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Antibacterial activities of clove *Syzygium aromaticum* and turmeric *Curcuma longa* essential oil on some pathogen bacteria implicated in periodontal diseases

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Abstract. The antibacterial activities of the clove essential oil (C), turmeric essential oil (T) as well as clove and turmeric (CT) essential oil were analysed against some bacterial pathogens implicated in periodontal diseases using agar well diffusion. The bacteria include *Klebsiella oxytoca*, *Bacillus subtilis*, *Pseudomonas flourescens*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Enterobacter* sp., *Proteus mirabilis* and *Escherichia coli*. At 15% concentration of the essential oils, both clove essential oil and the essential oil of clove and turmeric (CT) exhibited pronounced and varying degrees of growth inhibition zones against the bacteria (8.3±0.33-22.6±1.53 and 7.3±0.33-22.3±1.45). The MIC value for clove essential oil, tumeric essential oil and clove and turmeric (CT) essential oils ranges from 0.9-7.5 %, respectively. The result shows that in general, clove essential oil has significantly greater zone of inhibition (mean) than tumeric essential oil. The bactericidal rate of clove essential oil against *Klebsiella oxytoca* and *Bacillus subtilis* was also determined. From our study, we can conclude that clove essential oil has more prevailing and sustainable antibacterial properties than turmeric essential oil even at a considerable low percentage. We recommend that clove essential oil not only has very promising potential for a broad-spectrum antibiotic drug against periodontal pathogenic bacteria. In addition, it can be used as an effective source of natural herbal antibiotics.

Keywords: Antibacterial; Essential oil; Periodontal diseases.

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Introduction

Periodontal diseases are multifactorial infections elicited by a complex of bacterial species that interact with host tissues and cells causing the release of a blood array of inflammatory cytokines, chemokine and mediators. Some of these mediators lead to the destruction of the periodontal structures, including the tooth supporting tissues, alveolar bone and periodontal ligament (Bascones-Martinez, 2004). The most common forms of periodontal diseases are gingivitis and periodontitis (Peterson, 2005). About 20% of the population of the world is affected by these diseases (Petersen, 2008). Periodontal pathogens include *Aggregatibacter*

actinomycetemcomitans, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Peptostreptococcus nucleatum*, *Eubacterium* species, beta-hemolytic *Streptococci*, a variety of enteric rods and *Pseudomonas*, *Enterococci*, *Staphylococci* and possibly yeasts (Ram, 2005). The pathogenic microflora of the periodontal pocket must be suppressed in order to maintain the health of the periodontal tissues (Cobbs, 1996). Modalities of treatment of periodontal diseases may be non-surgical approach or surgical approach (Bruce et al., 1997). Scaling and root planning has been effective in immediately decreasing the microbial load of the periodontal pocket but recolonization of the same can occur as early as 60 days after scaling and root planning.

In addition to non-surgical treatment, the use of antimicrobial agents, both systemic and topical has been increasing because of the realization that periodontal diseases is not merely an overgrowth of bacteria, but a shift in bacterial species (Slots, 2004). Systemic administration of drugs has been also useful in treating periodontitis but it involves a relatively high dose with repeated intake over a prolonged period of time to achieve the required inhibitory concentrations in the sulcular fluid (Slots et al., 2004).

During the last two decades there has been an increasing trend in search for new plant derived drugs containing the medically useful alkaloids, glycosides, polyphenolics, steroids, terpenoids derivatives. *Curcuma longa* (tumeric) is a tropical perennial herb related to the Zingiberaceae Family (Chattopadhyay, 2004). The rhizome has traditionally been used as a coloring agent spice in Asian cuisine and is well known for its medicinal properties (Luthra et al., 2001). The active component in tumeric is curcuminoids which include mainly curcumin (diferuloyl methane), demethoxycurcumin and bisdemethoxycurcumin (Chattopadhyay, 2004). Curcumin is the most important fraction which is responsible for the biological activities of turmeric. It is soluble in ethanol and acetone and has a melting point of 184 °C (Joe et al., 2004). The extracts of tumeric roots has traditionally been used as an insect repellant, for rheumatism, skin diseases, body ache, intestinal worms, diarrhea, intermittent fever, hepatic disorders, urinary discharge and colic inflammatory disorders (Villages et al., 2008). The oil extracted from tumeric had been reported to have antibacterial activities against *Escherichia coli* (Norajit et al., 2007) and to have anti-fungal activities (Behura et al., 2000).

