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


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Key words: Thyme dimethyl sulphoxide, Essential oil

http://dx.doi.org/10.12692/ijb/10.2.105-110 Article published on February 28, 2017

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, antioxidiant; 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 ingريدient 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 fascilitis, 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 (BaCl₂2H₂O) 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.

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

*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 08mm 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 Thymus Vulgaris 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 *Thymus 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**


