

Ocimum gratissimum: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties

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Abstract: *Ocimum gratissimum* is a well-known plant used in the Indian system of medicine. Folklore medicine claims its use in headache, fever, diarrhoea, pneumonia etc. Research carried out using different *in vitro* and *in vivo* techniques of biological evaluation supports most of the claims. This review presents the ethnobotanical, natural product chemistry, pharmacological, clinical and toxicological data of the plant.

Key Words: *Ocimum gratissimum*, antifungal, antibacterial, essential oil.

1. INTRODUCTION

This review emphasizes the traditional use and clinical potentials of *Ocimum gratissimum*. Through this review, authors hope to attract the attention of natural product researchers throughout the world to focus on the explored potential of *O. gratissimum*.

This review has been compiled using references from major database such as Chemical Abstracts, Medicinal and Aromatic Plants Abstracts, Pubmed, Duke's Phytochemical and Ethnobotany database, United States Patent and Trademark Office (USPTO) Patent Full Text and Image database.

The available information on *O. gratissimum* has been divided into six sections, that is, ethnopharmacology, morphology, phytochemistry, pharmacological, clinical and toxicological studies. The ethnopharmacological section has been further subdivided into two sections, viz. traditional uses and alternative and complementary medicinal uses. The reports in which *O. gratissimum* have been used as a domestic remedy by common men without prescription for the treatment of various ailments have been discussed under traditional uses. The subhead "Alternative and Complementary medicinal uses" highlights *O. gratissimum* as medicine prescribed by medical practitioners for the treatment of various ailments.

O. gratissimum is a herbaceous plant which belongs to the Labiatae family. The plant is indigenous to tropical areas especially India and it is also in West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan [1]. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called

"effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya" [2].

2. ETHNOPHARMACOLOGY

2.1. Traditional Uses

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion [3]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea [2]. In the Savannah areas decoctions of the leaves are used to treat mental illness [4]. *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [5]. Brazilian tropical forest inhabitants use a decoction of *O. gratissimum* roots as a sedative for children [6]. People of Kenyan and sub Saharan African communities' use this plant for various purposes like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum [7]. In India, the whole plant has been used for the treatment of sunstroke, headache, influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity [8-10].

The tribals of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids [11]. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis [12].

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The infusion of *O. gratissimum* leaves is used as pulmonary antisepticum, antitussivum and antispasmodicum [13].

2.2. Alternative and Complementary Medicinal Uses

Among the various species of *Ocimum*, *O. gratissimum* finds extensive use clinically throughout the world. Formulations of the leaf essential oil of *O. gratissimum* (*Ocimum* oil) have been incorporated in a variety of bases as topical antiseptics and for use in the treatment of minor wounds, boils and pimples [14].

Ijeh I.I. (2005) reports that *O. gratissimum* and *Xylopia aethiopica* in combination are used in the preparation of potions and teas for women during peuperium [5].

3. MORPHOLOGY AND MICROSCOPY

3.1. Morphology

O. gratissimum is a shrub up to 1.9m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubescent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface. Ordinary trichomes are few, while the long ones up to 6-celled are present on the margins mostly; the short ones which are 2 celled, are mostly found on the lamina. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is upto 5mm long, campanulate and 5-7 mm long, greenish-white to greenish-yellow in colour. Nutlets are mucilaginous when they are wet [15] (Fig. (1)).

3.2. Microscopy

On the 2 surfaces of the leaf epidermal cells are typical of irregular contours, and diacytic stomata, secretory glands most abundant in the leaf, are also present in simple pluricellular hairs on the leaf veins. The cross section shows the epidermis monoestratificada (beam), a layer of parenchyma fenced in sub-epidermal position, followed by parenchymal pond, and finally the epidermis monoestratificada lower [16].

3.3. Powder Microscopy

Powder study was carried out by Lic Dinah Garcia (1998) [18]. On examination following characters were observed (Fig. (2)).

4. PHYTOCHEMISTRY

Maria gorette de Vasconcelos Silva *et al.* worked on compositions of essential oil from *Ocimum* species obtained by steam, microwave and supercritical CO₂ extraction. The report reveals the following components by GC-MS analysis (Table 2) [23].

Thin layer chromatography of the fractions isolated from *O. gratissimum* leaves indicated the presence of fairly polar compounds. Analysis of one of the components by mass spectra showed the presence of an unknown compound with a molecular mass of at least 353 daltons containing carbon, hydrogen, oxygen and possibly nitrogen. A tentative molecu-

lar formula, as deduced from computer analysis, is either C₂₁H₃₇O₄ or C₁₉H₃₅N₃O₃ [25].

a)



b)



Fig. (1). a) Flowering tops of *O. gratissimum*. b) Whole plant *O. gratissimum*.

Cristiana M. Murbach Freire *et al.* carried out the experiment to determine the percentage composition of the essential oil obtained from fresh leaves of *O. gratissimum* in different seasons throughout the year. Percentage of the chemical constituents obtained in different seasons throughout the year has been shown in the (Table 3) [6].

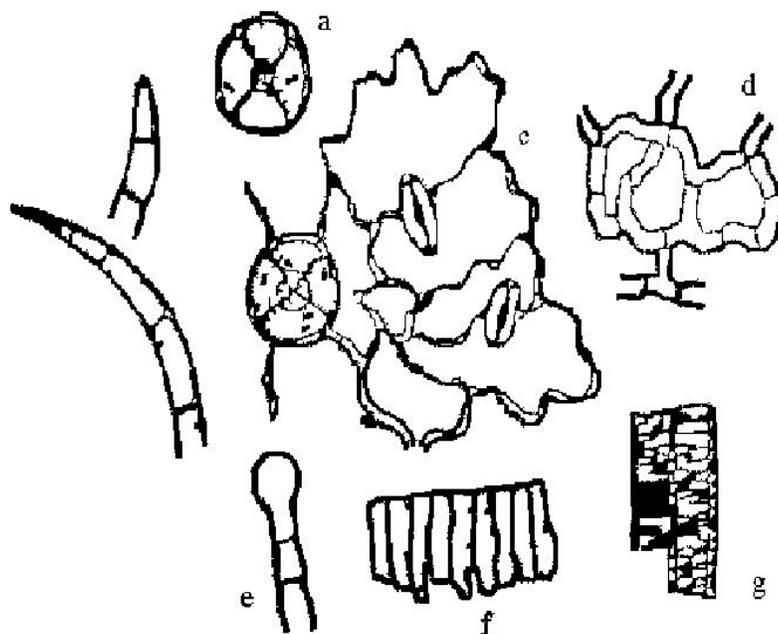


Fig. (2). Powder microscopy of *O. gratissimum*. a) Pelos and non-glandular uniseriate pluricellular. b) gland view from the top of the head with 4 cells radiant. c) fragment of skin with glands and stomata. d) of leaf tissue lagoon. e) hair with glandular stalk multicellular. f) parenchyma fence. g) tracheal reticular.