Syzygium aromaticum is an ever green tree which belongs to the Family Myrtaceae, that has numerous medicinal properties. It is often referred to as clove bud which has a shaft and a head. Clove is mostly used as spice and its oil is used essentially in Indian Ayurveda and Chinese medicine for treatment of many ailments. Clove is a plant derived spice that has been traditionally used as a natural medicine for the treatment of various ailments including dental diseases. Major component in clove oil which imparts aroma is eugenol, a phenyl-propanoid class of chemical compound. It contributes 72-90% pleasant, sweet aromatic fragrances to the clove bud. The traditional use of clove is supported by its many properties which have been described in numerous scientific reports highlighting its anti-oxidant, hypotensive, dental analgesic (Alitonou et al., 2012), anti-bacterial (Pinto et al., 2009), anti-inflammatory and anti-fungal (Sessou et al., 2009) activity, apart from the synergistic antimicrobial activity of the essential oil with other plants (Hadizadah et al., 2009) which allows it to be considered with great potential for dental application (Ferreira et al., 2010; Keles et al., 2011).

This study is aimed at comparing the efficacy of *Curcuma longa* in combination with *Syzygium aromaticum* essential oils on microorganisms implicated in periodontal diseases.

Objectives

a) To determine the sensitivity pattern of the bacteria isolates to the essential oils.

b) To determine the Minimum and Inhibitory concentration and Minimum Bactericidal Concentration of the essential oils on the bacterial isolates.

c) To determine the Bactericidal rate of the essential oils on a Gram negative and Gram positive representative of the test isolates.

Materials and methods

Collection of samples

Curcuma longa (tumeric) rhizomes and *Syzygium aromatic* (clove) flower bud were purchased at Lafenwa market in Abeokuta.

Microorganisms

Clinical isolates from periodontal pus used for this study were obtained from the Department of Microbiology, University College Hospital, Ibadan, Oyo State. These bacterial isolates include strains *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Klebsiella edwardsii*, *Escherichia coli*, *Enterobacter* sp., *Citrobacter* sp. and *Pseudomonas flourescens.*

Preparation of essential oils (hydro-distillation)

Curcuma longa (Tumeric) rhizomes and flower buds of *Syzygium aromaticum* (Clove) were crushed using a blender and poured into a round bottom flask and subjected to hydro-distillation using Clavenger's apparatus. The distillation units consists of heating mantle, round bottom flask (3,000 mL), condenser and separating funnel. The plant materials were washed, blended and immersed in double their volume of distilled water was heated to 78 °C for turmeric and 60 °C for clove. The oil released was carried by the steam rising out of the flask into the condenser on the top and the condensed liquid that dropped into the condensate were removed with a Pasteur pipette and stored at a temperature of 4 °C in tightly closed glass vials wrapped in Aluminium foil to avoid exposure to light and oxygen. The clove essential oil and turmeric essential oil were separated from water by separatory funnel and dried with anhydrous Na_2SO_4 and weighed (Guenther, 1949).

Sensitivity testing of the essential oils on the bacterial isolates

The sensitivity testing or antibacterial activity of the essential oils were determined using agar-well diffusion method as described by Irobi et al. (1994) with some modifications. The bacterial isolates were first grown in nutrient broth for 18h before use. 0.2 mL of the standardized test isolates (10^6 cfu/mL of 0.5 McFarland Standards) was sub-cultured on to Mueller-Hinton agar (Lab M). The medium was allowed to set and wells were bored into the agar medium using a sterile 6 mm cork borer. The wells were filled up with prepared solutions of the essential oils and care was taken not to allow solutions to spill on the surface of the medium. The plates were allowed to stand on the laboratory bench for 1 h to allow proper inflow of the solutions into the medium before incubating the plates in an incubator at 37 °C for 24 h. The plates were later observed for the zones of inhibition.

Determination of Minimum Inhibitory Concentration (MIC) of the essential oil on the bacterial isolates.

The MICs of the essential oils were determined using the method described by Akinpelu and Kolawole (2004). Two-fold dilution of the essential oils were prepared and 2 mL of the different concentrations of the solutions was added to 18 mL of pre-sterilised molten nutrient agar to give final concentrations regimes of 0.391 mg/mL to 25.0 mg/mL. The medium was poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old standardized bacterial cultures. The plates were later incubated at 37 °C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of the essential

oil that prevented the growth of the bacteria.

Determination of Minimum Bactericidal Concentration (MBC) of the essential oils on bacterial isolates

The MBCs of the essential oils were determined in accordance with the method of Olorundare et al. (1992). Samples were taken from prepared plates with no visible growth in the MIC assay and subculture onto freshly prepared nutrient agar medium and later incubate at 37 °C for 48 h. The MBC was taken as the lowest concentration of the essential oils that did not allow any bacterial growth on the surface of the agar plates.

