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# ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF BASIL AND THYME ON CARIOGENIC BACTERIAL STRAINS: A SYSTEMATIC STUDY

**[1]T.M. OBUOTOR, *[1]W.E. IKE, [1]A.O. KOLAWOLE, [1]F.J. SALAKO, [2]C.O. IGBOKWE, [3]C.O. FASUYI**

[1]Department of Microbiology, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria

[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

[3]Department of Health Sciences and Social Work, Western Illinois University, Macomb, Illinois, U.S.A.
***Corresponding Author**: ike.williams62@yahoo.com, ikewe@funaab.edu.ng, **Tel**: +234 806 718 5598

## ABSTRACT

Dental caries has remained the most prevalent and costly oral infectious disease globally. Although several techniques have been employed to prevent this biofilm-dependent disease, many have failed including the use of available synthetic drugs. This current study investigated the antibacterial activity of the essential oils (EOs) of *Ocimum citriodorum* (Basil) and *Thymus vulgaris* (Thyme) against clinical bacteria of oral origin. The chemical composition and antimicrobial attributes of the essential oils obtained from the aerial parts of the plants have been well studied. Nine bacterial strains comprising, *K. pneumonia*, *E. coli*, 2 strains of *P. aeruginosa*, *B. licheniformis*, *S. aureus*, *L. acidophilus*, and 2 strains of *S. mutans* were been study. Essential oils were extracted by hydro-distillation Clevenger apparatus. Inhibitory zone dimension, and minimal inhibitory and minimal bactericidal concentrations (MIC/MBC) were congruently determined using well-diffusion, and broth microdilution methods. EO of *Ocimum citriodorum* showed significant activity at 64 µl/mL dose against *B. licheniformis* and the 2 strains of *S. mutans*, *B. licheniformis*, *L. acidophilus*, *E. coli*, but the 2 strains of *S. mutans*, and *S. aureus* were highly susceptible at 128µl/mL dose as their MIC/MBC varied from 0.20 to 1.60 µl/mL, and 0.20 to 3.13 µl/mL respectively. For *Thymus vulgaris* EO, at 64 µl/mL only *B. licheniformis* and *S. aureus* were susceptible. However, all the isolates except *E. coli* and the 2 strains of *S. mutans* were sensitive at 128 µl/mL dose. The MIC/MBC values ranged from 0.20 to 0.40 in both cases. The use of essential oils from basil and thyme leaves may be a plausible alternative in the prevention and treatment of cariogenic pathogens.

**Keywords**: Antibacterial activity, Cariogenic bacteria, Essential oils, *Ocimum citriodorum*, *Thymus vulgaris*

## INTRODUCTION

For centuries, plants have been widely useful in a variety of purposes, including treatment of infectious diseases, preservation of food, and perfume production. Presently, the increasing resistance of microorganisms to available antimicrobials as well as emerging and re-emerging diseases have narrowed the therapeutic options for infections caused by microorganisms (Sakkas and Papadopoulou, 2017). In order to counter the emergence of the drug resistance phenomenon, new antimicrobials need to be developed. Plant essential oils are currently explored as a promising substitute, and to date, many plant essential oils have been documented to exhibit considerable antimicrobial activity. This activity which is associated with their ability to synthesize aromatic compound, has been attributed to the presence of phenols or oxygen-substituted derivatives **(**Yamazaki *et al.* 2004**)**.

The term essential oils (EOs) was first used in the 16$^{th}$ century by Paracelsus to name the active components of drugs (Edris, 2007). EOs which are mixture of volatile constituents produced by aromatic plants as secondary metabolites, have been reported to protect such plants against predators, microorganisms or adversities of weather conditions (Bakkali *et al.*, 2008, Sá *et al.*, 2013). Approximately 3,000 out of the 100,000 well-known secondary metabolite are essential oil based compounds, of which about 300 are used commercially in the food, cosmetics, and pharmaceutical industries (Rota *et al.*, 2004, Freires *et al.*, 2015). About 500 compounds including terpenes, terpenoids, and aliphatic and aromatic molecules such as aldehydes and phenols are the main components of essential oils (Freires *et al.*, 2015).

Considering the different classes of EOs, it is possible that their antimicrobial potency may be connected to several modes of action rather than a single mechanism targeting the microbial cell. However, important properties including hydrophobicity, disturbance of the cytoplasmic membrane, disruption of the electron flow, active transport, and coagulation of cell contents, have been described as plausible mechanisms of antimicrobial activities (Burt, 2004).

