimum camphora* Guerke) belongs to family Lamiaceae. It is a native of Kenya and distributed in East Africa, India, Thailand, Uganda and Tanzania [1,2,3]. It is extensively grown in the Tropics [3]. In India it is cultivated on a small scale especially in West Bengal, Assam, Tamil Nadu, Karnataka, Kerala and Dehradun. Commonly the plant is called as camphor Basil, African blue basil and in Ayurveda as Karpura Tulasi [4,5].

Morphologically it is a perennial aromatic evergreen undershrub (Figure 1) with pubescent branchlets having pale green leaves which are glandular, ovate or oblong in shape, base is acute, deeply serrated, pubescent on both surfaces, oppositely arranged and about 3-7 cm in length including petioles which are 4 to 12 mm long, 1 to 2.5 cm wide; indumentum of long white adpressed hairs or sometimes glabrous above; petiole 4-10 mm [1,6-9]. Stems are brownish green, round-quadrangular, much branched, woody with epidermis sometimes peeling off in strips below, arising from a large woody rootstock, with white glandular hairs, becoming denser in the inflorescence-axis, with sparse sessile glands [1]. The older stems tend to lose their hairs near their bases and the bark becomes shredded and conspicuous when the dry stems are broken [6]. Inflorescence is vertical, flowers are purplish white in simple or much branched racemes [8,9]; bracts usually deciduous, forming a small coma, ovate, entire, cuspidate; pedicel 3 mm, erect, slightly curved [1].

Traditional Claims

The plant has traditionally been used in different parts of the world for various ailments (Table 1). *O. kilimandscharicum* is employed as an indigenous medicine for a variety of ailments like cough, bronchitis, viral infections, foul ulcers, anorexia and wounds [10]. The leaves of *O. kilimandscharicum* are

acrid, thermogenic, aromatic, insecticidal, antiviral, appetizing and deodorant and are useful in cough, bronchitis, foul ulcers and wounds, ophthalmopathy and vitiated conditions of 'vata'^[44]. The plant has reported to have various central nervous system (CNS) activities. The plant has shown neurotoxic, antineuralgic, CNS stimulant, tranquilizer, anti-alzheimerian and sedative effects^[44].



Figure 1: Morphology of *Ocimum kilimandscharicum* plant

Phytochemistry

The plant has been investigated for its phytochemical profile.

Volatile oil

The plant is characterized by the presence of essential oil with high amount of camphor. Lowman and Kelly^[6] separated the volatile oil from *O. kilimandscharicum* and reported a yield of 0.5 to 1%. The volatile

oil from *O. kilimandscharicum* reported to mainly dominated by monoterpenes and oxygenated monoterpenes followed by sesquiterpenes and oxygenated sesquiterpenes and some miscellaneous compounds (Figure 1a, 1b, 1c)^[21,23-30]. Recently, More than 40 compounds have been identified form essential oil of the leaves of *O. kilimandscharicum*^[30].

Variation in the constituents of volatile oil with change in season and place of collection was observed (Table 2 and 3). Majority of studies identified camphor as major component of volatile oil of *O. kilimandscharicum* ranging 45.9%-78.3% of oil ^[24,26-33].

Table 1: Traditional use of *O. kilimandscharicum* in different parts of the world

Sr. No.	Location	Plant Part	Traditional use	Reference
Africa				
1.	East Africa	Leaves	Used to protect foodstuffs from pests; to treat cold & cough, abdominal pains, measles and diarrhea: insect repellents against mosquitoes	[12,13]
2.	North-Eastern Tanzania	Leaves	Fresh or smoke of the leaves is used as mosquito repellent at night	[14]
3.	Central Kenya	Whole plant	Anti-malarial	[15]
4.		Whole plant	Grain protectant; Mosquito repellent: Analgesic and antipyretics; Nasal congestion, colds, flu, Insect bites, General aches and pains (western Kenya)	[16-19]
	Kenya	Leaves	Infusion for curing measles: Infusion mixed with <i>Lippia ukamensis</i> treats flu and stomach-ache	[20]
		Leaves	crushed leaves are snuffed or vapours of boiling leaves are inhaled to treat congested chest, cough and cold	[20]
5.	Rwanda	Whole plant	In eye infections	[21]
India				
1.	West Bengal	Leaves	Decoction in eyes diseases	[22]
		Whole Plant	Spasmolytic, antibacterial, insecticidal, mosquito repellent	[22]

