Antibacterial effect of crude herbal, aqueous extracts and oil of Thyme on Staphylococcus aureus in laboratory

Z.K.Yousif , S.S.Hussein

Department of Medical Microbiology , College of dentistry , Al-Anbar university , Al-Anbar , Iraq

(Received: 11 / 3 / 2013---- Accepted: 18 / 6 / 2013)

Abstract

Objective: The aim of this study was to establish the antibacterial activity of the extracts of Thymus vulgaris against staphylococcus aureus in vitro.

The antibacterial activity of different concentrations in (0.25%,0.5%,1%,2%) of the dried leaves and flowering tops and oil of Thymus vulgaris were examined on fifty five isolates of staphylococcus aureus that isolated from different samples (gingival , saliva and dental caries swabs) from patients of dental clinics of College of Dentistry in Al-Anbar University .

Material and method

Antimicrobial effects of thyme was detected by four different methods by adding crude extracts of thyme directly to the nutrient agar medium, disc diffusion method saturated with 20mg/ml aqueous extract, disc diffusion method and agar diffusion method with different concentration of thyme oil (0.25%,0.5%,1%,2%) .

Results

The results show that 5 mg/ml crude extracts of the dried leaves and flowering tops of thyme was lowest concentration that inhibition growth of bacteria it's taken as the Minimum Inhibition concentration (MIC) . Discs saturated with aqueous extracts of the dried leaves and flowering tops of thyme did not show any effect , but the oil of thyme showed inhibitory effects against staphylococcus aureus in both methods used in the study. The antibacterial effects at 0.25%, 0.5%, 1% and 2% concentration of oil using the disc diffusion method against fifty five isolates of staphylococcus aureus oil showed antibacterial activity against all the bacteria used in this assay . The thyme oil at 2% concentration were more effective than of the other concentrations .

Conclusion

It is concluded that the use of crude extracts of the dried leaves and flowering tops of thyme was best than use of thyme oil and oil was best that than aqueous extracts against staphylococcus aureus it is need to more study on thyme in vivo.

Key Words: Antibacterial , Thymus vulgaris , Staphylococcus aureus .

Introduction

Thyme (Thymus vulgaris) , Family : Libiatae . The common names common thyme , Garden thyme and Whooping cough herb . The habitat native to the Mediterranean region and widely cultivated in Europe and the United States . It prefers limy , sandy and well drained soil with sufficient sunlight . Thyme is a perennial plant with numerous procumbent stems , 6 to 12 inches high , covered with fine hair and pale brown bark. The leaves are small , opposite , sessile and gray – green with slightly rolled edges . The small, blue – purple flowers are two – lipped and grow in dense, whorled clusters , blooming from May to September. The medicinal parts is leaves and flowering tops – dried and the chemical composition is Volatile oils , Flavonoids , Ursolic acid , Thymol , Caffeic acid , Oleanolic acid , Tannins , Labiatic acid [1] . Thyme is antispasmodic and expectorant in the respiratory system, it is beneficial in the treatment of bronchial coughs, laryngitis, and wooping cough. like many other herbs with a high content of volatile oil, thyme has strong antibacterial properties. In addition, the herb has hypotensive (sedative) and cardiotonic characteristics. Thymol, a major constituent of Thyme's volatile oil, is powerful, and should not be used internally unless directed by a physician [2] . Thyme contains a large concentration of voiatile oil . Normally, the primary component of that oil is thymol, but actually concentration may vary greatly. Other constituents include carvacol, tannin, flavonoids, caffeic acid Labiatic acid, ursolic acid and oleanolic acid. These oils have antioxidant properties. A lipid fraction has been found to have anti -cancer properties [3] . Thymol also provides the distinctive , strong flavor of the culinary herb thyme, also produced from Thymus vulgaris [4] . Thymol is only slightly soluble in water at neutral PH, but it is extremely soluble in alcohols and other organic solvents.

It is also soluble in strongly alkaline aqueous solutions due to deprotonation of the phenol [5]. Thymol and carvacrol are now known to kill bacteria and fungi, making thyme well suited for such purposes. The bee balms (Monarda fistulosa and Monarda didyma) , North American wildflowers , are natural sources of thymol. The Blackfoot Native Americans recognized this plant's strong antiseptic action, and used poultices of the plant for skin infections and minor wounds9. A tea made from the plant was also used to treat mouth and throat infections caused by dental caries and gingivitis [6] . Thymol has microbial activity because of its phenolic structure , and has shown antibacterial activity against bacterial strains including Aeromoans hydrophila and Staphylococcus aureus [7] . This antibacterial activity is caused by inhibiting growth and lactate production, and by decreasing cellular glucose uptake [8].

