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Research Article

ANTIVIRAL, ANTIPROTOZOAL, ANTIMALARIAL AND INSECTICIDAL ACTIVITIES OF*OCIMUM GRATISSIMUM L.*

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ABSTRACT

Ocimum gratissimum has been used in traditional medicine for curing various ailments in tropical countries. The plant with unique bioactive compounds possesses a significant medicinal value. Among numerous curative properties the plant shows strong inhibition against human viruses and parasitic protozoans. The presence of eugenol makes it a safe mosquito repellent and anti-malarial agent. Moreover, the plant causes mortality of agricultural insects in both store seed and field crops. The researches have also proved the inhibitory action of plants against various plants and human nematodes harboring alimentary canal. Thus, there is a need to explore the potentiality of this plant to discover biological drug formulation with relevant action against plant and animal microbes, malaria parasites and nematodes. This systematic review presents the antiviral, antiprotozoal, antimalarial, insecticides and nematicidal activities of the plant for developing a standard therapeutic system.

Keywords: *Ocimum gratissimum,Antiviral, Antiprotozal, Antimalarial, Insecticidal, Nematicidal*

INTRODUCTION

The use of traditional medicine in health care and disease control is in practice in developing countries and is expanding rapidly in various other countries.[1] These countries are using medicinal plants for the treatment of multiple diseases, including cancer, infectious and parasitic diseases. Medicinal plants, mainly contain bioactive compounds in the form of secondary metabolite [2] which performs an active role to protect plants against microorganism infections and predator insects and can help in developing new biological products to combat plant and animal diseases.[3,4] The use of plant extracts as antiviral [5-7] antiprotozoal [8-11] and anthelmintic agents, have also been justified through numerous studies.

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Today, most of the existing anti malarial drugs are taken from plant products.[2] These are readily obtainable, cheaper, biodegradable, non-toxic, and possess a broad-spectrum target-specific action against vector mosquitoes.[12] In recent years, various researches have been conducted on the traditional medicine of plant origin to evaluate their therapeutic potentials.[13-15]

Ocimum gratissimum an important tropical plant has been used in traditional medicine since ancient times.[16,17] The plant, belonging to family Lamiaceae commonly known as lemon basil or clove basil, is an erect aromatic shrub, polymorphic, branched perennial 50-200cm long[18] with opposite, ovate-lanceolate leaves, having dotted glands on the lower surface. The inflorescence is sparingly branched with dense calyx on the outer region. The mouth being held in private by a lower lip with small concealed white corolla (Fig. 1). The plant is distributed in Tropical Africa; Eastern Asia – Indonesia,

Malaysia, Vietnam, Cambodia, Laos, Thailand, India, Bangladesh, Sri Lanka and Nepal. The plant generally occurs wild along disturbed land and lake shores[19] and is also cultivated in various countries.[20] The plant produces essential oil and used in culinary and medicinal purposes like curing fever, antiseptic, antispasmodic, insect repellent, killing internal parasites etc.[20-22] There are numerous studies that have been carried with

Fig. 1: Ocimum gratissimum

Ocimum gratissimum proving its antimicrobial, insect repellent, antimalarial, insecticides and pesticide activities.[23-29] This comprehensive review aims to focus the main antiviral, antiprotozoal, antimalarial and nematicide properties of the plant for future studies as an alternative medicine.

Antiviral activities

The literature on antiviral activities of clove basil oil reveals potent activities against virus pathogens causing human diseases. There are various literatures that provides the potentiality of Eugenol, a principal constituent of clove basil plant, as an antiviral agent.[30-32] Some study suggests the antiviral activities of plant oil with commercial importance and traditional uses.[33] Nevertheless the further study emphasizes in carrying more researches regarding the use of clove basil oil in encountering the lethal and highly infectious human viruses.[34] There are various studies that justified the antiviral action of the plant on human lethal viruses such as cytomegalovirus (CMV), murine CMV (MCMV)[6], hepatitis C virus[35] herpes simplex-1 (HSV-1) and herpes simplex -2 (HSV-2) viruses.[26,36] In vitro studies of the plant leaf extract exhibits potent inhibition of HIV-1 and HIV-2 replication with antiviral indices value 110. Further, it also expresses cytotoxicity inhibiting reverse transcriptase and proviral DNA copying of HIV-1, justifying the antiviral potential of the plant.[6] Moreover the plant also possesses effective curative properties for treating measles in children resulted from Paramyxovirus [37]. Depending on research, there should be a special concern on investigations that encourage the use of herbal essential oil that is economical with less side effects showing significant inhibitory activity against human viruses.[7]

