



RESEARCH ARTICLE

Antibacterial potential of Clove & Thyme extracts against *Streptococcus spp.* In culture media

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Abstract

The present study was undertaken with an objective to investigate the *antistreptococcus spp.* potentials of both clove flowers and thyme leaves alcoholic extracts prepared in different concentrations (25 , 50 & 100 mg/ml) and compared their activity with effectiveness of standard reference antibiotic (Ciprofloxacin (5µg) , Ampacillin (30µg) , Cloxacillin (30µg) by measuring the zones of inhibition produced after incubation on Muller-Hinton agar .

The agar well diffusion method was used for this purposes. The result indicated that the two plants extracts inhibition significantly the growth of streptococcus with vary degree depends on the plant type & used concentration. The ethanolic extract of the Clove flowers showed more therapeutic effect (14.33 – 36.33) mm in diameter on the tested bacteria while ethanolic extract of Thyme leaves displayed little inhibitory activity between (10.33 – 16.18) mm compared with the activity of standard reference antibiotic ciprofloxacin , ampacillin & cloxacillin (22 , 15.33 , 22.21 mm) respectively.

As well as the current study indicated that the zone of inhibition on the tested bacteria increased significantly ($p \leq 0.05$) as the concentration of both plant extracts increased.

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Introduction

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased expressively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Calixto,2000) A major part of the total population in developing countries still used traditional folk medicine obtained from plant resources (Fransworth,1994) due to the emergency of multiple drug resistant microorganism has become a major cause of failure of the treatment of infectious diseases (Gibbons,2005) so this demands to search for new antibacterial drug agents , in particular from plants sources (Kelmanson *et al.*,2000;Ho *et al.*,2001) Many efforts have been done to discover new antibacterial compounds from various kinds of sources such as soil, microorganisms, animals, and plants. One of such resources is folk medicine, while systematic screening of medicinal plants may result in the discovery of novel effective compounds (Janovska *et al.*,2003) There are several plant products in pre-clinical evaluation while others show promising anti-microbial activities in *in vitro* and *in vivo* assays. Therefore, plants are considered the most potential and preferred choice in developing alternative antibiotics of natural origin (Paclma *et al.*,1998). Cloves (*Syzygium aromaticum* (Linn.) (Syn. *Eugenia aromatica*) belongs to Myrtaceae family , is an aromatic tree, native to Indonesia and used as a spice in cuisines in many parts of the world (Chaieb *et al.*,2007 ; Hema *et al.*,2010). Cloves have been used by humans for medicinal applications from over two thousand years , being chewed to alleviate the pain of toothache and are also widely used to disinfect root canals in temporary fillings (Duke,1985) common clove and its oil possesses a wide range of biological activities and therapeutic effectiveness

including antimicrobial , antioxidant , insecticides , antispasmodic , anticarminative , antiseptic , antiemetics , analgesic and Kidney reinforcement (**Liu et al.,1997 ; Kamel et al.,2007**) Thyme (*Thymus vulgaris* L.) belonging to the Lamiaceae family , is a pleasant smelling perennial shrub , which grows in several regions of the world (**Davis,1982**) there are 350 species of the thyme cultivated all over the world (**Stahl-Biskup & Saez,1987**) common thyme contains 0.8-2.6% volatile oil consisting of highly variable amount of phenol , monoterpene hydrocarbons and alcohols. thymol is normally the major phenolic compounds present in common thyme (**Naghdi & Makkizadeh,1998**) the beneficial effects of common thyme well known from ancient times and consumption of its extract is recommended all over the world (**Alkerle,1993**) common thyme and its oil possesses a wide range of biological activities including antioxidant , antihelmintic , antispasmodic , antiviral , antifungal , antiseptic , antirheumatic , expectorant and carminative effect (**Davis,1982; Stahl-Biskup & Saez,1987,Goze et al.,2009**) from the light of the above the present study was aimed to study the antistreptococcus effectiveness of numerous concentration of ethanolic extract of clove and thyme in culture media.

Material and methods:

Plant materials:

Flowers of clove and leaves of thyme were purchased from the local markets in Al-Diwanyia city. Our department of physiology and pharmacology has identified the studied plants.

Preparation and plants extract :

The flowers clove and thyme leaves were dried at (45°C) indoors under subdued light with good ventilation after that crushed to a coarse powder by using a mechanical blender, take a bout 100g of powdered were added to 500 ml of 70% ethanol alcohol and put the mixture in soxhlet apparatus during at 50° C for 48 hours . After that, resulting extracts were filtered using whatman No1 filter paper and concentrated to dryness in rotary vacuum evaporator in the room temperature and the recipient was used by several concentration for each extracted plant (25 , 50 , 100 mg/ml) .

