



RESEARCH PAPER

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Antimicrobial activity of Thyme (*Thymus vulgaris*) essential oil cultivated in Quetta, Balochistan, Pakistan

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Abstract

The aim of the present study was to assess the antimicrobial efficiency of thyme (*Thymus vulgaris*) essential oil against different microbial strains viz. *E. coli*, *Salmonella*, *staphylococci*, *Proteus*, *Pseudomonas*, and *Streptococcus* strain; verified strains were collected from Bolan Medical Complex Hospital, Quetta. Thyme essential oil was extracted by using Clevenger type apparatus at BARDC Quetta and antimicrobial effect was measured by dissolving with Dimethyl sulphoxide (DMSO), using by disc diffusion technique. Results shows that the best inhibitory zones were observed by dissolving thyme oil with DMSO and the best zone was observed against *E. coli* (22 mm) while reduced zone was observed against *Streptococcus* (08 mm). This study explores its highly valuable contribution in medicinal usage. It will also improve the socio economic status of the farmers while used as replacement for some non-profitable crops by local farmers

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Introduction

Medicinal plants have great contribution to health care all over the world, in Asian region particularly identification and recognition of traditional medical value of medicinal plants which have significant power of healing. Due to antibiotic resistance and failure of chemotherapy by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity because of plants safety and cost effectiveness (Hammer *et al.*, 1999). Plants as a source of medicine have been used by all cultures from ancient times to the present day. Traditional medicines as a primary health care, about 80% of the world's people are depended. Plants extracts are the active principle of the traditional therapy (Murray and Shaw, 2000).

Thyme plants are perennials, belonging to the mint family Lamiaceae, and exist in various shapes and colors. It produces valuable essential oils, depending on the nature of land where it has been grown. Thyme essential oil is water distilled from the leave, flower, dried or fresh plant. It is shown that Antibacterial, antiviral, antioxidant; antifungal and insecticidal properties have been possess by the Essential oils (Burt, 2004; Kordali *et al.*, 2005). Essential oils are good source of biologically active compounds, has been used in food preservation agents (Faid *et al.*, 1995) aromatherapy (Buttner *et al.*, 1997) and fragrance industries (Van de Braak and Leijten, 1999).

Thyme has bactericidal and fungicidal effects and its alcoholic extracts are expectorant (Hornok, 1992). Thymol the active ingredient of thyme oil has antibacterial/antimicrobial activity against *Aspergillus*, *Cryptococcus neoformans*, *Saprolegnia*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* (WHO). The active ingridient Thymol has successful result against virus (warts, epithelial tumors) and worms like tapeworm and round worm (Trattler, 1985).

According to Food and agriculture Organization of UN about 32% world's food supply was lost to spoilage or waste in 2009.

Essential oils and extracts from several plant species are able to control microorganisms related to skin and food spoilage, including Gram-negative and Gram-positive bacteria. As our environment is badly contaminated by different microorganisms, it spoils the vegetables and fruits, deteriorates the quality of milk and complicates the wound or infections and mainly Gram Positive and Gram Negative bacteria are responsible for food spoilage. Folk medicine describes *thyme-vulgaris* as antiseptic, sedative, antipyretic, cramps and dermatitis treatment. Chemically the essential oils (mainly contain thymol and caracole), flavonoids, tannins and triterpenes (Adam *et al.*, 1998; Sartoratto *et al.*, 2004).

Escrecia coli, gram-ve rod shape with high mortality rates worldwide and the most common bacterial enter pathogens importantly causes of neonatal meningitis and the agent frequently associated with bloody, watery and Traveler's diarrhea, can also contaminant fruits and vegetables that can easily cause an outbreak (Chen and Frankel, 2005). Similarly species of *Salmonella* cause enteric fever. Typhoid fever, septicemia such as osteomyelitis and enter colitis. *Enterobacter* is opportunistic pathogen that causes particularly pneumonia and UTI. *Streptococcus* cause a variety of infections, *S. pyogene* is leading bacterial cause of pharyngitis, cellulitis, impetigo, necrotizing fasciitis, streptococcal shock syndrome, rheumatic fever and acute glomerulonephritis. *Staphylococcus aureus* cause abscesses, various pyogenic infections, toxic shock syndrome, found in human respiratory tract and on skin and is the common pathogen associated with staphylococcal food poisoning (salads) ready to eat products (Le Loir *et al.*, 2003). The aim of this study is to investigate the *in vitro* antibacterial properties of thyme essential oil (*Thymus vulgaris*) against *E. coli*, *Salmonella*, *staphylococci*, *Proteus*, *Pseudomonas*, and *Streptococcus* strains.

