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REVIEW ARTICLE

Phytochemistry and pharmacological properties of *Ocimum gratissimum* (L.) extracts and essential oil - A critical review

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ABSTRACT

The plant *Ocimum gratissimum* is well-known from the ancient Indian medicine system. *O. gratissimum* has wide variety of therapeutic applications. Folk medicine says that it can help with headaches, fevers, diarrhoea, pneumonia, and other ailments. *O. gratissimum* contains several bioactive constituents widely used as food additives, food colorants, pharmaceuticals, pesticides, and fragrances. This review discusses up to this point data on the phytochemical composition, pharmacological studies of *Ocimum gratissimum* extracts and oil from numerous locations worldwide. Pertinent data of *O. gratissimum* was earned from numerous electronic scientific databases, and additional information was obtained from books, thesis and different relevant websites. The yield of the *O. gratissimum* essential oil (OGEO) varied between 0.12% and 1.66% on a dry basis, depending on the variety, plant parts and extraction methods used. OGEO was predominantly accumulated phenylpropenes, (55.7%-57.3%) followed by sesquiterpenes (27.5% - 38.1%), and monoterpenes (4.0%-16.1%). Eugenol, germacrene-D, β-ocimene, 1,8-cineole, β-selinene, caryophyllene, γ-murolene, p-cymene, thymol, γ-terpinene, α-thujene and β-myrcene are major constituents of OGEO from various origins. These compounds are chief bioactive substances responsible for pharmacological activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, gastrointestinal, insecticidal, and larvicidal activities.

Keywords: *Ocimum gratissimum*; Essential oil; Eugenol; β-ocimine; Phytochemistry; Biological activities

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INTRODUCTION

In India, *Ocimum gratissimum* (L.) is known as Ram tulsi and belongs to the Lamiaceae family. It is a native of Asia, with most of its distribution and cultivation taking place in India, Sri Lanka, Nepal, Nigeria, and West Africa (Nadkarni, 1999). Cough, cold, stomach pain, anxiety, headache, and bronchitis are treated with this plant's leaves in teas and infusions (Rabelo et al., 2003; Matasyoh et al., 2007). In several countries, *O. gratissimum* has been widely used in traditional medicine. Also, it is used for medical, condiment, and culinary purposes worldwide. The plant is used to treat epilepsy, high fever, and diarrhoea in Nigeria's coastal areas (Effraim et al., 2003). Decoctions of the leaves are used to treat mental illness in the Savannah areas (Akinmoladun et al., 2007). It's also used to treat fungal infections and fever, colds, and catarrh in Nigeria (Ijeh et al., 2005). *O. gratissimum* roots decoction is used as a sedative for children in the Brazilian tropical forest (Istiana et al., 2006). In India, *O. gratissimum* plant has been used to treat Sunstroke, headache, influenza, as a diaphoretic, antipyretic, and for its anti-inflammatory function (Gupta et al., 2002).

Essential oils are extracted from plants using hydrodistillation, steam distillation, microwave, ultrasound-assisted, and supercritical fluid methods (Azwanida, 2015; Ashokkumar et al. 2020a; Ashokkumar et al., 2020b). The hydrodistillation process is the most widely used by researchers worldwide, owing to the lower cost of the Clevenger apparatus and the use of water as a solvent (Ashokkumar et al., 2020c). The yield of essential oil from *O. gratissimum* varies between 0.21 and 0.70 per cent (Dubey et al., 2000; Matasyoh et al., 2007; Joshi, 2013; Ashokkumar et al., 2020d). Several studies on the OGEO have been conducted worldwide (Matasyoh et al., 2007; Joshi, 2013; Matasyoh et al., 2007; Padalia and Verma, 2011). Phynelypropene (eugenol & methyl eugenol), sesquiterpenes (germacrene D & caryophyllene, γ- muurolene), monoterpenes (γ-ocimene), and other constituents are abundant in the essential oil of *O. gratissimum* (Matasyoh et al., 2007; Padalia and Verma, 2011; Joshi, 2013). According to many scientific reports, *O. gratissimum* has antioxidant, antimicrobial, anti-inflammatory, anthelmintic, cardiovascular, antimutagenic, antidiarrheal and others (Offiah and Chikwendu, 1999; Aguiyia et al., 2000; Lahlou et al., 2004; Trevisan et al., 2006; Joshi, 2013; Ajayi et al., 2014; Gontijo et al., 2014; Aderibigbe and Idowu, 2020). This review investigates the information regarding phytochemical compositions and their potential pharmacological properties of *O. gratissimum*. Nevertheless, additional advanced research studies are needed to understand the mechanism of bioactive constituents and their consumption in animals and humans, which can help protect human beings from various diseases.