Table 1. List of Biologically Active Compounds that have been Isolated from *O. gratissimum*

Class	Part Used	Compounds	References
Essential oil	Plant	Thymol, eugenol, methyl chavical	[1]
Essential oil	Plant	Gratissimol	[17]
Mucilage	Seed	Pentoses, hexoses, uronic acid and lipids	[18]
Phytochemical evaluation	Plant	Alkaloids, tannins, flavonoids and oligosaccharides	
Essential oil	Leaves	Eugenol, methyl eugenol, cis-ocimene, trans-ocimene, pinene, camphor, germacrene- D, trans-caryophyllene, farnesene and l-bisabolene	[7]
Essential oil	Leaves	Eugenol, bisabolone and thymol	[19]
Essential oil	Seed	Thymol and eugenol	[20]
Essential oil	Plant	thymol, p-cymene, γ terpene and trans sabiene hydrate	[21]
Essential oil	Plant	Eugenol, 1,8 cineole, linalool, methyl chavicol , methyl eugenol	[22]
Essential oil	Aerial parts	Eugenol, linalool, limonene, methyl eugenol, β -caryophyllene, farnesene, α -terpineol, β -salinene, methyl isoeugeneol, geraniol, α -copaene, bisabolol, α -pinene, p-cymene, fenchone, cubenene, camphene, T-cadinol, γ -eudesmol, sabinene, myrcene, β -bisoboline, α -humelene and β -elemene.	[24]
Essential oil	Aerial parts	Eugenol	[26]
Essential oil	Leaves	Citral, ethyl cinnamate, eugenol, linalool and thymol	[27]
Volatile oils	Leaves	Thymol, γ -terpinene, p-cymene, limonene, terpinolene and 1,8-cineole	[28]
Phytochemical evaluation	Aqueous extract	Tannins,steroids,triterpinoids,carbohydrates,	[29]
Essential oil	leaves	oleanolic acid	[30]

Table 2. Percentage Chemical Constituents Obtained by Different Methods from *O. gratissimum* Leaf

Constituents	Steam Distillation %	Hydrodistillation Using Microwave Oven %	Supercritical CO ₂ %
α -pinene	0.7	-	-
β -pinene	2.6	-	-
1,8-cineole	21.6	22.6	5.6
Ocimene	4.1	1.7	-
α -terpineol	0.7	1.6	-
Eugenol	54.0	34.6	73.1
β -caryophyllene	5.3	10.5	5.6
α -humulene	0.8	1.6	0.8
γ -muurolene	2.3	4.7	1.6
β -selinene	5.5	15.3	7.7
α -selinene	2.6	6.1	2.4

Table 3. Percentage Composition of the Essential oil Obtained from the Fresh Leaves of *O. gratissimum* in Different Seasons Throughout the Year

Class	Compound	Autumn	Winter	Spring	Summer
Phenylpropanoid	Eugenol	56.10	50.99	46.55	44.89
Monoterpenes	1,8-cineole	16.83	33.67	27.13	32.58
	α -pinene	0.84	0.39	0.15	0.62
	β -pinene	2.61	1.35	0.84	2.12
	Sabinene	0.66	0.29	0.17	0.67
	Myrcene	0.92	0.23	0.15	0.68
	Linalool	-	1.69	1.06	1.80
	α -terpineol	0.95	1.61	1.38	1.28
		Except the major 1,8-cineole	5.98	5.56	3.75
Sesquiterpenes	β -elemene	0.18	0.22	0.42	0.24
	Trans-caryophyllene	4.08	1.41	5.29	2.63
	α -humulene	0.61	0.28	0.8	0.4
	Germacrene D	1.43	0.95	2.42	1.15
	Germacrene A	0.16	0.15	0.36	0.21
	α -selinene	1.76	0.90	2.29	1.19
	β -selinene	5.72	2.69	7.41	3.50
Not identified		7.15	3.18	3.58	6.04

Variation of chemical composition of the essential oil of *O. gratissimum* eugenol type was studied for 11 h during the daytime. Microwave oven technique was used for the serial extraction and the obtained oils were analysed by GC/MS. A considerable variation was observed in the eugenol yield,

98% at 12.00 a.m. to 11% at 05.00 p.m. These results show the influence of the solar light on eugenol production and can be useful to indicate the optimal time for collection of the plant (Table 4) [31].

Table 4. Daytime Hourly Variation of the Chemical Composition of *O. gratissimum* Leaf Essential Oil

Constituents	Time of Collection in h.										
	08.0	09.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00	17.00	18.00
β -pinene	3.7	-	-	-	-	-	-	2.0	-	2.7	1.3
Mircene	1.6	-	1.0	-	-	-	-	-	-	0.8	-
1,8-cineole	52.1	32.4	27.4	7.1	-	2.5	-	60.7	32.5	75.5	22.5
Linalool	1.5	-	7.9	-	-	1.4	-	-	-	-	-
4-terpineol	0.6	-	2.3	-	-	2.5	-	-	-	-	-
α -terpineol	1.1	-	1.8	-	-	1.8	-	-	-	-	-
Eugenol	14.0	32.4	29.0	90.6	98.0	91.9	80.3	27.5	67.0	11.4	39.5
β -elemene	0.4	-	8.3	-	-	-	-	-	-	-	-
β -caryophyllene	4.4	-	1.3	-	-	-	7.9	2.9	-	-	11.6
β -malilene	4.8	-	12.2	-	-	-	11.5	2.4	-	-	13.4

Table 1 and Fig. (3) gives the list of few biologically active compounds and their structures that have been isolated from *O. gratissimum* respectively.

5. PHARMACOLOGICAL STUDIES

5.1. Antimicrobial and Antifungal Activity

Honey is reported to have wound healing properties. Study was carried out to investigate the effect of honey as well as those of surfactants on the antibacterial activity of the essential oil of *O. gratissimum*. The antibacterial activity of dispersions of ocimum oil (2%) in methanol, honey, a macrogol blend, nonionic and ionic emulsifiers were assessed by cup-plate method using type bacteria and wound isolates. Honey enhanced the antibacterial activity of ocimum oil to a greater extent than the macrogol blend. The activity of ocimum oil emulsion in cetrimide (cationic) was lower than obtained for cetrimide solution. Emulsion of the oil in sodium lauryl sulphate (anionic) exhibited a slightly higher activity than the solution of the surfactant alone. Although Tween® 20 (nonionic) and aqueous methanol had no activity, the emulsion of the oil in Tween® 20 showed lesser activity than the oil solution in methanol. Honey's inherent antibacterial activity, surfactant charge interaction and the effect of emulsification were adduced to the observed differences in antibacterial activity of the ocimum oil formulations. Findings indicated that honey was a suitable base for ocimum oil especially in the treatment of infected wounds [32].

An investigation of antifungal activity of the essential oil obtained by steam-distillation (1.1% w/w) of the aerial parts of *O. gratissimum* and of an ethanolic extract from the steam-distillation residue was carried out using the agar diffusion method. The results revealed that the essential oil inhibited the growth of all fungi tested, including the phytopathogens, *Botryosphaeria rhodina*, *Rhizoctonia* sp. and two strains of *Alternaria* sp., while the extract from the residue was inactive. The antifungal activity of eugenol was evaluated against a species of *Alternaria* isolated from tomato and

Penicillium chrysogenum. The minimal inhibitory concentrations of eugenol were 0.16 and 0.31 mg/disc for *Alternaria* sp. and *P. chrysogenum*, respectively [26].

Cryptococcal infection had an increased incidence in last few year's due to the explosion of acquired immunodeficiency syndrome. *O. gratissimum* has been reported earlier with *in vitro* activity against some bacteria and dermatophytes. *In vitro* activity of the ethanolic crude extract, ethyl acetate, hexane, chloroform fractions, essential oil, and eugenol of *O. gratissimum* was studied using an agar dilution susceptibility method towards 25 isolates of *Cryptococcus neoformans*. All the extracts of *O. gratissimum* studied showed activity *in vitro* towards *C. neoformans*. Based on the minimal inhibitory concentration values the most significant results were obtained with chloroform fraction and eugenol. It was observed that the chloroform fraction inhibited 23 isolates (92%) of *C. neoformans* at a concentration of 62.5 μ g/ml and eugenol inhibited 4 isolates (16%) at a concentration of 0.9 μ g/ml [19].