Materials and methods

Study area

This study was conducted at Balochistan Agriculture Research and Development Centre (BARDC) Brewery Road Quetta and at the Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan (UOB) Quetta, Pakistan.

Extraction of essential oil

About 70gm of *Thyme vulgaris*, aerial parts were carried, dried at room temperature and were subjected for 3hr to Neo-Clevenger type apparatus for water distillation (Werl Lab-Germany). In a sterile vial the essential oil was collected and with anhydrous sodium sulphate the EO was dried for 24 hr and stored at 4°C after filtration.

Test microorganisms

A total of six (06) numbers of different verified microbial strains *Escherichia coli*, *Proteus*, *Pseudomonas*, *Salmonella*, *Staph aureus* and *Streptococcus* spp were taken from Microbiology Laboratory. Bolan Medical Complex Hospital (BMCH),

Culture media, apparatus sterilization

At 180°C for 2 hours, the glass wares were washed with detergent, rinsed with water, were air dried, then wrapped in aluminum foil and sterilized in hot air oven. Similarly all the culture media were autoclaving at 121°C for 15 minutes after preparation with manufacturer's specification.

Preparation of culture medium

Eosin Methylene Blue agar (wright), Brain Heart infusion (BHI) Agar (Oxoid) and Mueller Hinton Agar (Oxoid), As per manufacturer's specification, all the said media's were mixed with distilled water in 500 ml quantity and were autoclaved at 15 lb/in² pressure per square inch (PSI) for 15 minutes at 121°C and allowed to cool down to 45°C.

The solid media were aseptically and allowed to solidify in Petri plates. To confirm sterility, the plates were incubated for 24 hours at 37°C Sterile media were stored at 4°C.

McFarland standard

Specified amounts of sulfuric acid and barium chloride were mixed together for making McFarland standard. Barium sulfate precipitates were form by mixing the two compounds as a result turbidity were caused.

By mixing 9.95 ml of sulfuric acid (H₂SO₄) concentration of 1% with 0.05 mL of 1.175% barium chloride dihydrate (BaCl₂·2H₂O) a 0.5 McFarland standard was achieved.

Preparation of normal saline

Normal saline were prepared by mixing distilled water (100ml) with sodium chloride (0.87g) in conical flask of 500ml the solution were autoclaved at 121°C for 15 and stored the solution at 4°C.

Preparation of filter paper Disc

All the filter paper discs were prepared from Whatman #6 each of 06 mm in diameter and at 121°C for 15 minutes the paper discs were autoclaved.

Screening of antibacterial activity

Few colonies of tested organisms were picked up by sterile loop and inoculated in 05 ml tubes containing Normal saline and matched with 0.5 McFarland nephelometer turbidity standards as described by (Saeed *et al.*, 2006). A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate entire surface of Muller Hinton Agar (MHA) plate.

The essential oils were mixed in dimethylsulfoxide (DMSO). Under aseptic conditions using Laminar flow cabinet with 0.2 micron Hepa Filters, sterilized discs (Whatmann # 6) were impregnated with 15 µl of different concentrations (1:1, 1:2, 1:4) of the respective essential oils and DMSO were placed on the agar surface as recommended by National Committee for Clinical Laboratory Standard, (2002).

The inoculated plates were incubated at 37°C for 24 hours. Paper discs moistened with aqueous DMSO were placed on the seeded petriplate as a vehicle control. Studies were performed in duplicate, and the mean values were calculated.

Results and discussion

Thyme oil showed great effect against selected microbial strains. The findings of the study are summarized in Table 1.

Table 1. Antimicrobial activity of thyme essential oil diluted with DMSO against different clinical microbial isolates.

Bacterial Spp.	Mean Zone of Inhibition (mm)				
	Oil 01:00 15µl	Concentration			DMSO* 00:01 15µl
		01:01	01:02	01:04	
<i>E. coli</i>	31	22	19	16	0
<i>Salmonella</i>	25	17	13	10	0
<i>Staphylococcus</i>	25	21	15	13	0
<i>Proteus</i>	17	13	10	8	0
<i>Streptococcus</i>	10	8	7	7	0
<i>Pseudomonas</i>	11	10	8	7	0

*DMSO= Dimethyl sulphoxide

Best inhibitory zone were observed against *E. coli*, the pure oil (15µl) zone was 31mm while diluted with DMSO were 22mm, 19mm and 16mm, respectively. The second best zones were against *Salmonella* and *Staphylococcus* and were observed as 25mm for each with pure 15µl after 24 hrs., while diluted with DMSO the observation for *Salmonella* were 17mm, 13 mm and 10mm and for *Staphylococcus* the observed zones were 21mm, 15mm and 13mm, respectively. Moderates zones of pure thyme oil were observed against *Proteus* (17mm) and *Pseudomonas* (11mm) strains and diluted oil with DMSO the zones were 13mm, 10mm and 08mm, respectively for *Proteus*. Diluted zones for *Pseudomonas* were 10mm, 08 mm and 07mm respectively. Reduced zones were observed for *Streptococcus*, against pure oil were recorded 10mm and diluted with DMSO the zones were recorded 08mm and 07mm consequently.