O. GRATISSIMUM ESSENTIAL OIL (OGEO) AND ITS COMPOSITION

The EO yield from *O. gratissimum* varied between 0.12% and 1.66%, on a dry basis, depending on the variety, plant parts and extraction methods used (Table 1). The EO estimation by various methods is summarized in Table 1. The profiling of EO of aerial parts of *O. gratissimum* sampled from Western Ghats of southern India predominantly exhibited eugenol (54.42%), germacrene D (15.43%), β-ocimene (12.37%), caryophyllene (4.59%), and γ-muurolene (3.05%), (Ashokkumar et al., 2020d). However, the Brazil grown leaves of OGEO chiefly contained eugenol, 1,8-cineole, and β-selinene. Furthermore, Benin grown aerial parts of *O. gratissimum* essential oil was predominant in p-cymene, thymol, γ-terpinene, α-thujene and myrcene (Table 2). OGEO was predominantly accumulated phenylpropenes, (55.7%- 57.3%) followed by sesquiterpenes (27.5% - 38.1%), monoterpenes (4.0%-16.1%), (Joshi, 2017; Ashokkumar et al., 2020d). The yield of minor constituents of OGEO include β-pinene (0.1%), borneol (0.1%), carvenone (0.1%), α-humulene (0.1%), elemol (1.2%), caryophyllene oxide (0.15%), α-thujene (0.17%), γ-elemene (0.2%), γ-terpinene (0.2%), α-pinene (0.2%), camphor (0.3%), humulene (0.3%), δ-Cadinene (0.4%), sabinene (0.5%), linalool (0.5%), β-bourbonene (0.5%), α-copaene (0.6%), cis-verbenol (0.7%), terpin-4-ol (0.7%), isobornylformate (0.9%), isoledene (1.0%), (Joshi, 2017; Ashokkumar et al., 2020d). The molecular structures of major essential oil constituents isolated from *O. gratissimum* are shown in Figure 1.

PHARMACOLOGICAL PROPERTIES OF OGEO AND EXTRACTS

The OGEO and extracts have various pharmacological properties including antioxidant, antibacterial, antifungal, insecticidal, acaricidal and other miscellaneous activities that are summarized in Table 3.

Antioxidant activity

Antioxidants are naturally or artificially derived compounds that could prevent the free radical formation and suppress chronic and degenerative diseases by scavenging free radicals (Haliwell, 2000). Natural antioxidants derived from herbal sources are currently gaining popularity (Velioglu et al., 1998).

Table 1. Yield of essential oil from *O. gratissimum*

Technique or method	Plant parts	Oil yield (%)	Authors
Hydro-distillation	Leaves	1.66	Melo et al. (2019)
Hydro-distillation	Aerial parts	0.60	Ashokkumar et al. (2020d)
Hydro-distillation	Aerial parts	0.65	Padalia et al. (2014)
Hydro-distillation	Aerial parts	1.10	Verma et al. (2016)
Hydro-distillation	Leaves	0.12 – 0.78	Matasyoh et al. (2008)
Hydro-distillation	Aerial parts	0.65-0.78	Kpadonou Kpoviessi et al. (2012)
Steam distillation	Leaves	1.33	Ibeh et al. (2017)

Table 2. Major essential oil composition of *Ocimum gratissimum* L.