The antibacterial activity of different extracts from the leaves of *O. gratissimum* was tested against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Salmonella typhimurium*, pathogenic bacteria that cause diarrhoea. Extracts evaluated included cold water extract, hot water extract and steam distillation extract. Only the steam distillation extract had inhibitory effects on the selected bacteria and the minimum inhibitory concentration ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium*, and 0.001% for *S. typhi* [12].

Largely widespread in tropical countries, *O. gratissimum* has been claimed to possess for many uses in folk medicine. Antifungal activities were carried out by the agar dilution method using five chemotypes. Out of these five chemotypes, ethyl cinnamate showed better activity and was active against dermatophytes and *Scopulariopsis breicaulis*, causing skin mycosis and onychomycosis; against *Cryptococcus neoformans*, implicated in HIV disease and against *Mala-*

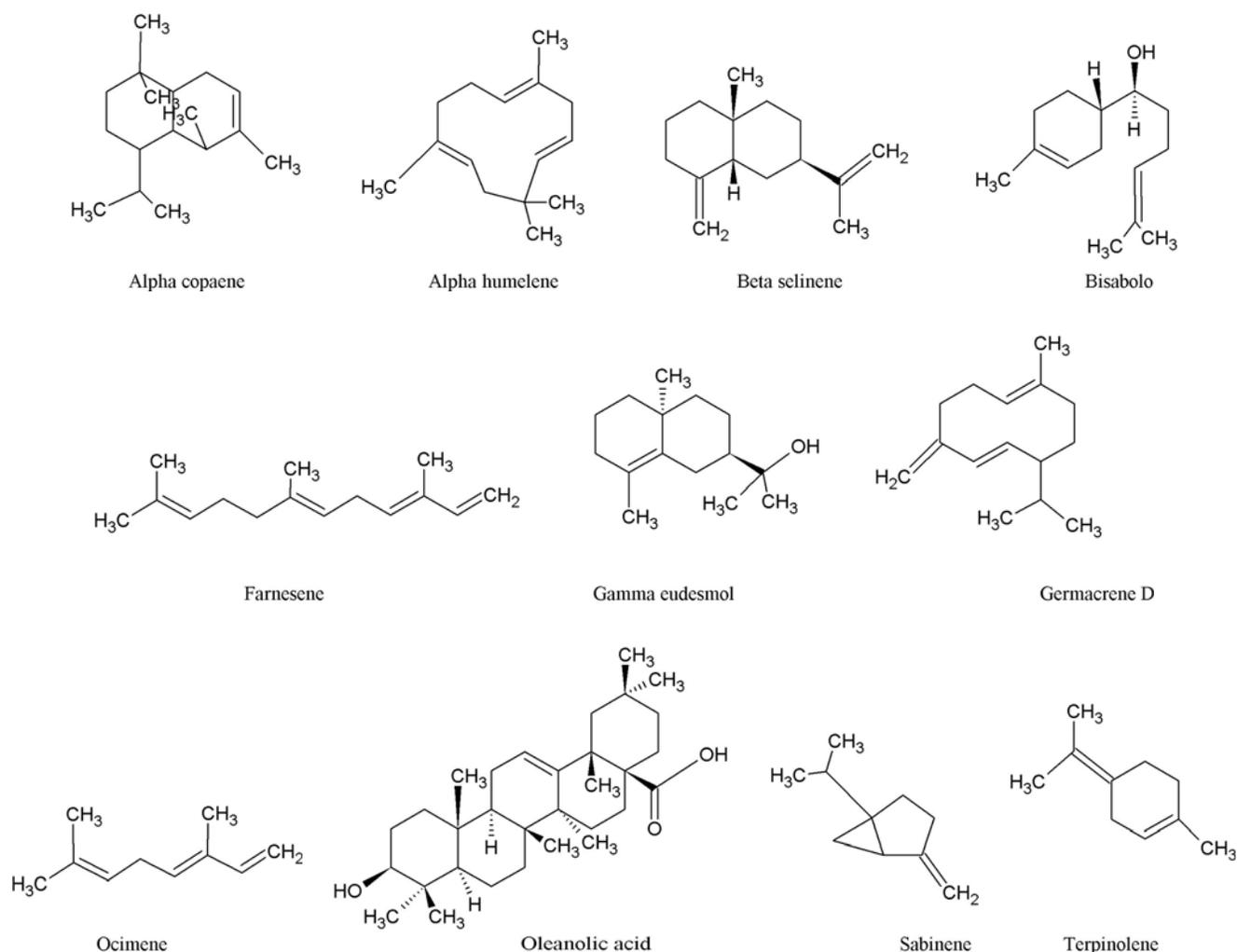


Fig. (3). List of structures of few biologically active compounds that have been isolated from *O. gratissimum*.

ssezia pachydermatis, found in the dog's otitis externa. Due to these polyvalent performances and the sweet fragrance of this natural product, *O. gratissimum* essential oil containing a high level of ethyl cinnamate seems especially suitable for dermatology and cosmetology [27].

An exhaustive study was performed on stem bark parts by Kabir O Akinyemi *et al.*, (2005) [33]. They attributed antimicrobial activity to the aqueous and ethanolic extract of *O. gratissimum*. Both the extracts were active against *S. aureus* and MRSA. They act as bacteriostatic at lower concentration and bactericidal at higher concentration. Minimum inhibitory concentration and minimal bactericidal concentration were found to be between 18.2 to 24.0 $\mu\text{g/ml}$ and 30.4 to 37.0 $\mu\text{g/ml}$ respectively. Their results offer a scientific basis for the traditional use of water and ethanol extracts of *O. gratissimum* against MRSA-associated diseases [34].

The essential oil of *O. gratissimum* inhibited *S. aureus* at a concentration of 0.75 mg/ml. The essential oil was also active against members of the family Enterobacteriaceae. The minimal inhibitory concentrations (MICs) for *Shigella flexineri*, *Salmonella enteritidis*, *Escherichia coli*, *Klebsiella* sp., and *Proteus mirabilis* were at concentrations ranging from 3 to 12 $\mu\text{g/ml}$. The minimum bactericidal concentration of the essential oil was within a twofold dilution of the MIC

for this organism. The compound that showed antibacterial activity in the essential oil of *O. gratissimum* was identified as eugenol [33].

Lima *et al.* (1993) tested *in vitro* antifungal activity of thirteen essential oil obtained from plants against dermatophytes. Of the tested oils, *O. gratissimum* was found to be the most active, inhibiting 80% of the dermatophyte strains tested and producing zones greater than 10 mm in diameter [35].

Hydro-distilled volatile oils from the leaves of *O. gratissimum* from Meru district in Eastern Kenya were evaluated for antimicrobial activity. The antimicrobial activities of the essential oils were evaluated against both Gram positive (*S. aureus*, *Bacillus* spp.) and Gram negative (*E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, *P. mirabilis*) bacteria and a pathogenic fungus *Candida albicans*. The minimum inhibitory concentration of oil for gram negative bacteria ranged from 107 to 750 mg/ml and 93.7 to 150 mg/ml for gram positive bacteria. The minimum inhibitory concentration for the fungus *C. albicans* was 50 mg/ml. The minimum inhibitory concentration values for chloramphenicol ranged from 22.5 to 31.3 mg/ml. The oil had pronounced antibacterial and antifungal activities on all the microbes [7].