Basil (*Ocimum basilicum*) of the family Lamiaceae (Labiatae) belongs to the family Lamiaceae. Basil leaves which is commonly used, contain essential oil of 0.2-1%, with the major constituents being linalool and estragole (methyl chavicol), in addition to o-cymene, citral, alpha-pinene, camphene, beta-pinene, geraniol, and geranial (Lachowicz et al., 1998, Opalchenova and Obreshkova, 2003). According to Rattanachaikunsopon and Phumkhachorn (2010), the major constituents of basil oil vary considerably, based on genetic factors, geographical origins, nutritional status, the extracted plant parts (stem, leaf, and flower), and the extraction method (Rattanachaikunsopon and Phumkhachorn, 2010). The medicinal uses of basil as an inhibitor of acid-resistant bacteria in foods have been documented (Lang and Buchbauer, 2012, Freires *et al.*, 2015, Sakkas and Papadopoulou, 2017). It forms the major proportion of ingredients used in many dental preparations and oral solutions due to its antiseptic properties (Matiz *et al.*, 2012, Sakkas and Papadopoulou, 2017). Reports have, however, shown that methyl eugenol and estragole are accountable for cytotoxic damages and cancerogenesis associated with basil oil (Sakkas and Papadopoulou, 2017). In food products, based on the Council of Europe guidelines, methyl eugenol must not be detectable, while estragole must not exceed the limit of 0.05% mg/kg (Schulz *et al.*, 2003).

Thyme (*Thymus vulgaris*) belongs to the family Lamiaceae and has 928 species which have been identified in Europe, Northern Africa, Asia, Southern America, and Australia (Sakkas and Papadopoulou, 2017). There are different chemotypes based on the chemical composition. For instance, in Southern France, there are oil chemotypes of species *T. vulgaris* with thymol as the prevailing components, whereas in Spain, it has been reported to have a chemotype containing 1, 8-cineol as the predominating component (Sakkas and Papadopoulou, 2017, Nabavi *et al.*, 2015, Badi *et al.*, 2004). Due to its properties as an antioxidant, antibacterial,

antifungal and as preservative in food, thyme essential oil represents one of the ten most globally and commercially sought after. Its antibacterial effects, which are linked to the presence of their phenolic, carvacrol and thymol components are remarkable (Sakkas and Papadopoulou, 2017). In a retrospective study, carvacrol shows the most effective potency against *Listeria monocytogenes,* and this is followed by thymol, eugenol, cinnamaldeyde, and isoeugenol (Yamazaki *et al.*, 2004). In another study where oregano and thyme essential oil were comparatively analyzed, the two oils demonstrated considerable antibacterial activity against Gram-positive and Gram-negative bacteria such as *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Aeromonas sobria*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Serratia marcescens* (Burt and Reinders, 2003). In this current study, we aimed to investigate the potency of lemon basil and thyme essential oils on bacteria isolates recovered from dental caries of patients attending dental facilities in Abeokuta.

## MATERIALS AND METHODS

*Plant material collection and identification*

The *Thymus vulgaris* and the *Ocimum citriodorum* leaves used in this study were purchased from a local market in Sango Otta, Ogun state. These wild plants are commonly found in the communal lands in the Northern part of Nigeria (Figure 1). These plants are not endangered protected species consequently, specific permission was not needed for their collection and investigation. These plant species were identified by a botanist at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, and voucher specimens were deposited in the Herbarium.

### Preparation of plant materials and extraction of essential oils

The purchased *Thymus valgaris* and the *Ocimum citriodorum* plants were shade dried and milled into powders. The fine powders (300 g) of each plant were added to 3000 ml distilled water (with vegetal/extraction solvent rate = 1/100 (w/v) in a 4 litre round bottom flask and subjected to range of 45 to 55°C using Clavenger type apparatus. The resulting volume of oil was quantified in milliliter, dried over anhydrous sodium sulfate and stored in an amber vial at 4°C until used.

*Isolation and identification of cariogenic bacteria*

*Culture method.*

Samples of Saliva collected from the State Dental Center, Ijaye, Abeokuta, were vortex mixed, diluted ten-fold with sterile water and grown on agar plates. The following media were used targeting various dental associated pathogenic bacteria; Mitis Salivarius (MS) agar supplemented with potassium tellurite *(Streptococci*), de Man, Rogosa, and Sharpe (MRS) agar (*Lactobacilli),* Mannitol Salt agar (MSA) (*Staphylococcus aureus*) and MacConkey for other pathogens. Appropriate amounts of sterilized semi-solid media were poured on to Petri-plates and were allowed to solidify at room temperature. To each plate, 100µL of the diluted sample was spread evenly and incubated at 37°c for 18 to 24 hours. Bacterial colonies were observed and deduced based on colony morphology, shape, and color.

*Biochemical tests to confirm the identity of isolates*.