Origin	Plant parts	Major constituents	Yield (%)	Authors
Western Ghats, South India (Kerala)	Aerial parts	eugenol	54.4%	Ashokkumar et al. (2020d)
		germacrene D	15.4%	
		β-ocimene	12.4%	
		caryophyllene	4.6%	
		γ-muurolene	3.1%	
Brazil	Leaves	Eugenol	74.3%	Melo et al. (2019)
		1,8-cineole	15.2%	
		β-selinene	2.8%	
North India	Aerial Parts	eugenol	78.0%	Padalia et al. (2014)
		germacrene D	4.4%	
Peninsular India (Karnataka)	Aerial Parts	eugenol	53.0%	Verma et al. (2016)
		caryophyllene oxide	7.2%	
		(Z)-β-ocimene	3.5%	
Kenya	Leaves	eugenol	68.8 %	Matasyoh et al. (2008)
		methyl eugenol	13.2%	
Portugal	Aerial Parts	Thymol	48.1%	Martins et al. (1999)
		p-cymene	12.5%	
Colombia	Leaves	eugenol	43.2%	Benitez et al. (2009)
		1,8-cineole	12.8%	
		β-selinene	9.0%	
Benin	Aerial Parts	*p*-cymene	28.1–53.8%	Kpadonou Kpoviessi et al. (2012)
		thymol	3.3–29.1%	
		γ-terpinene	1.1–10.9%	
		α-thujene	3.4-10.8%	
		myrcene	4.2–8.3%	