The antibacterial effect of *O. gratissimum* extracted from the aromatic plant was investigated against *Listeria monocytogenes* serotype 4a. Agar well diffusion and tube dilution methods were used and the data recorded demonstrated antibacterial activity of the essential oil against the test bacteria. The bacterium was grown at 37°C in a chemically defined or a complex medium, containing essential oil obtained from *O. gratissimum*. At concentrations from 20 to 250 µg/ml, the essential oil progressively inhibited the bacterial growth. The bacteria cultivated on chemically defined medium were more sensitive to essential oil at concentrations of 50, 62.5 and 100 µg/ml in relation to those cultivated in complex medium at 37°C. The agar well diffusion was also evaluated. The results yielded a zone of inhibition of 25mm. These established a good support to the use of this plant in herbal medicine and a base for the development of new drugs and phyto-medicine [36].

The antibacterial activity of different extracts from the leaves of *O. gratissimum* was tested against *S. aureus*, *E. coli*, *S. typhi* and *S. typhimurium*, pathogenic bacteria that cause diarrhoea. The extracts evaluated included cold water extract, hot water extract and steam distillation extract. Only the steam distillation extract had inhibitory effects on the selected bacteria. The MIC ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium*, and 0.001% for *S. typhi* [12].

Effects of leaf extracts of *O. gratissimum* on spore germination and mycelial reduction of the most commonly occurring fungal pathogen causing soft rot of yam tuber were investigated. Fungi isolated from rotted yams were *Aspergillus niger*, *A. flavus*, *Fusarium oxysporium*, *Rhizopus stolonifer*, *Botryodiplodia theobromae* and *Penicillium chrysogenum*. The ethanol leaf extract was most effective followed by cold-water and hot water extracts [37].

Essential oils extracted by hydrodistillation from local plants in Benin, western Africa were evaluated *in vitro* and *in vivo* for their efficacy against *Fusarium verticillioides* infection and fumonisin contamination. *O. gratissimum* was found to be the most effective *in vitro*, completely inhibiting the growth of *F. verticillioides* at lower concentrations over 21 days of incubation. These oils reduced the incidence of *F. verticillioides* in corn and totally inhibited fungal growth at concentrations of 8, 6.4, and 4.8 µl/g, respectively, over 21 days. At the concentration of 4.8 µl/g, these oils did not affect significantly fumonisin production. However, a marked reduction of fumonisin level was observed in corn stored in closed conditions. The oils adversely affected kernel germination at 4.8 µl/g and therefore cannot be recommended for controlling *F. verticillioides* on stored corn used as seeds, when used at this concentration [38].

Hexane extract of *O. gratissimum* leaves and eugenol were investigated for *in vitro* antifungal activity, using agar dilution technique against dermatophytes. The extracts (hexane, chloroform fractions, the essential oil and eugenol) produced antifungal activities against *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum* and *T. mentagrophytes*. The hexane fraction and eugenol were the most active. Hexane fraction inhibited the growth of 100% of dermatophytes at a concentration of 125 µg/ml, while eugenol inhibited the growth of 80% of dermatophytes at this same

concentration. These results show that extracts of *O. gratissimum* are active *in vitro* against human pathogenic dermatophytes [39].

A study was carried out to determine the repellent activity of *O. gratissimum* volatile oil against *Simulium damnosum* (blackflies). A 12 month field study was conducted in three onchocerciasis endemic communities (Idomido, Obio camp, and Ikot Adaha) in Ini Local Government Area of Akwa Ibom State, Nigeria. The results revealed that topical application of 20% v/v concentration of the oil with liquid paraffin as a base reduced the biting rate of *S. damnosum* by 90.2, 81.6, and 79.7%, in Idomido, Obiocamp, and Ikot Adaha respectively. The oil gave protection against the bite of *S. damnosum* for at least 3 h. A total of 710 adult *S. damnosum* were caught by individuals treated with Ocimum oil, as against 4296 caught by the control group. When the flies caught by the treated individuals were dissected none of them were infected with microfilariae of *Onchocerca volvulus* [40].

O. gratissimum leaves from Cameroon are a potential source of essential oil. Bioactivities were tested on the insect pest *Sitophilus zeamais*, which is the major pest of stored maize. Insecticidal activity was tested by putting 20 adult representatives of *S. zeamais* with 20 g of maize grains powdered with various mixtures of essential oil and kaolin (5 and 10%). The tested essential oils of *O. gratissimum* protected 74 % of the test-material against the *S. zeamais* population after 4 days. A direct application of the *O. gratissimum* on the test insects, was found to be 85.7% by knock down effect [28].

The effect of the essential oil of *O. gratissimum* on *Herpetomonas samuelpessoai*, a nonpathogenic trypanosomatid was observed. Parasites were grown at 28 or 37°C, in a chemically defined or a complex medium, containing essential oil obtained from *O. gratissimum*. At concentrations from 20 to 250 µg/ml, the essential oil, progressively inhibited the protozoan growth. The inhibitory concentration (IC₅₀), in defined and complex media, at 28°C was 100 and 91 µg/ml, respectively. Cells cultivated in a chemically defined medium were more sensitive to essential oil at concentrations of 50, 62.5 and 100 µg/ml in relation to those cultured in complex medium at 37°C. In addition, ultrastructural and enzymatic alterations of the trypanosomatid were also evaluated. *H. samuelpessoai* exposed to 100 µg/ml of essential oil, in chemically defined medium at 28°C for 72 h, presented considerable ultrastructural alteration, mainly at mitochondrial level, as showed by transmission electron microscopy. Furthermore, cells cultivated in the presence of 100 µg/ml of essential oil showed a decrease of activity of the succinate cytochrome c reductase enzyme, a typical mitochondrion marker, as compared to untreated cells [41].

All the bacteria were susceptible on a different scale to the undiluted oils. The inhibition zone of the undiluted oil of *O. gratissimum* is more extensive than that of the other oil. The most susceptible strains are *B. cereus* and *E. faecalis*. The least sensible strains are *B. subtilis*, *C. glutamicum* and *E. coli*, while the other ones show a medium susceptibility. The susceptibilities of the strains changed with the dilution of the essential oils with Tween 80. Using a dilution of 1/30 of essential oils all the strains have practically no susceptibil-

ity any more, expect *B. subtilis*. The pure, undiluted essential oils of fresh leaves of *O. gratissimum* showed the most extensive inhibition zones and are, therefore, very effective antimicrobial systems. Table 2 shows the Inhibition zone (mm) using the essential leaf oil of *O. gratissimum* [13].

Alabi, D.A. *et al.* carried out the testing of four botanicals for fungitoxic property. It was observed that *O. gratissimum* exhibited mild activity compared to the other three [42].

Hot and cold water leaf extracts of *O. gratissimum* were effective in reducing the spore germination and radial growth of *Colletotrichum lindemuthianum in vitro* and the growth of the pathogen *in vivo* [43].

Mbata *et al.* showed that *O. gratissimum* oils have properties that can inhibit the growth of *psychrophils* and heat resistant organisms and suggested that the plant and its derivatives can be used for the primary purpose of flavouring foods and for antimicrobial activities [36].

5.2. Ovicidal Activity

The ovicidal activity of the essential oil of *O. gratissimum* and its main component eugenol was evaluated against *Haemonchus contortus*, a gastrointestinal parasite of small ruminants. The oil and eugenol were diluted in Tween 20 (0.5%) at five different concentrations. In the egg hatch test, *H. contortus* eggs were obtained from the faeces of goats experimentally infected. At 0.50% concentration, the essential oil and eugenol showed a maximum eclodibility inhibition. These results suggest a possible utilization of the essential oil of *O. gratissimum* as an aid to the control of gastrointestinal helminthiasis of small ruminants [44].