Confirmation for the bacterial isolates were done by Gram staining, catalase tests, oxidase test, urease utilization, arginine dehydrogenase test, and sugar fermentation tests (mannitol, sorbitol, inulin, lactose, raffinose, and melibiose). Aerobic growth was tested by inserting inverted Durham tubes into test tubes containing broths where the bacteria were inoculated. The presence or absence of air bubbles (gas) at the bottom of inverted Durham tubes was considered as an indicator of aerobic or anaerobic respiration, respectively (Damtie and Mekonnen, 2020). Hemolysis test was also conducted by inoculating the isolates on to blood agar medium.

*Determination of the antibacterial activities of the EOs*

Susceptibility tests against the bacterial isolates were accomplished using agar-well diffusion method on Muller-Hinton agar. A colony of the test organism was picked using a sterile wire loop and was inoculated into 5 ml of normal saline in a test tube. This was then standardized to 0.5 McFarland using a colorimeter. A sterile swab stick was used to inoculate the suspension onto

the surface of a gelled Mulluer-Hinton agar and six wells were made using sterile cork borer (6 mm diameter). The essential oils were diluted in 3% DMSO to the doses of 16 µL/mL, 32 µL/mL, 64 µL/mL, and 128 µL/mL and dispensed into each of the wells. The fifth and sixth wells were filled with 3% DMSO and 3% $H_2O_2$ as negative quality control (NQC) and positive quality control (PQC), respectively.

*Determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC).*

The MIC was determined using the agar dilution method which is described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (EUCAST, 2000). To determine the MIC, 20 mL of Mulluer-Hinton agar (MHA) was used in 9 cm Petri dishes for agar dilution. Nineteen mL of molten MHA was added to 1 mL of each EO dose to make the total 20 mL. MHA was prepared as recommended by the manufacturers and was cooled to 50 °C in a water-bath. EOs of *Thymus vulgaris* and the *Ocimum citriodorum* were prepared into doses of 0.195 mg/ml, 0.391 mg/ml, 0.782 mg/ml, 1.563 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml in 25-30ml containers (ten containers for each selective medium).

Nineteen mL of molten MHA was added to each container and mixed thoroughly, and thereafter poured into pre-labeled sterile Petri dishes on a level surface. The plates were allowed to dry at room temperature to avoid drops of moisture on the surface of the agar. Bacterial suspensions grown in Tryptic Soy broths supplemented with 0.2% glucose (McGhie, *et al.*, 1977, Bokhout, *et al.*, 1996) and incubated for 18-24 hours were inoculated on the dry plates. The wells were loaded with the different doses of the EOs and incubated without inverting the plates at 37°C for 18-24 hours. The MIC (the lowest concentration of the extracts that completely inhibited visible growth) was judged by the naked eyes. The MBC (the lowest concentration of the extracts that completely killed all bacteria) was determined by taking scratches from MIC tests and trying to grow bacteria on new agar plates. In the case of the growth of colonies from scratches taken from the MIC test plates, the MIC was not considered as MBC and vice-versa.

## STATISTICAL ANALYSIS

Data were expressed as the mean ± SEM for each group. A computer program (SPSS version 20) was used for statistical analysis. Differences among the groups were analysed using one-way analysis of variance (ANOVA) followed by LSD postHoc multiple comparisons. P values $< 0.05$ were considered statistically significant.

*Ethical consideration*

This study did not require ethical clearance as samples were obtained by the laboratory staff of the State Dental Center, Ijaye, Abeokuta. No contact whatsoever was made between the patients and the researcher.

# RESULTS

*Identification of cariogenic bacteria*

The bacteria isolates identified included: *Klebsiella pneumoniae, Bacillus licheniformis, Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Pseudomonas aeruginosa* and *Escherichia coli,* with two *Pseudomonas aeruginosa* designated as *Pseudomonas aeruginosa* 1 *and Pseudomonas aeruginosa* 2, and two *Streptococcus mutans,* designated as *Streptococcus mutans*-1 *and Streptococcus mutans*-2. They all produced yellow colors by fermenting the sugars. They all grew in broth supplemented with arginine and produced brick red color. The Gram stain of the isolates revealed Gram-positive for *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Bacillus licheniformis*, and *Streptococcus mutans*-1 and -2. However, *Pseudomonas aeruginosa*-1 and -2, *Bacillus licheniformis*, *Klebsiella pneumoniae* and *E. coli* appeared rod shaped. Reaction to hemolysis revealed positivity for *Bacillus licheniformis*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, and *E. coli*, whereas *Pseudomonas aeruginosa*-1 and -2, *Streptococcus mutans*-1 and -2, and *Staphylococcus aureus* appeared hemolytic negative (Table 1).