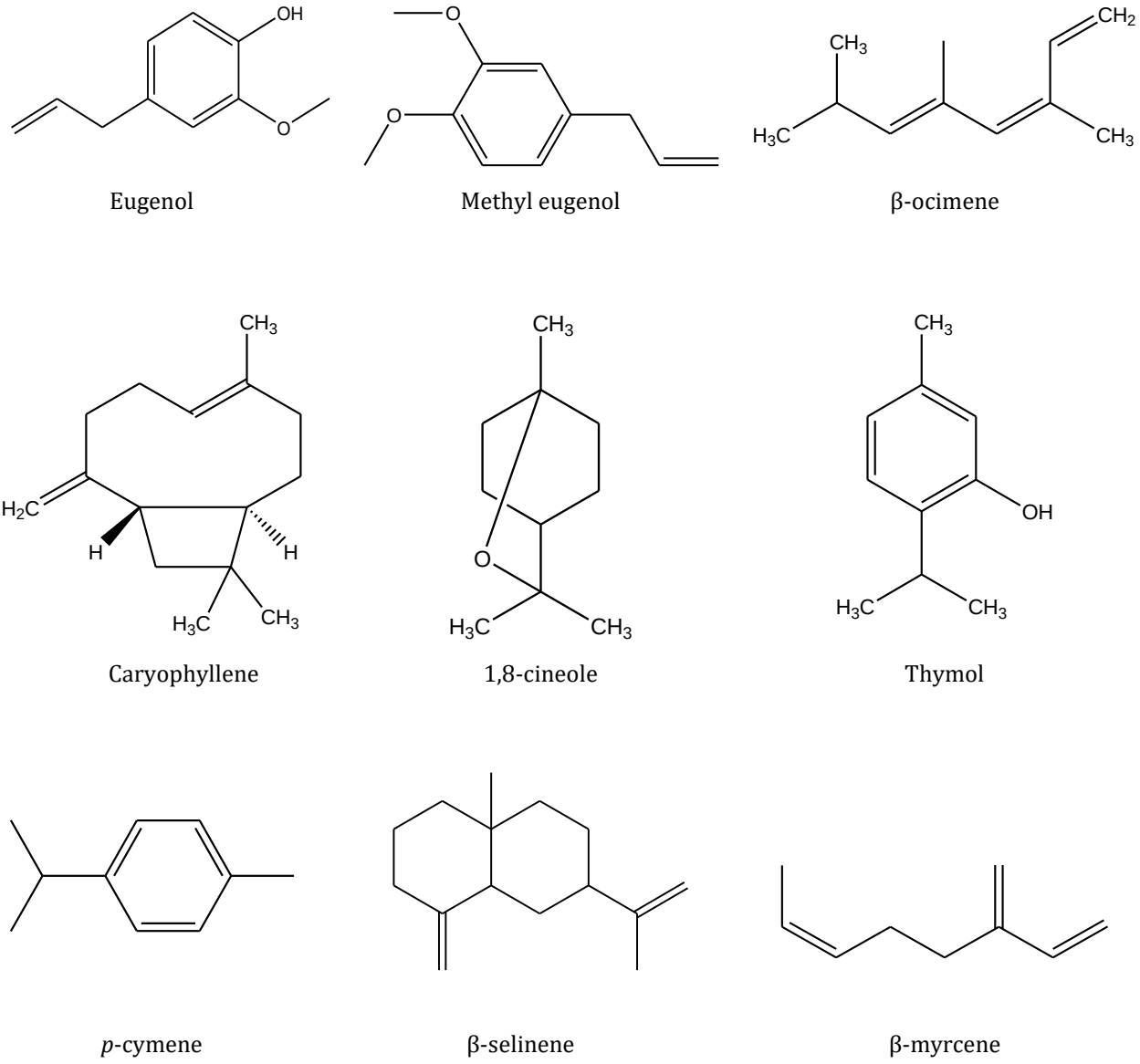

Figure 1. Molecular structures of major essential oil constituents isolated from *O. gratissimum*

The leaves and aerial parts of *O. gratissimum* are rich sources of antioxidant substances that neutralize free radicals by preventing other components' oxidation. In vitro, antioxidant assays such as DPPH, iron chelating, ABTS, nitric oxide, and hydroxyl radical scavenging indicated that *O. gratissimum* as a potent free radical scavenging activity with IC_{50} values of 470, 330, 133, 83 and 260 g ml^{-1} (Venuprasad et al., 2014) In another study, DPPH and ABTS models, OGEO demonstrated comparative antioxidant activity with IC_{50} values of 23.66 and 23.91 g ml^{-1}, respectively. This study also noted that eugenol had marginally lower antioxidant activity than OGEO. In contrast, *O. sanctum* oil had very low antioxidant activity (Joshi et al. 2013) Yung-Wei et al. (2013) studied the antioxidant and cytoprotective activity of *Ocimum gratissimum* extracts against hydrogen peroxide-induced toxicity in human HepG2 cells and results showed that 66.7 µg ml^{-1} concentration reduced up to 80% of the free radicals.

Antimicrobial activities

In vitro antifungal activity of OGEO (MIC: 0.06 - 0.25 mg/ml; MFC: 6.25- 12.50 mg ml^{-1}) studied against *Candida albicans*. The best inhibitory effect was noted @ 0.24 mg ml^{-1} (Kpadonou Kpoviessi et al.,