Antiprotozal activities

There are numerous studies on the efficacy of clove basil oil as an antiprotozoal agent. The use of plant volatile oil in traditional medicine as a protozoan controlling agent is given in the literature [8]. In the last three-four decades plenty of work has been done to analyze the clove basil oil in controlling leishmaniasis, a human cutaneous disease caused by Sand fly and other harmful protozoan parasites. Some study reports that clove basil oil inhibits the growth and modifies ultrastructure of mitochondria of *Herpetomonas samuelpessoai*, a non pathogenic trypanosomatid protozoan.[38] The in vivo test of plant oil in concentration between 200-500mg/kg in mice, against the growth of *Plasmodium berghei* a parasitic protozoa causing malaria, exhibit a strong percentage of inhibition ranging from 55-77.8%[39] whereas some other observation reported moderate action

of plant leaf extract against *Plasmodium falciparum*.[40]

An investigation using transmission electron microscopy on peritoneal macrophages of mouse observed that plant oil with chief constituent as eugenol when treated with promastigotes and amastigotes of *Leishmania amazonensis*, alters cell division and modify mitochondrial structure, minimizes association of promastigotes and macrophages, and increases production of nitric oxide by infected macrophages without any cytotoxicity.[24] According to some study methanolic extracts of clove basil oil was found effective against promastigotes *Leishmania chagasi*[41,42] and *L. amazonensis*, proving antileishmanial activity for treating several infectious and inflammatory diseases.[41] The steam distilled concentration dependent assay of essential oil expressed an effective control of *L. chagasi* promastigotes with IC50 values 75µg/ml, and modifies the whole viability, including cell structure, lipid accumulation in the cytoplasm and rise in acidocalcisome of the protozoan.[43] The minor compounds of the plant oil such as myrcene with IC50 value 2.24 ± 0.27µg/mL, limonene having IC50 value 4.24 ± 2.27µg/mL, citronellal with IC50 value 2.76 ± 1.55µg/mL, and ethanol crude extracts of the plant collected in full blossoming stages with leaves having IC50 value1.66 ± 0.48 µg/ml and seeds selected with IC50 value1.29 ± 0.42 µg/ml shows effective inhibition of *Trypanosoma bruceibrucei*, which causes vector-borne disease in vertebrates, including humans. Further, the plant oil and non-volatile extracts also show inhibition of *Plasmodium falciparum* to some extent.[26]

There are various studies that record the credentials of the use of Ocimum oil with eugenol as a chief ingredient with antiprotozoal activities.[9,10,44] In past four-five years, various studies, including the one in which the plant oil tested against protozoans Leishmania sp. and *Trypanosoma cruzi* a causal organism of leishmaniasis and trypanosomiasis, shows significant inhibition against the protozoans.[45] An in vitro investigation regarding efficacy of clove basil oil along with five other essential oils obtained through hydro distillation at a concentration necessary to suppress 50% of amastigotes growth IC (50) and trypomastigote forms LC(50) of *T. cruzi*, causal organism of chagas disease, shows a significant inhibition of the parasite mainly due to the presence of chemical substances monoterpenes and sesquiterpenes in all the products.[46] A study in Cameroon contends that Nefang a polyherbal anti-malarial folklore medicine with O. gratissimum leaves as an important constituent, exhibit an excellent action against multi-drug resistant *Plasmodium falciparum* along with in vivo suppression of P. berghei and P. chabaudi malaria parasites.[47] Accordingly, when the evaluation of ten plant species against bloodstream strains of *Trypanosoma brucei rhodesiense* was carried out, it was noted that the ethyl acetate leaf extract of *O. gratissimum* expressed highest activity IC(50) value of 2.08 0.01 µg/ml and a high selective index of 29 against the protozoan.[48] Moreover, a recent study on use of lemon basil oil against *L. amazonensis* also provided evidence of the leishmanicidal activity of the plant.[11]