Table 2: Variation with place of collection in major phytoconstituent of volatile oil

Country	Major constituent of volatile oil	% Age of major constituent	Reference
US (Indiana)	Linalool (leaves)	42.94	[23]
	Linalool (flowers)	58.85	
Rwanda	1,8-cineole	62.00	[21]
South India	Camphor	57.87	[34]
Tanzania	Camphor (Leaves and flowering tops)	52.4	[25]
Western Ghats	Camphor (leaves)	45.90	[30]

Table 3: Variation with season of collection in major phytoconstituent of volatile oil

Season of collection	Major constituent of volatile oil	%age of major constituent	Reference
Winter	Camphor	48.90	[35]
Summer	Camphor	58.90	[35]

Phenolics and flavonoids

Phenolic compounds quantified in the methanol extract of leaves *O. kilimandscharicum* include rosmarinic acid, lithospermic acid, vanilic acid, p-coumaric acid, hydroxy benzoic acid, syringic acid, caffeic acid, ferulic acid and sinapic acid^[36].

Three flavonoids, quercetin, quercetin-3-O-rutinoside (rutin) and luteolin-5-O-glucoside (galuteolin), were isolated from the 80% hydro-methanolic extract of leaves of *O. kilimandscharicum*^[37,38].

Triterpenoids

Ursolic acid (a pentacyclic triterpenoid), cadinol (a sesquiterpenoid) and thymol (a monoterpene) were quantified in aerial parts of *O. kilimandscharicum*^{[8],[38]}.

Miscellaneous compounds

Proteins, carbohydrates, volatile oils, fatty acids, flavonoids, tannins, saponins, steroids and alkaloids have been detected in the aqueous extract^{[10],[18]}. Eugenol, β -sitosterol and stigmasterol were separated from chloroform, methanol and acetone extract of *O. kilimandscharicum*^[38].

The structures of these compounds are given in Figure 2.

Pharmacological Activities

Antimicrobial activity

Table 4: Activity of volatile oil/extract of *O. kilimandscharicum* against microorganisms

Volatile Oil/Extract/Constituents	Active Against	Active Dose/ Minimum Inhibitory Concentration	Reference	
Ethanol extract of whole plant	Gram negative bacteria: <i>Escherichia coli</i>	Inhibited growth at 50, 100, 150, 200, 250 and 300 mg/ml	[41]	
	<i>Escherichia coli</i>	1.55mg/ml; NIL; 31.50 μ g/ml	[25,42,43]	
	<i>Enterobacter cloacae</i>	2.97 mg/ml	[25]	
	<i>Klebsiella pneumonia</i>	2.70 mg/ml	[25]	
	<i>Pseudomonas aeruginosa</i>	2.50 mg/ml; 27%; 62.50 μ g/ml	[25,42,43]	
	Gram negative bacteria	<i>Klebsiella sp.</i>	Not active	[42]
		<i>Shigella dysenteriae</i>	250 μ g/ml	[43]
		<i>Vibrio cholera</i>	125 μ g/ml	[43]
		<i>Shigella flexneri</i>	62.50 μ g/ml	[43]
		<i>Salmonell abaetetuba</i>	Inhibited growth at 250 and 300 mg/ml	[41]
Gram positive bacteria		<i>Staphylococcus aureus</i>	2.85 mg/ml	[25]
	<i>Staphylococcus epidermidis</i> ,	3.35 mg/ml	[25]	
	<i>Streptococcus mutans</i> ,	Not active	[25]	
	<i>Streptococcus viridans</i>	Not active	[25]	
	<i>Bacillus subtilis</i>	15.62 μ g/ml	[43]	
	<i>Micrococcus luteus</i>	62.50 μ g/ml	[43]	
	<i>B. cereus</i>	25%; >2640mg/ml	[42,44]	
Eugenol;Cadinol	Multidrug-resistant (MDR) and methicillin-resistant <i>Staphylococcus aureus</i>	2-128 μ g/ml	[38]	
	<i>Candida albicans</i>	Not active	[25]	
	<i>Candida tropicalis</i>	Not active	[25]	
	<i>Candida glabrata</i>	Not active	[25]	
Volatile Oil	<i>Candida sp.</i>	Active from 31.3 μ g/ml – 1000 μ g/ml	[45]	
	Fungus	<i>Malassezia furfur</i>	128 μ g/ml	[40]
		<i>Rhizoctonia solani</i>	730 ppm	[46]
Volatile Oil, camphor	<i>Choanephora cucurbitarum</i>	1200 ppm	[46]	
Volatile Oil, camphor				