2012). Ethanolic extract of *O. gratissimum* was tested antimicrobial effects against *Actinobacillus actinomycetemcomitans* in human dental plaque. The 0.6% concentration of extract have potential antimicrobial activity compared with 0.2% chlorhexidine and dimethyl sulfoxide (DMSO), positive and as and negative control, respectively (Eswar et al., 2016). In another study, the minimum inhibitory concentration and minimum bactericide concentration of 0.24 mg ml^{-1} and 0.95 mg ml^{-1} respectively of the OGEO had significant antibacterial activity (Kpadonou Kpoviessi et al., 2012). Joshi (2017) studied the antibacterial potential of OGEO and eugenol against 13 bacterial species like *S. aureus, S. epidermidis, S. faecalis, Micrococcus flavus, Micrococcus luteus, Bacillus subtilis, E. coli, Klebsiella pneumoniae, Serratia marcescens, Proteus vulgaris, P. mirabilis, Pseudomonas aeruginosa,* and *Salmonella typhimurium.* The MIC concentration of eugenol were 0.33 – 3.33 mg ml^{-1} and significant inhibitory activity observed @ 1.04 mg ml^{-1}against *S. aureus.* The MIC concentration essential oil was 0.29 -1.51 mg ml^{-1} and a significant inhibitory effect noted @ 0.29 mg ml^{-1} against *S. marcescens.*

Insecticidal activity

The insecticidal activity of OGEO was tested against *Sitophilus zeamais* (a major stored pest of maize) by mixtures OGEO (5%) and kaolin (10%). Results remarked that OGEO on the test insects was possessed an 85.7% knockdown effect (Jirovetz et al., 2005). The OGEO was substantially more active on target insects @ LC_{50}/ LD_{50} of 39.6 mg l^{-1} on *C. quinquefasciatus*, 72.2 µg $adult^{-1}$ on *M. domestica* and 30.2 µg $larva^{-1}$ on *S. littoralis* (Benelli et al., 2019). Other insecticidal and acaricidal activities were also summarized in Table 3.

Miscellaneous activities

Anxiolytic activity

O. gratissimum extract has shown potential lipid peroxidation with an IC value of 735 µg ml^{-1}. Also, *O. gratissimum* extract found to possess a substantial anxiolytic effect at a dosage of 400 mg kg^{-1} body weight as analyzed by open field and elevated plus-maze tests in mice (Venuprasad et al., 2014).

Gastrointestinal activity

In another study, the ovicidal activity of OGEO and its predominant constituent eugenol was evaluated against *Haemonchus contortus*, a gastrointestinal parasite of small ruminants. *H. contortus* eggs were obtained from faces of goats experimentally infected and used for egg hatch test. OGEO and eugenol at a dosage concentration of 0.50% remarked maximum eclodibility inhibition (Pessoa et al., 2002). This study also suggested that a possible utilization of the OGEO and eugenol to control gastrointestinal helmintosis of small ruminants.

Antinociceptive activity

Alabi et al. (2019) investigated the antinociceptive activity of traditional analgesic drug polyherbal-TADP (100, 200 and 400 mg kg^{-1}) in the hot plate test and acetic acid-induced nociception in mice. TADP (200 and 400 mg kg^{-1}) significantly extended the latency time in the hot-plate test. Dose-dependent inhibition was observed at a TADP concentration of 100–400 mg kg^{-1}.

Antiviral activity

Eugenol from *O. gratissimum* has reported that antiviral activity, which inhibits the HSV-1,2 replication. The bioactive constituent thymol also destructs the virion of HSV-1 (Tshilanda et al., 2020). Other miscellaneous activities were summarized in Table 3.

CONCLUSION

In this review, we discuss what we know about the phytochemistry and pharmacological properties of *O. gratissimum*, a plant that has long been used to treat a range of ailments in ancient and modern India. Cough, cold, stomach pain, anxiety, headache, and bronchitis have all been treated with *O. gratissimum*, according to conventional Indian medical treatizes. *O. gratissimum* is reliable over a long history, and it is now essential to know if new pharmacological trials on *O. gratissimum* are available to determine the conventional uses. We show that several recent *in vitro* and *in vivo* pharmacological studies have confirmed *O. gratissimum* traditional usage.

More than 75 secondary metabolites have been isolated from *O. gratissimum* based on currently available knowledge, with eugenol being the most significant bioactive compound that has shown many potential health benefits. Furthermore, the beneficial constituents of O. gratissimum extracts and OGEO have been thoroughly investigated (Table 3). However, there are gaps in the research studies on O. gratissimum, and we have made suggestions for some topics that should investigate further.