Antimalarial activities

Malaria brought about by the bites of mosquitoes is a life-threatening disease, accounting for 90% of global cases in the African region, 7% in South-East Asia and 2% in Eastern Mediterranean Region.[49] Although there is a rapid development in active ingredients or new insecticides, but the pesticide increasing resistance in parasites is a matter of grave concern. In these circumstances, the bio-products can ensure non-toxicity to meet the environmental safety standards and public health. There are numerous works on the use of lemon basil as mosquito repellent and insect mortality. The synergistic formulation of essential oil of the leaves and peels of *Ocimum gratissimum* with *Eucalyptus globulus, Cymbopogon citratus, Azadirachta indica, Citrus sinensis* and *Hyptis suaveolens* expresses strong repellent activities and can be used as an effective bioproduct.[50] Indigenous communities of Massissip region in Ngumba Cameroon apply smashed plant leaves on the skin as a mosquito-repellent.[51] The lotion prepared by volatile oil with olive oil base exhibited highest repellencies whereas palm kernel and olive oil bases shows mosquito bite protection and expressed fast knockdown and paralyzing effect on the few mosquitoes expressing mosquito-repellent and

mosquitocidal potentials on human.[52] O. gratissimum petroleum ether extracts at 50% concentration causes 100% mortality of the larvae of Culex mosquito species.[53] Some study observed methanol[54], whereas other reported n-hexane extract of the plant with an effective larvicide activity against *Culex quinquefasciatus* credited to presence of caryophyllene oxide, along with ethyl acetate and methanol leaf extract being effective against *Culex gelidus* larval and adult mosquitoes.[55] According to a study plant powder, oil and wax candle formulation of *O. gratissimum* results in 100% mortality of adult *Anopheles gambiae* mosquito.[56] The leaf essential oil possesses larvicide activity against *Aedes albopictus* which is credited due to chemical compounds 3-allyl-6-methoxyphenol, 4-(5-ethenyl-1-azabicyclo (2, 2, 2) octan-2), 1-(2, 5-dimethoxyphenyl) -propanol and 1-(1-hydroxybutyl) -2, 5-dimethoxybenzene in the concentration of 19.30 %, 16.82 %, 12.23 %, and 5.53 % respectively.[57] The mixture of *Lantana camara* leaves and *O. gratissimum* with ethyl acetate followed by methanol mixture of these plants shows synergistic effect[58] and in another experiment the combination of hexane fraction of the two plants and mixture of ethyl acetate (*O. gratissimum*) and hexane (*L. camara*) yielded synergistic actions. However, the same author also reported that the combination of ethyl acetate and hexane (*O. gratissimum*) and ethyl acetate (*L. camara*) shows antagonistic effects against *Aedes aegypti* mosquito.[59] Various studies observed that crude essential oils[3,60] and essential oils with liquid paraffin base, exhibits larval mortality or even repellent action against *Aedes aegypti* mosquitoes.[61] Further, this concept got strengthened by a study that demonstrated that n-hexane leaf extract contains terpenes, saponins, tannins, steroids and phenol possessing high larvicide mortality against mosquito larvae.[62] Another study suggests that the chloroform extract also has pupicidal and adulticidal activity against *Aedes aegypti* mosquito, which is credited to the presence of phenolic compounds Hentriaconate, tetracosahexane, Hepta 2-1 trimethyl, Benzopyran, Hexamethyl and Dihydro tetramethyl trimethyl acetate.[63]

