

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *OCIMUM GRATISSIMUM L.*

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ABSTRACT

The development of antibiotic resistance in microorganisms is a global challenge for the clinicians, pharmacist and research scientists leading to the development of new medicinal formulations that are effective and easily consumable. The plant yielding essential oil with chief constituent as eugenol has been identified as an important compound with strong inhibition of bacteria, and storage fungi. *Ocimum gratissimum* is an aromatic shrub occurring in warm tropical regions has been used in traditional medicine in India to cure various ailments in general and as an antimicrobial agent in particular. The literature surveyed reveals that the plant oil exhibit strong potentiality against gram negative and gram positive bacteria along with diverse human and plant fungi. As a natural product, the plant oil is safer as food preservatives and stored grain protectant and has wide application in cosmetics and perfumery industries. This comprehensive review is attempted to provide the valuable information on antimicrobial activities of the plant essential oil that will be used to explore and develop a standard therapeutics system for the management of clinical and multiple drug resistant microorganisms.

Keywords: *Ocimum gratissimum*, Essential oil, Antibacterial, Antifungal

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INTRODUCTION

Multiple drug resistance (MDR) and synthetic food—a great concern to human health

The rise in antibiotic-resistant microbes in last five decades or more has emerged as a big crisis and a serious threat in every region of the world [1]. The increasing ineffectiveness in the antibiotic activities used for controlling plant and animal pathogen [2-4] has become a big challenge among researchers, clinicians and policymakers. Antimicrobial synthetic products used in food industries are even not safer as for the human health is concerned, leading to chemical toxicity [5]. Although the new science of molecular-microbial interaction helps in understanding and resolving the antibiotic-resistant complex mechanism, but the more success lies in discovery of new medicines to control infections in this twenty-first century resistance era [1] and also to provide valuable information on medicinal plant, their parts used and methods of preparation for treating various diseases [6]. Altogether these and many more allied issues have drawn curiosity in searching for a new drug formulation [7,8] that are safer as food preservative [5] and can control diseases and food contamination caused by microbial activities.

Plant essential oil-an alternative to MDR microbes

The plant essential oils (EO) complex mixtures of mono-and sesquiterpenoids with hundreds of constituents [9] derived as secondary metabolites from aromatic and other higher plants [4, 10-11] can be a better alternative in protecting from infections and combating antimicrobial drug-resistant (ADR) pathogens [8]. Natural products have no or fewer side effects [12, 13] compared to synthetic chemicals that are blended with food products [13]. In recent years, scientific research has focused more in discovering and identifying local plant species with bioactive compounds as an essential oil, their characterization, extraction and purification processes and application in drug industries [14]. Presently 25 to 50% of drugs are available from plants [15] and numerous other aromatic plants are under evaluation for their valuable essential oil [13]. The traditional use of plant essential oil in folk medicine, fragrance, food flavouring and preventing food spoilage and their antimicrobial activities in plant and animal models is well documented [4, 16-17]. Most important thing that the researchers insisted is in restoring the bioactive compounds of essential oils by

combining them with other products (like antibiotics), for generating synergistic actions of the obtained products [9, 18-19].

***Ocimum gratissimum*-exploring potentiality as an essential oil yielding plant in tropics**

Ocimum gratissimum L. commonly known as clove basil belonging to family Lamiaceae is an important aromatic and medicinal plant existing wild or cultivated in various tropical and subtropical parts of the globe. The plant possesses two unique features firstly it contains essential oil with diversity in chemical composition and water stress tolerance capacity. Plant oil is considered the Mother Nature's chemical factory [20] shows a unique composition of alcohols, aldehydes, ketones, esters, lactones, oxides, peroxides [21], tannins [22] and flavonoids [23]. The plant obtained from various regions in Africa mainly contain eugenol, thymol, methyl eugenol, camphor, (Cis)-ocimene, (trans)-ocimene, β -pinene, gramicidin D, (trans)-caryophyllene, Γ -terpinene and p-cymene [24-26]. Brazilian and Asian races contain phenylephrine (eugenol) as the chief constituent [27-29] along with sesquiterpene (caryophyllene), monoterpenes (β -ocimene) [25], bornyl acetate and some non terpene components [30]. There is scientific evidence about the eugenol which is a hydroxy phenylpropene possessing significant antimicrobial activity against bacteria and fungi mainly human pathogens, multi-drug resistant and food spoilage microorganism [31]. Moreover, plant flavonoids and tannins which are an important constituent of *O. gratissimum*, possess unique antibacterial activities against the plant and human bacterial pathogens as reported in earlier studies either in the same or other plants oil [32-34].