Determination of bactericidal rate of the essential oils on the test isolates

The bactericidal rate was determined using the method described by Odenholt et al. (2001). Experiment was determined using each of the active essential oil on the viability of *Bacillus subtilis* representing Gram positive and *Klebsiella oxytoca* representing Gram negative organisms. Viable counts of the test organisms were initially determined. A 0.5 mL volume of known cell density (by viable counts 10^6 cfu/mL) from each organism suspension was added to 4.5 mL of different concentrations (7.5%, 3.75%, 1.875% and 0.9%) of the essential oil. The suspension was thoroughly mixed and held at room temperature (28-30 °C) and the bactericidal rate was determined over a period of 2 h. Exactly 0.5 mL of each suspension was withdrawn at appropriate time (5, 10, 15, 20 and 25 min) intervals and transferred to 4.5 mL nutrient broth (Oxoid Ltd) recovery medium containing 3% “tween 80” to neutralize the effects of the antimicrobial compounds carry-over from the test suspensions. The suspension was shaken properly then serially diluted up to 10^{-5} in sterile physiological saline. Exactly 0.5 mL of the final dilution of the test organism was transferred into pre-sterilised nutrient agar (Oxoid Ltd) at 45 °C and plated out. The plates were allowed to set and incubated upside down at 37 °C for 72 h. Control experiment was set up without the inclusion of antimicrobial agent. Viable count were made in three duplicates for each sample. Decrease in the viable counts indicated killing by the antimicrobial agents.

Results and discussion

Extract obtained from the plant materials

The essential oil obtained from the rhizome of *Curcuma longa* (turmeric) was light yellow in colour and had a spicy odour. The essential oil obtained from *Syzygium aromaticum* (clove) flower was transparent and light cream in colour with a very strong relaxing spicy odour. The essential clove of *Allium sativum* (garlic) was light yellow in colour with a pungent odour.

Antimicrobial activities exhibited by the essential oils against the test isolates

Table 1 show inhibition zones for the essential oils against some pathogenic bacteria implicated in periodontal diseases. The antimicrobial effect of the essential oils varied depending on the chemical components of the oil and the specific microorganism tested. Among the essential oils, clove essential oil had the highest antimicrobial activity (22.6±1.53) against the test isolates than the combination of clove and turmeric essential oils (22.3±1.45) while turmeric essential oil exhibited no antimicrobial activity against the test isolates. The results from the well diffusion method indicated that *Klebsiella oxytoca* is the most resistant microorganism to all the essential oils showing the lowest zone of inhibition (8.3±0.33) for clove essential oil and no inhibition zones for the othe essential oils. *Bacillus subtilis* and *Escherichia coli* (a) were the most sensitive microorganisms to clove essential oil showing the largest inhibition zones of 22.0±1.53 and 22.0±1.16, respectively, while *Pseudomonas aeruginosa* (b) had the largest inhibition zone for the combination clove and turmeric essential oil with an inhibition zone of 22.3±1.45.

Table 1. The Sensitivity patterns exhibited by clove essential oil, turmeric essential oil and the combination of clove and turmeric essential oils against the test isolates.

	Isolate	Zones of inhibition (mm)		
		CT (15%)	C (15%)	T (15%)
1	*Pseudomonas aeruginosa* (a)	7.3±0.33	10.0±0.57	0±0.0
2	*Citrobacter sp* (a)	13.3±0.88	13.3±0.33	0±0.0
3	*Peudomonas aeruginosa* (b)	22.3±1.45	20.7±0.33	0±0.0
4	*Klebsiella edwardsii*	12.3±0.33	16.0±1.16	0±0.0
5	*Bacillus subtilis*	10.3±0.88	22.6±1.53	0±0.0
6	*Citrobacter* sp.	20.0±1.73	14.0±1.00	0±0.0
7	*Escherichia coli* (a)	15.7±0.33	22.0±1.16	0±0.0
8	*Proteus mirabilis* (a)	15.0±0.00	15.7±1.20	0±0.0
9	*Enterobacter* sp.(a)	12.0±0.57	16.7±0.67	0±0.0
10	*Escherichia coli* (b)	15.7±0.33	18.0±1.16	0±0.0
11	*Klebsiella oxytoca*	0.0±0.00	8.3±0.33	0±0.0
12	*Pseudomonas .flourescens*	20.0±0.00	10.0±0.57	0±0.0
13	*Proteus mirabilis* (b)	12.0±1.15	17.3±0.33	0±0.0
14	*Enterobacter* sp.(b)	20.7±2.18	14.7±0.88	0±0.0

C = clove; T = turmeric; CT= clove and tumeric.