Numerous experiments have shown that *O. kilimandscharicum* inhibits various microbial organisms (Table 4). Essential oil from *O. kilimandscharicum* exhibited antibacterial activity against both gram positive and gram negative bacterial strains^[39] although this activity was weak in comparison to essential oils from *O. americanum*, *O. basilicum* 'Genovese' and *O.x citriodorum*^[26]. Verma et al., compared the efficacy of essential oil of *Ocimum gratissimum* L. and *Ocimum kilimandscharicum* Guerke against four gram positive bacterial strains, *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC435), *Enterococcus faecalis* (MTCC 439) and *Streptococcus mutans*, by disc diffusion assay and reported higher activity index for *O. gratissimum* than *O. kilimandscharicum*^[29].

Volatile oil was also found active some fungal strains like *Rhizoctonia solani* and *Choanephora cucurbitarum*. In combination with cinnamon oil (1:1), essential oil of kapur tulusi showed antifungal activity with 4 µg/ml as minimum inhibitory concentration [40].

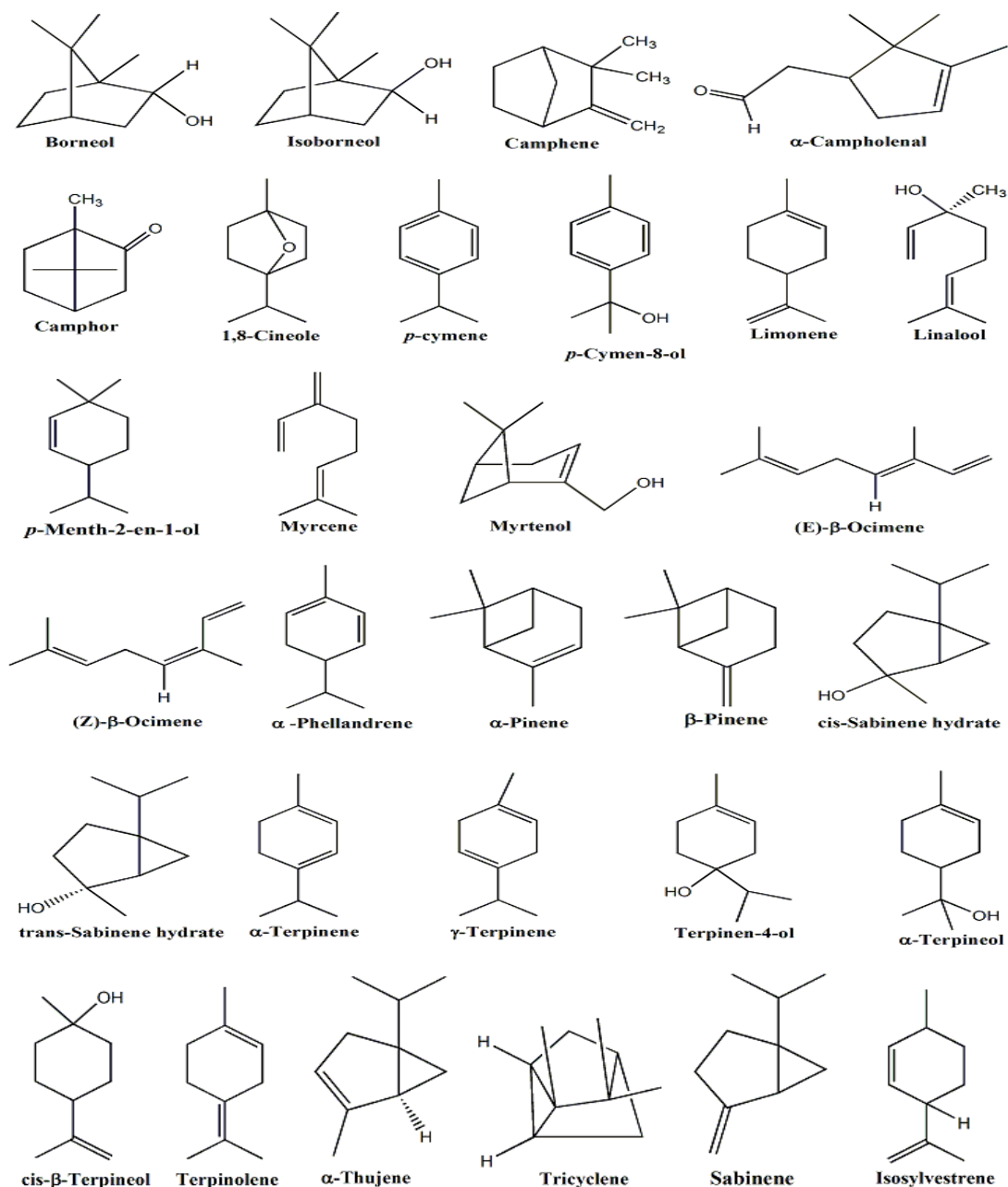


Figure 1a: Monoterpenes and oxygenated monoterpenes identified from essential oil of *O. killimandscharicum*

Anti-diarrhoeal activity

Aqueous extract of leaves at three different dose levels (100, 200 and 400 mg/kg, p.o. in rats and corresponding doses in mice) against castor-oil induced diarrhoea model and castor oil induced enteropooling assay in rats; and charcoal meal test/intestinal motility test in mice. The extract (100 and 200mg/kg) showed increased defecation, reduced cumulative faecal weight and reduced intestinal fluid accumulation in rats while in mice extract at a dose 280mg/kg caused reduction in the distance travelled by charcoal in intestine. The results showed anti-diarrhoeal activity of leaves which may be due to its anti-motility and anti-secretory effects[47].

Antimelanoma and radioprotective activity

The 50% hydro methanolic leaf extract (200 mg/kg bw) significantly lowered the tumour volume and increased survival rate of mice showing antimelanoma potential and also showed partial protection to bone marrow, increases reduced glutathione level and GST activity against lethal irradiation doses of gamma radiation^[48].