Antimicrobial effects of the essential oils, mainly the constituents of the essential oil (EO) were assessed from variety of medicinal plants (Soylu *et al.*, 2007). Essential oil of the *Thymus vulgaris* is known for antimicrobial effects against fungi and vides range of microorganisms (Arras and Usai, 2001). The higher antimicrobial effects were found against fungi (Hammer *et al.*, 1999) against pathogenic microbes (Bouhdid *et al.*, 2010; Piskernik *et al.*, 2011) against spoilage bacterial microbes against mold and yeast (Tserennadmid *et al.*, 2011) (Tyagi and Malik, 2012).

In the present study the inhibition zone diameter (IZD) of 10mm to 31mm were observed with 15µl essential oil and 12mm inhibition zone diameter was consider be the effective.

Against *E. coli* the inhibition zone was observed as 21 mm and 31 mm using pure thyme oil with constant quantity (15µl), indicated higher activity against *E.Coli* bacteria the mean zone of inhibition against *Pseudomonas* were recorded as 10 mm, showing moderate effect against the said bacteria because no effect was reported by Abu-Darwish *et al.* (2012) against *Pseudomonas aeruginosa*. The mean Inhibitory zones of *Streptococcus* were observed 10mm, indicating low effect against the said tested microorganism. Against *Salmonella*, the mean inhibition diameter zones were observed about 25mm, indicating the strong inhibitory effect. The mean inhibition zones against *Pseudomonas* were 11mm, showing also a low effect respectively. The mean inhibition diameter zones against *Proteus* were observed 17mm, which indicate an effective efficiency of the thyme oil.

The efficiency of thyme was observed as effective against all the tested microorganism and our these findings corroborate with the finding of (Imelouane *et al.* 2009; Klaus *et al.*, 2008) reported the strongest effects of *thyme* oil about >45mm and (0.33mg mL⁻¹, against *E. coli* bacteria. The zones of inhibition against *Pseudomonas* are in line with the finding of Al-Fatimi *et al.* (2010) who also reported that 15 mm zone of Yemen thyme species. The efficiency of *Thymu Vulgariss* observed in our study against *Streptococcus*, *Salmonella*, *Pseudomonas* and *Proteus* were inline with the finding of (De Martino *et al.* 2009) and (Azaz *et al.*, 2004).

The findings of our study proved that the antimicrobial effects of EOs extracted from Quetta,

Pakistan species of *Thymu vulgaris* is as effective and comparable to the one observed in other species of *Thyme* essential oils like the findings of (Hyun *et al.*, 2015, Akgul and Kivanc, 1988; Nelson, 1997).

Conclusion

The findings of the study suggesting that thyme essential oils represents antibacterial effects against pathogenic microorganisms and can be as effective as modern medicine and safe alternative to treat infectious diseases. Some more research is needed to explore its active compounds. Further investigation needs to create awareness among farmers to cultivate such valuable, natural medicinal herbs to improve their socio-economic status.