First, studies on metabolites' structural characterization in *O. gratissimum* leaves and aerial parts are highly restricted, based on currently available phytochemistry reports. Second, very little research has been done to date on how to preserve OGEO's shelf-life quality.

Table 3. The activities of *O. gratissimum* extracts and essential oil components

Pharmacological activities	Extract/ essential oil	*In vitro*/ *In vivo*	Target/ Model	Control(s)	IC 50/ Dosage	Results / Remarks	Reference
Antibacterial activity	Essential oil	*In vitro*	*Staphylococcus aureus*	Positive: Doxycycline	MIC : 0.24mg ml^{-1} MBC: 0.95mg ml^{-1}	Moderate antibacterial activity	Kpadonou Kpoviessi et al. (2012)
Antibacterial activity	Essential oil	*In vitro*	*E. coli*	Positive: Doxycycline	MIC: 0.48mg ml^{-1}	Noteworthy antibacterial activity	Kpadonou Kpoviessi et al. (2012)
Antibacterial activity	Essential oil	*In vitro*	*S. aureus, S. epidermidis, S. faecalis, Micrococcus flavus, M. luteus, Bacillus subtilis, E. coli, Klebsiella pneumoniae, Serratia marcescens, Proteus vulgaris, P. mirabilis, P. aeruginosa, Salmonella typhimurium*	Positive: Erythromycin & Amikacin	MIC: 0.29 to 1.51 mg ml^{-1}	Best inhibitory effect @ 0.29 mg/ml against *S. marcescens*	Jhoshi, (2017)
Antibacterial activity	Eugenol	*In vitro*	*S. aureus, S. epidermidis, S. faecalis, Micrococcus flavus, M. luteus, Bacillus subtilis, E. coli, Klebsiella pneumoniae, Serratia marcescens, Proteus vulgaris, P. mirabilis, P. aeruginosa, Salmonella typhimurium*	Positive: Erythromycin & Amikacin	MIC: 0.33 to 3.33mg ml^{-1}	Best inhibitory effect @ 1.04 mg/ml against *S. aureus*	Jhoshi, (2017)
Antifungal activity	Essential oil	*In vitro*	*Candida albicans*	Positive: Nystatin Negative: Solvent	MIC: 0.06 to 0.25 mg ml^{-1} MFC: 6.25 to 12.50 mg ml^{-1}	Best inhibitory effect @ 0.24 mg/ml against *Candida albicans*	Kpadonou Kpoviessi et al. (2012)
Antioxidant activity	Essential oil	*In vitro*	DPPH ABTS	- -	IC_{50}: 23.66 µg ml^{-1} IC_{50}: 23.91 µg ml^{-1}	Significant antioxidant activity observed	Jhoshi, (2013)