The highest MIC (Figure 1) exhibited by Clove essential oil against the strains of *Pseudomonas aeruginosa*, *Citrobacter* sp., *Klebsiella edwardsii*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter* sp., *Klebsiella oxytoca* and *Pseudomonas flourescens*, were 3.75%, 0.9%, 3.75%, 1.8%, 0.9%, 1.8%, 1.8%, 7.5%, and 0.9%, respectively, while the highest MBC (Figure 2) exhibited against the isolates were 3.75%, 7.5%, 3.75%, 1.8%, 1.8%, 1.8%, 1.8%, 7.5%, and 0.9%, respectively. The highest MIC (Figure 1) exhibited by the combination clove and turmeric essential oil against the strains of *Pseudomonas aeruginosa*, *Citrobacter* sp., *Klebsiella edwardsii*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter* sp., *Klebsiella oxytoca* and *Pseudomonas flourescens*, were 3.75%, 3.75%, 3.75%, 7.5%, 3.75%, 3.75%, 3.75%, 7.5%, and 3.75%, respectively, while the highest MBC (Figure 2) exhibited by the same essential oil on the same organisms, were 7.5%, 3.75%, 7.5%, 7.5%, 3.75%, 3.75%, 7.5%, 7.5%, and 7.5%, respectively.

The bactericidal rate of clove essential oil against *Bacillus subtilis*

The bactericidal rate of clove essential oil against *Bacillus subtilis* at 1x MIC, 2x MIC and 3x MIC are shown in Figure 3. The percentage of the organisms killed by the essential oil at 1x MIC in 5 min of contact was 42.6% while the percentage of the cell killed at 10 min was 92%. When the contact time was increased to 15 min the percentage of the cell killed was 100% till the 25th minute. When the MIC was doubled, the percentage of organisms killed by the clove essential oil within 5 min of contact time was 86.9%. At 10 min of contact time, the percentage of the cell killed was 96% which increased to 100% at 15 min of contact time. At 3x MIC of the essential oil, the percentage of the organism killed increased to 100% at just 5 min of contact time.

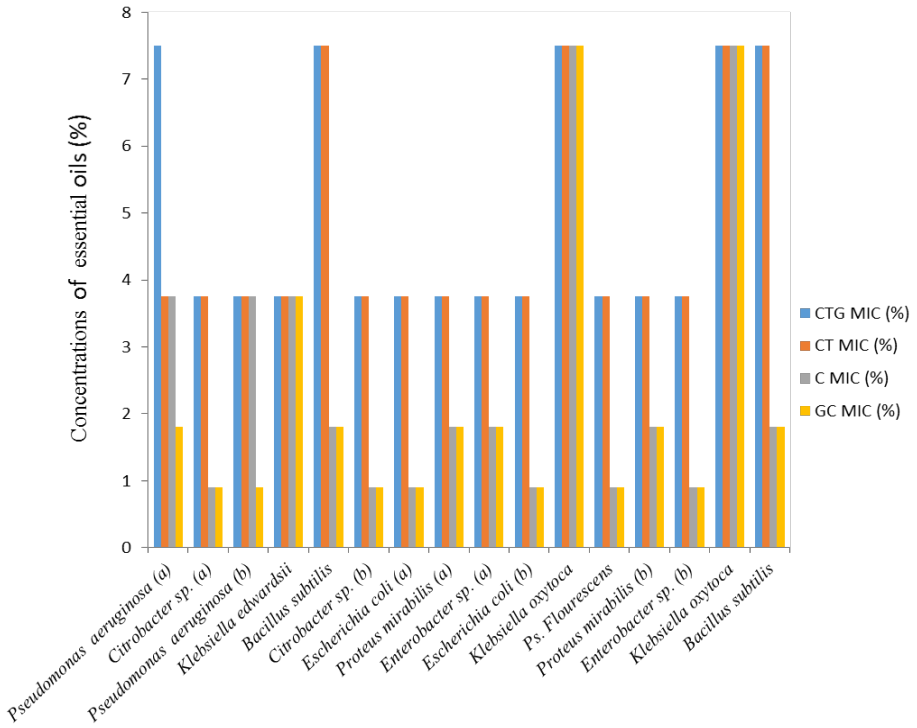

Figure 1. The minimum inhibitory concentrations exhibited by the essential oils of clove, turmeric and garlic against the test isolates.

