An in Vitro Study to Evaluate Antibacterial Effect Thymus Vulgaris Essential Oil Against Porphyromonas Gingivalis in Kurdistan– Iraq

Hama Rash A[1], Kareem S[2], Hamaamin H [3], Qadr K[3], Salih S[4], Azeez S[2], Sha A[5]

[1]Lecturer, Protected Agriculture Department, Bakrajo Technical Institute, Sulaimani Polytechnic University, Sulaimani, Iraq, [2]Lecturer, Dental Nursing Department, Sulimani Technical Institute, Sulaimani Polytechnic University, Sulaimani, Iraq, [3] Lecturer, Department of Biology, College Of Science / University of Sulaimani, Sulaimani, Iraq, [4]Assistant Lecturer, Food Science and Quality control department, College of Agriculture engineering. University of Sulimani, Sulimani, Iraq, [5] Lecturer, Periodontics Department, College of Dentistry, University of Sulimani, Sulimani, Iraq

Abstract

Purpose: Porphyromonas gingivalis is a keystone among perio-pathogengenic bacteria to initiate periodontitis and treatment is better when the periodontal pathogens including Porphyromonas gingivalis are totally eliminated. The essential oil of Thymus vulgaris has many bioactive constituents having antimicrobial activity against many bacteria. This study investigates the antibacterial effect of this extracts against Porphyromonas gingivalis. Methods: Sub gingival plaque samples were collected from individuals having periodontitis with the probing pocket depth of at least 6mm; Conventional microbiological tests and molecular technique used for confirmation of Porphyromonas gingivalis, hydrodistillation method was used for extraction of essential oil. Antibacterial activity of the extract against Porphyromonas gingivalis has been determined by using two fold serial dilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Result: the essential oil extracted from Thymus vulgaris demonstrated antimicrobial effect against Porphyromonas gingivalis growth. Conclusion:The extract was able to demonstrate inhibitory and bactericidal activity against Porphyromonas gingivalis. Further studies should focus on the use of Thymus vulgaris essential oil in the treatment of periodontitis.

Keywords: *Porphyromonas gingivalis, Thymus vulgaris, minimum inhibitory concentration, periodontitis.*

Introduction

Thymus vulgaris is herb with an evergreen status and it is native to the Mediterranean and southern Europe area [1]. The family Lamiaceae consists of approximately 150 plant genera with roughly 2800 species throughout the world, among which Thymus vulgaris, known as common thyme popularly [2]. This plant has been used traditionally as a culinary component, to give flavor to cheeses [3,4] and liqueurs and meats [5,6]. Now a day is a common ingredient of bouquet garni [7]. In addition to its usefulness in foods, this plant is a well-known as a medicinal herb that has been used many years ago in treatment of alopecia, dental plaque, dermatophyte infections, bronchitis, cough, inflammatory skin disorders, and gastrointestinal distress [8]. The major constituents of T. vulgaris essential oil are thymol (23%–60%), γ-terpinene (18%–50%), p-cymene (8%–44%), carvacrol (2%–8%), and linalool (3%–4%) [9]. T. vulgaris essential oil has antibacterial, antifungal, and anti-inflammatory effects, accounting for the medicinal uses of T. vulgaris [8]. The terpenoids and some hydrocarbons that are present in essential oils are responsible for their anti-microbial effect [10]. For these purposes, researchers are searching for new antimicrobial agents, through the use of natural plant oils.

Porphyromonus gingivalis is major periodontal pathogens and one of the main etiological factors in the inflammatory episode in initiation and progression of periodontal disease [11]. The therapeutic roles of Plant-derived essential oil products have been widely used in

the treatment of periodontitis [12]. The data concerning the efficacy of this extracted oil against periopathogens is limited in Iraq. The objective of this work is to determine the antibacterial activity of the extracted essential oil from T. vulgaris against the growth of clinical isolates of porphyromonous gingivalis.

Materials and Methods

Setting: this study was approved by Ethics Committee sulimani technical institute, sulimani polytechnic University/ kursitan- Iraq.

Plant row material and extraction of essential oil

The T. vulgaris was collected in June-July, 2019 from the Piramagron Mountain, 50 km far from Sulimani city in north of Iraq. The plant was identified in College of agriculture/ University of Sulimani. The essential oil was extracted from the herb by hydrodistillation method; 100g of thyme soaked in 350 ml of distilled water in a conical flask and left for 5 hours using a Clevenger apparatus according to [13]. The essential oil was collected after decantation and drying of the oils over anhydrous sodium sulfate, the oil stored under refrigeration (−4 ◦C) until its use.

