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ANTIBACTERIAL ACTIVITIES OF *THYMUS SERPYLLUM* ESSENTIAL OIL

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The antibacterial activity of the essential oil of *Thymus serpyllum* Linn. varied against gram positive bacteria (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *S. aureus*, *Vibrio cholera*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Shigella ferrarie*). The oil showed activity even at 50µg concentration. The antibacterial activity was comparable with standard antibiotics, Amoxil, Streptomycin and Kanamycin at same concentration against some of the gram positive and gram negative bacteria. At higher concentrations all the bacterial strains were more sensitive to oil than the standard antibiotics. The minimum inhibitory concentration (MIC) and minimum bactericidal/bacteriostatic concentration (MBC) of oil ranged between 90-170 µg ml^{-1} against the susceptible bacteria.

Key words: *Thymus serpyllum*, Essential oil, Antibacterial activity, Gram positive and gram negative bacteria.

Introduction

Thymus serpyllum belonging to Lamiaceae family and locally known as 'Masho' that grows wildly in the hilly tracts of Ziarat and its surroundings in Balochistan. *T. serpyllum* has a very important place in Ayurvedic system of medicines because of the presence of two isomeric phenols, Thymol and Carvacrol in its essential oil (Sattar *et al* 1991).

The antimicrobial activities of essential oils have been known since the dawn of early medical practices to the present time of investigation (Qamar and Chaudhary 1991); Naqvi *et al* (1985, 1987) had studied and evaluated 163 plant species for their antimicrobial activities and found 30% of them active. Other workders Morris *et al* (1979), Syed *et al* (1986, 1987, 1991), Razia *et al* (1996), Yazdana *et al* (1997), Rahman and Gul (2000), Qasim and Rafiq (2001) have also reported anti-microbial activities of essential oils of different plant species against various microorganisms. Volatile oil is found to be much effective against gram positive and negative pathogenic bacteria (Rahman and Gul 2002, Qasim and Rafiq 2001).

The present work deals with the antimicrobial activity of the volatile oil of *Thymus serpyllum* against 7 gram positive and 3 gram negative bacteria. The minimum inhibition, bactericidal/bacteriostatic concentrations were also determined.

Materials and Methods

Extraction of essential oil. *Thymus serpyllum* plants were collected from hilly tracks of Ziarat valley, Balochistan, Pakistan. The aerial parts of the plants were brought to the laboratory and dried in air. The oil was obtained from the plants by steam distillation, 5 kg dried and crushed plants were steam distilled in Dean Starke head (Guenther 1952).

Test organisms. Standard cultures of gram positive bacteria (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *S. aureus*, *Vibrio cholera*) and gram negative bacteria (*E.coli*, *Salmonella typhi*, *Shigella ferrarie*) were procured from Drug Testing Laboratories, Government of Punjab, Pakistan and maintained on nutrient agar slants, stored in refrigerator and sub cultured after every 15 days.

Antibacterial activity. Antibacterial activity of the essential oil was checked by agar diffusion method (Murtaza *et al* 1994). Mueller and Hinton agar (Ericson and Sherris 1971) was used for testing the sensitivity of different strains towards the essential oil of *Thymus serpyllum*. Stock solution of the essential oil 1µg ml^{-1} and dilutions of the stock solution containing 50, 100, 150 and 200 µg were prepared in dimethyl sulfoxide (DMSO) and 100 µL of each dilution was added in their respective well and 100 µL DMSO for control. Amoxil, Streptomycin and Kanamycin were used as standard drugs (100 µg). The inhibition zones were recorded after incubating the plates at 37°C for 24 h.

Determination of MIC and MBC. Minimum inhibitory concentrations (MIC) were determined by the tube dilution method using Mueller Hinton broth (Ericson and Sherris 1971). Bactericidal and bacteriostatic concentration of the essential oil was determined by diluting 1 ml of the culture

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Table 1

Antibacterial activity of *Thymus serpyllum* oil compared with Amoxil, Streptomycin and Kanamycin

Name of organism	Different concentrations of *Thymus serpyllum* oil (mcg) used for zone of inhibition (Diameter in mm) 50	100	150	200	Control DMSO 100 µl	Standard antibiotic used (100 mcg) for zone of inhibition (Diameter in mm) Amoxil	Streptomycin	Kanamycin
Gram Negetive Bacteria								
Escherichia coli	12.60	30.50	45.00	52.00	+	19.50	28.50	21.25
Salmonella typhi	13.50	18.00	24.00	31.00	+	27.50	33.50	19.75
Shigella ferarie	13.30	17.00	25.00	33.50	+	25.00	28.50	25.50
Gram Positive Bacteria								
Bacillus megaterium	20.50	24.00	30.00	38.00	+	12.65	34.00	32.75
Bacillus subtilis	14.63	22.30	28.00	37.00	+	15.25	15.75	19.00
Lactobacillus acidophilis	11.50	19.20	25.00	33.00	+	14.00	31.50	22.25
Micrococcus leuteus	15.00	18.20	24.00	31.00	+	21.00	20.75	27.50
Staphylococcus albus	13.67	20.00	30.00	36.00	+	21.00	25.75	26.50
Staphylococcus aureus	11.60	21.00	30.00	37.00	+	17.25	19.50	23.75
Vibrio cholera	12.83	18.00	26.00	32.00	+	25.00	18.75	26.25

Table 2

Minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of *Thymus serpyllum* oil compared with Amoxil, Streptomycin and Kanamycin

