Assessment of Antimicrobial Effects of *Thymus Vulgaris* Leaves in Extracts of Methnol, Ethanol and N-Hexane against Pathogenic Bacterial Species

Hamad Ullah[1], Shandana Zulfiqar[1], Yusra Arshad[1], Muhammad Junaid Khan[1], SumariaSamad Dawar[1], SahibzadaMehmoodAhmad[2], Farah Shireen[2], Aamir Aziz[2], Muhammad Idrees[4],Abdul Rehman[3], Nazish Bilal[1], Sadia Bibi[1], Muhammad Asghar[5]

[1]Khyber Medical Univeritsy, IPDM, Peshawar Pakistan

[2]Department of Microbiology, Institute of Biological Sciences Sarhad University of Science and Information Technology, Peshawar

[3]Center of biotechnology and Microbiology, University of Peshawar

[4]Department of Bioetchnology, Univeristy of swabi

[5]Department of Microbiology KMC/KTH Peshawar

Corresponding author Muhammad Asghar, Email address:drazghar@gmail.com

Abstract

Antibacterial activity of *Thymus vulgaris* leaves was scrutinized in solvent i.e., methanol, ethanol and n-hexane contrary to *Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, E.coli* and *Klebsiellapneumoniae*over well diffusion method. Tremendous inhibitory effects of methanolic extract weredisplayed against *E. coli* and *Proteus vulgaris*(90% and 80% respectively). The extract on the other hand exhibited a good inhibitory effect against *Klebsiellapneumoniae,Salmonella typhi* and *Staphylococcus aureus* (72%, 64% and 60%correspondingly). Moreover, the inhibitory effect of ethanolic extract showed also a worth noticing results against *Proteus vulgaris,E. coli* (72% and 70%) and showed adequate result against *Salmonella typhi, Klebsiellapneumoniae*and *Staphylococcus aureus* (56%, 54% and 43% respectively).Furthermore, n-hexanoic extract disclosed good results against *E. coli, Klebsiella pneumonia* and showed average results contrary to*Salmonella typhi, Staphylococcus aureus* and *Proteus vulgaris* (65%, 63%, 52% and 36%).The *E. coli* was determined for the minimum inhibitory concentration.Precisely the MIC results were attained with *Thymus vulgaris* extract against *E. coli.* Results were affirmative for the inhibiting effects of the extracts of *Thymus vulgaris* against numerous bacterial species entailing its use as a resilient antibacterial agent providing a scientific ground for the advance research. the increased resistance of microorganisms to conventional chemicals and drugs is a serious and obvious worldwide problem, and research has been urged to identify new biocides with good activity. The

purpose of this work is to replace synthetic antibiotics with natural plant extract of *Thymus vulgaris*, a drug that replaces commercially available antibiotics.
Key words: *Thymus vulgaris*, Antibacterial activity, Minimal inhibitory Concentration

Tob Regul Sci.™ 2022; 8(1): 977-983
DOI: doi.org/10.18001/TRS.8.1.82

1. INTRODUCTION

Plants are far more valuable than people realise, as no human can thrive without them. Plants like Ayurveda, Unani, and other Chinese traditional herbal remedies like Asian Ginseng have been found to have a good effect on body chemistry[1]. Even today, 70-80% of the developing world consumes herbal remedies as an allopathic therapy with a high price tag [2]. The quantity of plant-derived medicines and health foods has been increased to meet demand [3],[4]. A large number of extracts have recently been identified as pharmacologically active substances [5],[6]. The plant kingdom has a wide range of structural variation in the utilisation of biochemicals termed pharmacophores[7].The plant that was chosen for study Thymus vulgaris belongs to the Lamiales order, Lamiaceae family, Thymus genus, and Thymus vulgaris specie. The leaves are linear/linear–lanceolate, ellipsoid or oblong, up to 6 0.5–2 mm in length, with a sharp apex, obtuse base tapering into a petiole, and revolute edges. The hairs on the top surface range from light grey to light brownish grey to light olive green. The inferior surface is equally dark in colour, pubertal, and punctuated with glands. Calyx is tubular, bilabiate, about 4 mm long, pubescent, 9-12 nerved, and has three tiny apexes on top. Two hairs, ascending and attenuating divisions, cover the bottom section. The purple and bilabiate corolla is almost twice as long as the calyx. Stamens are four and didynamous, and the stigma is bi-lobed. Thyme has an aromatic and sweet-smelling perfume. When the plants are in sprout, around 15 cm from the tallest points are harvested, and the cut stems are dried in the sun or in a well-ventilated shed or chamber.Thymus vulgaris (Lamiaceae), also known as thyme, is a pungent herb native to Southern Europe with a global distribution [8,9]. The Mediterranean and adjacent nations, Northern Africa, and portions of Asia are home to the plant. Egypt, Morocco, Algeria, Tunisia, and Libya are the countries involved. Thyme has been grown in Cameroon and South Africa for centuries, and people have utilised it as a spice, gourmet herb, and herbal medicine. Thymus vulgaris, sometimes known as thyme, is a replacement medicinal plant that contains antioxidant and antibacterial chemicals, as well as anti-inflammatory and immune system stimulating compounds [10-13].Despite the fact few studies have confirmed the health possessions of *Thymus vulgaris* in humans, there are some confirmations that the herb may aids indiminution menstrual soreness. Results revealed that both treatments were similarly beneficial in relieving pain. [14, 15].