5.3. Leishmanicidal Activity

Study carried out by Luize PS *et al.* showed that hydroalcoholic extract of *O. gratissimum* showed good leishmanicidal activity against *Leishmania amazonensis* compared to that of *Trypanosoma cruzi*. *O. gratissimum* showed inhibition of 91.5% at a concentration of 100 µg/ml. Along with leishmanicidal activity, haemolytic activity of the extract was also observed. At a concentration of 1000 µg/ml the extract showed 25% lysis of the cell, while no lysis was seen at a concentration of 500 and 100 µg/ml. At the end of 120 min there was increase in lysis of cell to 75% and no lysis was seen at concentration of 500 and 100 µg/ml [45].

The essential oils obtained by hydrodistillation from fresh leaves of *O. gratissimum* growing in Cameroon were analyzed. The effects of these oils on the growth of *Plasmodium berghei* were investigated. Oils showed significant antimalarial activities in the four-day suppressive *in vivo* test in mice. At concentrations of 200, 300 and 500 mg/kg of mouse per day, the essential oil of *O. gratissimum* at the same concentrations were 55.0 %, 75.2 % and 77.8 %, respectively. Chloroquine (10 mg/kg of mouse, positive control) had a suppressive activity of 100 %. [46].

5.4. Antidiarrhoeal Effect

The aqueous extract of the leaves of *O. gratissimum* was screened for antidiarrhoeal effects. The extract inhibited castor oil-induced diarrhoea in rats as judged by a decrease in the number of wet faeces in the extract-treated rats. In addition,

the extract inhibited the propulsive movement of the intestinal contents. On the isolated ileum of guinea-pig, the extract showed no direct action; however, it reduced the responses of the guinea-pig ileum to acetylcholine, nicotine and histamine. The findings suggested that the aqueous extract of the leaves of *O. gratissimum* might elicit an antidiarrhoeal effect by inhibiting intestinal motility, partly *via* muscarinic receptor inhibition [29].

The antidiarrhoeal activities of leaf extracts of *O. gratissimum* were investigated by disc diffusion and tube dilution methods. The extracts were active against *Aeromonas sobria*, *E. coli*, *Plesiomonas shigelloides*, *S. typhi*, and *Shigella dysenteriae*. The leaf extracts were most active against *S. dysenteriae* and least active against *S. typhi*. The sensitivity of the organisms measured in terms of zone of inhibition ranged from 8.00 to 19.50 mm. The minimum inhibitory concentrations were from 4 to 50 mg/ml, while the minimum bactericidal concentration ranged from 8.00 to 62 mg/ml [47].

The anti-diarrhoeal property of the aqueous extract of *O. gratissimum* was investigated in Wistar albino rats. The aqueous leaf extract of this plant, at various doses tested (25, 50 & 100 mg/kg body weight) displayed remarkable antidiarrhoeal activity evidenced by the reduction in the rate of defecation and consistency of faeces in albino rats. The protective role of *O. gratissimum* extract at 100 mg/kg body weight was comparable to that of the reference drug, diphenoxylate (50 mg/kg body weight). *O. gratissimum* extract mimicked the action of adrenaline and noradrenaline on isolated guinea pig ileum by abolishing the acetylcholine induced contraction of the smooth muscles of ileum and also exhibited anti-inflammatory action against agar induced rat paw oedema in the dose range of 100 to 400 mg/kg body weight. Like phenylbutazone, the ability of the extract to block oedemogenesis was more manifest at the second phase after induction of inflammation of the reactions [48].

O. gratissimum leaf extracts have been extensively demonstrated to be effective against the various aetiological agents of diarrhoea, including *Shigellae*. Study investigated the effects of *O. gratissimum* essential oil at sub-inhibitory concentrations of 0.75 and 1.0 µg/ml on virulent and multi-drug-resistant strains of 22 *Shigella* isolates from Nigeria. Compared with untreated *Shigella* strains, *O. gratissimum* caused significant decreases ($p < 0.01$) in extracellular protease activity, o-lipopolysaccharide rhamnose content and incidence of invasiveness mediated as keratoconjunctivitis in guinea pig. The disparity in extracellular protease activity and o-lipopolysaccharide rhamnose between the two treatment groups was also found to be significant ($p < 0.05$), suggesting greater anti-virulent effects of *O. gratissimum* oil at 1.0 µg/ml. Antibiotic susceptibility testing revealed that the essential oil of *O. gratissimum* reduced the MICs of antibiotics to which *Shigellae* showed resistance by 9.8–53.1% and fluoroquinolones by 18.2–45.5%. The results of this study strongly suggest inhibition of extracellular protease and expression of O-LPS rhamnose in *Shigellae* by *O. gratissimum* [20].

5.5. Gastro Intestinal Tract

The relaxant action of the essential oil of *O. gratissimum* is likely to be due to a direct effect on the smooth muscle of the ileum rather than an indirect action on neurotransmitter release, because a full reversal of the contraction induced by

high (60 mM) KCl. Under these conditions, the plasmalemmal membrane of guinea pig enteric neurons is sufficiently depolarised to prevent the generation of action potentials. Additionally, essential oil of *O. gratissimum* was able to completely reverse ACh-induced tonic contractions, in a slightly less potent manner than in KCl-precontracted tissues, in agreement with a direct action of the essential oil on the smooth muscle. It is possible that the relaxant action of essential oil of *O. gratissimum* may be linked to a therapeutic sedative effect of the gastrointestinal tract. It is also possible that the combined effect of several chemical constituents of the plant is responsible for a final therapeutic effect. The principal chemical components identified in the present study were 1,8-cineole and eugenol. Further detailed studies on the components of essential oil of *O. gratissimum* are required to clarify the pharmacological action of this oil on the guinea pig ileum [49].

The effect of aqueous extract of the leaves of *O. gratissimum* on intestinal transit was determined in experimental rats. 10% extracts of powders were made and administered orally to rats at varying doses. Test rats were given the 10% extracts of *O. gratissimum* and control rats received saline instead of extracts. After 30 min, each animal was then given 1.5 ml of a dye solution orally. 1 h after administering the dye each rat was sacrificed and the intestine carefully dissected out. The length of the intestine and the transit point of the orally administered dye were then measured. The transit point was calculated as a percentage of the total length of the intestine. The extracts of *O. gratissimum* caused a reduction in the transit points of the dye. The reduction in transit point, and hence the increase in transit time by both extracts indicates that the plants could be useful at appropriate doses in the control of diarrhoea [50].

The medicinal plant *O. gratissimum* is widely encountered in the Northeast of Brazil where it is used to treat digestive problems. Leaves have an essential oil (EOOG) content whose chemical composition varies according to the time of plant collection. Madeira *et al.* have compared the effects of the EOOG, collected at 08:00 a.m. (EOOG₈) and at 12:00 a.m. (EOOG₁₂), on the relaxation of guinea-pig isolated ileum. Both EOOG₈ and EOOG₁₂ (30–300 µg/ml) reversibly relaxed the spontaneous tonus of the guinea-pig ileum in a concentration-dependent manner, with similar IC₅₀ values. The magnitude of the decrease in resting tonus was similar to that of the recognised smooth muscle relaxant papaverine. EOOG₈ and EOOG₁₂ relaxed 60 mM KCl-precontracted preparations similarly (38.33±9.91 µg/ml and 35.53±6.70), whereas a significantly more potent relaxant effect of EOOG₁₂ compared to EOOG₈ was observed when tissues were contracted using 10 µM acetylcholine. The principal constituents of the essential oil, eugenol and cineole, also relaxed KCl-precontracted preparations, although they were less potent than EOOG. Results showed that the essential oil extracted from the leaves of *O. gratissimum*, collected at different time periods, exerts significant relaxant effects on isolated guinea-pig ileum which may underlie the therapeutic action of the plant [51].