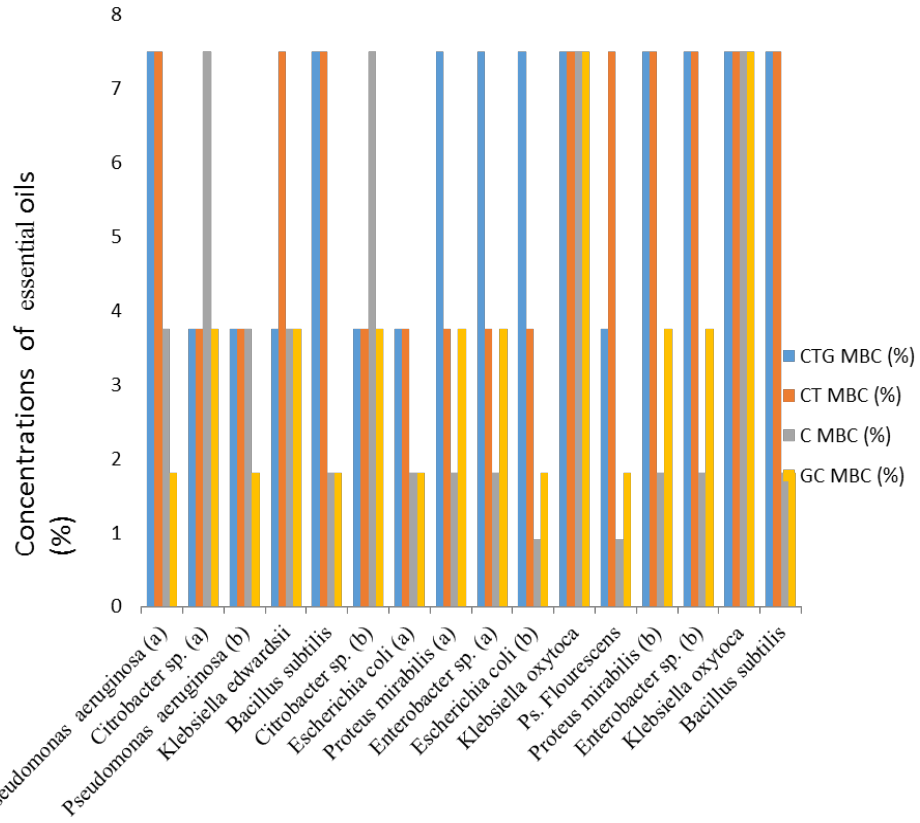

Figure 2. The minimum bactericidal concentrations exhibited by the essential oils of clove, turmeric and garlic against the test isolates.

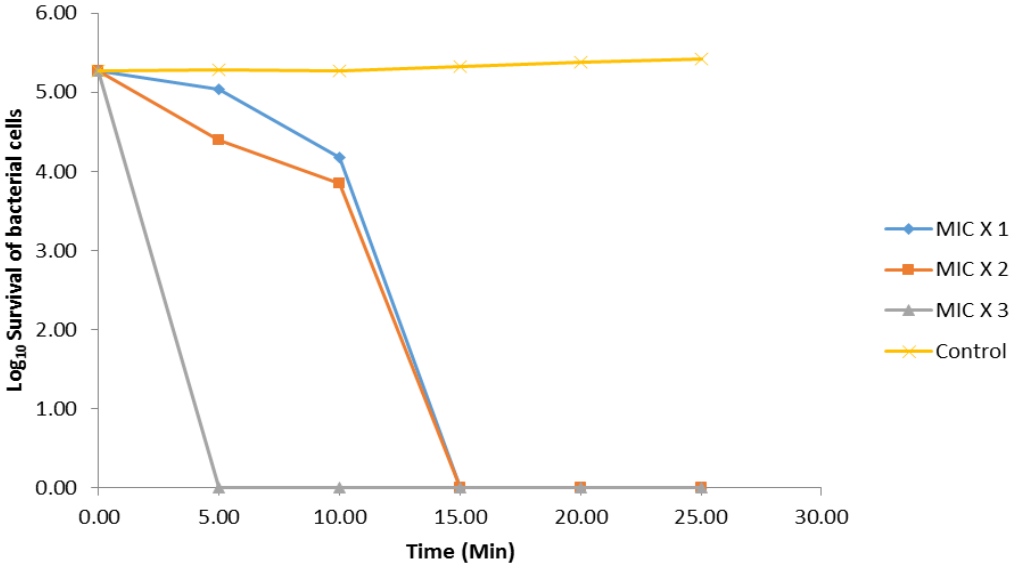

Figure 3. The bactericidal rate of *Bacillus subtilis* cells by clove essential oil at 1x MIC (—◆—), 2x MIC (—■—), 3x MIC (—▲—) and control (—✕—). Each point represents the mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the oil.