2. MATERIALS AND METHODS

On February 2019, dried Thymus vulgaris (leaves) were purchased from a commercial zone Hyper Mall in Peshawar, Pakistan, and packed by Rossmoor Company. Thymus vulgaris shade dried leaves were gathered and finely powdered with a weight of 500 g. The powdered substance was then infused for 14

days at 28°C with daily shaking in methanol, ethanol, and n-hexane. In addition, the leaf material was filtered out of the various solvents, yielding blackish green crude extracts weighing 90g.

2.1 ANTIBACTERIAL ASSAY

The antibacterial experiment was carried out to determine the inhibitory impact of Thymus vulgaris leaves extract in various solvents, including methanol, ethanol, and n-hexane, on *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi,* and*Klebsiellapneumoniae*. As test organisms, *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi, and Klebsiellapneumoniae* were obtained from the Microbiology lab, Institute of Biological Sciences, Sarhad University of Science and Information Technology Peshawar's culture bank. Antibacterial testing was carried out utilising the well diffusion technique. Fresh bacterial cultures were injected for 24 hours on sterile nutrient agar plates, then 6 mm wells were bored in the solidified agar. Further on DMSO stock solution with 3 mg/µl concentration was prepared, from which 100 µL was transferred into each well [12].

2.2 Controls

As a positive control, amoxicillin was used at a dosage of 0.5 mg/l in DMSO, which had strong antibacterial properties. The sterile DMSO served as the negative control. The petri plates with positive and negative controls were then stored for 1 hour in a laminar flow hood. The inoculation plates were then incubated at 37°C for 24 hours. Finally, inhibitions were calculated using the formula shown below.;

$$\boldsymbol{PercentInhibition} = \frac{\boldsymbol{Zoneofinhibitionofsample\ (mm)}}{\boldsymbol{Zoneofinhibitionbycontrol\ (mm)}} x\ 100$$

2.3 Minimum Inhibitory Concentration (Mic) Assay

Minimum inhibitory concentrations (MICs) were calculated as the lowest concentration of antimicrobial drugs required to prevent bacteria from replicating for 24 hours. The test organism for MIC was Escherichia coli. One of the most fundamental laboratory techniques is the broth diffusion method, which is used to evaluate an agent's antibacterial activity against microbes with the purpose of demonstrating that if the MIC level is low, the quantity of microbial growth with a minute concentration of a medicine is reduced. The MIC value is critical for determining the efficacy of an antibiotic dosage for a certain illness (13). The MIC test is critical for preventing antibiotic resistance in microorganisms. As a result, MIC ratings assist in improving patient outcomes and reducing the spread of drug-resistant microbial strains.

3. RESULTS AND DISCUSSIONS

3.1 Antibacterial Assay

The following graphical data illustrates the zones of percent inhibitions for the extractsof*Thymus vulgaris* in methanol, ethanol and n-hexane against *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi*and *Klebsiellapneumoniae,* as shown in fig1 to 3.