*Antibacterial activity of Ocimum citriodorum EO against bacterial isolates*

The EO of *Ocimum citriodorum* resulted in a dose-dependent inhibition against all the bacterial isolates. *Bacillus licheniformis* had the highest zone (Table 2) followed by *Streptococcus mutans*-2, *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus acidophilus*, *Streptococcus mutans*-1, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-1 and -2 in decreasing order. Out of the four different doses of *Ocimum citriodorum* EO (16 µL/mL, 32 µL/mL, 64 µL/mL, and 128 µL/mL) used against these bacterial isolates, 128 µL/mL dose resulted in significant zone of inhibition except for *Pseudomonas aeruginosa*-1 and -2 with mean inhibition zone of 10 and 12 mm congruently.

The highest mean zones of inhibition observed were 40.5 ± 1.0 mm against *Bacillus licheniformis*, 23.0 ± 0.5 mm against *Streptococcus mutans*-2, 22.0 ± 0.5 mm against *Staphylococcus aureus* and 20.0 ± 0.5 mm for *Escherichia coli* at the dose of 128 µL/mL; 19.1± 0.5 mm, 18.0 ± 0.0 mm, 16.0 ± 0.2 mm, 12.0 ± 0.0 mm, and 10.0 ± 0.0 were recorded for *Lactobacillus acidophilus*, *Streptococcus mutans*-1, *Klebsiella pneumoniae, Pseudomonas aeruginosa*-2 and -1 correspondingly. At 64 µL/mL dose, a mean inhibition zone of 25.0 ± 0.5 mm was recorded against *Bacillus licheniformis*, as 19.7 ± 0.7 mm was against *Streptococcus mutans*-2, and 18.0 ± 0.0 mm was for *Staphylococcus aureus.* The other isolates, however, exhibited inhibition zones lower than 18 mm. At concentrations of 32 µL/mL and 16 µL/mL, all the isolates showed a range of inhibition zones between 7 and 14 mm. The mean minimum inhibition zone at the dose of 32 µL/mL lied between 8 and 20 mm with *Pseudomonas aeruginosa*-1 being the least (08.0 ± 0.7 mm) and *Streptococcus mutans*-1 being the highest (14.3 ± 0.3 mm). *Pseudomonas aeruginosa*-1 recorded 07.0 ± 0.0 mm as the least zone of inhibition, and 09.7 ± 0.3 mm against *Bacillus licheniformis* as the highest zone of inhibition at concentration of 16 µL/mL (Table 2).

*Antibacterial activity of Thymus vulgaris EO against bacterial isolates*

All the isolates except *Streptococcus mutans*-1 and *Escherichia coli* were inhibited by the different doses of the EO; 16 µL/mL, 32 µL/mL, 64 µL/mL, and 128 µL/mL in a dose dependent pattern (Table 2). Concentration of 16 µL/mL represented the smallest range of inhibition. The test EO at the dose of 16 µl/mL was observed to show no mean inhibitory zones against *Streptococcus mutans*-1 and *Escherichia coli* (00.0 ± 0.0 mm), the mean maximum inhibitory zone was recorded against *Staphylococcus aureus* (14.3 ± 0.9 mm). At concentration of 32 µL/mL, mean zero inhibition zone (00.0 ± 0.0 mm) was recorded against *Streptococcus mutans*-1 and *Escherichia coli* but the maximum (18.7 ± 0.7 mm) was detected against *Staphylococcus aureus.*

At concentration of 64 µL/mL, the EO inhibited isolates with mean inhibition zones better than result obtained at 32 µL/mL concentration. At this dose, *Streptococcus mutans*-1 and *Escherichia coli* remained resistant to the oil as *Staphylococcus aureus* maintained susceptibility with highest mean susceptibility zone (26.0 ± 1.0 mm) followed by *Bacillus licheniformis* (22.3 ± 0.3 mm), *Klebsiella pneumoniae* (17.7 ± 0.3 mm), *Pseudomonas aeruginosa*-2 (14.7 ± 0.9 mm), *Lactobacillus acidophilus* (14.3 ± 0.3 mm), *Pseudomonas aeruginosa*-1 (12.7 ± 0.3 mm), and *Streptococcus mutans*-1 (10.1 ± 0.0 mm). At the dose of 128 µl/mL, *Thymus vulgaris* EO inhibited all the isolates except *Streptococcus mutans*-1 and *Escherichia coli* with mean inhibition zones significantly higher than the rest doses and the positive control (3% $H_2O_2$). Mean inhibition zone for *Staphylococcus aureus* stood at 47.0 ± 1.0 mm as the maximum mean inhibition zone; the positive control of 32.0 ± 1.0 mm resulted in inhibition zone higher than the maximum mean inhibitory zones for 16, 32, and 64 µl/mL doses. However, in all the tests, the negative control (3% DMSO) resulted in no inhibition against all the isolates.