Insecticidal activity

O. gratissimum exhibit an effective control of larval and adult insects as evidenced in various in-vitro and field experiments. The plant can be used as a good natural protection agent for food commodities and its oil followed by constituents eugenol and β-(Z) -ocimene shows fumigant toxicity and repellent action against agricultural insects, *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Oryzaephilus surinamensis* (L.), *Callosobruchus chinensis* (L.) and *Rhyzopertha dominica* (F.).[64] According to a report, 1% of essential oils diluted with acetone cause 98% mortality of *Sitophilus zeamais* Motsch insect, after 24 h of treatment.[23] The other studies also reported that time and duration of the application of plant oil play a decisive role in inhibiting the insects and causes mortality of adult weevils up to 74 % after 4 days[65] and further the mortality decreases of 50% after 8 days.[66] The formulations based on plant oil and modified clays can act as a good bio-insecticide which was experimentally proved in a study which reports the strong affinity of essential oil with modified Cameroonian montmorillonite clay that persists even up to 107 days effecting maize weevil *S. zeamais*.[67] There are investigation that suggests that 25 µl/vial of *O. gratissimum* causes 80% mortality of adult cowpea seed beetle *Callosobruchus maculate* after 12 hours of treatment and when 1 g of plant powder is used, 50% lethal concentration was noted to be 116 µl/g of the oil which compared more sensitive to the male than to female adults after 48 hourS. Further the use of essential oil at the concentration of 30 µl reduces egg hatching rate up to15%. The plant oil at the rate of 400 µl also provided complete protection from the beetle even for more than 3 months duration under storage.[68] A study observed that a mixture of *Vernonia amygdalina* and *Ocimum gratissimum* in the ratio of 50%: 50% applied at the rate of 5% per 30 g of cowpea seeds, causes 33-80% mortality of *C. maculatus*, reduces egg count and impose least the adult emergence of the beetle at 24, 48 and 72 hours after treatment.[69]

Table 1: Antimicrobial activities of *O. gratissimum* against Virus, Protozoa, Malaria Vector, Insects and Nematodes

Target organisms	References
Virus	
i. Cytomegalovirus (CMV)	[5]
ii. Murine cytomegalovirus (MCMV)	[5]
iii. HIV-1	[6, 32]
iv. HIV-2	[6]
v. Herpes simplex-1(HSV-1)	[7,25,37]
vi. Herpes simplex-2 (HSV-2)	[7,37]
vii. Hepatitis C virus	[36]
viii. Paramyxovirus	[38]
Protozoa	
i. *Leishmania amazonensis*	[10,24,42]
ii. Trypanosoma brucei brucei	[26]
iii. Plasmodium falciparum	[26,41,48]
iv. *Herpetomonassamuelpessoai*	[39]
v. Plasmodium berghei	[40,48]
vi. Plasmodium chabaudi	[48]
vii. Leishmaniachagasi	[42-44]
viii. Trypanosoma cruzi	[46,47]
ix. Trypanosoma brucei rhodesiense	[49]
Malaria Vector	
i. *Culex quinquefasciatus*	[54-56]
ii. Culex gelidus	[56]
iii. Anopheles gambiae	[57]
iv. Aedes albopictus	[58]
v. Aedes aegypti	[3,60-64]
Insects	
Agriculture insect	
i. *Tribolium castaneum* (Herbst)	[65]
ii. Sitophilus oryzae (L.)	[65]
iii. Oryzaephilus surinamensis (L.)	[65]
iv. Callosobruchus chinensis (L.)	[65]
v. Rhyzopertha dominica (F.)	[65]
vi. Sitophilus zeamais	[66-68]
vii. Callosobruchus maculatus	[69,70]
viii. Acanthscelides obtectus	[71]
ix. Plutella xylostella	[72]
x. Brevicoryne brassicae	[72]
xi. Aphis craccivora	[73,74]
Flies	
i. Musca domestica	[75]
ii. Simulium damnosum	[76,77]
Nematodes	
i. *Haemonchus contortus*	[78]
ii. Meloidogyne incognita	[27,80,82]