Tested bacteria

Tested Microorganism was obtained from the Department of Biology, College of science/ University of Sulimani. Clinical strain was previously isolated from plaque of sub gingival deep pockets from patients having periodontitis according to [14].

MIC and MB determination

The antimicrobial effect of essential oil of thyme by detecting MIC was evaluated against a common oral pathogens in initiation of periodontitis by two fold serial dilution method, in which the stock solution of the tested material was prepared (800µL/1 ml) by dissolving the essential oil in (tween-80). For MIC, twelve dilutions of the essential oil were prepared by using Muller Hinton broth medium - two-fold serial dilution method. The procedure done by 1 ml from the stock solution was added into the first tube which contained 1 ml of broth. Then1 ml of broth from the first tube was transferred into the second tube. The serial dilution was repeated for the tested agent till reach 0.2 µL /ml, thus the concentrations of serial dilution was—400,200, 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, 0.2. µL /ml respectively.

To each of the above 12 prepared MIC tubes with different concentrations, 100µL of the earlier prepared suspension of bacterial (5*10 CFU/ml) was added to the tubes. Tubes were sealed with cotton and incubated for ≥48 h at 37ºC in an anaerobic jar by using AnaeroGen® system Oxoid gas pack and observed for turbidity. The minimum concentration of the essential oil in the tube determined by which does not show any turbidity [Figure 1].

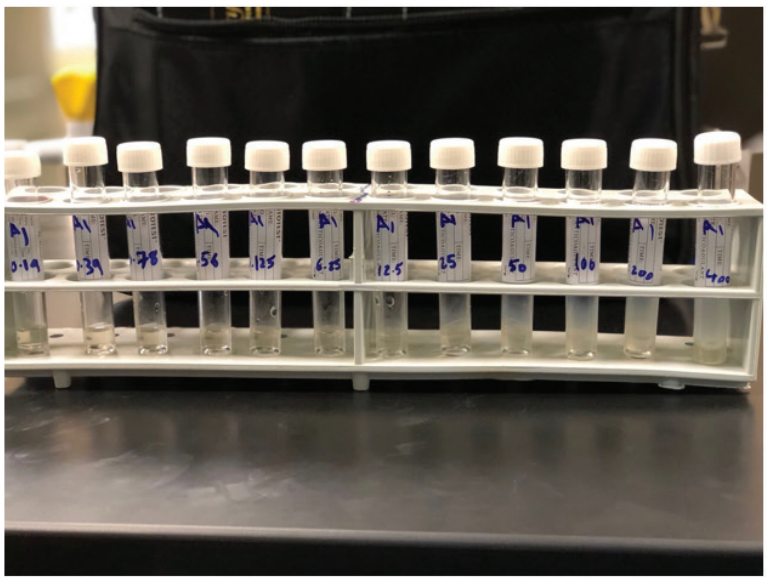
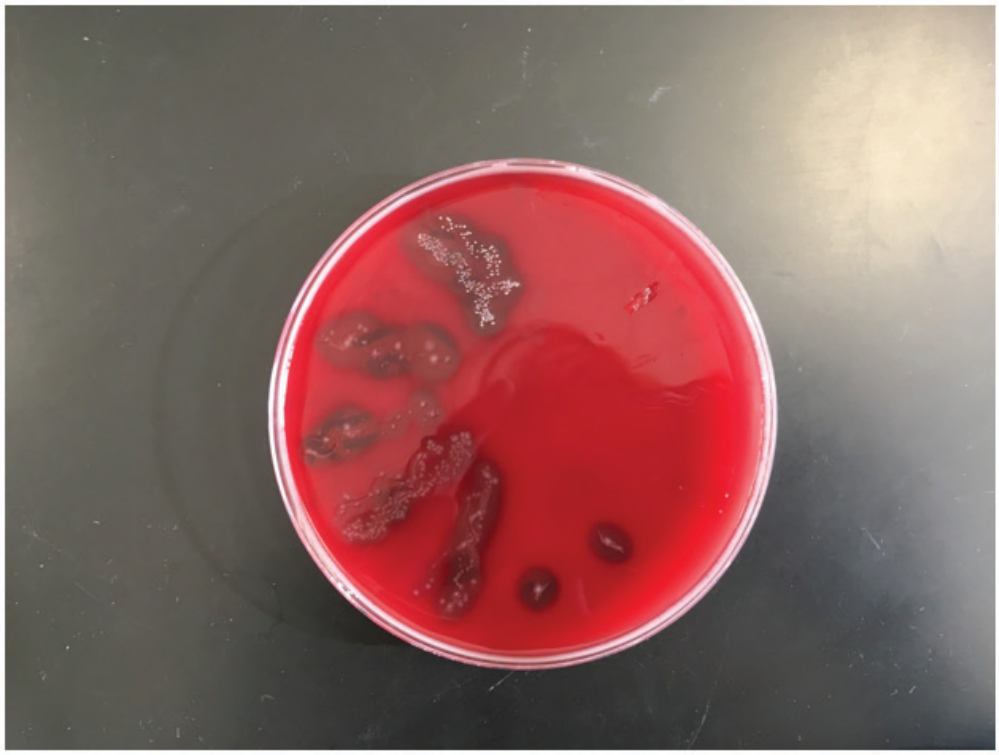