The bactericidal rate of clove essential oil against *Klebsiella oxytoca*

The bactericidal rate of clove essential oil against *Klebsiella oxytoca* at 1x MIC, 2x MIC and 3x MIC was also studied and shown in Figure 4. The percentage of the organism killed by clove essential oil at 1x MIC in 5 min was 91.5%. The percentage increased to 92.9% at 10 min of contact time and at 15 min the percentage of the cell killed increased to 100%.

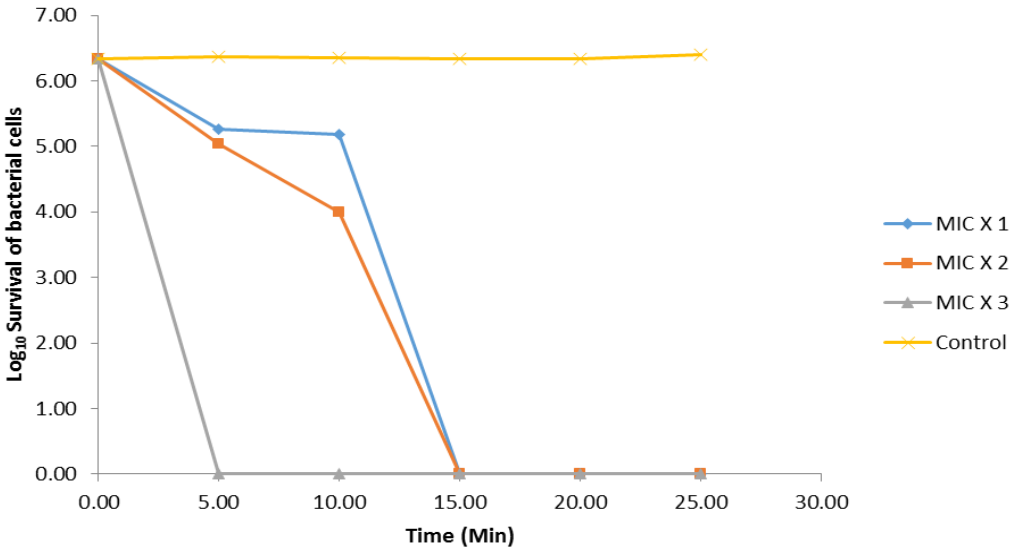

Figure 4. The bactericidal rate of *Klebsiella oxytoca* cells by clove essential oil at 1x MIC (—◆—), 2x MIC (—■—), 3x MIC (—▲—) and control (—✕—). Each point represents the mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the oil.

Discussion

At a concentration of 15%, clove essential oil (C) inhibited all the 14 isolates used for these study. This agrees with the work of Petersen et al. (2005) who observed significant bactericidal action of clove essential oil against all the strains of *Escherichia coli* used for the study. Shabnam et al. (2012) also reported that clove essential oil at 10%, significantly suppressed the microbial growth of all tested fungal and bacterial strains. The combinations of clove and tumeric essential oils (CT) inhibited 92.8% of the isolates. This is similar to the work of Mahfuzul et al. (2008) who reported that the combination of clove and cinnamon essential oils was effective against both Gram-positive and gram-negative food-spoilage bacteria. Tumeric essential oil (T) did not inhibit any of the 14 isolates used for this study. This suggests that clove alone was active in the combination of clove and turmeric. Therefore, Clove essential oil was found to possess a good antimicrobial property and no synergistic effect was found in clove and turmeric (CT) essential oil. However, this research is in disagreement with the study carried out by Shawket (2013), who reported that tumeric essential oil exhibited 100% antimicrobial activity against *Staphylococcus aureus* tested. The results of this study showed that the essential oil of clove was more effective than tumeric essential oil against the pathogenic bacteria implicated in periodontal disease used for the study. All the bacteria isolates were resistant to tumeric essential. Clove essential oil had the highest activity against *Bacillus subtilis*. Eugenia et al. (2009) investigated the essential oil of clove and observed that it contains 85.3% of eugenol which is an antimicrobial compound having a wide spectrum of antimicrobial effects against both gram negative and gram positive bacteria (Burt et al., 2003; Nanasombat et al., 2005). According to Gurdip et al. (2006), the antibacterial activity of essential oil is considered significant if the MIC of the essential oil is below 50%. The minimum inhibitory concentration and the minimum bactericidal concentration exhibited by the combination of CT essential oils and clove essential oil were far below 50 % as observed in this study. This agrees with the work of Mahfuzul et al. (2008) who reported 15% as the lowest minimum inhibitory concentration of clove essential oil for almost all the test organisms.