The studies related to the efficacy of essential oil of *Ocimum gratissimum* against gram-negative [25-28], gram-positive bacteria [25-26, 35], fungi [30, 36], and its regular consumption as spice without affecting the activities of conventional antibiotics [37], makes it a strong antimicrobial herbal product throughout the tropics.

Moreover, the plant shows drought tolerant capacity without undergoing any change in cell structure and composition, thus making it another strong candidate along with other plants to combat drought conditions [38]. The use of plant volatile oil in inhibiting microorganism of economic importance is well approved and justified in the literature. This systematic review is attempted to explore the antimicrobial potentiality of clove basil oil for wide application in food and drug industries.

Antimicrobial activities of *Ocimum gratissimum*

The aromatic properties of *O. gratissimum* and their use for various ailments dates long back in the ancient past history is mentioned in great Indian Medicinal epic 'Charaka Samhita', 'Sushruta Samhita' [39] and 'Materia Indica' [40]. Different mechanism has been applied to test the efficacy of plant oil to inhibit microbes *in vitro* and *in vivo*.

Antibacterial activities of *Ocimum gratissimum*

The essential oil (EO) of the plant shows a high range of antibacterial activity against animal and human pathogenic gram positive and gram negative bacteria (table 1). The essential oil expresses strong inhibitory

action against oral swab bacteria *K. pneumonia* and *P. vulgaris*, thus proving effective mouthwashes [41].

According to a study, the plant oil was found effective against human pathogenic *Staphylococcus aureus* (Rosenbach) causing skin infection and *Staphylococcus choleraesuis* (Smith) causing food infection and non-pathogenic *Bacillus subtilis* (Ehrenberg) Cohn with the minimal inhibitory concentration (MIC) value 1, 1.10 and 0.60 mg/ml respectively [42]. The plant extract is a good anti-diarrheal agent showing a remarkable inhibition of *E. coli* [43-44], *Shigella sp.* and *Salmonella sp* [43] *Salmonella enteritidis* [26], *Shigella flexineri* [25, 28] *Salmonella typhi*, *Shigella dysenteriae*, *Aeromonas sobria*, and *Plesiomonas shigelloides*, with strongest action against *S. dysenteriae* [44].

Table 1: Antimicrobial activities of *O. gratissimum* oil against bacteria and fungi

Microbes	Sources
A. Bacteria	
i. Gram Negative	
1. <i>Staphylococcus choleraesuis</i>	[26]
2. <i>Escherichia coli</i>	[19, 25-26, 43-48, 51, 53-56]
3. <i>Aeromonas sobria</i>	[44]
4. <i>S. typhi</i>	[25, 50-51]
5. <i>S. marcescens</i>	[28]
6. <i>P. aeruginosae</i>	[25]
7. <i>P. mirabilis</i>	[25, 47]
8. <i>K. pneumonia</i>	[25, 41, 49]
9. <i>Klebsiella sp.</i>	[26]
10. <i>Shigella sp.</i>	[43, 46, 83]
11. <i>S. dysenteriae</i>	[44]
12. <i>Plesiomonas shigelloides</i>	[44]
13. <i>Shigella flexineri</i>	[26, 29]
14. <i>Salmonella sp.</i>	[43, 46]
15. <i>Salmonella enteritidis</i>	[2, 26]
16. <i>Salmonella typhimurium</i>	[51, 54]
17. <i>Salmonella enterica</i>	[25]
18. <i>Pseudomonas aeruginosa</i>	[47, 56]
19. <i>Proteus vulgaris</i>	[26, 41]
20. <i>Vibrio cholerae</i>	[49]
ii. Gram-Positive	
1. <i>Enterococcus faecium</i>	[42]
2. <i>Enterococcus faecalis</i>	[48]
3. <i>Staphylococcus aureus</i>	[19, 25, 42, 46-47, 50-51, 53-54, 57-58, 84]
4. <i>Bacillus subtilis</i>	[42, 48]
5. <i>Bacillus sp.</i>	[25]
6. <i>Bacillus cereus</i>	[29]
7. <i>Listeria monocytogenes</i>	[25, 31, 58]
8. <i>Listeria innocua</i>	[58]
9. <i>Streptococcus faecalis</i>	[42, 56]
10. <i>Streptococcus agalactiae</i>	[86]
B. Fungi	
i. Human pathogenic fungi	
1. <i>Basidiobolus haptosporus</i>	[61]
2. <i>B. ranarum</i>	[61]
3. <i>Trichophyton rubrum</i>	[61]
4. <i>T. mentagrophytes</i>	[61]
5. <i>Tinea versicolor</i>	[60]
6. <i>Candida albicans</i>	[25, 47, 54, 62-64],
7. <i>C. krusei</i>	[57, 63]
8. <i>C. parapsilosis</i>	[63-64]
9. <i>C. tropicalis</i>	[63]
10. <i>Cryptococcus neoformans</i>	[62, 64-65]
11. <i>Aspergillus flavus</i> ,	[64]
12. <i>A. fumigatus</i>	[64]
13. <i>A. niger</i>	[64]
14. <i>Microsporum canis</i>	[66]
15. <i>M. gypseum</i>	[19]
16. <i>Trichophyton rubrum</i>	[19]
17. <i>T. mentagrophytes</i>	[19]
ii. Plant pathogenic fungi	
1. <i>Rhizoctonia sp.</i>	[69]
2. <i>R. Solani</i>	[67]
3. <i>Botryosphaeria rhodina</i>	[69]