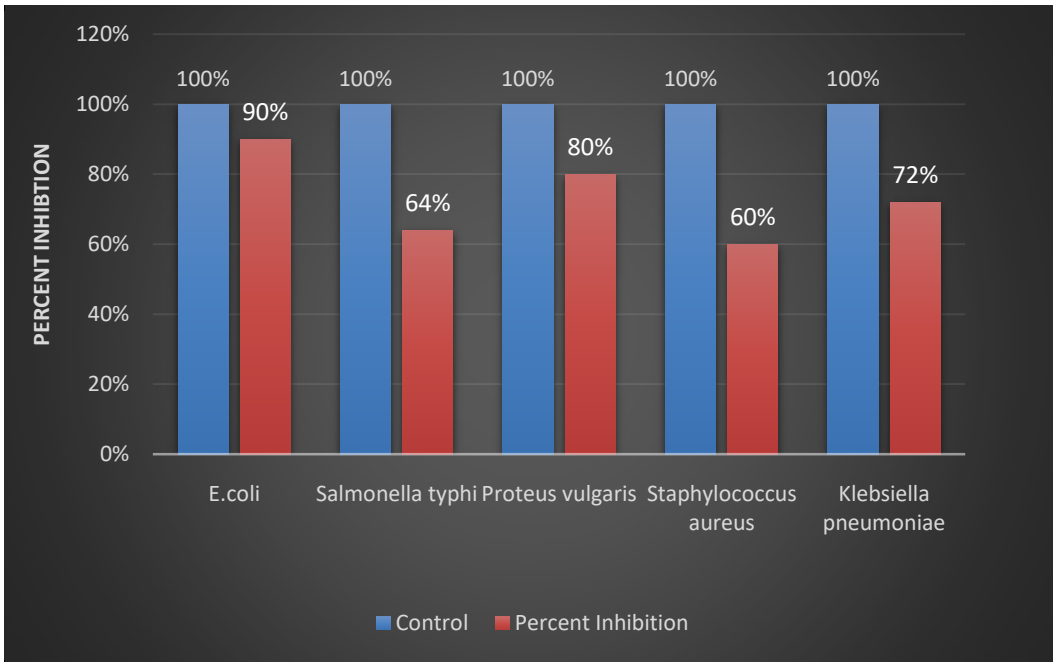

Fig 1: Inhibitory effect of methanolic extracts against test bacterial species

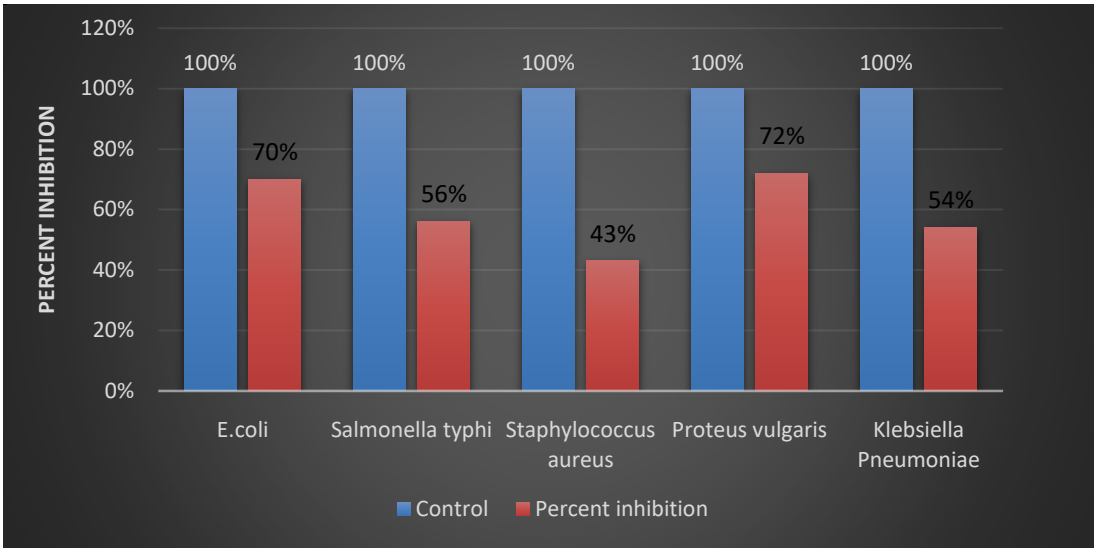

Fig 2: Inhibitory effect of ethanolic extract against test bacterial species.