5.6. Wound Healing

Persistent microvascular hyperpermeability to plasma proteins is a characteristic feature of normal wound healing.

Evan's blue dye (20mg/kg body weight) in normal saline was administered intravenously through marginal ear vein of experimental rabbits (n=5). Each animal served as its own control. One hour after Evan's blue dye administration, 0.1ml each of *O. gratissimum* oil, histamine dihydrochloride (30µg/ml) and normal saline were randomly administered by intra-dermal injection at the prepared sites on each of the animals. Increase in vascular permeability was assessed by dye effusion test. Analysis of the differences in vascular permeability between treatment groups showed that, *O. gratissimum* oil, in intensity and duration, was significantly (p<0.05) more effective in increasing cutaneous capillary permeability over a 24 h period after treatment. The ability of *O. gratissimum* oil in increasing vascular permeability may be one of the factors that contribute to its wound healing property [52].

5.7. Anti-Inflammatory

The following study report the inhibitory effect produced by chemical constituents of essential oils of three plants used in traditional medicine as anti-inflammatory and analgesic drugs, *in vitro*, on soybean lipoxygenase L-1 and cyclooxygenase function of prostaglandin H synthase (PGHS), the two enzymes involved in the production of mediators of inflammation. The essential oils were extracted from plants *O. gratissimum* along with two more plants. Among the three essential oils, *O. gratissimum* inhibited the two enzymes, cyclooxygenase function of PGHS and lipoxygenase L-1, with an IC₅₀= 125 µg/ml and 144 µg/ml [53].

5.8. Analgesic Activity

The pharmacological activities of aqueous extracts of *O. gratissimum* was screened for isolated rabbit jejunum (IRJ); rat stomach strip (RSS); and analgesic properties in mice. The extract caused a dose-dependent inhibition of the rabbit jejunum spontaneous pendular movement. The blocking effect on acetylcholine induced contraction was non-competitive in the rat stomach strip since maximum contractions were suppressed and no parallel shift was observed in the curve. The result of the analgesic study showed that the extract evoked a prolongation of reaction time of 85% over 20 min observation time with no overt signs of toxicity. The results suggest the presence of analgesic and spasmolytic activities [54].

5.9. Antimutagenic

Obaseiki-Ebor *et al.* investigated the antimutagenic activity of *O. gratissimum* leaves extract along with other three edible vegetable plant. *O. gratissimum* showed inhibitory activity against *S. typhimurium* [55].

6. CYTOTOXIC ACTIVITY

Cytotoxic study was carried out on oleanic acid isolated from leaves of ethanolic extract of *O. gratissimum*. Effective dose of the compound at 50% concentration (ED₅₀) to be tested against a panel of six human solid tumor cell lines viz. human lung carcinoma (ED₅₀ 3.16 µg/ml), human breast carcinoma (ED₅₀ 2.46 µg/ml), human colon adenocarcinoma (ED₅₀ 3.12 µg/ml) human renal carcinoma (ED₅₀ 3.13 µg/ml), human prostate adenocarcinoma (ED₅₀ 2.58 µg/ml)

human pancreatic carcinoma (ED₅₀ 3.47 µg/ml), and yellow fever mosquito larvae *Aedes aegypti*. (LC₅₀ 4.4 µg/ml) [30].

The essential oils isolated from the leaves of *O. gratissimum* were tested for their cytotoxic activity against P388 leukemia cells. The IC₅₀ of the Cymbopogon oil was found to be 5.7 µg/ml while that of Ocimum oil was 10.8 µg/ml. The mixture of the oils (1:1 v/v) showed an IC₅₀ value of 10.2 µg/ml with no synergism in the cytotoxic activity [27].

6.1. Antihypertensive Effect

Intravenous treatment of conscious deoxycorticosterone acetate DOCA-salt hypertensive rats with the essential oil of *O. gratissimum* (EOOG) induced a hypotensive effect that seems related to an active vascular relaxation. To corroborate this hypothesis, the present study examined the vascular effects of EOOG and its main constituent, eugenol (EUG). In conscious DOCA-salt hypertensive rats, the EOOG-induced hypotension was reversible and remained unchanged by intravenous pretreatment with propranolol (2 mg/kg). In isolated aorta preparations with intact endothelium from DOCA-salt hypertensive rats, EOOG (1-1000 µg/mL) and EUG (0.006-6 mM) relaxed the phenylephrine-induced contraction similarly with IC₅₀ = 226.9 µg/mL and 1.2 (0.6-2.1) mM, respectively. Vasorelaxant effects of EOOG were significantly altered by removal of the vascular endothelium IC₅₀ = 417.2 µg/mL. In a calcium-free medium, the CaCl₂-induced contractions were significantly reduced and even abolished by EOOG at 300 and 1000 µg/mL, respectively, whereas EOOG (1000 µg/mL) did not have any significant effect on caffeine-induced contractions. Similar results were obtained with EUG (1.8 and 6 mM) on both CaCl₂ and caffeine-induced contractions, respectively. The data suggest that hypotensive responses to EOOG in DOCA-salt hypertensive rats are due to an active vascular relaxation, which is partly dependent upon the integrity of the vascular endothelium and seems predominantly mediated through an inhibition of plasmalemmal Ca₂⁺ influx rather than Ca₂⁺-induced Ca₂⁺ release from the sarcoplasmic reticulum [56].

The cardiovascular effects of intravenous treatment with the essential oil of *O. gratissimum* (EOOG) and its main constituent, eugenol (Eug) were investigated in the experimental model of deoxycorticosterone acetate (DOCA-salt)-hypertensive rats. In both conscious DOCA-salt hypertensive rats and their uninephrectomized controls, intravenous bolus injections of EOOG (1-20 mg/kg) or Eug (1-10 mg/kg) induced dose-dependent hypotension and bradycardia. Treatment with DOCA-salt significantly enhanced the maximal decreases in mean aortic pressure (MAP) elicited by hexamethonium (30 mg/kg, intravenous) as well as the hypotensive responses to both EOOG and Eug without affecting the bradycardia. However, the enhancement of EOOG-induced hypotension in hypertensive rats remained unaffected by intravenous pretreatment with either hexamethonium (30 mg/kg) or methylatropine (1 mg/kg). These results show that intravenous treatment with EOOG or Eug dose-dependently decreased blood pressure in conscious DOCA-salt hypertensive rats, and this action is enhanced when compared with uninephrectomized controls. This enhancement appears related mainly to an increase in EOOG-induced vascular smooth relaxation rather than to enhanced sympathetic nervous system activity in this hypertensive model [57].

6.2. Cardiovascular Effect

The cardiovascular effects of intravenous administration of the essential oil of *O. gratissimum* (EOOG) were investigated in rats. The present study examined: (i) whether the autonomic nervous system is involved in the mediation of EOOG-induced changes in mean aortic pressure (MAP) and heart rate (HR); and (ii) whether these changes could be attributed, at least in part, to the actions of eugenol, the major constituent of EOOG. In both pentobarbitone-anaesthetized and conscious rats, intravenously administered bolus injections of EOOG (1-20 mg/kg) elicited immediate and dose-dependent decreases in MAP and HR. These responses to EOOG were of the same order of magnitude irrespective of whether the animal was under general anaesthesia. Pretreatment of anaesthetized rats with bilateral vagotomy did not significantly modify the EOOG-induced dose-dependent hypotension, whereas it significantly reduced the bradycardia at the highest dose used. In conscious rats, intravenous injections of bolus doses (1-10 mg/kg) of eugenol also elicited immediate and dose-dependent decreases in MAP and HR. Intravenous pretreatment of conscious rats with either methylatropine (1 mg/kg) or hexamethonium (30 mg/kg) significantly reduced the EOOG-induced dose-dependent bradycardia without affecting the hypotension. These data show, for the first time, that intravenous administration of EOOG to either anaesthetized or conscious rats induces an immediate and significant hypotension and bradycardia, which appear to be due, at least in part, to the actions of the major constituent of EOOG, eugenol. This may suggest that the hypotensive activity of EOOG results from its vasodilatory effects directly upon vascular smooth muscle [58].