4.	<i>Penicillium chrysogenum</i>	[69]
5.	<i>Alternaria</i> sp.	[69]
6.	<i>Alternaria padwickii</i>	[36]
7.	<i>Bipolaris oryzae</i>	[36]
8.	<i>P. citrinum</i>	[71]
9.	<i>C. sphaerospermum</i>	[71]
10.	<i>Aspergillus flavus</i>	[58, 70]
11.	<i>A. niger</i>	[48, 70]
12.	<i>A. ochraceus</i>	[70]
13.	<i>A. parasiticus</i>	[70]
14.	<i>A. fumigatus</i>	[58]
15.	<i>Fusarium solani</i>	[70]
16.	<i>F. moniliforme</i>	[75]
17.	<i>F. verticillioides</i>	[72-73]
18.	<i>F. oxysporum</i>	[68, 70]
19.	<i>F. graminearum</i>	[70]
20.	<i>Mucor</i> spp	[70]
21.	<i>Sclerotium rolfsii</i>	[74]
iii.	Food contamination fungi	
1.	<i>Aspergillus</i> spp	[78]
2.	<i>A. flavus</i>	[76]
3.	<i>A. ochraceus</i>	[77]
4.	<i>Penicillium</i> spp	[78]
5.	<i>Penicillium expansum</i>	[77, 82]
6.	<i>P. verrucosum</i>	[77]
7.	<i>Fusarium</i> spp	[78]
8.	<i>Scopulariopsis</i> spp	[78]

There are studies that supported this hot water and ethanol leaf extracts inhibiting *Staphylococcus aureus* [45-46], *E. coli*, *Salmonella* sp. and *Shigella* sp. [46] whereas other work supported metabolic leaf extract showing inhibitory action against *E. coli* [47-48], *Bacillus subtilis* [48], *S. aureus*, *P. aeruginosa* and *P. mirabilis* [47]. The inhibition of *E. coli* and *B. subtilis* bacterial isolates using ethyl acetate extract of *O. gratissimum* was also reported [48]. Another study justifies aerial parts of the hexane fraction of the plant having anti-diarrheal activity against *V. cholera* [49]. Some observation reported aqueous extracts suppressing *S. typhi* strains with MBC and MIC values of 55 and 40 mcg/ml [50]. Various other observations found that the essential oil processed through steam distillation expresses high inhibition of *S. aureus*, *E. coli*, *S. typhimurium* and *S. typhi* [51], or even obtained through hydro-distillation shows strong efficacy against *Bacillus* sp. *S. typhi*, *P. aeruginosae*, *P. mirabilis*, *K. pneumonia* [25,52] along with *S. aureus* and *E. coli* [25,52-54], *S. typhimurium* [54], *Salmonella enteritidis* [2] and also restrict extracellular protease enzyme kinetics of *E. Coli* [55].