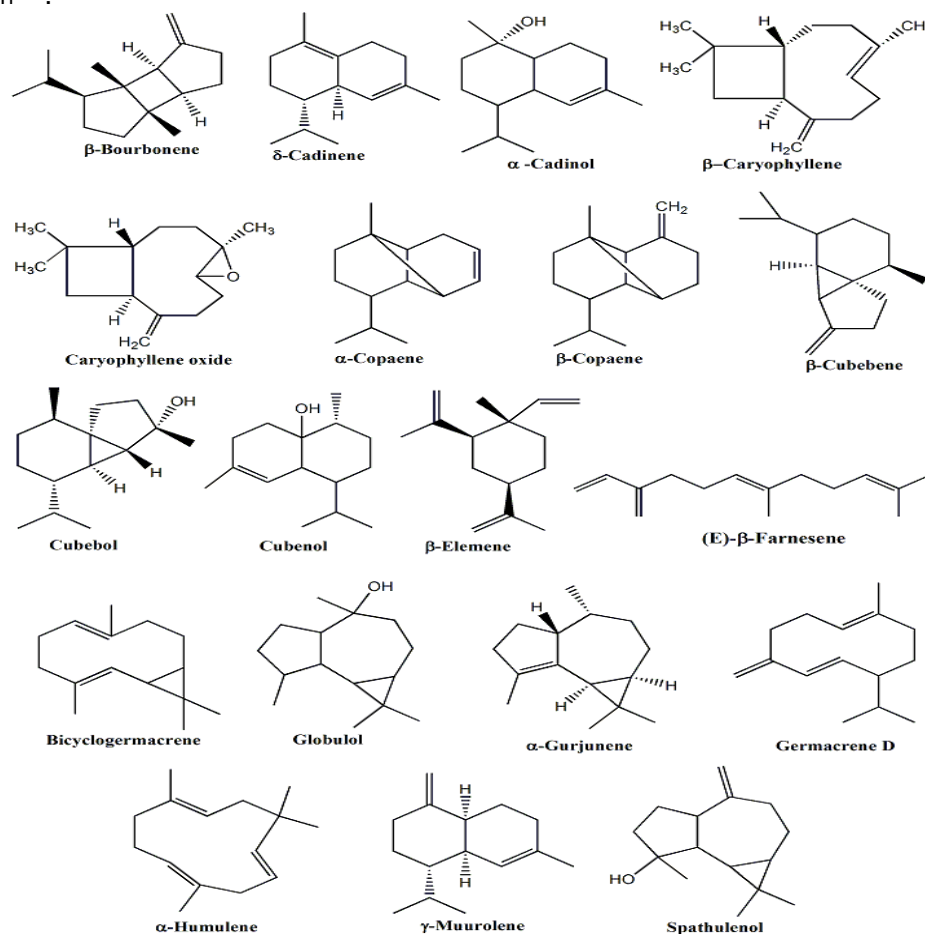


Figure 1b: Sesquiterpenes and oxygenated sesquiterpenes identified from essential oil of *O. killandscharicum*

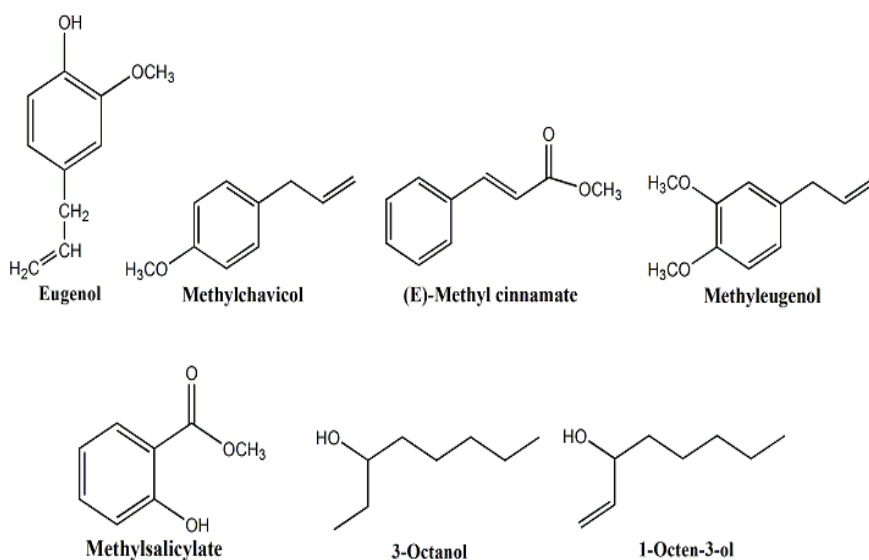


Figure 1c: Miscellaneous compounds identified from essential oil of *O. killandscharicum*

Antinociceptive Activity

The antinociceptive activity of ethanolic leaf extract of *O. kilimandscharicum* was reported. The extract showed significant dose dependent (100, 200, 400 and 800 mg/kg b.w.) activity in the radiant tail-flick test in mice [18].