Thus, the doses 128 µl/mL and 16 µl/mL were found to demonstrate the most and least inhibitor concentrations against the isolates. In most cases, the positive control resulted in inhibition zones higher than different doses. Overall, 128 µl/mL concentration of both EOs appeared to be the most potent concentration than other doses (Table 2).

*MIC and MBC of Ocimum citriodorum* **and** *Thymus vulgaris EOs against bacterial isolates*

The MIC and MBC of the test EOs were determined for all isolates except for *Streptococcus mutans*-1 and *Escherichia coli* which were found to show no zones of inhibition with regard to *Thymus vulgaris* EO. For *Ocimum citriodorum*, the MIC and MBC values ranged from 0.20 µl/mL to 1.60 µl/mL and 0.40 µl/mL to 3.13 µl/mL respectively (Table 3). Same MIC and MBC values were determined for *Klebsiella pneumoniae*, *Streptococcus mutans*-2, *Lactobacillus acidophilus*, and *Escherichia coli*. *Klebsiella pneumoniae* was 0.80 µl/mL, *Streptococcus mutans*-2 was 0.20 µl/mL as *Lactobacillus acidophilus*, and *Escherichia coli* showed 0.40 µl/mL. Similarly, the *Pseudomonas aeruginosa* strains (1 and 2) demonstrated the same MIC (0.20 µl/mL) and MBC (0.40 µl/mL). High MIC (1.60 µl/mL) and MBC (3.13 µl/mL) values were recorded against *Streptococcus mutans*-1 and *Staphylococcus aureus* congruently (Table 3).

The *Thymus vulgaris* EO was also very active against the isolates as *Klebsiella pneumoniae*, *Streptococcus mutans*-2, *Lactobacillus acidophilus*, and *Pseudomonas aeruginosa*-2 strain showed the same MIC and MBC values (0.20 µl/mL) (Table 3). For *Bacillus licheniformis*, the MIC and MBC values were found to be the same (0.40 µl/mL), while the MIC and MBC values for *Pseudomonas aeruginosa*-1 strain were 0.20 µl/mL and 0.40 µl/mL correspondingly (Table 3). However, values of MIC and MBC for *Streptococcus mutans*-1 and *Escherichia coli* could not be determined because they were resistance to *Thymus vulgaris* EO.

## DISCUSSION

Essential oils have been proven to contain certain antimicrobial properties that may be significant in resolving the problems associated with microbes such as bacteria, viruses, and protozoa (Upadhyay *et al.*, 2010, Chouhan Sonam., *et al.* 2017, Khalil Noha., *et al.*, 2018). Many are known to produce antimicrobial agents that have the ability to track infectious diseases. While some can synthesize chemicals that can induce chemotherapeutic mechanisms, others have molecular constituents capable of causing the death of bacteria (Man Adrian., *et al.*, 2019, Ahmad *et al.*, 2021). Apart from the emergence of antimicrobial resistance among microorganisms, especially bacteria, poor selective toxicity has also been added as major drawback to laboratory-prepared antibiotics. In both cases, however, natural antimicrobial compounds have shown to possess the ability to circumvent antimicrobial resistance and kill bacteria selectively (Ahmad *et al.*, 2021, Frieri Marianne., *et al.*, 2017, Yelin and Kishony, 2018).

This study shows that the EO of *Ocimum citriodorum* leave purchased from Sango Otta market of Ogun State showed antimicrobial activity against all the oral pathogenic bacteria. However, not all diameter of inhibition zones were recorded as susceptible. Susceptibility were recorded against *Bacillus licheniformis*, *Streptococcus mutans*-2, and *Staphylococcus aureus* at concentrations of 64 µl/mL and 128 µl/mL. Similarly, *Streptococcus mutans*-1, *Lactobacillus acidophilus*, and *Escherichia coli* were susceptible at dose of 128 µl/mL. Although *Klebsiella pneumoniae* and the two strains of *Pseudomonas aeruginosa*-1 and -2 showed zones of inhibition, the diameter recorded were not comprehensive enough to suggest susceptibility (Upadhyay *et al.*, 2010). Results showed that the activity of *Ocimum citriodorum* EO was similar for both Gram-positive and Gram-negative pathogens. In this present study, the MIC/MBC doses of *Ocimum citriodorum* EO at 1.60/3.13 µl/mL was observed to be effective against *Staphylococcus aureus* than the dose of EO of *Thymus vulgaris* at 0.20/0.40 µl/mL. This result is similar to previous report by Matiz et al. (2012). However, in the case of *Pseudomonas aeruginosa*-1 the same MIC/MBC doses of 0.20/0.40 µl/mL was found to be effective in the case of *Ocimum citriodorum* and *Thymus vulgaris* EOs. This finding is in tandem with what was reported by Sakkas and Papadopoulou (2017). The least doses of MIC/MBC observed in *Thymus vulgaris* EO against *Pseudomonas aeruginosa*-2 validates the report that *Thymus* species exhibit antibacterial actions against multiple targets in a bacteria. Gram-positive strains demonstrated elevated MIC/MBC ratio than Gram-negative strains.