6.3. Immunostimulatory Effect

Immunostimulatory activity of ethanolic leaf extract of *O. gratissimum* was investigated in albino rats using immunologic/haematologic indices. The rats were dosed orally with standard inoculum of *E. coli* (NCIB 86) of 1x10⁷ cfu/ml. The extent of infection was carried out by checking the haematologic indices before, during and after treating the infection with ethanolic extract of *O. gratissimum*. The animals were divided into four groups. The first group was dosed with 8 ml of the standard inoculum for two days. The second group was dosed with the standard inoculum and treated with 250 mg/ml of *O. gratissimum* ethanolic leaf extract. The third group was dosed with the extract alone while the fourth group was given normal saline and this serve as the control. The infected rat that was not given the extract showed a WBC count of 4,800 mm³ before infection and increased to 13,800 mm³ during infection and later decreased to 2,400 mm³ after oral administration of the extract. The Packed Cell Volume (PCV) was 57% before infection, 47% during infection and 35% after treatment. The neutrophil and lymphocyte percentage in the differential count were 48 and 51% before infection, 62 and 37% during infection and 74 and 26% after treatment of infection respectively. For the rats treated with extract, it showed a WBC count of 5,000 mm³ before infection, which decreased to 3,000 mm³ during infection and 1,700 mm³ after infections. It had a PCV, neutrophil and lymphocyte value of 55, 47 and 52% before infection, 50, 42 and 58% during infection and 33, 44, 56% after infection. The rats given the extract of *O. gratissimum*

alone showed a value of 4,400 mm³, 48, 41 and 58% for the WBC, PCV, neutrophil and lymphocyte before infection, a value of 3,200 mm³, 63, 43 and 57% during infection and a value of 2,100 mm³, 25, 42 and 56%, respectively after infection. The control showed only a significant increase in WBC with a value of 4,000 mm³ before infection, to 6,100 mm³ after infection and back to 4,400 mm³ after infection. The urinalysis showed a pH value of 5, was negative for glucose, ascorbic acid, ketone, nitrite, protein and bilirubin, normal for urobilinogen and negative blood value for all the groups before infection. The infected rat without administration of extract showed a pH of 7 and became positive for ketone, nitrite, protein and bilirubin urobilinogen and blood value of Ca. 250 during infection while others remain the same. After infection, the pH turned to 6, became negative for other parameters except protein and bilirubin while the treated rats remain negative. The ethanolic leaf extract of *O. gratissimum* was found effective in inhibiting/preventing the disease condition after infection and was capable of reducing excessive breakdown of red blood cells and neutralizing toxin produced by the organism [59].

6.4. Antidiabetic Effect

The hypoglycemic effects of the aqueous leaves extract of *O. gratissimum* was investigated in streptozocin-induced diabetic rats. The extract was administered once at the dose of 250, 500 and 1000 mg/kg body weight. The aqueous extract at the dose of 500 mg/kg significantly lowered blood glucose level ($P < 0.05$) of the diabetic rats by 81.3% after 24 h of extract administration. Preliminary phytochemical screening revealed the presence of reducing sugars, cardiac glycosides, resin, tannins, saponins, glycosides, flavonoids, glycerin and steroids. The median lethal dose (LD50) in rats was calculated to be 1264.9 mg/kg body weight. The leaves extract of *O. gratissimum* was reported to possess antidiabetic activity in streptozocin-induced in diabetic rats [60].

6.5. Hepatoprotective Effect

Aqueous extract of the leaves of *O. gratissimum* were used to evaluate the hepatoprotective and diuretic effects. Extracts were administered orally by means of polythene cannula to male rabbits. The drug given at dose of 0.4g/kg body weight showed increase in luminal diameter of the collecting duct. At 0.8 g/kg body weight further increase in luminal diameter was observed. Marked increase in the luminal diameter of the renal tubules was observed when the extract dose was increased to 1.6g/kg body weight, showing a dose response effect of the extract on the structure of the kidney, thus indicating the use of *O. gratissimum* as a diuretic. The structure of the liver also showed dose-dependent changes when exposed to various doses of the extract. At a dose of 0.4g/kg body weight of the extract, there was a generalized edema/hypertrophy of the hepatocytes resulting in a marked widespread, sinusoidal congestion. About 80% of the hepatocytes showed cytoplasmic compaction and disintegration, with some apoptotic bodies as well as nuclear pyknosis. Kupfer cells were many and were trapped within the sinusoids indicating a degenerative/necrotic process. Increasing the dose of extract to 0.8g/kg body weight produced similar results. There was a reduction in all the parameters observed. There was less hepatocytic edema/hypertrophy

resulting in slightly widened sinusoidal spaces. Hepatocytes showed reduced cytoplasmic compaction and disintegration with less prominent apoptotic bodies. In addition there was mild leukocyte infiltration and compaction was observed in the hepatocytes with mild tissue lesion or damage as compared with the 0.4g/kg treated group. The group of animals treated with 1.6 g/kg of the extract depicted a reestablishment of the normal structure of the liver. Hepatocytes showed no sign of oedema hypertrophy resulting in sinusoids with larger (normal) diameter thereby indicating the usefulness of *O. gratissimum* as an hepatoprotective agent [2].

6.6. Hair Loss

Hair loss is one of the most feared side effects of cancer chemotherapy. Preliminary study investigated by Orafidiya *et al.* showed the efficacy of the leaf essential oil of *O. gratissimum* (Ocimum oil) in promoting hair growth in cyclophosphamide-induced hair loss. Shaved sites, 4 cm², were created on the flanks of 6 groups each of 7 freshly weaned 4-week old rats. Four groups (groups 2, 4, 5 and 6) were treated with 30 mg/kg cyclophosphamide i. p. daily to simulate changes seen in human chemotherapy-induced hair loss. Ocimum oil was administered topically alone (group 3) or in combination with cyclophosphamide in groups 2, 4 and 5 for 14 days and in group 6 for 8 days. Group 1 received no test substance. Tissue biopsies were obtained from 2 rats selected at random from each group on treatment day 9 for histological examination. Surviving animals were further observed for 7 days after treatment. Histopathology and gross morphologic observations for hair re-growth at shaved sites revealed active follicular proliferation in Ocimum alone and cyclophosphamide + Ocimum oil treated groups. Ocimum oil may, therefore, be capable of enhancing normal hair growth and promoting follicular proliferation in cyclophosphamide-induced hair loss [61].

6.7. Antioxidant Capacity

The antioxidant capacity of essential oils obtained by steam hydrodistillation from five species of the genus *Ocimum* were evaluated using a high-performance liquid chromatography-based hypoxanthine/xanthine oxidase and DPPH assays. The yield of oils from the leaves of the five species was variable with the greater amount obtained from *O. gratissimum* (3.5%). In the hypoxanthine/xanthine oxidase assay, strong antioxidant capacity was evident in all the oils. Antioxidant capacity was positively correlated ($r = 0.92$, $p < 0.05$) with a high proportion of compounds possessing a phenolic ring such as eugenol, while a strong negative correlation ($r = -0.77$, $p > 0.1$) with other major volatiles was observed. These correlations were confirmed to a large extent in the DPPH assay. The data generated with *Ocimum* species indicates that essential oils obtained from various herbs and spices may have an important role to play in cancer chemoprevention, functional foods, and in the preservation of pharmacologic products [62].