O. gratissimum essential oil was reported to exhibits significant inhibition of organisms that cause urinary tract infections (UTI) as evidenced in various studies. The result shows that essential oil possesses strong action against UTI *Serratia marcescens* [28] *Enterococcus faecium* [42], *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* sp. which may be credited to the presence of eugenol [26]. Some report proposes that the hexane fraction of the aerial parts of the plant shows antibacterial action against *K. pneumoniae* causing pneumonia and UTI [49] whereas another study illustrates methanolic plant extract being most effective against *Enterococcus faecalis* [48]. The strong inhibitory action of ethanolic leaf extract of the plant against UTI *Staphylococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* was also reported [56]. The time of collection of plant parts and flowering shows a significant impact on disease curing process. Plant oil collected during a pre-flowering stage at 7 am shows high activity against *S. aureus* with MIC 0.24 mg/ml [57]. The concentration-dependent plant oil exhibit strong antibacterial action against food contaminating *Bacillus cereus* [29] and ethanolic leaves extract in 250 mg/ml of plant oil proved best in inhibiting food borne pathogenic bacteria *Listeria monocytogenes* [31]. Similar results were also obtained by other studies that exhibit an effective inhibition of *Salmonella enterica* [30], *L. monocytogenes* [25, 58], along with two other bacterial strains *Listeria innocua* and *S. aureus* [58]. Further, the observation discloses that *L. innocua* stained with

carboxyfluorescein diacetate had a decline in fluorescence intensity (FI) of cells exposed to plant oil compares to unexposed cells [58].

Antifungal activities of *Ocimum gratissimum*

Human pathogens

O. gratissimum essential oil exhibits an effective fungicidal activity against a wide range of human fungal pathogens as evidenced in various literatures (table 1). Plant oil with five chemotypes is very common folk medicine and among this ethyl cinnamate is very popular Indian chemotype with antifungal activities [59]. Dried or fresh plant samples are utilized to treat superficial mycosis in young children with strong anti-dermatophyte activity against infected scalp and hand [60]. The plant extract has potentiality in suppressing the growth of *Basidiobolus haptosporus*, *B. ranarum*, causing subcutaneous infection in children below ten years, along with *Trichophyton rubrum* and *T. mentagrophytes* that cause skin, feet and fingernail infections in human [61].

Literature survey discloses that essential oil shows potent antifungal properties. There are various studies providing evidence of the use of plant oil in inhibiting *Candida albicans* the causal organism of skin infection in humans [54, 62-64] and *Aspergillus* molds including *A. flavus*, *A. fumigatus* and *A. niger* [64]. The methanolic [47] and in some cases hydro-distilled leaf extract was also found effective against this fungus [25, 52]. A study suggests that plant oil has potentiality in suppressing other groups of *Candida* genus like *C. parapsilosis* [63, 64], *C. krusei* and *C. tropicalis* providing a shield to HIV patients against candidiasis infection [63]. The crude plant oil [62, 64] and even the eugenol and chloroform fraction of crude extracts of plant exhibit an effective action against *Cryptococcus neoformans* isolate that causes central nervous and lung disorders, showing potentiality in protecting AIDS patients against *Cryptococcal* infection [65]. According to another study, the hexane fraction and eugenol extract of the plant leaf at a concentration of 125-micron ml (-1) suppresses 100% and 80% growth of dermatophytes *Microsporum gypseum*, *M. canis*, *Trichophyton mentagrophytes* and *T. rubrum* [66].

Plant pathogens

O. gratissimum essential oil is a strong antifungal agent against plant pathogens as proved in various studies. As a natural product, they can be utilized to the management of a variety of pests in vegetable, cereals and stored grains. Several studies observed that plant oil exhibit a remarkable inhibition of *A. niger* causal organism of black

mold in vegetable and fruit [45], *R. Solani*, a soil-borne plant pathogenic fungus [67] and *F. oxysporum* f. sp lycopersici and eight other phytopathogenic fungi [68]. The ethanolic extract of aerial parts of the plant provides evidence of suppressing plant pathogens, *Rhizoctonia sp.*, *Botryosphaeria rhodina* and two strains of *Alternaria sp.* (*Alternaria sp.* (A1) and *Alternaria sp.* (A2)-tomato wilt fungi) which are mainly due to the presence of eugenol a phenolic compound with fungitoxic properties. Further, this eugenol tested for anti-fungal action expresses significant control of *Alternaria sp.* (A1) and *Penicillium chrysogenum* [69]. The fresh leaves oil expresses strong efficacy against mycoflora *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *Fusarium solani*, *F. oxysporum*, *F. graminearum* and *Mucor sp* with a minimal fungicidal concentration ranging between 5.5 to 8.0 $\mu\text{l/ml}$ [70], while other study supported methanolic leaf extract being effective against *A. niger* [48]. The plant extract can act as a promising fungitoxic agent against molds *P. citrinum* and mycoflora *C. sphaerospermum* [71], whereas the plant essential oil shows remarkable inhibition of *Alternaria padwickii* and *Bipolaris oryzae*, the seed-borne fungi of rice, remaining active even up to six days of storage [36]. The plant oil with eugenol as the main ingredient exhibits a strong anti-fungal action against *F. verticillioides* [72, 73] and also suppresses fumonisin contamination level in closed conditions in Maize [73]. *O. gratissimum* leaf ash shows strong potentiality in inhibiting germination of *Sclerotium rofsii*, a soil-borne facultative pathogen in wheat [74].