Name of organism	*Thymus serpyllum*			*Amoxil*			*Streptomycin*			*Kanamycin*		
	MIC mg ml^{-1}	Control DMSO	MBC	MIC mg ml^{-1}	Control DMSO	MBC	MIC mg ml^{-1}	Control DMSO	MBC mg ml^{-1}	MIC DMSO	Control	MBC
Gram Negetive Bacteria												
E. coli	90.00	+	Bactericidal	125.00	+	Bactericidal	72.00	+	Bactericidal	85.80	+	Bactericidal
S. typhi	135.00	+	Bactericidal	75.00	+	Bactericidal	105.00	+	Bactericidal	90.00	+	Bactericidal
S. ferrarie	170.00	+	Bactericidal	80.00	+	Bactericidal	110.00	+	Bactericidal	95.00	+	Bactericidal
Gram Positive Bacteria												
B. megaterium	115.00	+	Bactericidal	205.00	+	Bactericidal	85.50	+	Bactericidal	90.00	+	Bactericidal
B. subtilis	150.00	+	Bactericidal	220.00	+	Bactericidal	82.20	+	Bactericidal	72.50	+	Bactericidal
L. acidophilus	155.00	+	Bactericidal	145.00	+	Bactericidal	72.50	+	Bactericidal	75.30	+	Bactericidal
M. leuteus	90.00	+	Bactericidal	70.00	+	Bactericidal	80.50	+	Bactericidal	125.00	+	Bactericidal
S. albus	110.00	+	Bactericidal	200.00	+	Bactericidal	95.00	+	Bactericidal	105.00	+	Bactericidal
St. aureus	170.00	+	Bactericidal	180.00	+	Bactericidal	80.00	+	Bactericidal	92.50	+	Bactericidal
V. cholera	115.00	+	Bactericidal	100.00	+	Bactericidal	75.00	+	Bactericidal	85.30	+	Bactericidal

from the MIC tube as well as 1 ml from above and below the MIC tubes. Diluted broth culture 0.1 ml was transferred to nutrient agar plates and incubated at 37°C for 18-24 h for the determination of growth.

Results and Discussion

The essential oil of *T. serpyllum* was found active against all the gram positive (*B. megaterium, B. subtilis, L. acidophilus, M. leuteus, S. albus, S. aureus, V. cholera*) and gram negative (*E.coli, S. typhi, S. ferrarie*) bacterial strains. The activity of the essential oil varied with the organisms and the concentration of the essential oil against standard antibiotics (Amoxil, Streptomycin and Kanamycin) is shown in Table 1. The dose dependency of antimicrobial activity of Labiatae members has also been reported by Paris and Paris (1978).

The essential oil showed much better activity against *E.coli* among gram negative bacteria and even better than standard antibiotics. The activity of oil against *E.coli* at 100 µg con-

centration was 36.06, 6.55 and 30.32% better than the standard antibiotics Amoxil, Streptomycin and Kanamycin, respectively. When the concentration of oil was doubled i.e., 200µg, the activity was far better than the standard antibiotics (100µg concentration). Qasim and Rafiq (2001) have reported the anti-bacterial activity of Labiateae plant *Carum copticum* against *E.coli.*

The antibacterial activity against gram positive bacteria was also comparable with standard drugs. At 50µg concentration oil showed 62.05% better activity against *B. megaterium* as compared to Amoxil. Whereas at 100µg concentration *B. megaterium, B. subtilis, L. acidophilus* and *S. aureus* were most sensitive (21.72 to 89.72%) to oil than Amoxil (100µg).

The activity of oil against *B. subtilis* was 29.37% better than Streptomycin at same concentration (100µg). At 150µg concentration , oil showed 15.66-77.77 % greater activity against *B. subtilis, M. leuteus, S. albus, S. aureus* and *V. cholera* than Streptomycin except *B. megaterium* and *L. acidophillus*. The oil (200µg conc.) had far better antibacterial activities against all the susceptible gram positive bacteria with respect to standard antibiotics (100µg conc.).

The oil showed 17.36% greater activity against *B. subtilis* than Kanamycin (100µg). At 150µg concentration oil was 47.36, 12.35, 13.20, 26.31% better active against *B. subtilis, L. acidophilus, S. albus* and *S. aureus*, respectively than Kanamycin (100µg). The antibacterial activity of oil at 200µg was 13.81, 11.29% higher than the drug (100µg) against *B. megaterium* and *M. leuteus*, respectively. *Carum carvi*, member of Labiatae family reported to be active against *S. aureus* (Qasim and Rafiq 2001). Shahnaz *et al* (1992) have also reported the antimicrobial activities of Labiatae plant *Salvia santolinifolia* Boiss, which is responsible of infection of skin, hair and nails.

The minimum inhibition concentration (MIC) and minimum bactericidal/bacteriostatic concentration (MBC) of oil and standard antibiotics are given in Table 2. The data showed that the MIC value of oil against gram positive and negative strains varied from 90-170 µg ml^{-1} whereas the MIC value for Amoxil was 75-125 & 70-220, Streptomycin 72-110 & 72-95 and Kanamycin 85-95 & 72-125 µg ml^{-1} against gram positive and negative susceptible bacteria, respectively. All these concentrations were bactericidal and not bacteriostatic.

All the above said activities against the susceptible microorganisms are because of the presence of phenolic compounds thymol (2-4%) and carvacrol (50-55%) (Guenther 1952) in the essential oil of *Thymus serpyllum* Linn.

Conclusion

It can safely be concluded from the above results that *Thymus serpyllum* Linn. plant as a whole or its parts can be utilized as an antiseptic, anthelmintic and carminative drug.

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