Thymol has been used in alcohol solutions and in dusting powders for the treatment of tinea or ringworm infections , and was used in the United States to treat hookworm infections . It is also used as a preservative in halothane , an anaesthetic , and as an antiseptic in mouthwash. When used to reduce plaque and gingivitis, thymol has been found to be more effective when used in combination (synergism effect) with chlorhexidine than when used purely by itself [9].

Thymol is also the active antiseptic ingredient in some toothpastes, such as Euthymol. Thymol is caused by thymol's ability to alter in the hyphal morphology and cause hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall [10] . Additionally , thymol is lipophilic , enabling it to interact with the cell membrane of fungus cells , altering cell membrane permeability permitting the loss of macromolecules [11] .

Patients and Methods

Seventy swabs were taken from both sexes of patients from dental clinic –Department of Operative , college of dentistry, Al-Anbar University in Ramadi city during the period from October 2011 to Janauray 2012 . Out of seventy patients , fourty one (58.5%) of males and twenty nine (41.4%) of females oral specimen are taken from (50) secondary school students (26 dental caries and 24 caries free) and (20) adult patients from both sexes in clinic of Dentistry College. The ages of the students (males) are between 15-18 years and females are 12-17 years (table1) while the ages of patients (males) are between 25-60 years and females are 40-45 years (table 2) . Fifty five isolates of Staphylococcus aureus are obtained from oral specimens 17 gingival swab (9 males and 8 females) , 7 saliva (males) and 46 dental caries swab (26 students 18 males and 8 females) and (20) patients 7 males and 13 females) .

TABLE (1) Oral specimens types of students

Type of sample	Number of males	Age of males (years)	Mean of age (years)	% of sample number	Number of females	Age of females (years)	Mean of age (years)	% of sample number
Gingival swab	9	15-18	16.5	12.8	8	12-17	14.5	11.4
Saliva sample	7	15-17	16	10	0	-	-	-
Caries swab	18	15-18	16.5	25.7	8	12-15	13.5	11.4

TABLE (2) Oral specimens types of patients

Type of sample	Number of males	Age of males (years)	Mean of age (years)	% of sample number	Number of females	Age of females (years)	Mean of age (years)	% of sample number
Caries swab	7	25-60	42.5	10	13	40-45	42.5	18.6

Fifty five isolates of Staphylococcus aureus are obtained from oral specimens as shown in table -3 .

TABLE (3) The percentages of staphylococcus aureus isolated from oral specimens

Type of oral specimen	No. of staphylococcus aureus isolates	The percentages of staphylococcus aureus isolates
Gingival swab	31	56.36 %
Saliva sample	9	16.36 %
Dental caries swab	15	27.27 %

Materials and Methods

Swabs were cultivated soon on Nutrient agar, blood agar and incubated at 37°C aerobically for 24 hrs. Bacterial isolates were identified using Direct Gram stained smears and biochemical test as described by [12] . Bacterial isolates were kept frozen in glycerol brain heart infusion (10%) to be used for antibacterial activity assay which carried out by using Nutrient agar and Muller – Hinton agar (MHA Himedia).

Bacterial standardization: Bacterial inoculation was standardizes according to McFarland suspension [13]. Tube no. 5 contain 1 × 105 Cfu /ml.

Determination of antibacterial activity of crude extracts of thyme

The minimum inhibitory concentration (MIC) were determined by adding the grind of dried leaves and flowering tops of thyme at different concentration (0.5, 1, 2, 3, 4, 5 mg/ml) to the nutrient agar medium after sterilization it and allowed to cooled at 100°C for 10 min . The plates were inoculated with bacteria as following: The inoculums size of each test organism was adjusted to suspension of 105 cells. A 1 ml of 24 hours over night culture of bacteria were added to 125 ml of melted cooled (with Thyme concentration) test agar and after through mixing ,

approximately 20 ml of this inoculated agar were poured in to 10 cm diameter pre sterilized Petri dishes and allowed to solidify. Incubation was at 37°C for 18 hours. The lowest concentration that inhibition growth of bacteria was taken as the MIC.