References

- Abu-Darwish MS, Al-Ramamneh E, Kyslychenko VS, Karpiuk UV.** 2012. The antimicrobial activity of essential oils and extracts of some medicinal plants grown in Ash-shoubak region-South of Jordan. *Pak J Pharm Sci* **25(1)**, 239-246.
- Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M.** 1998 Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of agricultural and food chemistry* **46(5)**, 1739-1745.
- Akgul A, Kivanc M.** 1988. Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi. *International journal of food microbiology* **6(3)**, 263-268.
- Al-Fatimi M, Wurster M, Schroder G, Lidequist U.** 2010. In vitro antimicrobial, cytotoxic, and radical scavenging activities and chemical constituents of the endemic *Thymus laevigatus* (Vahl). *Rec Nat Prod* **4(1)**, 49-63.
- Arras G, Usai M.** 2001. Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *Journal of Food Protection* **64(7)**, 1025-1029.
- Azaz AD, Irtem HA, Kurkcuoğlu M, Can Baser KH.** 2004. Composition and the in vitro antimicrobial activities of the essential oils of some *Thymus* species. *Zeitschrift für Naturforschung C* **59(1-2)**, 75-80.
- Bouhdid S, Abrini J, Amensour M, Zhiri A, Espuny M, Manresa A.** 2010. Functional and ultrastructural changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Cinnamomum verum* essential oil. *Journal of applied microbiology* **109(4)**, 1139-1149.
- Burt S.** 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. *International journal of food microbiology* **94(3)**, 223-253.
- Buttner M, Willeke K, Grinshpun S.** 1997. Sampling and analysis of airborne microorganisms. *Manual of environmental microbiology* 2.
- Chen HD, Frankel G.** 2005. Enteropathogenic *Escherichia coli*: unravelling pathogenesis. *FEMS Microbiology Reviews* **29(1)**, 83-98.
- De Martino L, De Feo V, Nazzaro F.** 2009. Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils. *Molecules* **14(10)**, 4213-4230.
- Faid M, Bakhy K, Anchad M, Tantaoui-Elaraki A.** 1995. Almond paste: physicochemical and microbiological characterization and preservation with sorbic acid and cinnamon. *Journal of Food Protection* **58(5)**, 547-550.
- Hammer KA, Carson C, Riley T.** 1999. Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology* **86(6)**, 985-990.
- Hornok L.** 1992. Cultivation and processing of medicinal plants. John Wiley & Sons Ltd.
- Hyun JE, Bae YM, Song H, Yoon JH, Lee SY.** 2015. Antibacterial effect of various essential oils against pathogens and spoilage microorganisms in fresh produce. *Journal of Food Safety* **35(2)**, 206-219.

- Imelouane B, Amhamdi H, Wathelet J-P, Ankit M, Khedid K, El Bachiri A.** 2009. Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *Int J Agric Biol* **11(2)**, 205-208.
- James E, Reynolds F.** 1996. Martindale: The extra pharmacopoeia. Royal Pharmaceutical Society, London 1738.
- Klaus A, Beatovic D, Niksic M, Jelacic S, Nedovic V, Petrovic T.** 2008. Influence of ethereal oils extracted from Lamiaceae family plants on some pathogen microorganisms. *Zbornik Matice srpske za prirodne nauke (Serbia)*. DOI:10.2298/ZMSPN 0815065K.
- Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A.** 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of agricultural and food chemistry* **53(24)**, 9452-9458.
- Le Loir Y, Baron F, Gautier M.** 2003. *Staphylococcus aureus* and food poisoning. *Genet Mol Res* **2(1)**, 63-76.
- Mitscher LA, Drake S, Gollapudi SR, Okwute SK.** 1987. A modern look at folkloric use of anti-infective agents. *Journal of natural products* **50(6)**, 1025-1040.
- Murray V, Shaw D.** 2000. WHO Monographs on Selected Medicinal Plants, Volume 1. Health and Hygiene **21(3)**, 129.
- Nelson R.** 1997. In-vitro activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. *Journal of Antimicrobial Chemotherapy* **40(2)**, 305-306.
- Piskernik S, Klančnik A, Riedel CT, Brøndsted L, Mozina SS.** 2011. Reduction of *Campylobacter jejuni* by natural antimicrobials in chicken meat-related conditions. *Food Control* **22(5)**, 718-724.
- Saeed S, Naim A, Tariq P.** 2006. In vitro antibacterial activity of peppermint. *Pakistan Journal of Botany* **38(3)**, 869.
- Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG (2004)** Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology* **35(4)**, 275-280.
- Soylu S, Yigitbas H, Soylyu E, Kurt W S.** 2007. Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. *Journal of applied microbiology* **103(4)**, 1021-1030.
- Standards N.** 2002. Reference Methods for Broth Dilution Antifungal Susceptibility testing of Yeast: Approved Standar, National Committee for Clinical Laboratory Standards.
- Trattler R.** 1985. Better Health through Natural Healing. 1985 Thorsons Publishers. ISBN 7225-1382-8.
- Tserennadmid R, Tako M, Galgoczy L, Papp T, Pesti M, Vagvolgyi C, Almassy K, Krisch J.** 2011. Anti yeast activities of some essential oils in growth medium, fruit juices and milk. *International journal of food microbiology* **144(3)**, 480-486.
- Tyagi AK, Malik A.** 2012. Bactericidal action of lemon grass oil vapors and negative air ions. *Innovative Food Science & Emerging Technologies* **13**, 169-177.
- Van de Braak S, Leijten G.** 1999. Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam **116**.
- WHO I WWF.** 1993. Guidelines on the conservation of medicinal plants. Gland & Geneva, Switzerland.