Plant oil has shown promising inhibitory action against three food spoilage and mycotoxin fungi, *F. moniliforme*, *Aspergillus fumigatus* and *A. flavus* and remains stable even in changing pH environment hence making it an important food preservative [75]. A study reported that the plant oil possess shelf life enhancer property and can act as an anti-aflatoxigenic agent against aflatoxin and fungal contamination of spices with the potentiality to restrict aflatoxin B1 (AFB1) formation of *A. flavus* [76]. The volatile oil also exhibits an effective food preservation property as reflected by various researchers. The pH and concentration-dependent study conducted on fungicidal activity of EO on mycotoxigenic strains each from *P. expansum*, *A. ochraceus* and *P. verrucosum* reveals a strong activity against these strains [77]. The investigation on efficacies of six essential oils against *Aspergillus*, *Penicillium*, *Fusarium* and *Scopulariopsis* genera for preservation of cheese wagashi reported *O. gratissimum* as the second best essentials oil in suppressing these molds [78].

Synergistic properties of essential oil in combination with other products

The characteristic feature of the clove basil essential oil to freeze the activity of microorganism that developed resistance against the antibiotic drug should be exploited in discovering new formulations in which the sovereignty of the natural product and highly influential antibiotics should be sustained. In this context, various studies are undertaken focusing on developing a blended product with more synergism. The combination of clove basil volatile oil with antibiotics [79] and other biologically active substances shows an effective synergism resulting strong therapeutic potentiality of the product. In traditional practice, it is reported that a combination of honey and clove basil oil results in a highly synergetic product that possesses a strong wound healing capacity in humans [80]. Plant oil possesses the anti-septical property and with hydrophilic bases exhibits an effective antibacterial action against pimples, boil and wound [81]. Another research reports that the mixture of various fractions of plant essential oil against two strains of fungus *Penicillium expansum* MRC 6935 and MRC 6939, the causal organism of the rotting of vegetables and fruits (like apples), reported that the obtained product shows strong synergistic effects against strain MRC 6935 with 0.77 NDRcfu (Number of Decimal Reduction of colony forming units). This effect can be used in enhancing the antimicrobial action of essential oil slowing down the concentrations of the oil required in producing an antimicrobial effect without impact on the edible product [82].

According to a study, the combination of antibiotics fluoroquinolones and *O. gratissimum* oil forms an efficient synergistic anti-diarrheal product against *Shigella* bacteria that causes diarrhea in humans [83]. The hexane extracts of the plant in

combination with norfloxacin enhance and modifies activities of antibiotics against multiresistant strains of bacteria *S. aureus* [84]. Plant oil and eugenol combined with antibiotics amikacin and erythromycin were deemed to be synergetic against *E. coli* and *S. aureus*. Further, this combination with antibiotic Gentamicin also develops a synergetic product with high efficacy against these microorganisms [85]. Combination of eugenol with biologically produced silver nanoparticles of *Fusarium oxysporum* (AgNPbio) exhibited a strong synergistic action against *Streptococcus agalactiae* a bacteria present in maternal vaginal causing infection in infants [86]. Nefang, a polyherbal anti-malarial folklore medicine of Cameroon of which *O. gratissimum* leaves are a major constituent, exhibit an excellent *in vitro* action against multi-drug resistant *Plasmodium falciparum* along with *in vivo* suppression of *P. berghei* and *P. chabaudi* malaria parasites [87].

CONCLUSION

In conclusion, *O. gratissimum* essential oil is a boon for producing novel drugs and food preservatives with antimicrobial activities. They are worth in combating antibiotic resistant bacteria and fungal pathogens. As a safe natural product, they are a better substitute for synthetic drugs and are highly recommended for food, fragrance and pharmaceutical industries. In the present situation, the emphasis should be given more to discover antimicrobial elements from plant oil for developing effective medicines that can nullify the effect of antibiotic-resistant microbes. Moreover, the focus should be developing new blended products by combining antibiotics or other organic compounds with the clove basil oil which shows unique synergistic property with wide applications in medicines and food industries.

CONFLICT OF INTERESTS

Declared none

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