Determination of antimicrobial activity of aqueous extracts of thyme grind

1- Disc diffusion method

One ml of Staphylococcus aureus suspension (1 × 105 cfu/ml) was diffused evenly on MH agar plates and kept in incubator at 37°C for 2 hours to be dry . Then sterile filter paper discs impregnated with 100 µl of aqueous extracts of thyme in the concentration (0.25 , 0.5 , 1 , 2 , 3 , 4 , 5 mg/ml) were prepared which the aqueous extracts of grind dried leaves of thyme was prepared as the following: 50 ml of boiling distil water was poured into 1 gm of crushed of thyme leaves in a beaker. The mixture was allowed to stand for 30 minutes and filtered , this extracts were sterilized by passing through a Millipore filter which the best way to sterilize the extracts weather aqueous or alcoholic is by filteration using milipore filter size (0.2 µm) to keep the integrity of antimicrobial components of the plants extracts . An equivalent of 20 mg dried the leaves of thyme per ml of distil water aqueous extracts were obtained . The aqueous extracts of thyme dried leaves were freshly prepared in order to prevent loose the components of the seeds during long storage . Control assay discs impregnated with sterile water . Assay discs were placed on the surface of the inoculated Muller-Hinton agar . Plates were incubated at 37°C for 24 hours [14].

2- Gel diffusion method

One ml of Staphylococcus aureus suspension (1 × 105 cfu/ml) was diffused on MH agar plates. Circular wells (6mm × 3mm) were cut in the agar culture media and full with 100 µl aqueous extracts of grind of dried leaves and flowering tops of thyme, control well was filled with sterile water [15] .

Determination of antibacterial activity of thyme oil

1- Disc diffusion method

The disc were saturated with 20 µl of thyme oil obtained from captain company (CAP PHARM) in concentrations (0.25%, 0.5%, 1%, 2%, 5%) concentrations of oil diluted in sun flower oil . Sun flower oil was also used as control . The plates were incubated at 37°C for 18 -24 hours [16]. The diameter (mm) of inhibition zones of thyme oil was measured . Sterilize filter paper disc with diameter 6 mm were impregnated with 0.1 ml of sterilize thyme extracts concentration those were allowed to dry for 10 minutes in an open sterilize petridish using an septic procedures . Control assay disc impregnated with sterilize water assay disc were placed on the surface of the inoculated brain heart infusion agar. plates were incubated at 37°C for 18 – 24 hours .

2- Gel diffusion method

One ml of Staphylococcus aureus suspension (1 ×106 cfu/ml) was diffused on MH agar plates . Circular wells (6mm × 3mm) were cut in the agar culture media and full with 20 µl of thyme oil , control well was filled with sterile sun flower oil .

Determination of antibiotic sensitivity of Staphylococcus aureus

The disc diffusion method was used to detect the antibiotic sensitivity antibiotic disc were used for comparison i.e five standard antibiotic discs were used for comparison i.e Rifampin (RA) (5 µg/ml) , Gentamicin (CN) (10 µg/ml) , Trimethoprim (TMP) (5 µg/ml) , Penicillin (P) (10 µg/ml) and Cephalexin (CL) (30 µg/ml). The antimicrobial activity of various samples was determined by agar diffusion technique . Microbial sensitivity testing was done on Muller-Hinton agar medium. The diameter of inhibition zones was measured in mm [17] .

Results

In this study we investigated the antibacterial effects of crude extracts, aqueous extracts of dried leaves and flowering tops and oil of thyme on fifty five isolates of Staphylococcus aureus .

Determination of antibiotic sensitivity of Staphylococcus aureus

The five standard discs were used for comparison the result show 100% sensitivity to CN (Gentamicin) while all strains 100% resisted to CL (Cephalexin) . Table 4. The results showed that Minimum Inhibition Concentration (MIC) of thyme crude extracts was 5 mg/ml to all isolated bacteria were tested .The aqueous extracts did not show any effect while the oil of thyme at 2% concentration of thyme were more effective table (5) than other concentrations . The crude extract of dried leaves and flowering tops of thyme was most effective against Staphylococcus aureus .

TABLE (4) : The sensitivity test of Staphylococcus aureus on Muller - Hinton agar plates at 37 C° after 18 hrs incubation

Antibiotic disc	Mean of inhibition zone in (mm)	The percentage of resistance isolates %	The percentage of sensitive isolates %
Gentamicin(10µg/ml)	30	0.0	100
Penicillin(10 µg/ml)	25	95	5
Rifampin(5µg/ml)	20	88	12
Trimethoprim(5µg/ml)	13	50	50
Cephalexin(30µg/ml)	Resist	100	0.0

TABLE (5) The concentration of Thyme oil and inhibition zone of disc diffusion and well diffusion methods test of Staphylococcus aureus on Muller – Hinton agar plates at 37°C after 18 hrs incubation

Concentration of oil %	(Disc diffusion) Inhibition zone in (mm)	(Well diffusion) Inhibition zone in (mm)
10%	12	12
20%	13	13
30%	14	14
40%	20	20
50%	26	26
100%	30	30

Discussion

The overuse of antibiotic drugs has led to the extensive antibiotic resistance in human pathogenic bacteria , which highlights the research need on new antimicrobial agents [18 , 19] .

