# ANTIMICROBIAL ACTIVITIES OF THE VOLATILE OILS OF *OCIMUM BACILICUM* L. AND *OCIMUM GRATISSIMUM* L. (LAMIACEAE) AGAINST SOME AEROBIC DENTAL ISOLATES

**AHONKHAI I, AYINDE BA*, EDOGUN O AND UHUWMANGHO MU****

*Department of Pharmaceutical Microbiology, *Department of Pharmacognosy, **Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*

**ABSTRACT**

Essential or volatile oils of plants have been variously reported to have many medicinal applications. Their probable uses against oral microbes have received little attention. Oral swabs obtained from eighteen dental patients at the University of Benin teaching Hospital, Benin City, Nigeria, led to the isolation of twenty nine bacteria. Using standard methods, the microorganisms were identified as *Streptococcus viridians* (16; representing 55.17%), *Staphylococcus albus* (9; 31.04%), *Klebisiella pneumonia* (2; 6.90%), *Pseudomonas aeruginosa* (1, 3.45%) *and Proteus vulgaris* (1, 3.45%). The antimicrobial activities of the volatile oils of *Ocimum basilicum* L. and *O. gratissimum* L. were evaluated on the twenty nine organisms using agar diffusion and agar dilution methods. In the susceptibility tests, the volatile oils of *O. basilicum* and *O. gratissimum* independently inhibited the growth of *Klebisiella pneumonia* at a concentration of 0.51% in the agar; *Streptococcus viridians* and *Staphylococcus albus* at 1.10% and *Pseudomonas aeruginosa* at 10.0%. *Proteus vulgaris* was inhibited at 0.53% by the volatile oil of *O. gratissimum* and 0.67% by *O. basilicum*. Separate incorporation of the volatile oils into tooth pastes (2 and 5 %), the volatiles oils showed antibacterial activities comparable to a commercial tooth paste (which contains *O. basilicum* 0.01% among others) against most resistant organisms. As components of mouth washes, the volatile oils completely inhibited the growth of organisms at a concentration of 0.5%.

**Keywords**: Antimicrobial activities, *Ocimum basilicum, Ocimum gratissimum*

## INTRODUCTION

Volatile oils are one of the groups of secondary metabolites produced by some plants. They are found in some plants in both the angiosperm and gymnosperms in which they constitute materials of odoriferous nature. Medicinally, they are used in aromatherapy, as insect repellents, larvicidal and insecticidal agents (Aisien *et al.*, 2004; Ayinde and Odigie, 2001; Gbolade *et al.*, 1999). There are also reports on the hypotensive effects of volatile oils obtained from *Mentha villosa, Alpinia zerumbet* and *Ocimum gratissimum* (Lahlou 2000; Lahlou *et al.*, 2003; Interaminense *et al.*, 2005) while others like *Cymbopogon citratus, Citrus sinensis* and *Citrus maxima* have been reported to possess remarkable antimicrobial effects against some bacteria and fungi (Onawumi and Ogunlana, 1988; Ahonkhai and Ayinde, 2005).

Many bacterial have been implicated in different dental ailments and disease conditions. For example, certain *Streptococcus* species like *S. mitis, S. sanguis*, S. *mutans* and *S. salvarius* have been reportedly involved in tooth pulp infection, dental plaque and root surface caries (Robert and Bryan, 1977). Various Lactobacillus species have also been associated with caries and are thought to be important secondary dental caries (vanHoute, 1994). Quite a number of synthetic antimicrobial agents are known to be of use in reducing the population of the non-desirable oral microbes although to varying degrees of success. There is need to explore natural herbs with probable antimicrobial potentials against dangerous oral microbes. This work therefore was aimed at examining the probable antimicrobial effects volatile oils of *Ocimum bacilicium* L. and *O. gratissimum* L. could show against some aerobic dental isolates. It is of chemotaxonomic importance as the two plants evaluated belong to the same genus.

## MATERIALS AND METHODS

### *Collection of the plant materials and extraction of the volatile oils*

*Ocimum bacilicum* and *O. gratissimum* leaves were collected from cultivated sources in Ugbogiobo village, Benin City, Nigeria. The volatile oil of each plant was extracted by distillation method using Clavenger-type apparatus for 4 hours (African Pharmacopoeia, 1986; British Pharmacopoeia, 1988). After collection, the volatile oils were measured to determine their yields and each was kept in a refrigerator maintained at 4°C until needed. At the end of the extraction period, 7.4ml of the volatile oil was obtained from 4.20 kg of the fresh leaves

*Corresponding author:* e-mail: baayinde01@yahoo.com, baayinde@uniben.edu

of *O. bacilicum* while 1.78 kg of *O. gratissimum* leaves produced 10ml of the oil. These were 0.18 and 0.56% respectively.