Antioxidant activity	Eugenol	*In vitro*	DPPH ABTS	- -	IC_{50}: 27.16 µg ml^{-1} IC_{50}: 32.16 µg ml^{-1}	Eugenol showed slightly weaker antioxidant activity compared to essential oil of *O. gratissimum*	Jhoshi, (2013)
Anthelmintic activity	Acetone extract	*In vitro*	Adult *H. placei* nematodes using adult worm motility assay	Negative: Normal Saline	IC_{50}: 5-60 mg ml^{-1}	Best anthelmintic activity recorded @ 56.04 mg/mL against H. placei	Segun & Sunday (2020)
Anti-inflammatory activity	Hydroethanolic extract	*In vivo*	Rats anesthetized by intraperitoneal injection of 25 mg/kg of thiopental sodium	Positive: - Negative: Distilled water	100, 200 or 400 mg kg^{-1} b.w	The inhibition by the extract was not dose dependent as it was 15.2, 26.7 and 22.4% for 100, 200 and 400 mg/kg respectively	Ajayi, et al. (2014)
Anti-inflammatory activity	Aqueous extract	*In vivo*	Carrageenan-induced paw oedema in rats.	Negative: Distilled water	100, 200 and 400 mg kg^{-1}	Concentration 400 mg kg^{-1} significantly reduced malondialdehyde concentration and increase glutathione level in the carrageenan-induced rat paw	Alabi et al. (2019)
Antinociceptive activity	Essential oil	*In vivo*	Swiss albino mice (25-30g) induced pain. Writhing and formalin test	Positive: Indomethacin	30, 100, 300mg kg^{-1} (p.o)	Dose dependent inhibition observed. OGEO possessed antinociceptive properties in the writhing and formalin test	Rabelo et al. (2003)
Antinociceptive activity	Aqueous extract	*In vivo*	Acetic acid-induced nociception in mice. Hot plate test	Negative: Distilled water	200 and 400mg kg^{-1}	TADP (200 and 400 mg kg^{-1}) was significantly extend the latency time in the hot-plate test. Dose dependent Inhibition was observed at TADP concentration of 100–400 mg kg^{-1}.	Alabi et al. (2019)
Gastroprotective activity	Methanolic extract	*In vivo*	Stress induced ulcer in rats	-	200, 400, 800 mg kg^{-1}	Significantly reduced ulcer indices in dose dependent manner	Akah et al. (2007)

Hypoglycemic activity	Methanolic extract	*In vivo*	Alloxan-induced diabetic rats	Negative: Normal saline	400 mg kg^{-1}	Intraperitoneal treatment with 400 mg/ kg of methanolic extract significantly reduced in blood sugar level in both normal and diabetic rats by 56 and 69%, respectively	Aguiyia et al. (2000).
Antidiabetic activity	Aqueous extract	*In vivo*	Intraperitoneal administration of (65 mg/kg), Type 1 Diabetes mellitus (DM 1) rats	Negative: Distilled water	Not reported	The blood glucose concentration of all the diabetic groups was significantly raised compared to normal control. Treatment with OG and insulin showed potent antioxidant activity	Okon and Umoren (2017)
Insecticidal activity	Essential oil	*In vivo*	Cockroach (*Nauphoeta cinerea)* nymphs (20 days of age) were used	-	LC_{50}: 50 to 1000 µg of oil per ml of air.	Mortality rates were observed every 12 hours for 1 day. OGEO has substantial insecticidal properties at lethal concentration (LC50) of 516 µg ml^{-1}	Rodrigues et al. (2020)
Acaricidal activity	Essential oil	*In vivo*	*Rhipicephalus microplus* (Acari: Ixodidae)	-	LC_{50}: 0.84 mg ml^{-1} LC_{50}: 1.58 mg ml^{-1}	December (LC_{50}: 0.84 mg ml^{-1}) and September (LC_{50} :1.58 mg ml^{-1}) oils obtained in the dry season were the most active. Study remarked that seasonal variation in the chemical composition of the OGEO influences its acaricidal activity	Silva Lima (2018)

Note: -, Not reported; MIC, Minimum inhibition concentration; MBC, Minimum bactericide concentration; MFC, Minimum fungicidal concentration; IC_{50}, Inhibitory concentration; LC_{50}, Lethal concentration 50%

Third, some biological activity studies used very high dose concentrations, others lacked comparison with standard positive and negative controls, and others lacked the determination of MIC values. Fourth, *O. gratissimum* has numerous therapeutic effects on antioxidant, anti-inflammatory, antibacterial, antifungal, insecticidal, acaricidal, and other miscellaneous activities. These studies were performed only in animal and cell models, and clinical investigations have rarely been implemented in humans. Future studies need to focus on studying structural characterization of metabolites, shelf-life quality of OGEO, proper experimental setup conduct with negative or positive control and correct MIC values, and finally, clinical investigation implemented with humans is essential.

DISCLOSURE STATEMENT

The author declares no competing interests

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