Extracts from the leaves of *O. gratissimum* were investigated for their phytochemical constituents and for antioxidant activity. Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in both methanolic and aqueous extracts. The methanolic extract of *O. gratissimum* had a DPPH scavenging activity of 84.6% at 250

µg/ml and a reductive potential of 0.77 at 100 µg/ml. These values were comparable with those of gallic acid, 91.4% at 250 µg/ml and ascorbic acid, 0.79 at 60 µg/ml as standards for DPPH scavenging activity and reductive potential, respectively. These findings suggest the rich phytochemical content of *O. gratissimum* and its good antioxidant activity [4].

6.8. Suspending Activity

Mucilage extracted from *O. gratissimum* seeds were subjected to toxicity studies for its safety and preformulation studies for its suitability as a suspending agent. Zinc oxide suspensions were prepared and compared with different concentrations of *O. gratissimum* mucilage, tragacanth and sodium CMC. The mucilage extracted is devoid of toxicity. The mucilage was found to be a superior suspending agent to tragacanth and was comparable to sodium CMC. Studies indicate that the extracted mucilage may be a good pharmaceutical adjuvant, specifically a suspending agent [63].

6.9. Central Nervous System Activity

Cristiana M. Murbach Freire *et al.* carried out the study to investigate whether seasonal variations in composition of essential oil of *O. gratissimum* are accompanied by changes in pharmacological properties; using experimental procedures to investigate the central nervous system activity. The essential oils obtained in each season were capable of increasing the barbiturate-induced sleeping duration. The greatest effect was obtained with the preparation from autumn, and the least effect was observed with that from winter, which was not active in the lesser dose administered. Eugenol was the most abundant compound in the essential oil from each season, with the greatest relative percentage detected in autumn (56.10%). The greatest activity (enhanced 7.9 times in relation to their TW group) was observed in the preparation from autumn, which had 16.83 % 1,8-cineole. Essential oil collected in spring (27.13 %) increased 3.1 times the duration of sleep, in summer (32.58%) the increase was 3.0-fold while in winter (33.67 %) the increase was 2.5 times. The phytoconstituent 1,8-cineole is a monoterpene that has stimulant activity upon CNS. Thus, it is possible to suggest that the decrease in the amount of this compound facilitates increase in sleeping time [6].

7. ANTICONVULSANT ACTIVITY

The experimental models used to evaluate the anticonvulsant activity, MES and PTZ tests, are assumed to identify anticonvulsant drugs effective against generalized tonic-clonic partial seizures and generalized clonic seizures, respectively. The anticonvulsant activity observed in the essential oil from *O. gratissimum* extracted in the Spring could be also related to the higher amount of sesquiterpenes. In fact, synergic effect among compounds could not be discarded, since minor compounds such as linalool and myrcene present sedative and/or anticonvulsive effects [64].

7.1. Nematicidal Activity

Nematicidal activity was attributed to eugenol reported to be earlier as major component of the *O. gratissimum* oil by Chatterje *et al.* 1982 [65].

7.2. Disintegrating Activity

Ravikumar *et al.* carried out study on the disintegrant properties of seed mucilage of *O. gratissimum*. It was found that the disintegration time of tablet in oral cavity was found between 43-68s and 45 -65s for *O. gratissimum* mucilage powder, and seed powder respectively. Wetting time was found between 40-88 s and 38-75 s for *O. gratissimum* mucilage powder and seed powder respectively. All designed formulations using *O. gratissimum* mucilage powder and seed powder showed rapid dissolution and percent cumulative drug release at the end of 5 min was 75-97% for all formulations. The conventional marketed tablet of Metformin HCl required around 35 min. for same amount of drug to be released [66].

CLINICAL STUDIES

A recent study tested a range of concentrations of *O. gratissimum* oil in comparison to 10% benzoyl peroxide and a placebo, over a period of 4 weeks, for the reduction of acne lesions in a population consisting mainly of students. *O. gratissimum* oil was incorporated at concentrations of 0.5%, 1%, 2% and 5% v/v in four different bases (polysorbate 80, cetomacrogol, petrolatum and alcohol) resulting in 16 parallel experimental groups. The numbers of lesions were counted daily by investigators throughout the test period and the time taken to achieve a 50% reduction relative to pre-treatment was noted for each subject. Preparations containing 2% and 5% *Ocimum* oil in alcohol and 5% in cetomacrogol were significantly more active than benzoyl peroxide ($P < 0.05$), while 2% oil in cetomacrogol had similar activity to the reference product. The most active 5% preparations produced skin irritation but the authors considered a 2% preparation in cetomacrogol to be suitable for the management of acne [67].

Another clinical study was carried out by the same author using a combination of *O. gratissimum* and Aloe vera gel. The study was carried out as follows. 84 subjects presenting with clinically significant *Acne vulgaris*, were randomly assigned into seven groups and treated with different test preparations (2% v/v *Ocimum* oil lotion containing graded concentrations (0-100%) of aloe gel, placebo or control preparations). Samples tested were applied to the face after washing morning and evening. The numbers of inflammatory lesions (papules and pustules) were counted prior to application and daily for 4 weeks. The efficacy of the preparations was rated in terms of product activity (1/D), which is the reciprocal value of the number of days taken to achieve 50% reduction in lesion count. It was found that Aloe vera gel enhanced the antiacne properties of *Ocimum* oil. The oil or its combination with aloe vera gel was found to be effective than 1% Clindamycin in the treatment of *Acne vulgaris* [68].

TOXICOLOGY

Oral and intraperitoneal acute toxicity and the sub chronic intraperitoneal toxicity of the essential oil of *O. gratissimum* was investigated. The acute toxicity test involved oral and intraperitoneal administration of graded doses of *Ocimum* oil prepared as a 4%v/v emulsion to 2 groups each of 30 rats and mice. LD₅₀ and LD₁₀₀ were determined for both routes and species. In the sub chronic tox-

icity study, 25 male Sprague-Dawley rats were randomized into 4 test groups and a control. Organs and blood samples were taken for analyses after a 30 days treatment period. A dose dependent sedative effect of Ocimum oil was observed during the acute toxicity study in mice and rats and in the subchronic test in mice and rats. Evidence of treatment, route, and dose-dependent toxicity were detected in both studies. Change in weight of the testes, hearts, kidney, intestines and lungs of the rats were statistically insignificant. Data analysis of blood biochemical, haematological and histopathological findings showed significant differences between control and treated groups and revealed that Ocimum oil is capable of invoking an inflammatory response that transits from acute to chronic on persistent administration. While the study revealed that Ocimum oil might be better tolerated when orally for systemic delivery, the oil has toxic potentialities that should not be overlooked [69].

It was also reported that *O. gratissimum* can affect macrophage functioning and can also be hepatocarcinogenic [70].

CONCLUSION

This review has covered the morphology and microscopy, natural products, pharmacology and clinical studies of the plant *O. gratissimum*. *O. gratissimum* is a plant with much potential and is useful in many diseases. Hence this article will be useful to those researchers interested in validating the hidden truth which has not been scientifically validated

Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases [71-73]. Numerous drugs have entered the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies have been done on a large number of Indian botanicals, a considerably smaller number of marketable drugs or phytochemical entities have entered the evidence-based therapeutics.

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