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Research Article

ANTIBACTERIAL ACTIVITY OF AERIAL PARTS OF *THYMUS SERPHYLLUM* LINN AGAINST CLINICALLY IMPORTANT BACTERIAL STRAINS

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ABSTRACT

In the present research work *in vitro* antibacterial activity of methanolic extract of aerial parts of *Thymus serphyllum* L. growing wild in Kashmir Himalaya was evaluated by agar well diffusion method and broth dilution assay against nine human pathogenic bacterial strains, known to cause serious infections. The extract was also screened for the presence of various bioactive phytoconstituents present in the plant. The extract in the present study possess appreciable potential of inhibiting the growth of all the bacterial strains at all tested concentrations (30, 60 and 90 mg/ml). The highest sensitivity was exhibited against *Staphylococcus epidermidis* MTCC- 435 and *Staphylococcus aureus* with mean zones of inhibition 20.66 and 20 mm respectively at the concentration of 90 mg/ml. *Salmonella typhi* showed the least activity with mean zone of inhibition of 10.00 mm at the concentration of 30 mg/ml. The MIC value ranged between 1.56 to 12.56 mg/ml. The phytochemical analysis of the crude extract revealed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, cardiac glycosides, terpenes, steroids and carbohydrates. Anthraquinone glycosides were absent. The present study clearly indicate that the crude methanolic extract of *Thymus serphyllum* from high altitude of Kashmir Himalaya (2350 m) shows significant antibacterial activity in concentration dependent manner.

Keywords: *Thymus serphyllum*, Kashmir Himalaya, Antibacterial activity, Agar well diffusion method, MIC.

INTRODUCTION

Bacterial resistance to antibiotics is a major therapeutic problem and the pace at which new antibiotics are being produced is slowing[1]. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[2]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[3]. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines[4]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[5]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[6]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases[7].

Thymus serphyllum L. belongs to the family Lamiaceae. It is commonly known as wild thyme. The genus Thymus is a well known aromatic perennial herb[8], is widely distributed in temperate zones, comprises about 350 species worldwide[9]. *Thymus serphyllum* is a small much branched and strongly scented shrub. It bears tiny purple coloured flowers with evergreen leaves 3-8 mm long[10]. Thymus species are well known as medicinal plants because of their biological and pharmacological properties[11]. In traditional medicine, leaves and flowering parts of Thymus species are widely used as tonic and herbal tea, antitussive and carminative as well as treating colds, coughs, sore throats, cystitis, insomnia bronchitis, and indigestion[12 13]. The active ingredients of *Thymus serpyllum* are volatile oil containing thymol, carvacrol, cineole, borneol, linalool, and pinene; flavonoids, apigenin and luteolin; tannins. *Thymus serphyllum* is known to have antimicrobial activity[14], antihelminthic, antioxidant, strongly antiseptic, antispasmodic, carminative, deodorant, diaphoretic, expectorant, sedative and antiseptic property[15].

MATERIALS AND METHODS

Plant Material:

Thymus serphyllum was collected at flowering stage from Tangmarag area of Kashmir Himalaya at an altitude of 2350 m (a.s.l) by conducting field trips. The collected plant material was properly identified at the Centre of Biodiversity and Plant Taxonomy, University of Kashmir and a specimen Voucher was deposited in Kashmir University Herbaria (KASH) for further reference.

Preparation of extract:

The aerial parts of the plant were properly cleaned and dried under shade for one week. After drying, the material was chopped and then grinded to powder. Dried plant powder was then packed in Soxhlet apparatus and extracted with methanol at 50-65 ^{o}C. The extract was then filtered through Whatmann filter paper No. 1. The pellet was discarded and the supernatant was collected and concentrated under reduced pressure at 35-45°C using Buchi rotavapor (R-215). The extract was then dried, labelled and stored at 4^{o}C in storage vials for experimental use[16].

Antibacterial Activity:

Microorganisms tested:

Microbial cultures of nine different species of both Gram positive and Gram negative bacteria were used for determination of antibacterial activity. Four bacterial strains viz. *Proteus vulgaris* MTCC- 321, *Staphylococcus epidermidis* MTCC- 435, *Pseudomonas aeruginosa* MTCC- 1688 and *Bacillus subtilus* MTCC-441 were standard laboratory isolates obtained from Microbial Type Culture Colletion, Chandigarh (India). The rest five bacterial strains were clinical isolates obtained from Department of Microbiology, Sheri Kashmir Institute of Medical Sciences- Srinagar (India). All the bacterial strains were sub-cultured at 37°C on Mueller-Hinton agar (Himedia) slants every fifteen days and stored at 4°C.