Figure 1 different concentration of essential oil of thyme

The determination of minimum bactericidal concentration (MBC) of thyme oil was done by choosing the concentrations that showed no bacterial growth during the evaluation of the MIC. Then a sample by micropipette was taken from the chosen tubes and spread on a blood agar plate using a spreader and incubated anaerobically. After 48 h at 37ºC, the plates that showed no growth of bacteria were identified as minimum bactericidal concentration [figure 2].



Results

Essential oil of thyme showed a significant inhibitory effect with mean MIC of 6.25 µL /ml. While, Mean MBC for the extract was 12.5 µL /ml as shown in (Table 1).

Table 1 MIC and MBC of thyme oil

Essential oil	MIC against P.gingivalis	MBC against P.gingivalis
Thymus vulgaris	6.25 µL /ml	12.5 µL /ml

Discussion

Thymos is the name Greek that means small because of the fragrance of the plant, whicht belongs to over 300 species herbs and shrubs that are native to Europe and Mediterranian. It is one of the Hippocrates 400 simple remedies. Essential oil of Thymus Spp, vulgaris-Labiatae/ Lamiaceae) is got from the leaves and flowering tops of the herb. Its main constituents include 20-40% thymol and carvacrol with borneol, cineol, linalool, menthone, B-cymene, pinene and triterpenic acid. Thyme oil is a tonic stimulant used in stomachic and digestive system diseases such as gastritis, enterocolitis and mouth thrush. It was used in treatment of respiratory infections like asthma and bronchitis. It was useful in treatment of gout and rheumatic arthritis, for joint pains, backache and sciatica. Thyme oil is also used in urinary and genital

infections such as endometritis, prostrates and vaginitis [1,2,15]. Thyme oil exhibits antibacterial activity and has been useful in dental practice [16]. One of the components of thyme, which is thymol, has inhibitory effect on growth of oral bacteria that aid in decreasing dental caries [17,18]. Study by Sköld-Larsson et al., demonstrated that use of a dental varnish containing thymol aid in reducing the amont of Streptococcus mutans in supragingival plaque near brackets [19]. Thymol is used in mouthwashes because of its antibacterial activity such as Listerine mouth wash [20] Thyme oil also has antimicrobial effect against S. aureus, E. coli and C. albicansat [21-23]. Porphyromonas gingivalis is an anaerobic gram negative rod that is most prevalent etiological bacteria in periodontitis. Data on the inhibitory activity of Thymus vulgaris oil on this periodontopathic bacterium (p. gingivalis) is limited. A study by fani et al., exhibited antibacterial effect of thyme oil against p. gingivalis at (32 mg/mL) [24], while another studies showed higher concentration (62.5 mg/mL) [25]. This study represents the first reported study in determination the antibacterial activity of essential oil of Thymus vulgaris in Kurdistan-Iraq against clinically isolated periopathogenic bacteria (P. gingivalis). In our study the minimum inhibitory concentration was 6.25 µL /ml. This difference with other studies may be due to harvesting time, climatic conditions, geographical origin, and sex of cultivars that affect the chemical compositions of essential oil which are responsible for their medicinal effects or their biological activity which varies from one region to another [26]. MBC of the extract was 12.5 µL /ml against the P. gingivalis, indicated a bactericidal effect of the essential oil. The difference between bactericidal or bacteriostatic effect is clinically important in that bactericidal agents may be more effective in the treatment of diseases [27].

Conclusion

In this study, we report for the first time, the antibacterial activity of essential oil of Thymus vulgaris in Kurdistan region –Iraq against periopathogen p.gingivalis which was clinically isolated previously in vitro models. The extract of showed a significant antibacterial activity, therefore, it can be used as a natural adjuvant antimicrobial agent in treatment of periodontitis.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bakrajo technical institute and all experiments were carried out in accordance with approved guidelines.

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