According to a report 4% solution of the ethanolic leaf extracts causes 28.80 % mortality of beans weevil *Acanthscelides obtectus* 1.50 hours after treatment and rate of toxicity increases with the concentration and time of the exposures.[70] The detergent and water extracts of plant shows significant inhibition of cabbage diamondback moth, *Plutella xylostella*, and *Brevicoryne brassicae* aphid and is comparable to synthetic insecticide.[71] Some study found that hot solvent extraction of aerial parts of the plant and root causes of 73.33 and 86.66% mortality of groundnut aphid *Aphis craccivora* Koch.[72] The acetone extract of the plant also shows an influential activity against cowpea aphid, *Aphis craccivora* causing mortality, life span reduction and decrease in reproduction.[73] Plant oil diluted in 2% acetone expresses 100% repellency against housefly *Musca domestica* L. and is comparable to pesticides dimethyl phthalate, N, N-diethyl-meta-toluamide, malathion, thymol, pyrethrum extract, piperonylbutoxide and mineral oil.[74] The plant oil with liquid paraffin base shows a strong repellent against *Simulium damnosum* blackflies.[75] An investigation reveals that plant oil at 20% concentration with liquid paraffin as the carrier applied on forearms and legs gave protection up to three hours against blackfly, *S. damnosum* s.l., the insect vector of human onchocerciasis.[76]

Nematicidal activities

There are numerous studies on inhibitory properties of lemon basil extracts against helminths and their herbal use. A study reports that *O. gratissimum* oil 0.50% concentration shows ovicidal activity against *Haemonchus contortus* a parasite present in gastrointestinal of small ruminants[77] and the eugenol isolated from the plant also possess nematicidal properties.[77,78] The aqueous extract of the plant at the rate of 20,000 mg/kg increases the yield and reduces Meloidogyne incognita pathogenicity in cowpea by inhibiting egg hatching 40% - 63.7% and juvenile mortality ranging from 82% - 93.8% of the root-knot nematode[79], whereas water extract at the concentration of 20 ml exhibited nematicidal activity against M. incognita and found comparable with carbofuran by increasing fruit production, controlling root damage and the growth of okra.[27] The powdered extract of *Azadirachta indica* and *O. gratissimum* possess nematicidal properties and when used in combination shows synergistic action against root knot nematode of a pepper plant as compared to be used individually and the soil treated with these plants has potentiality to kill other plant parasitic nematodes.[80] In vitro study suggest that plant essential oil at zero percent concentrations affects the survival of the eggs of *M. incognita* root nematodes of tomato in 48 hours and at higher concentration inhibits hatching or causes mortality.[81]

A study reports that more than 1000 ppm concentrations of the plant cause 50% mortality of brine shrimp nauplii and were significantly comparable to piperazine drug. The plant extract also shows toxicity against parasitic Haemonchus infective L3 stage nematode. The in vitro study further reveals the inhibitory action of plant with IC50s of 8 and 10 micro/ml against recombinant Onchocerca and Ascaris GSTs (glutathione-S-transferases).[82] The methanolic leaf extract based on dose and duration shows haematinic and haemopoietic activities which were justified by an experiment inducing phenylhydrazine anaemia in Albino wistar rats. The result reveals a significant decrease in the mean value of the red blood cell (RBC), an increase in the RBC indices, a slight increase in the mean values of white blood cell (WBC) and increase in lymphocytes.[83]

CONCLUSION

There are various scientific studies on the uses of lemon basil as antimicrobial, antimalarial, nematicidal and insecticides agent. As most of the mentioned researches have been conducted using a crude preparation, few of them use ethanol or acetone base, but the chemical studies are less mentioned, thus requiring a need of controlled clinical trial. The plant potential in inhibiting the HIV virus, mosquito repellent and larvicidal activities makes it a novel bio-

product. The use of the plant and their essential oils in the form of insecticides is safer than synthetic pesticides. Most importantly, it is economical and easily available in tropical countries this special focus should be placed on the discovery of new drug formulations using these natural products to combat the microorganisms and insects.

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