Antibacterial activity assay:

In the present research work, the antibacterial activity of methanolic extract of *Thymus serphylum* was determined by agar well diffusion method as adopted by Perez *et al*[17]. Each microorganisms were grown overnight at 37°C in Mueller-Hinton Broth. Ten microlitres (10µL) of standardized inoculum (0.5 Mac-Farland) of each test bacterium was inoculated on molten Mueller-Hinton agar, homogenized and poured into sterile Petri dishes. The Petri dishes were allowed to solidify inside the laminar hood. A standard cork borer of 5mm in diameter was used to make uniform wells into which was added 30µl essential oil diluted in DMSO. Standard antibiotic kanamycin (30µg/disc) was used as positive control and DMSO as negative control. The plates were then incubated at 37 ± 1°C for 24h. The zone of inhibition was measured to the nearest size in mm with the help of standard scale[18]. The experiments were carried in strict aseptic conditions so as to achieve consistency. The experiments were carried out in triplicates and results were calculated as mean ± SD.

Determination of minimum inhibitory concentration (MIC):

Minimum inhibitory concentrations (MICs) are considered to be the 'gold standard' for determining the susceptibility of organisms to antimicrobials. The MIC of methanolic extract of *Thymus serphylum* was determined by the method as developed by Jennifer[19]. Dilution ranges (50 - 0.78 mg/ml) of methanolic extract from the selected plant material were prepared from stock solution by serial dilution technique. 20 ml of sterile molten Muller Hinton Agar to each dilution mixed properly and poured into 90 mm Petri plates and

allowed it to cool under laminar air flow before streaking with 1-2 µl of 0.5 Mc-Farland standard inoculums to each plate. Plates were incubated at 37± 1°C for 24 hours. The lowest concentration of the extract at which there was no visible growth of microorganisms was considered as minimum inhibitory concentration (MIC).

Photochemical Screening:

Photochemical screening for major bioactive constituents was done by using standard qualitative photochemical methods[20-21].

RESULTS AND DISCUSSION

The methanolic extract of *Thymus serphylum* exhibited varying degree of antibacterial activity against the tested bacterial strains (table- 1). The bacterial strains used were clinical and laboratory isolates. All these bacterial species are known to cause serious human infections. From clinical point of view, *Klebsiella pneumonia* causes neonatal nosocomial infection[22]. *Escherichia coli* cause's septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs[23]. *Salmonella typhi* causes serious public health problem in developing countries and represents a constant concern for the food industry[24]. *Shigella dyssenteriae* cause shigellosis. *Staphylococcus aureus* causes dermatitis and sialadenitis. *Proteus vulgaris* causes bacteremia, sepsis and urinary tract infections[25-26]. The most antibacterial sensitivity was shown by *Staphylococcus epidermidis* MTCC- 435 with mean zone of inhibition of 20.66 mm at the concentration of 90 mg/ml, while as *Salmonella typhi* showed the least activity with mean zone of inhibition of 10.00 mm at the concentration of 30 mg/ml. The minimum inhibitory concentration of methanolic extract of *Thymus serphylum* ranged between 1.56 to 12.56 mg/ml (table-2). The extract in the present study exhibited broad spectrum antibacterial activity which was comparable to the standard antibiotic drug (kanamycin). The Gram positive bacterial strains were found to be slightly more sensitive than Gram negative bacterial strains. It may be due to the absence of lipo-polysachride layer in Gram positive bacteria that might function as a barrier to the phytocemical substances that are responsible for antibacterial activity[27-28]. The plant extract being active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against multidrug resistant microorganisms. The phytochemical screening of methanolic extract of aerial parts of *Thymus serphylum* raveled the presence of alkaloids, flavonoids, phenolics, saponins, tannins, cardiac glycosides, terpenes, steroids and carbohydrates (table-3). Anthraquinone glycosides were found to be absent. These phytochemicals (Secondary plant metabolites) are responsible for the biological activities and are known to have antimicrobial, antioxidant activities[29-30]. The present study reports the presence of diverse phytochemicals in the plant than earlier reports by Kavita *et al* [14]. The present research work supports the resourcefulness of the plant in terms of presence of phytochemicals and antibacterial potential of the plant from higher altitude region of Kashmir Himalaya.

Table 1: Zone of inhibition (mm) at various concentrations of methanolic extract of *Thymus serphyllum* against selected bacterial strains**.**