Antioxidant activity

The antioxidant activity of methanol extract of leaves was evaluated for antioxidant properties by a battery of in-vitro assays viz. DPPH assay, iron (III) to iron (II) reduction, superoxide anion scavenging activity and β -carotene-linoleic acid bleaching assays. The results showed significant antioxidant activity of extract in all assays which was attributed to phenolic content as increase in total phenolic content showed an increase in free radical scavenging activity[36].

Water, methanol and ethanol extracts of leaves showed free radicals scavenging action by DPPH method with IC₅₀ values of 95.66, 96.76, and 82.22 (μ g/ml), respectively[49]. Significant increase in reduced glutathione level and glutathione S-transferase activity in the liver, lung and stomach of Swiss albino mice was exhibited by 50% hydro methanolic extract (200 mg/kg bw) of leaves of *O. kilimandscharicum*[48].

Antiplasmodial activity

Dichloromethane extract of leaves and twigs of *O. kilimandscharicum* has been reported to have in-vitro antiplasmodial activity with IC₅₀ value of 1.547 μ g/ml and 0.843 μ g/ml for chloroquine resistant and chloroquine sensitive clones, respectively[50].

The oil exhibited larvicidal activity against *Culex quinquefasciatus* larvae with LC₅₀ value of 323.6ppm[45].

Mosquito repellent

Leaves and seeds of *O. kilimandscharicum* showed repellency against *Anopheles gambiae* and *Anopheles funestus* by thermal expulsion method in semi-field experimental huts, while potted plant did not show any result[51]. In combination with *Lippia uckambensis*, *O. kilimandscharicum* repelled 54.8% mosquitoes by thermal expulsion[17]. Smoke from burned plant material in experimental huts caused an increase in exophily behavior, blood-feeding inhibition and high deterrence and feeding inhibition rates against *Anopheles gambiae*, *Anopheles arabiensis* and *Culex quin-quefasciatus* (malarial vectors)[52] and showed mortality by decreasing feed intake[14]. Volatile oil also showed significant protection efficiency to human volunteers against the above mentioned three mosquito species[14]. Volatile oil mixed with glycerine and/or liquid paraffin caused feed inhibition, 87.9 and 84.7%, respectively, in *Anopheles gambiae* s.s.[53]. Essential oil of plant also showed oviposition deterrence in *Anopheles gambiae* s.s.[54].

Pesticidal activity

Powdered leaves and essential oil protected maize and sorghum grains against *Sitophilus zeamais* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and caused 100% mortality after 48 h[16]. Bekele and Hassanali reported the toxicity of essential oil of *O. kilimandscharicum* and its major components (camphor, limonene, 4-terpeneol, 1,8-cineole, camphene and trans-caryophyllene). Essential oil and blend of its major components showed 100% mortality against *Sitophilus zeamais* (1.44 mg per cm²; LC₅₀ at 0.76 mg per cm²) and *Rhyzopertha dominica* (0.8 mg per cm²; LC₅₀ at 0.7 mg per cm²) in Petri dish assay. Three major component in combination (camphor, limonene and 1,8-cineole) showed maximum 82% toxicity. Further, drop in activity of 5-component blend was reported when camphor was removed in subtraction assay and individual compounds (amount present in dose causing 100% mortality) were ineffective except camphor against *Rhyzopertha dominica*[31]. In another study, camphor was evaluated against the beetles (*Sitophilus granarius*, *S. zeamais*, *Tribolium castaneum* and *Prostephanus truncatus*) using contact toxicity, grain treatment and repellency assays. Camphor caused maximum mortality of 93% at 100mg/disc against *S. granarius*, *S. zeamais* and *P. truncatus* and 70% against *T. castaneum*. Dose of 60 micro gram/insect and 1mg caused 100% mortality among all beetles on topical application and grain treatment, respectively. Also, it inhibited the development of eggs, larvae and pupae, oviposition and subsequent progeny production of *S. granarius* and *S. zeamais*[12].