The bactericidal effect of the clove essential oil on *Bacillus subtilis* and *Klebsiella oxytoca* in this study showed that the rate at which clove essential oil eliminates the bacteria cells increases with increase in the concentrations of the essential oil and contact time. This corroborate the work of Pankey and Sabbath (2004) who observed that the *in vitro* time kill studies found daptomycin to be rapidly bactericidal against the majority of the organisms tested, killing 99.9% of bacterial within 20 min. The rate at which this essential oil eliminated these organisms at a very short period and contact time is the most accepted definition of the bactericidal activity in antibiotics (Pankey and Sabath, 2004). *In vitro* bactericidal assays are expressed as the rate of killing by a fixed concentration of an antimicrobial agent and are among the most reliable methods for determining tolerance (Nostro et al., 2001).

Conclusion

Clove essential oil was found to be active against all the bacterial isolates. The ability of the clove essential oil to inhibit and kill such multi-resistant gram negative and gram positive bacterial isolates implicated in periodontal diseases used for this study at low concentration and at minimal contact time shows the effectiveness of the clove essential oil. This study, therefore, establishes that clove essential oil can be used as a potential source of broad spectrum antimicrobial drug in the treatment of periodontal diseases. Therefore, different formulations

could be prepared from this essential oil for clinical trials. Such formulation could be an efficient therapy to kill or inhibit microbial populations in periodontal diseases and thus enhances periodontal health.

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Conflict of interest statement

Authors declare that they have no conflict of interests.

References

Akinpelu, D. A.; Kolawole, D. O. Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum.). **Science Focus**, v. 7, p. 64-70, 2004.

Alitonou, G. A.; Tchobo, F. P.; Avlessi, F.; Yehouenou, B.; Yedomonhan, P.; Koudoro, A.; Sohounhloue, D. K. Chemical and biological investigations of *Syzygium aromaticum* essential oil from Benin. **International Journal of Biological Science**, v. 6, No. 3, p. 1360-1367, 2012.

Bascones-Martinez, A. Periodontal diseases as bacterial infection. Medicine. **Oral Pathology Oral Bulletin**, v. 9, Suppl. 101-7, p. 92-100, 2004.

Behura, C.; Ray, P.; Rath, C. C.; Mishra, R. K.; Ramachandraiah, O. S.; Charyulu, J. K. Antifungal activity of essential oils of *Curcuma longa* against five rice pathogens *in vitro*. **Journal of Essential Oil-Bearing Plants**, v. 3, No. 2, p. 79-84, 2000.

Bruce, L. P.; William, F. A. America Academy of Periodontology: treatment of gingivitis and periodontitis (position paper). **Journal of Periodontology**, v. 68, p. 1246-53, 1997.

Burt, S. A.; Reinders, R. D. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. **Journal of Applied Microbiology**, v. 36, p. 162-167, 2003.

Chattopadhyay, I.; Biswas, K.; Bandyo Padhyay, U.; Banerjee, R. K. Turmeric and curcumin: Biological actions and medicinal applications. **Current Science**, v. 87, p. 44-53, 2004.

Cobb, C. M. Non-surgical pocket therapy-mechanical. **Annual Periodontal Review**, v. 1, p. 443-490, 1996.

Eugénia, P.; Vale-Silva, L.; Cavaleiro, C.; Salgueiro, L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. **Journal of Medical Microbiology**, v. 58, No. 11, p. 1454-1462, 2009.

Ferreira, O. G.; Cardoso, S. V.; Borges, A. S.; Ferreira, M. S.; Loyola, A. M. Oral histoplasmosis in Brazil. **Oral Medicine**, v. 93, p. 654-659, 2010.

Guenther, E. **The essential oil**: history, origin in plant production analysis. New York: Van Nostrand, 1949. v. 1.

Gurdip, S.; Sumitra, M.; De Lampasona, M. P.; Cesar, C. Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. **Journal of Science of Food and Agriculture**, v. 86, p. 111-121, 2006.

Hadizadah, I.; Peivastegan, B.; Hamzehzarghani, H. Antifungal activity of essential oil from some medicinal plants of Iran against *Alternarea alternata*. **Annual Journal of Applied Science**, v. 6, No. 5, p. 857-861, 2009.

Irobi, O. N.; Moo-Young, M.; Anderson, W. A. Antimicrobial activity of Annato (*Bixa orellana*) extract. **International Journal of Pharmacognosy**, v. 34, p. 87-90, 1994.