This plausibly explains the decrease in the susceptibility values recorded against Gram-positive strains.

Numerous studies in the literature have shown that Thyme (*Thymus vulgaris*) possesses antimicrobial ability against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Bacillus* (Anwar *et al.*, 2015). For many decades past, *Thyme* EOs have been known to demonstrate antibacterial properties, and the Gram-positive bacteria are generally susceptible to these EOs than the Gram-negative strains (Damtie and Mekonnen, 2020). *Bacillus licheniformis*, *Staphylococcus aureus*, and *Lactobacillus acidophilus* all of which are Gram-positive bacteria thus were highly inhibited by EO of *Thymus vulgaris* in this study except the two strains of *Streptococcus mutans*. The major constituents of thyme EOs usually result in antibacterial activities against cariogenic pathogens (Botelho *et al.*, 2007). *Thymus vulgaris* discovered to be thymol and carvacrol chemotypes (Damtie *et al.*, 2019) in this study, therefore inhibited all the bacteria strains but *Streptococcus mutans* -1 and -2 at a dose of 128 µl/mL. *Bacillus licheniformis* and *Staphylococcus aureus* were inhibited at a dose of 64 µl/mL, but only *Staphylococcus aureus* was found to be susceptible at 32 µl/mL concentration.

Similarly, the Gram-negative pathogens researched in this study also showed high susceptibility rate against *Thymus vulgaris* EO exclusive of *Escherichia coli*. It has been shown that the major components of the *Thymus vulgaris* EO, phenol (thymol and/or carvacrol) (Damtie *et al.*, 2019) was plausibly responsible for the effective antibacterial potency against cariogenic pathogens. The antibacterial activities of this EO may have been from the bioactivities of their major components or the interactions of all their components (Zengin and Baysal, 2014). In a reviewed study by Bassolé and Juliani (2012), thymol and carvacrol interactions majorly result in synergistic and or additive activities against pathogenic bacteria. The MIC/MBC values of the essential oil of *Thymus vulgaris* were found to be relatively lower than *Ocimum citriodorum* EO.

While the MIC/MBC values of *Ocimum citriodorum* EO were found ranging from 0.20 µl/mL to 1.60 µl/mL and 0.20 µl/mL to 3.13 µl/mL respectively, the values for *Thymus vulgaris* EO ranges from 0.20 µl/mL to 0.40 µl/mL in both cases. *Thymus vulgaris* EO has also been reported to inhibit the growth of bacterial strains from the respiratory diseases with MIC/MBC range of 0.20 µl/mL to 0.80 µl/mL; our results were similar (Sienkiewicz *et al.*, 2012). The same correlation was confirmed of another study conducted in Poland where a commercially purchased *Thymus vulgaris* EO exhibited significantly higher antimicrobial properties against oral pathogens (Lapinska *et al.*, 2020).

Conversely, due to the bactericidal and fungicidal activities of many essential oils, their use in pharmaceutical, food, and cosmetics cannot be overemphasized as alternatives to synthetic chemical products. Essential oils and some of their components are widely used in perfume production, sanitary, dental, and agricultural products, as well as additives and preservatives in food apart from its usefulness as natural remedies (Sienkiewicz *et al.*, 2012). Investigations on the mechanism of action of essential oils and their components have been carried out in animals at the levels of *in vitro* and *in vivo* analyses (Ben Salah *et al.*, 2009, Ohkawara *et al.*, 2010, Schmitt *et al.*, 2010). Published analytical monographs are available (National Pharmacopeia, European Pharmacopeia, ISO, WHO, Council of Europe) to ensure the basic information about essential oils such as source, concentration of active components, and therapeutic doses. Due to cytotoxic capacity based on their pro-oxidant property, essential oils can be deployed as excellent antiseptic and antimicrobial agents. Essential oils are usually devoid of long-term genotoxic risks. Some of the oils have been reported to exhibit strong and clear antimutagenic property that could be connected to anticarcinogenic activity.

In spite of all their advantages, essential oils may be toxic not only to bacteria, fungi, or viruses but also against human body where they can exert adverse effects when overdosed. In eukaryotic cells, high doses of essential oils can elicit depolarization of the mitochondrial membranes via membrane potential reduction, interference

with $Ca^{2+}$ cycling and other ionic channels, as well as reduction of pH gradient (Sienkiewicz *et al.*, 2012). For example, report has shown that essential oils from plant families Apiaceae Rutaceae, Polygonaceae, and Hypericaeae contain photoactive molecules, furocoumarins which have the ability to cause damage to cellular macromolecules, and in some cases, formation of covalent adducts in DNA, proteins, and cellular membrane (Sienkiewicz *et al.*, 2012).