Assay of antibacterial activity:

Initially isolated bacteria of *streptococcus spp* was obtained from the Microbiology Laboratory, Al-Diwanyia hospital /Al-Diwanyia. The antibacterial activities of the both clove and thyme extracts were carried out using the agar well diffusion method . The extracts dissolved in diluted ethanol 20% . The bacterial strains were sub-cultured in nutrient agar medium and a few fresh colonies of the microorganism were suspended in sterile normal saline (0.85% NaCl). The turbidity was matched with 0.5 McFarland standard tube . Bacterial lawn was spread onto the surface of Muller-Hinton agar plates. The plates were allowed to dry for a few minutes at room temperature. Three wells on the growth medium with diameters of 6 mm were made using a sterile pasture pipette and filled up with 100 µL from each concentration of plant extracts. The plates were kept at room temperature to diffuse the extract , and were then incubated at 37 C ° for overnight. The plates were examined for any zones of growth inhibition. Negative Control plates (diluted ethanol 20%) and standard reference antibiotics plates (Ciprofloxacin (5µg) , Ampicillin (30µg) , Cloxacillin (30µg)) were also prepared for the antibacterial activity screening. Each experiment was repeated three times and the mean zone of inhibition diameter was calculated.

Statistical analysis :

Data were expressed as mean ± S.E.M . Statistical reading and comparison among the groups was performed by one way analysis of variance (ANOVA) using SPSS program version 10 followed by Least significant differences (LSD) test with a p- value ≤ 0.05 was considered significant.

Result & discussion:

Since the discovery of the first antibiotic, penicillin, the need for antimicrobial agents is yet to be satisfied. This has been attributed to the emergence of antibiotic resistant strains of microorganisms (**Davis,1994**). As a result, there is a continuous search for antimicrobials from plant sources. These plants are used either alone or in combination with known antibiotics for treating bacterial infections (**Collin & Pareicia,1970**). So the present study was execute to investigate the antibacterial potential effects of two local plant extracts (Clove & thyme) which prepared in several concentration against the growth and multiplication of streptococcus isolates in culture media.

The ager well diffusion method used to assess the anti- streptococcus effects of the ethanolic plants extracts. Both plants extracts inhibits the growth of *streptococcus spp*. in culture media and producing variety degree of zone of inhibitions around pores this depends mainly on the type of plant extract (Clove or thyme extracts) and used concentration (25 , 50 & 100 mg/ml)

The results of the antibacterial screening of two plant studied in different concentrations have been summarized in table (1) these data revealed that The zone of inhibition against tested bacteria by two studied plant increased significantly ($p \leq 0.05$) with increasing concentration of plant extracts this may be attributed to the increase in concentration of extract accompanied by increase in the active ingredient (or ingredients) which are present in the ethanolic extract of both plants.

Both extracts (Clove & thyme) inhibited the growth of *streptococcus spp.* producing clear zone of inhibition. The ethanolic extract of clove flowers showed stronger anti-streptococcus activity and the respective mean diameter of zones of inhibition were (14.33±0.84 , 19.21±0.78 , 36.33±0.66) mm at concentrations (25 , 50 & 100) mg/ml respectively While the ethanolic extract of thyme leaves exhibited a moderate inhibitory effect against tested bacteria and their respective mean diameter of zones of inhibition were (10.33±0.95 , 13.86±0.63 , 15.18±1.62)mm at concentrations (25 , 50 & 100) mg/ml respectively. The clove and thyme extracts are known to possess antimicrobial effectiveness and are used in various food preparation as flavour enhancers and in herb medicine (Nzeako *et al* ,2006) the results of clove extracts in our study are in harmony to study performed by (Cari & Wu,1996) that recorded active constituents in clove oil were found to possess antibacterial activity against *streptococcus mutans* in addition to the other periodontal oral pathogens included in study (*Actinomyces viscosus* , *porphyromones* & *prevotella intermedia*). It has also been reported that the ethanolic extract of clove have potentially inhibition effectiveness against the growth and multiplication of *S.aureus* in culture media (Betoni *et al.*,2006).

the results of thyme extracts in our study are in harmony to study performed by (oral *et al.*,2007) that recorded active constituents in thyme water extract were found to possess antibacterial activity against food borne pathogenic bacteria specially gram positive bacteria. The oil of thyme and its different components are becoming increasingly popular as a naturally occurring antimicrobial and also as an antioxidant agent (Dursun *et al.*, 2003). The previous results recorded that Thyme revealed broad antibacterial activity by inhibiting the growth of both gram-positive and gram-negative bacteria. However, gram positive bacteria appeared to be more sensitive than the gram-negative organisms (Nevas *et al.*, 2004). The alcohol and ethanol extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *Bacillus subtilis*, *S. sonnei*, *E. coli* (Fan and Chen, 2001). Though the mode of action of the extracts is not well known , its active constituents present in two extracts include thymol , terpenes , eugenol , flavones ,glycosides of phenolic monoterpenoids and aliphatic alcohols among other elements, these active substances acting alone or in combination may result in a broad spectrum of antimicrobial activity exhibited for bacteria (Nzeako *et al* ,2006) .