Thyme is a plant which has been used in a variety of food preparation . In this work we showed the significant antibacterial activity of the crude and oil of thyme on Staphylococcus aureus . Some earlier studies have demonstrated thyme antibacterial activity against Staphylococcus aureus [20] . Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with biocides such as carvacrol . In addition , naturally – occurring biocidal agents such as thymol can reduce bacterial resistance to common drugs such as penicillin [21] . The aqueous extract of Thyme did not show any effect the reason for this is: enable effective value or pharmacological for many of herbs in the volatile oils spreading in the air unless it uses the cover above and during the process of crushing or grinding for the dried leaves and flowering tops of thyme so it lose volatile oils and thus less from effectiveness and pharmacological characteristics as previously determined by [14] . The antibacterial activity of Thymus vulgaris extracts may be due to presence of phenolic constituents (thymol and carvacrol) , which make up a larage percentage of the volatile oil [22 , 23] . Our results supported the result of advanced studies that used Thymus spp. Extracts as antimicrobial agent depend on presence of both thyme essential oil and thymol . Also , these studies suggested use of thyme as an antibiotic . Thymol is 25 times as effective as phenol , but less toxic [24,25,26,27] . Other experimental evidence suggested that the in vitro activity of thyme preparation are due to the presence of polymethoxy flavones that have antibacterial activity [28] .

Conclusion

The crude extracts of dried leaves and flowering tops of thyme was most effective against Staphylococcus aureus than the oil of thyme in laboratory , the aqueous extracts of Thyme in this study did not show any effect .

Acknowledgement

I would like to thank Al- Taee Karama T. Ass . Lectural. University of Al-Anbar College of Dentistry and the herbalist Mr. Al-Dulaimi Munadlhil Mohammed for there advice and support us in this study .

References

1- Bruni R., Medoco A., Andreotti E., Fantin C., Muzzoli M., Dehea M., Romagnol C., Sacchetti G. (2004) . Chemical composition and biological activities of Ishpingo essential oils , a traditional Ecuadorian spices from Ocotea quixos (Lam.) Kosterm. (Lauraceae) flower calices . Food Chem. 85 , 415-421 .

2- Oussalah M., Caillet S., Saucier L., Lacroix M., (2007) . Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria : E. coli O157:H7,Salmonella Typhimurium , Staphylococcus aureus and Listeria monocytogenes . Food Control 18, 414 – 420.

3- Dadalioglu I. and Everendilek G. (2004) . Chemical composition and antibacterial effects of essentials oils of Turkisse oregano , Bay laurel , Spanish lavender on common food borne pathogens . J. Agric. Food Chem. 52, 8255-8260 .

4- Vanden B. (1980) . Chemical and pharmacological investigation on thyme herba and its liqid extracts . Plant Medica , 39: 253 – 254 .

5- Rasooli I. and Mirmostafa SA. (2003) . Bacterial susceptibility to and chemical composition of essential oils from Thymus Kotschyanus and Thymus persicus . J Agric Food Chem Apr 9;51 (8) : 2200-5 .

6- Gouin S. (2004) . Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science and Technology 15, 330-347 .

7- Dorman H.J.D. and Deans S.G. (2000). Antimicrobial agents from plant: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308 - 316.

8- Evans J. and Evans J. D. (2000) Effect of thymol on ruminal microorganisms. Curr. Microbiol. 41,336.

9- Filoche S. K., Soma K. and Sissons C. H. (2005) . Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. Oral Micobiol Immunol, 20,221- 225 .

10- Numpaque M. A., Oviedo L. A., Gil J. H., Garcia C. M. and Durango D. L. (2011) . Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi Colletotrichum acutatum and Botryodiplodia theobromae. Trop. Plant Pathol. 36, 3-13] .

11- ^ Segvic Klaric M., Kosalec I., Mastelic J., Pieckova E., Pepeljnak S. (2007) . Antifungal activity of thyme (Thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings Lett . Appl. Microbiol. 44, 36-42 .