## CONCLUSIONS

The role and application of essential oils in the treatment of various human diseases, remarkably infectious diseases due to multidrug resistant bacterial strains, may be an alternative therapy to synthetic drugs that show adverse side effects. Both essential oils from *Ocimum citriodorum* and *Thymus vulgaris* showed strong activity against clinical strains belonging to *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., *Klebsiella* sp., *Streptococcus* sp., and *Lactobacillus* sp., isolated from the oral cavity at a dose of 64 and 128 µl/mL. *Ocimum citriodorum* EO showed lower activity against *P. aeruginosa* and *K. pneumonia*, similar lower efficacy was also observed for *S. mutans*-2 as no activity was seen against *S. mutans*-1 and *E. coli.*

**Table 1: Biochemical and physical identification of isolates obtained from oral cavities**

| Bacterium | Enzyme/Sugar/Amino Acid Fermentation | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram Stain | Cell Shape | Hemolysis | Gas Pdt | $H_2S$ Pdt | Methyl Red | Glucose | Lactose | Sucrose | Catalase | Coagulase | Oxidase | Urease | Citrate | Arginine | Inulin |
| *Pseudomonas aeruginosa*-1 | - | Rod | γ | - | - | - | - | - | - | + | - | - | - | + | + | - |
| *Streptococcus mutans*-1 | + | Cocci | β | - | - | - | + | + | + | - | - | - | + | - | - | + |
| *Staphylococcus aureus* | + | Cocci | β | - | - | + | + | + | + | + | + | - | + | + | + | - |
| *Bacillus licheniformis* | + | Rod | γ | + | + | - | + | + | + | + | - | + | + | + | + | - |
| *Klebsiella pneumoniae* | - | Rod | γ | + | - | - | + | + | + | + | - | - | - | + | - | - |
| *Streptococcus mutans*-2 | + | Cocci | β | - | - | - | + | + | + | - | - | - | + | - | - | + |
| *Lactobacillus acidophilus* | + | Cocci | α | + | - | - | + | + | + | - | - | + | - | + | - | - |
| *Pseudomonas aeruginosa*-2 | - | Rod | γ | - | - | - | - | - | - | + | - | - | - | + | + | - |
| *Escherichia coli* | - | Rod | α | + | - | + | + | + | + | + | - | - | + | - | - | - |

**Note: (+)** = ability to ferment or break down, produce gas, Gram-positive; (-) = not able to ferment or break down, produce gas, Gram-negative.

**Table 2: Antibacterial activity of the EOs of *Ocimum citriodorum* and *Thymus vulgaris* using agar well diffusion method compared to the negative and positive controls (mean ± SEM for three repetitions)**

| Treatments | Conc. (µl/mL) | Diameter zone of inhibition (mm) [mean ± SEM] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | K. *pneumoniae* | *B. licheniformis* | *S. mutans*-1 | *S. mutans*-2 | *S. aureus* | *L. acidophilus* | *P. aeruginosa*-1 | *P. aeruginosa*-2 | *E. coli* |
| ***Ocimum citriodorum*** | 16 | 07.0 ± 0.5 | 09.7 ± 0.3 | 08.3 ± 0.7 | 09.0 ± 0.5 | 09.0 ± 0.0 | 08.0 ± 0.0 | 07.0 ± 0.0 | 08.0 ± 0.0[b] | 09.0 ± 0.5 |
| | 32 | 10.3 ± 0.3 | 14.3 ± 0.3 | 10.3 ± 0.3 | 12.7 ± 0.7 | 11.0 ± 0.3 | 10.7 ± 0.3 | 08.0 ± 0.7 | 09.0 ± 0.5 | 10.0 ± 0.5 |
| | 64 | 12.7 ± 0.0 | 25.0 ± 0.0 | 14.3 ± 0.3 | 19.7 ± 0.7 | 18.0 ± 0.0 | 14.3 ± 0.3 | 09.0 ± 0.5 | 10.3 ± 0.3 | 15.0 ± 0.0 |
| | 128 | 16.0 ± 0.2 | 40.5 ± 1.0 | 18.0 ± 0.0 | 23.0 ± 0.5 | 22.0 ± 0.5 | 19.1 ± 0.5 | 10.1 ± 0.0 | 12.0 ± 0.0 | 20.0 ± 0.5 |
| ***Thymus vulgaris*** | 16 | 08.0 ± 0.0 | 09.0 ± 0.6 | 00.0 ± 0.0 | 07.0 ± 0.5 | 14.3 ± 0.9 | 08.3 ± 0.3 | 08.3 ± 0.7 | 09.7 ± 0.3 | 00.0 ± 0.0 |
| | 32 | 12.3 ± 0.3 | 12.7 ± 0.7 | 00.0 ± 0.0 | 09.0 ± 0.6 | 18.7 ± 0.7 | 09.3 ± 0.3 | 10.3 ± 0.3 | 10.3 ± 0.3 | 00.0 ± 0.0 |
| | 64 | 17.7 ± 0.3 | 22.3 ± 0.3 | 00.0 ± 0.0 | 10.1 ± 0.0 | 26.0 ± 1.0 | 14.3 ± 0.3 | 12.7 ± 0.3 | 14.7 ± 0.9 | 00.0 ± 0.0 |
| | 128 | 24.0 ± 0.5 | 31.0 ± 0.0 | 00.0 ± 0.0 | 12.0 ± 0.0 | 47.0 ± 1.0 | 24.0 ± 1.0 | 24.0 ± 1.0 | 19.0 ± 1.0 | 00.0 ± 0.0 |
| Controls | 3% $H_2O_2$ | 29.0 ± 1.0 | 30.0 ± 1.2 | 31.0 ± 0.0 | 28.0 ± 1.0 | 32.0 ± 1.0 | 29.0 ± 1.1 | 30.0 ± 1.0 | 28.0 ± 01 | 30.0 ± 0.0 |
| | 3% DMSO | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 | 00.0 ± 0.0 |