Joe, B.; Vijaykumar, M.; Lokesh, B. R. Biological properties of curcumin-cellular and molecular mechanisms of action. **Critical Reviews in Food Science and Nutrition**, v. 44, p. 97-111, 2004.

Keles, L. C.; Gianasi, F. M.; Souza, R. C.; Brito, B. L.; Schaab, E. H.; Souza, M. G.; Carvalho, T. C.; Martins, C. H.; Veneziani, R. C.; Cunha, W. R.; Crotti, A. E. Antibacterial activity of 15 deoxygo-yazensolide isolated from the stems of *Minasia alpestris* (Asteraceae) against oral pathogens. **National Production Research**, v. 25, No. 4, p. 326-331, 2011.

Luthra, P. M.; Singh, R.; Chandra, R. Therapeutic uses of Curcuma longa (Turmeric). **Indian Journal of Clinical Biochemistry**, v. 16, p. 153-160, 2001.

Mahfuzul Hoque, M. D.; Bari, M. L.; Vijay, K.; Kawamoto, S. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria* monocytogenes in ground chicken meat with their essential oils. **National Food Resource**, v. 72, p. 9-21, 2008.

Nanasombat, S.; Lohasupthawee, P. Antimicrobial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. **Journal of science Technology**, v. 5, p. 527-538, 2005.

Norajit, K.; Laohakunjit, N.; Kerdchoechuen, O. Antibacterial effect of five Zingiberaceae essential oils. **Molecules**, v. 12, No. 8, p. 2047-2060, 2007.

Nostro, A.; Roccaro, A. S.; Bisignano, G.; Marino, A.; Cannatelli, M. A.; Pizzimenti, F. C.; Cioni, P. L.; Procopio, F.; Blanco, A. R. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. **Journal of Medical Microbiology**, v. 56, p. 519-523, 2007.

Odenholt. I.; Lowdinand, E.; Cars, O. Pharmacodynamics of telithromycin *in vitro* against respiratory tract pathogens. **Chemotherapy**, v. 45, p. 23-29, 2001.

Olorundare, E. E.; Emudianughe, T. S.; Khasar, G. S.; Koteyi, S. A.; Irobi, D. N. Antibacterial properties of leave extract of *Cassia alata*. **Bioscience Research Communications**, v. 4, p. 113-117, 1992.

Pankey, G. A.; Sabath, L. D. Clinical relevance of bacteriostatic ver bacteriocidal mechanisms of action in the treatment of Gram-positive bacterial infections. **Journal of Clinical Infectious Diseases**, v. 38, No. 6, p. 864-870, 2004.

Petersen, P. E. The burden of Oral disease: challenges to improving Oral Health in the 21st century. **Bulletin of World Health Organisation**, v. 83, No. 3, 2005.

Petersen, P. E. **Continuous improvement of oral health in the 21st century**: the approach of the WHO Global Oral Health Programme. Geneva: WHO, 2003. Available from: <http://www.who.int/oral_health/media/en/orh_report03_en.pdf>. Accessed on: Dec. 21, 2016.

Pinto, E.; Vale-Silva, L.; Cavaleiro, C.; Salgueiro, L. Antifungal activity of the Clove essential oil from *Syzigium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. **Journal of Medical Microbiology**, v. 58, No. 11, p. 1454-1462, 2009.

Ram, T. E.; Slots, J. Local delivery of antimicrobial agents in the periodontal pockets. **Periodontology**, v. 10, p. 139-159, 2005.

Sessou, P.; Farougous, S.; Kancho, S.; Djenontin, S.; Alitonou, G. A.; Azokpota, P.; Youssao, I.; Sohomloue, D. Bio-efficacy of *Cymbopogon citratus* essential oil against foodborne pathogens in culture medium and in traditional cheese wagashi produced in Benin. **International Research Journal of Microbiology**, v. 3, No. 12, p. 406-415, 2012.

Shawket, D. S. Screening the antibacterial potency of *Curcuma longa* L. essential oil extract against boils causing *Staphylococcus* sp. **International Journal of Advanced Biological Research**, v. 3, No. 4, p. 490-500, 2013.

Slots, J.; Greenwell, H.; Fiorellini, J.; Giannoble, W.; Offenbacher, S.; Townsend., C.; Sheridan, P.; Genco, R. Systemic antibiotics in periodontics (position paper). **Periodontology**, v. 75, No. 11, p. 1553-65, 2004.

Villages, I. S.; Fidalgo, S.; Alacon, L. C. New mechanisms and therapeutic potential of curcumin for colorectal cancer. **Molecular Nutrition and Food Research**, v. 52, p. 1040-1061, 2008. http://dx.doi.org/10.1002/mnfr.200700280