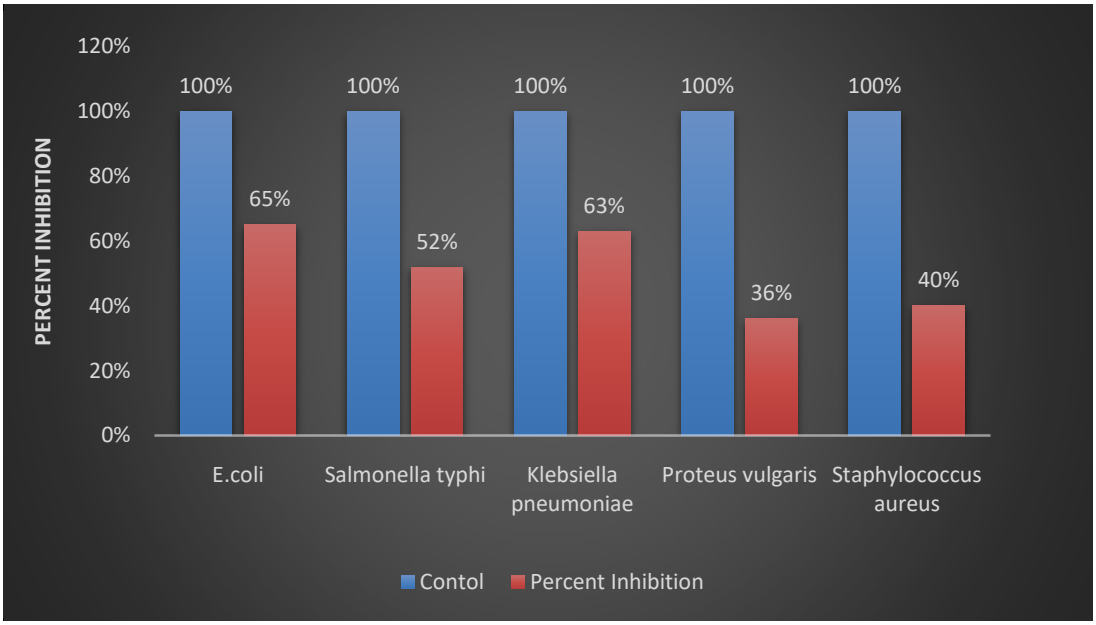

Fig 3: Inhibitory effect of n-hexanoic extract against test bacterial species.

3.2 Minimum Inhibitory Concentration Assay

Plant extracts were investigated for possible MICs at volumes preceding of 10 µg (10 to 100 µg/ml) against the selected bacterial specie by following protocol of (Galgani*et al.*, 1997). Autoclaved distilled water was prepared in autoclaved test tubes. The test samples were pipetted into the petri plates with nutrient agar and then inoculated with fresh bacterial culture. All the test plates were then incubated at 37°C for 24 h. After the incubation period, the results were assessed on the basis percent zone of inhibition by extract of methanol, shown in table 1.

Table 1: MIC shown against *E. coli* by different concentrations of methanolic extract.

Extract Concentrations	Percent Inhibitions
10 ml	30%
20 ml	35%
30 ml	45%
40 ml	55%
50 ml	60%
60 ml	60%
70 ml	70%
80 ml	70%
90 ml	80%
100 ml	95%