**Notes:** Diameter of cork-borer is 6mm; 3% DMSO represents negative control which did not show any activity; 3% $H_2O_2$ represents positive control which showed varied degrees of activity. The strength of activity is presented as resistant (>7 mm), intermediate (>12 mm), susceptible (>18 mm)

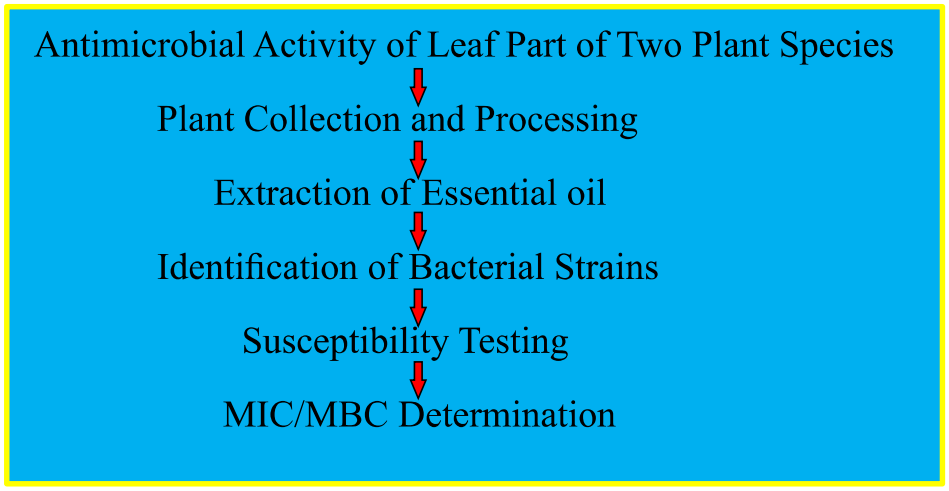
**Table 3: Antibacterial activity (MIC and MBC) of *Ocimum citriodorum* and *Thymus vulgaris* EOs against dental isolates**

| Bacterial isolates | *Ocimum citriodorum* EO (µl/mL) | | *Thymus vulgaris* EO (µl/mL) | |
|---|---|---|---|---|
| | MIC* | MBC** | MIC* | MBC** |
| *Klebsiella pneumoniae* | 0.80 | 0.80 | 0.20 | 0.20 |
| *Bacillus licheniformis* | 0.60 | 1.40 | 0.40 | 0.40 |
| *Streptococcus mutans*-1 | 1.60 | 3.13 | ND | ND |
| *Streptococcus mutans*-2 | 0.20 | 0.20 | 0.20 | 0.20 |
| *Staphylococcus aureus* | 1.60 | 3.13 | 0.20 | 0.40 |
| *Lactobacillus acidophilus* | 0.40 | 0.40 | 0.20 | 0.20 |
| *Pseudomonas aeruginosa*-1 | 0.20 | 0.40 | 0.20 | 0.40 |
| *Pseudomonas aeruginosa*-2 | 0.20 | 0.40 | 0.20 | 0.20 |
| *Escherichia coli* | 0.40 | 0.40 | ND | ND |

Notes: *MIC = Highest dilution (minimum concentration) showing no detectable growth

**MBC = Highest dilution (minimum concentration) which yielded no single colony on a solid medium.

ND = Not determined.




*Ocimum citriodorum*

*Thymus vulgaris*

**Figure 1: Experimental flow chart and aerial pictures of two plant species used in this research**

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