Wound Healing Activity

Aqueous extract (200 and 400m/kg b.w.) showed significant increase in skin breaking strength, granuloma breaking strength, wound contraction, dry granuloma weight and decreased in epithelization period in animals of the treatment groups. Histopathological studies of granulation tissue supported the reported activity. The result was correlated antioxidant effects as extract showed significant results when tested for L-hydroxy proline, hexose amine, malondialdehyde and ascorbic acid contents^[10].

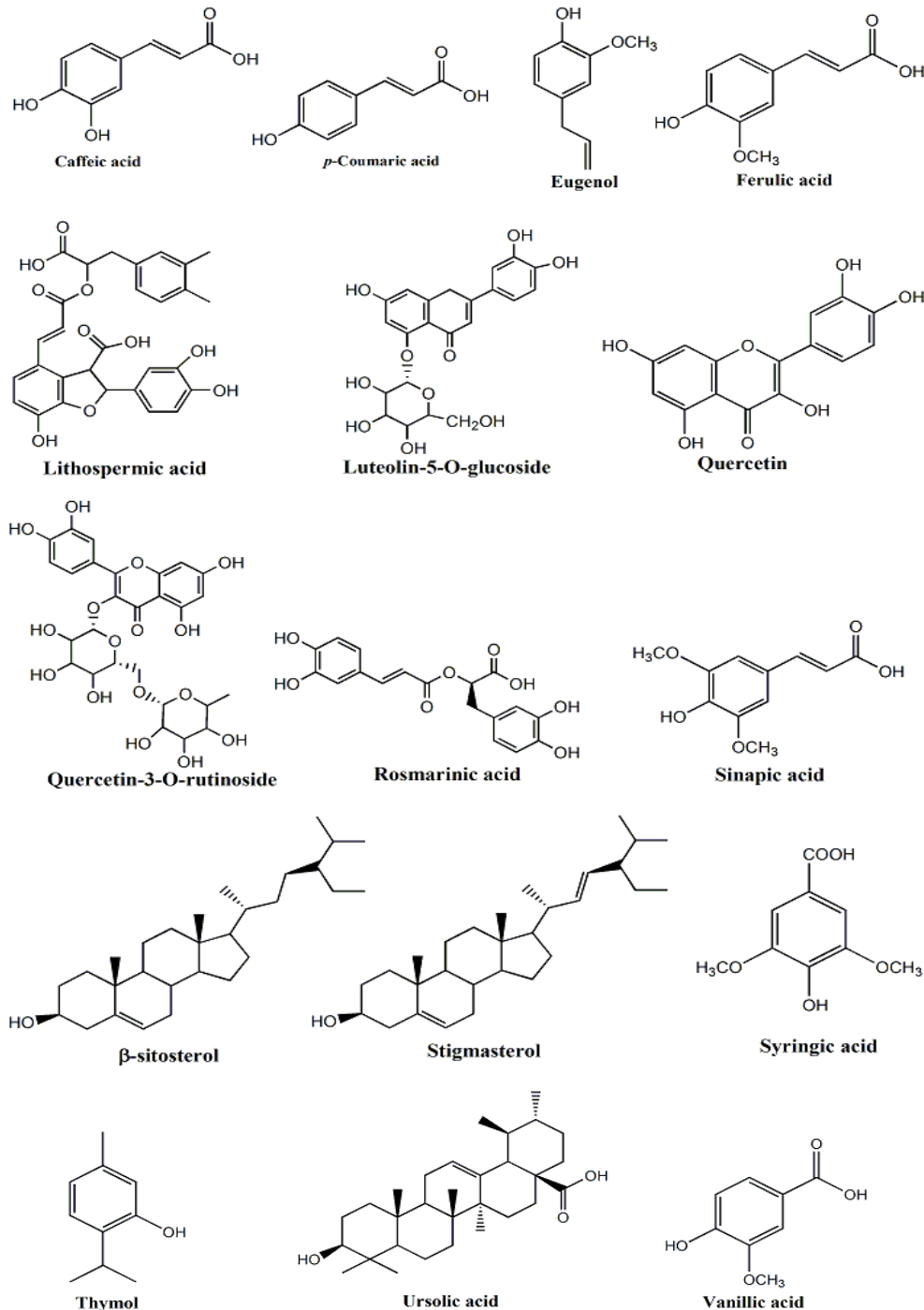


Figure 2: Compounds isolated from various extracts *O. kilimandscharicum*

Biotechnological Studies

- *Micropropagation*: An efficient method of plant regeneration from nodal segments was developed^[3,55]. A number of plantlets were grown from nodal explants of *O. kilimandscharicum* in vitro on MS basal media. The fully developed plantlets showed 81.13% survival when transferred

to plastic bags and regenerated plantlets showed genetic homogeneity with mother plant as confirmed by PCR-based RAPD markers^[3].

- Inoculation with *Meloidogyne incognita* (nematode infection) caused significant decrease in root length, shoot height and shoot fresh weight of *O. kilimandscharicum*. The significant decrease in oil yield was also noticed compared to untreated group^[56].

Marketed Formulation

Volatile oil obtained from the leaves of *Ocimum kilimandscharicum* is being used in the manufacturing of therapeutic products used in aromatherapy. 'Naturub' (available as balm and ointment) is a formulation developed from purified extracts of *O. kilimandscharicum*. Naturub® is manufactured by Muliru Farmers Conservation Group and certified and registered as the first natural product by the Pharmacy and Poisons Board of Kenya. It is used for alleviation of muscular pain relief, arthritis, flu, cold and chest congestion^[57].