Based on the present study, we can conclude the plants understudied of the flower of clove and leaves of thyme to be good sources of antimicrobial property.

Table (1) : the antibacterial effects of clove & thyme ethanolic extract against *streptococcus spp.* growth in culture media

Drug material	Concentration (mg/ml)	Zone of inhibition against bacteria organism (mm)
Clove extract	25 mg/ml	14.33±0.84A
	50 mg/ml	19.21±0.78B
	100 mg/ml	36.33±0.66C
Thyme extract	25 mg/ml	10.33±0.95D
	50 mg/ml	13.86±0.63A
	100 mg/ml	15.18±1.62A
Negative control (diluted ethanol)	20 %	0±0E
Positive control (standard reference antibiotic)	Ciprofloxacin (5µg)	22±0.73F
	Ampacillin (30µg)	15.33±0.91A
	Cloxacillin (30µg)	22.21±0.88F

The result represent means of six values ± standard error for mean

In each well , the sample volume was 100 µl

Values of the result of inhibition zone in mm diameter including the diameter of well (6mm) after 24 hours incubation period.

The similar letters denote to the non-significant differences among treatment while different letters denote to the significant differences under ($p \leq 0.05$).

Figure (1) : antibacterial effects of clove and thyme ethanolic extracts against *streptococcus spp.* Growth in culture media.

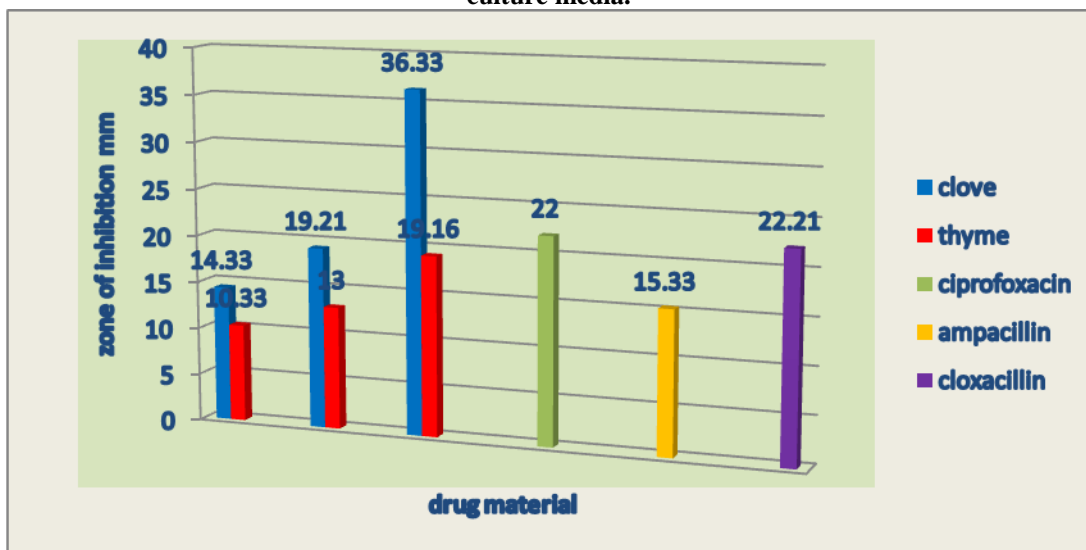
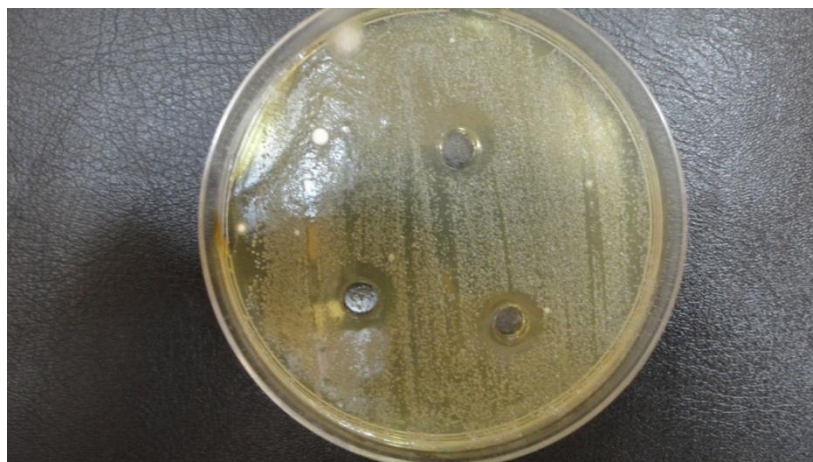


Figure (2) : antibacterial effect of clove ethanolic extract at 100mg/ml against growth of streptococcus in culture media



Figure (3) : antibacterial effect of thyme ethanolic extract at 100mg/ml against growth of streptococcus in culture media



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