***Sources of the micro organisms used***

After taking permission from the authorities of dental clinic, University of Benin Teaching Hospital (UBTH) Benin City, sterile swab sticks were used to collect swabs from the mouth of eighteen (18) patients comprising of males and females. They were diagnosed of various dental ailments like dental caries (9), periodontitis (5), aveola abscess (2), gingivitis (1) and propritis (1).

Each swab obtained was used to inoculate three different media namely blood agar, chocolate agar and Macconkey agar plates. The plates were aerobically incubated at 37°C for 24-48 hour for the organisms to grow.

The antimicrobial activities of the volatile oils were tested against each of the 29 isolates obtained using diffusion techniques on solid media. Different concentrations (0.5-10%) of the volatile oil in 20ml nutrient agar were made and incubated with a drop (using 0.04ml dropper pipette) of an overnight cultures of the bacteria. After absorption, the plates were incubated at 37°C for 24 to 48h.

***Preparation and antimicrobial activities of tooth pastes with the volatile oils***

Tooth pastes were formulated following standard laboratory procedures, however with exception of sodium

**Table 1**: Composite table relating the occurrence of dental diseases and the implicated micro organism(s)

| Dental diseases | Case Number | Type of growth | Number of isolates | Organisms implicated |
|---|---|---|---|---|
| Dental caries | 9 | Mixed | 2 | *Strept. viridians, K. pneumonia S. albus* |
| | | Single | 1 | *Strept. viridians, K. Pneumonia* |
| | | Mixed | 2 | *S. albus* |
| | | | | *S. viridans,* |
| | | Single | 1 | *Ps.aeruginosa, K. pneumonia* |
| | | Mixed | 3 | *S. albus, Strept.viridans* |
| | | Mixed | 2 | *S. albus Strept. viridans* |
| | | Mixed | 3 | *P. vulgaris* |
| | | Single | 1 | *Strept. viridans* |
| | | Single | 1 | *Strept. viridans* |
| Periodontitis | 5 | Single | 1 | *Strept. viridians* |
| | | Single | 1 | *Strept. viridians* |
| | | Single | 1 | *Strept. viridians* |
| | | Mixed | 2 | *Strept. viridians S. albus* |
| | | Mixed | 2 | *Strept. viridians S. albus* |
| Gingivitis | 1 | Single | 1 | *Strept. viridians* |
| Propritis | 1 | Single | 1 | *Strept. viridians* |
| Aveola abscess | 2 | Mixed | 2 | *Strept. viridians S. albus* |
| | | Single | 1 | *S. albus.* |

**Table 2**: Summary of the bacterial isolates with the associated dental diseases

| Bacterial species | Dental diseases | Occurrence of the bacteria | % occurrence of the bacteria |
|---|---|---|---|
| *Strept. viridans* | dental caries, periodontitis, gingivitis, and propritis | 16 | 55.17 |
| *S. albus* | dental caries and aveola abscess | 9 | 31.04 |
| *K. pneumonia* | dental caries | 2 | 6.90 |
| *Ps. aeruginosa* | denta caries | 2 | 3.45 |
| *P. vulgaris* | denta caries | 1 | 3.45 |

lauryl sulphate. The volatile oils of the two *Ocimum* species were made to constitute 2 and 5% of the total volume of the tooth pastes prepared.

The antimicrobial effects of the volatile oil containing pastes were carried out using ditch plate material. After filling the ditches with the tooth paste, isolates of five (5) of the most resistant organisms obtained from some of the mixed cultures (*K. pneumonia, S. albus, Ps. aeruginosa, Strept. viridans* and *P. vulgaris*) were streaked across the ditches at right angles. The plates were incubated at 37°C for 24 hour and 48 hour. A commercial tooth paste containing 0.01% of *O. bacilicum* as one of its components was used as standard. The diameters of the zones of inhibition were measured and recorded.

***Preparation of mouth washes with the volatile oils and their antimicrobial activities***

Mouth washes were prepared following prescribed standard formulation procedure (BP, 2003) but without peppermint oil. The volatile oil of each plant was incorporated into the mouth washes to make concentrations of 0.5, 1.0, 2.0, 5 and 10% w/v. The antimicrobial activities of the mouth washes were tested against five (5) most resistance isolates using the tube dilution method. Using 0.025ml dropper pipette, a drop of overnight broth culture of each organism was added to 0.1ml of each concentration of the mouth wash in sterile test tubes. Control tests were made by adding the overnight cultures of the organisms to 0.1ml of mouthwash containing none of the volatile oils. All the test-tubes were incubated at 37°C for 24 hour after which they were examined for growth by spreading the content of each tube on blood and chocolate agar plates. These were also incubated at 37°C for 24 hours and 48 hours.