Patented formulation

A herbal formulation containing 90% ethanol extract of *Zanthoxylum armatum* (fruit pericarp), *Spilanthes acmella* (flowers), *Potentilla fulgans* (roots) and *Hedychium spicatum* (Rhizomes) and volatile oil of *Zanthoxylum armatum* (fruits) and *Ocimum kilimandscharicum* and camphor separated from volatile oil of *Ocimum kilimandscharicum* (aerial parts) was prepared to treat tooth ache and related disorders and was patented by The Director General (DRDO)^[58]. In clinical trial, the patients got relief from pain within 2 to 10 minutes after applying the patented formulation.

Volatile oil is also a component of another patented formulation, Potable Liquid Disinfection ^[59].

CONCLUSION

Ocimum kilimandscharicum Guerke is a native shrub of Kenya and characterized by the high yield of camphor in volatile oil. Numerous traditional uses have been documented for this species. This review attempts to unite the relevant available information of the species. The experimental studies confirm the traditional claims of the species among various parts of the world. The essential oil of plant has found extensive use as mosquito repellent justifying its ethnobotanical claim. Tremendous efforts have been made to validate the traditional claims of *O. kilimandscharicum* but it remains surprising that none of the studies attempts to establish the relationship of pharmacological activity with secondary metabolites except for bactericidal actions. Thus, area of phytochemistry remains unexplored. Hence, a thorough biosystematic study of *Ocimum kilimandscharicum* may provide an effective natural compound(s) which can become a lead molecule(s) in drug discovery.

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