12- Makie and Mac Cartney (1996). Practical Medical Microbiology. 4 th. ed. New York Edinburgh London Madrid Melbourne San Francisco And Tokyo.

13- National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically – Sixth Edition: Approved Standard M7-A6 (2003) Wayne , PA , USA: NCCLS .

14- Mashhadian N. V. and Rakshandch , (2005) . Antibacterial and antifungal effects of Nigella sativa extract against S. aureus , P. aerogenosa and C. albicans Pak.J. med Sci. (1)21 .1: 47-52 .

15- Andrews J. M., (2001) . BSAC standardized disc susceptibility testing method.Antimicrob. Chemotherapy ; 48(suppl sl) :5-16.

16- Baydar H., Sagdic O., Ozkan G., Karadogan T. (2004) . Antibacterial activity and composition of essential oils from Origanum , Thymbra and Satureja species with commercial importance in Turkey . Food Control . 15: 169-172 .

17- Salman , Mohd Tariq , Khan , Rahat Ali and Shukla , Indu (2008) . Antimicrobial activity of Nigella sativa Linn. Seed oil against multi-drug resistant bacteria from clinical isolates . Natural Product Radiance,7(1): 10-14 .

18- Wagner H. and Ulrich-Merzenich G., (2009). Synergy research: Approaching a new generation of phytopharmaceuticals. Phytomedicine 16, 97-110.

19- Hemaiswarya S., Kruthiventi A.K. and Doble M. (2008). Synergism between natural products and antibiotics against infectious diseases. Phytomedicine 15, 639-652 .

20- Koluman A., Akan L.S., Cakirog F. P. (2009) . Occurrence and antimicrobial resistance of enterococci in retail foods. Food Control 20, 281-283.

21- ^ ab Palaniappan K. and Holley R.A. (2010) . Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria doi: 10. 1016/j.ijfoodmicro.04.001 .

22- Janssen A.M. Scheffer J.J.C. and Baerheim S.A. (1987) . Antibacterial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods . Pland Medica , 53: 395-398 .

23- Juven B.L., Kanner J., Schved F., Weisslowicz H. (1994) . Factors that interact with the antibacterial action of thyme essential oil and its active constituents . J. Appl. Bacteriol., 76: 626-631 .

24- Panizzi L., Flamini G., Cioni P.L. and Morreli I. (1993) . Composition and antimicrobial properties of essential oils of four Mediterranean lamiacease . J. Ethnopharmacol., 39(3): 167-170 .

25- Cosentino S., Tuberoso C.I., Pisano B., Satta M., Mascia V., Arzedi E. and Palmas F. (1999) . In vitro antimicrobial activity and chemical composition of Sardiian Thymus essential oils. Lett. Appl. Microbiol ., 29(2): 130-135 .

26- Marino M., Bersani C and Comi G. (1999). Antimicrobiol activity of the essential oils of Thymus vulgaris L. measured using a bioimpedometric method . J. Food . Prot ., 62(9): 1017-1023.

27- Dorman H.J. and Deans S. G. (2000). Antimicrobiol agents from plants: antibacterial activity of plants volatile oils. J. Appl. Microbiol., 88(2): 309-316 .

28- Ghazanfar S.A. (1994) . Handbook of Arabian medicinal plants. CRC press. Roca Raton .

التأثير المضاد البكتيري للعشبة الخام ، المستخلص المائي و الزيت لعشبة الزعتر البري ضد بكتريا المكورات العنقودية الذهبية في المختبر

زينب كامل يوسف ، سداد سلمان حسين

قسم الاحياء المجهرية الطبية ، كلية طب الأسنان ، جامعة الأنبار ، الانبار ، العراق

(تاريخ الاستلام: 11 / 3 / 2013 ---- تاريخ القبول: 18 / 6 / 2013)

الملخص

الهدف : هو لبيان التأثير المضاد البكتيري لمستخلصات عشبة الزعتر البري ضد المكورات العنقودية الذهبية في المختبر. حيث كان التأثير المضاد البكتيري بتراكيز مختلفة (٢٥ ،٠٪ ، ٥ ،٠٪ ، ١ ٪ ، ٢٪) للأوراق الجافة ، قمم الازهار النامية وزيت الزعتر وقد اختبرت على خمسة وخمسون عزلة من بكتريا المكورات العنقودية الذهبية التي عزلت من نماذج مختلفة وهي (مسحات لثة الاسنان ، مسحات اللعاب ، مسحات حشوات الاسنان) من المرضى المراجعين لعيادات طب الاسنان الموجودة في كلية طب الاسنان بجامعة الانبار .