## RESULTS AND DISCUSSIONS

From the eighteen (18) patients examined in this work, twenty-nine (29) different organisms were obtained. Based on the colonial appearance, morphological characteristics coupled with appropriate biochemical characteristics like catalase, coagulase, oxidase, citrate, indole, urea and motility tests (Cheesborough, 1984), the twenty-nine (29) different bacterial isolates were identified as *Strept. viridans* 16 (55.17%); *S. albus* 9 (31.04%); *K. pneumonia* 2 (6.9%); *Ps. aeruginosa* 1 (3.45%) and *P. vulgaris* 1 (3.45%).

Dental swabs obtained from nine (9) of the patients showed single organism isolate of the bacteria, seven (7) of which were *Strept. viridans* (implicated for dental caries, periodontitis, gingivitis, and propritis) while the remaining two were observed to be *S. albus* (implicated for dental caries and aveola abscess). The remaining nine patients had either a combination of these two bacteria or either of them with *P. vulgaris*, *K. pneumonia*, or *P. aeruginosa* (tables 1 and 2).

***Susceptibilities of the bacterial organisms to the volatile oil***

After 48 hours, at concentrations of 0.51% the volatile oils completely inhibited the growth of some of the bacterial isolates particularly those with single isolates. The resistant ones (*K. pneumonia, S. albus, P. aeruginosa, Strept. viridans* and *P. vulgaris*) especially among the mixed cultures/isolates were observed to be susceptible to the volatile oils from concentration of 0.53 to 10.0%. Only *P. aeruginosa* was observed to be completely inhibited at a concentration of 10.0% of the volatile oils (tables 2 and 3).

**Table 3**: Susceptibility of the twenty-nine (29) bacterial dental isolates to the volatile oils of *O.basilicum* and *O. gratissimum*.

| Sample No. | Organisms | MIC (%V/V) | |
|---|---|---|---|
| | | *O. basil-icum* | *O. gratissi-mum* |
| 1 | *Strept. viridans* | 0.67 | 0.53 |
| | *K. pneumonia* | 0.51 | 0.51 |
| 2 | *S. albus* | 0.51 | 0.51 |
| 3 | *Strept. viridans* | 0.51 | 0.51 |
| 4 | *Strept. viridans* | 0.50 | 0.50 |
| 5 | *Strept. viridans* | 0.50 | 0.51 |
| 6 | *Strept. viridans* | 0.50 | 0.50 |
| | *S. albus* | 0.67 | 0.53 |
| 7 | *Strept. viridans* | 0.50 | 0.50 |
| 8 | *Strept. viridans* | 0.50 | 0.50 |
| | *S. albus* | 1.10 | 1.10 |
| 9 | *Strept. viridans* | 0.50 | 0.50 |
| | *K. pneumonia* | 0.51 | 0.51 |
| | *Ps. aeruginosa* | 10.0 | 10.0 |
| 10 | *S. albus* | 0.50 | 0.50 |
| | *Strept. viridans* | 1.10 | 1.10 |
| 11 | *Strept. viridans* | 0.50 | 0.50 |
| | *S. albus* | 0.56 | 0.53 |
| 12 | *S. albus* | 0.51 | 0.50 |
| | *Strept. viridans* | 0.51 | 0.50 |
| | *P. vulgaris* | 0.67 | 0.53 |
| 13 | *S. albus* | 0.50 | 0.51 |
| | *Strept. viridans* | 0.50 | 0.50 |
| 14 | *Strept. viridans* | 0.50 | 0.50 |
| 15 | *Strept. viridans* | 0.53 | 0.53 |
| | *S. albus* | 0.50 | 0.50 |
| 16 | *Strept. viridans* | 0.51 | 0.51 |
| 17 | *S. albus* | 0.51 | 0.51 |
| 18 | *Strept. viridans* | 0.51 | 0.51 |

The volatile oils of the two plants constituted as 2 and 5% of tooth paste were observed to show significant antibacterial activities against the five most resistant organisms. Their antibacterial activities were comparable with the commercial tooth paste that contains volatile oil of *O. bacilicum* as one of its components (table 4).

When prepared as components of mouth washes, the volatile oil of *O. bacilicum* and *O. gratissimum* completely inhibited and growth of all the organisms at concentration of 0.5% compared to controls which showed no inhibition to the organisms (table 5).