4.DISCUSSIONS

These days a huge cause of infectious diseases are microbes and their resistance to antibacterial agents, which impetus the researchers to seek more about the antimicrobial substances. The recommended medicinal plants are proven to be having a strong antimicrobial activity, in order to formulate a drug, which will be effective in lower concentration. *Thymus vulgaris* has been used by Chinese since in 13th century as a very potent medicinal plant in combination with other homeopathic medicines. Alkaloids plants extracts exhibited inhibitory effects contrary to both fungal and bacterial pathogens. There are many types of inhibitory actions of alkaloids plants extracts against bacterial pathogens such as *Staphylococcus aureus, Klebsiellapneumoniae, Escherichia coli, Proteus vulgaris and Salmonella typhi.* The present experiment included the antibacterial activity of ethanolic, methanolic and n-hexanoic extract of *Thymus vulgaris* against selected bacterial culture. The result showed a conspicuous percent zone of inhibition which were formed from 9-20 mm. From the result, the extracts from the plants were found to have efficient antibacterial activity. It was interesting to note that the plant extracts from *Thymus vulgaris* showed antibacterial activity against all the test bacteria.The MIC values for *T. vulgaris* extracts on tested bacteria were in 10 to 100 mg/ml in concentration range which confirmed the inhibitory ability of these plant extracts. Such a trend was observed in the study conducted by in which the extract of *Thymus vulgaris* leaves indicated inhibitory effects against *Staphylococcus aureus, Klebsiellapneumoniae, Escherichia coli, Proteous vulgaris and Salmonella typhi*but their zones of inhibition up to some extentswere smaller because the extractsweused was 10 to 100 mg/ml concentration[1,2,3]. In another studies, the leaves extracts of *Thymus vulgaris*indicated effects against *Staphylococcus aureus, Klebsiellapneumoniae, Escherichia coli, Proteous vulgaris and Salmonella typhi*[4,5,6]. Other investigations conducted that the ethanolic and methanolic extract of *Thymus vulgaris* showed excellent antibacterial activity against*Klebsiellapneumoniae* and *Escherichia coli*[7,8,9]. Also, according to study, methanolic extract of *thymus vulgaris* leaves have efficient activity against *E. coli, Salmonella typhi* and *Pseudomonas aeruginosa* which was affirmed by spectroscopic and chromatographic technique that the antimicrobial properties were governed by 6 flavonoid namely Quercetin [10,11].Furthermore, in the research conductedalso showed MIC assay which they had performed in well plates using a twofold dilution series, with each *Thymus vulgaris* essential oil concentration replicated eight times per plate[12,13]. *Thymus vulgaris* extract was used as a liquid formulation and concentrations are expressed as (% v/v) against *Escherichia coli* and *Klebsiellapneumoniae.* The stock solution of DMSO was prepared to keep the extract's components unchanged while storing and also for its inert nature as carried out in research work[14,15]. The aim of this study was to replace synthetic antibiotics with natural plant extract *Thymus vulgaris*, a drug that could replaces commercially available antibiotics and to assess the antimicrobial activity of *T. vulgaris* extracts against four pathogenic bacteria. These bacteria were *Staphylococcus aureus, Escherichia coli*, *Proteus vulgaris, Salmonella typhi* and *Klebsiellapneumonia.*

5. CONCLUSION

From the result of current research study, it is concluded that extract of *Thymus vulgaris* can inhibit the growth of various pathogenic micro-organisms which can be used by medical sectors to formulate many efficient drugs to remediate many acute to chronic infections. These extracts can be efficiently used in medicines for their antibacterial activity and also in food for the amazing health benefits as spice.

REFERENCES

1. Spinella M. The psychopharmacology of herbal medicine: plant drugs that alter mind, brain, and behavior: MIT Press; 2001.
2. Yaniv Z, Bachrach U. Handbook of medicinal plants: CRC Press; 2005.
3. Fachini-Queiroz FC, Kummer R, Estevao-Silva CF, Carvalho MDdB, Cunha JM, Grespan R, et al. Effects of thymol and carvacrol, constituents of Thymus vulgaris L. essential oil, on the inflammatory response. 2012;2012.
4. Grabley S, Sattler IJMmodd. Natural products for lead identification: nature is a valuable resource for providing tools. 2003:87-107.
5. Silverman RB, Holladay MW. The organic chemistry of drug design and drug action: Academic press; 2014.
6. Williams JT, Ahmad Z. Priorities for medicinal plants research and development in Pakistan. 1999.
7. Dewick PM. Medicinal natural products: a biosynthetic approach: John Wiley & Sons; 2002.
8. Hosseinzadeh H, Karimi G, Niapoor M, editors. Antidepressant effect of Crocus sativus L. stigma extracts and their constituents, crocin and safranal, in mice. I International Symposium on Saffron Biology and Biotechnology 650; 2003.
9. Hashim S, Gamil MJB, Dar revolution of Press, Publication. Plants and herbs between the Iraqi folk medicine and scientific research. 1988.
10. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi HJFc. Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. 2007;18(12):1518-23.
11. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. 2009;58(8):1091-103.
12. Habbu P, Warad V, Shastri R, Madagundi S, Kulkarni VHJCjonm. Antimicrobial metabolites from marine microorganisms. 2016;14(2):101-16.
13. Dada OK, Akindele AJ, Morakinyo OA, Sofidiya MO, Ota DJCjonm. Hypoglycemic and antioxidant activities of the hydroethanolic leaf extract of Byrsocarpus coccineus Schumach. & Thonn.(Connaraceae). 2013;11(6):628-37.
14. Al-Bayati FA, Al-Mola HFJJoZUSB. Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. 2008;9(2):154-9.
15. Direkvand-Moghadam A, Khosravi AJJorimstojoIUoMS. The impact of a novel herbal Shirazi Thymus Vulgaris on primary dysmenorrhea in comparison to the classical chemical Ibuprofen. 2012;17(7):668.