S. No	Microorganism.	Zone of inhibition (mm).			
		Methanolic extract of Thymus serphylum			Standard Antibiotic (Kanamycin 30µg/disc)
		90 mg/ml	60 mg/ml	30 mg/ml	
1.	*Proteus vulgaris* MTCC- 321.	16.66±1.52	14.33±1.52	11.0 ± 1.00	29.00±1.00
2.	*Staphylococcus epidermidis* MTCC- 435.	20.66±1.52	17.00±1.00	13.00±1.00	30.33±0.57
3.	*Pseudomonas aeruginosa* MTCC- 1688.	17.66±1.52	15.0±1.00	12.16±1.52	28.66±1.52
4.	*Bacillus subtilus* MTCC- 441.	18.00±1.00	15.0±1.00	11.33±0.57	27.33±1.52
5.	*Salmonella typhi.*	15.66±2.51	13.33±1.52	10.00±1.00	27.33±1.14
6.	*Shigella dyssenteriae.*	17.66±1.52	14.66±1.52	12.33±1.52	29.33±1.52
7.	*Staphylococcus aureus.*	20.00±1.00	17.00±1.00	13.66±1.52	29.00±1.00
8.	*Klebsiella pneumonia.*	18.00±1.00	15.33±1.52	11.66±1.52	29.66±1.52
9.	*Escherichia coli.*	17.00±1.00	14.66±0.57	12.00±1.00	28.00±1.00

Values are mean zone of inhibition (mm) ± S.D of three experiments

Table 2: MIC value (mg/ml) of methanolic plant extract of *Thymus serphyllum* against different bacterial strains

Microorganism.	MIC (mg/ml).	Microorganism.	MIC (mg/ml).
Proteus vulgaris MTCC- 321.	3.12	*Shigella dyssenteriae.*	6.25
Staphylococcus epidermidis MTCC- 435.	1.56	*Staphylococcus aureus.*	1.56
Pseudomonas aeruginosa MTCC- 1688.	3.12	*Klebsiella pneumonia.*	3.12
Bacillus subtilus MTCC- 441.	6.25	*Escherichia coli*	12.56
Salmonella typhi.	12.56		

Table 3: Phytochemical screening of methanolic extract of *Thymus serphylum*

S. No	Phytoconstituents	Test	Result
1.	Alkaloids	Wagner's test	+
2.	Phenolics	phenol test	+
3.	Tannins	Ferric chloride test	+
4.	Cardiac glycosides	Keller-Killani test	+
5.	Anthraquinone Glycosides	Nitric acid test	-
6.	Terpenes	Salkwaski test	+
7.	Flavonoids	Shinoda test	+
8.	Saponins	Frothing test	+
9.	Steroids	Libermann-Buchard's test	+
10.	Carbohydrates	Benedict's/ Fehling's test	+

Key: (+) indicates presence, (-) indicates absence

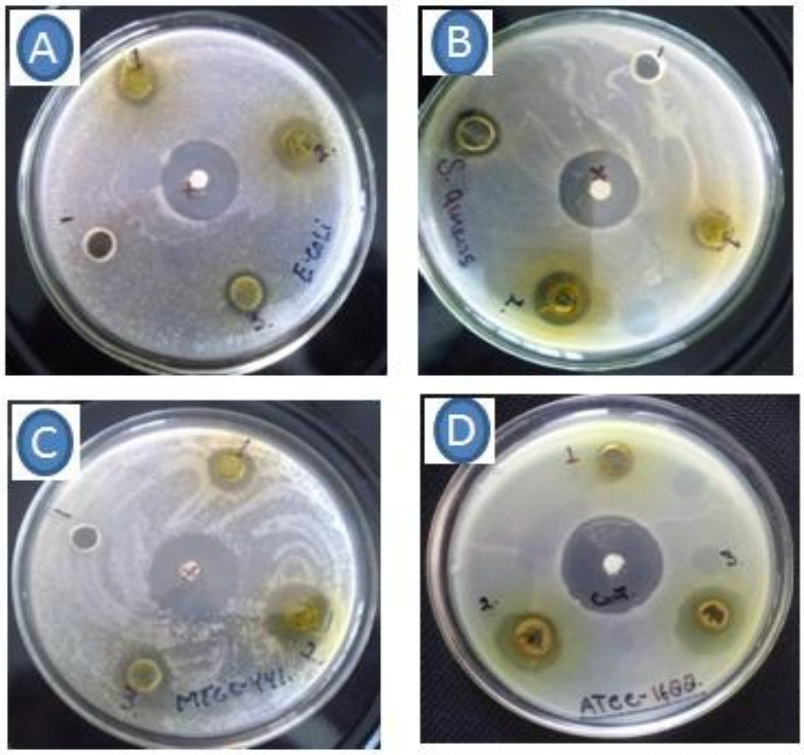

Figure: Showing Zones of inhibition against (A) *E. coli,* (B) *S. aureus* (C) *B. subtilus*-441 and (D) *P. aeruginosa*-1688 at various concentrations of plant extract

CONCLUSION

The results of the present study showed that the methanol extract can be an accessible source of promising therapeutic agents that can be used in combating infectious diseases caused by drug- resistant microorganisms. Further study is needed to isolate, structurally characterize the pure compounds and evaluate their antimicrobial activity against multidrug resistant microbial strains. The lead molecules will be further subjected to mechanistic studies and will be tested for the possibility of synergism.

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