Volatile oils constitute a group of plant secondary metabolites which can best be obtained (from the plant organs containing them) through hydro distillation. This method produces the volatile oils in their original form without being mixed with any other groups of secondary metabolites like alkaloids, tannins, saponins, anthraquinones, cardiac glycosides. They have been reported to have various medicinal applications. These range from hypotensive effects (Interaminense *et al*, 2005), their applications in aromatherapy, and in the manufacture of creams. Many have been reported to have insecticidal or insect repellents effects while there are some with potent antimicrobial effects against both fungal and bacteria (Aisien *et al*, 2004; Ahonkhai and Ayinde, 2005).

The volatile oils of *O. gratissimum* and *O. bacilicum* were established to produce inhibitory effect against the oral microbial flora tested. From the work, it was observed that dental caries was the most common dental ailment presented by the patients. Also, *Strept. viridans* was observed to be implicated in almost all the dental disease conditions.

The organism has been reported to be the dominant bacterial specie in the oral cavity and may be up to 98% of the total oral flora until the appearance of the teeth. It is also the most frequently encountered bacteria in plague, dental caries and periodontitis (Todar, 2002).

The presence of *Ps. aeruginosa* and *P. vulgaris* in two of the patients with dental caries may be as result of secondary infections since they were isolated with *Strept. viridans*. This may be responsible for their initial resistance to the volatile oils at lower concentrations. While *P. vulgaris* showed susceptibility to the volatile oils at a concentration of 0.67%, the growth of *Ps. aeruginosa* was completely inhibited at 10% concentration. The volatile oils were observed to be more potent as mouth washes than as components of tooth paste. This may be due to more contact or higher diffusion of the mouth washes into the agar while the other components of the tooth paste may have reduced the oils' diffusion into the agar medium to impact significant effects on the growth of the organisms. In all, the volatile

**Table 4**: Antibacterial effect of the tooth pastes formulated with volatile oils of the *Ocimum* species against the selected organisms.

| Organisms | Zones of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | FPn | FPob | | FPog | | Commercial tooth paste |
| | | 2% | 5% | 2% | 5% | |
| *Klebsiella pneumonia* | - | - | 6 | - | 6 | - |
| *Staphylococcus albus* | 1 | 3 | 5 | 3 | 5 | 5 |
| *Pseudomonas aeruginosa* | - | 1 | 3 | 1 | 3 | - |
| *Streptococcus viridans* | 2 | 2 | 3 | 3 | 6 | 4 |
| *Proteus vulgaris* | - | 2 | 5 | 3 | 5 | 2 |

Key: - = Indicates no zone of inhibition.
FPn = Formulated paste with no essential oil
FPob = Formulated paste + *O. basilicum*
FPog = Formulated paste + *O. gratissimum*
Dabur = Commercial herbal tooth paste containing 0.01% *O. basilicum* volatile oil (Marketed by Dabur International Limited, Douglas, UK)
% = Represents Essential Oil content (w/v)

**Table 5**: Susceptibility of selected bacterial isolates to the Mouthwash prepared with the Effective concentration (%) of EO of *Ocimum* species (a and b)* volatile Oils of the *Ocimum* species

| Bacterial isolates | 0.5% | | 1.0% | | 2.0% | | 5.0% | | 10.0% | | 10.00 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | a | b | a | b | a | b | a | b | a | b |
| *Klebsiella pneumonia* | - | - | - | - | - | - | - | - | - | - | | |
| *Staphylococcus albus* | - | - | - | - | - | - | - | - | - | - | | |
| *Pseudomonas aeruginosa* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Streptococcus viridians* | - | - | - | - | - | - | - | - | - | - | | |
| *Proteus vulgaris* | - | - | - | - | - | - | - | - | - | - | | |

*a = *O. basilicum* b = *O. gratissimum*

oil of *O. gratissimum* appeared to be more active than that of *O. basilicum.*

Volatile oil of *O. gratissimum* has been reported to have hypotensive effects, strong insect repellent effects and also showed significant antimicrobial effects against both fungi and bacteria (Interaminense *et al.*, 2005; Aisien *et al.,* 2004; Ahonkhai and Ayinde, 2005). The similarities in the activities of the two volatile oils indicate that they probably contain similar constituents (and may be to varying extents) as the two plants that produced them belong to same genus *Ocimum*. This observation is of chemotaxonomic value as it suggests that medicinal plants that belong to the same family are likely to have similar medicinal applications. This work has further established the natural potentials of *O. bacilicum* and *O. gratissimum* volatile oils as natural antimicrobial agents.

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