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Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v7i4.2121	Copyright © All rights are reserved by Corresponding Author
Vol. 7 No. 4, 2024: pp. 695-704	
www.readersinsight.net/pjmls	**Revised:** November 20, 2024 **Accepted:** December 16, 2024
Submission: April 21, 2024	**Published Online:** December 24, 2024

PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL INVESTIGATION ON *THYMUS VULGARIS* EXTRACT

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Abstract

Thymus vulgaris L., which has been used in traditional medicine for curing many diseases, has a rich history of curing different diseases. Thymus vulgaris was used for whooping cough, bronchitis and asthma. The current study was focused on the chemical content and biological activity of the methanolic extract obtained from Thymus vulgaris L. It has no glycosides, while it contains alkaloids, saponins, terpenoids, flavonoids, and tannins, highlighting the diverse nature of its chemical constituents. The study investigates the antioxidant capability of Thymus vulgaris L. using the inhibitory activity and comparison with Ascorbic Acid at different concentrations. As for the methanolic extract of thyme common (Thymus vulgaris L.), it exhibited significant ($p<0.05$) antioxidant activity, with percentage inhibitions ranging from 26.30% to 86.18%, which clearly shows its role as a natural antioxidant. In addition to its higher inhibition effects, Thymus vulgaris L continues to have the anticipated antioxidant properties which is evident in all the tested concentrations. The IC50 may be determined to be 158.2±1.13µg/ml for the Thymus vulgaris extract. Also, the acute toxicity bioassay based on the brine shrimp lethality demonstrates the dose-dependent action of the Thymus vulgaris extract on Artemia salina, the suspension-feeding brine shrimp. The assay carried out would result in the occurrence of a concentration-dependent response of Thymus vulgaris methanolic crude extract on brine shrimp (Artemia salina) and exhibit significant toxicity at relatively higher concentrations, having an LC50 of around 268 µg/ml. This sheds light on the extract's cytotoxic potential and relative toxicity compared to the standard drug Etoposide. Thymus vulgaris L. is also tested for its antibacterial activity along with the standard drug Ofloxacin against different strains of bacteria at various concentrations 25,50, and 75µg/ml. Thymus vulgaris extract showed significant ($p<0.05$) antibacterial activity against S. aureus, P. aeruginosa, E. coli, S. typhi, and B. subtilis growth, with stronger effects at higher doses.

Keywords: *Antioxidant activity, Brine shrimp lethality, Cytotoxicity potential, Methanolic extract, Phytochemical composition, Thymus vulgaris L.*

INTRODUCTION

Medicinal plants are becoming more popular because people are realizing how important they are for our well-being. Plants, in general, are crucial for the environment, providing essential services that support life for humans and other living organisms. Imagine a world without plants it would be difficult for any living being to survive. These herbs are like nature's health indicators (1). Plants are green biotic component of our environment, playing a vital role in nature (2).

Nowadays, infectious diseases are creating problems all around the world. They cause high rate mortality (3). People have always depended on plants to stay healthy and treat illnesses. Different parts of plants like roots, leaves, and seeds are used to make natural remedies (4).

In many parts of the world, especially in places that are still growing, people find traditional methods, using plants for medicine, really important for staying healthy (5). Pakistan has a lot of different plants, and its rich in all kinds of plant resources. This is because the country has different climates and different types of soil. Even though there are a lot of plants, only a small number of them have been looked

at closely in terms of their chemical makeup, according to a source (6). Balochistan, comprising a 44% of Pakistan's total land area, stands as the largest province in the country and is native habitat of many medicinal plants (7).

In the southwestern regions of Balochistan, a remarkable 114 plant species have been traditionally utilized for their medicinal properties. The local communities in these areas have relied on the inherent healing qualities of these plants, incorporating them into their traditional medicinal practices. This rich reservoir of plant-based remedies not only reflects the deep connection between nature and the local population but also highlights the valuable indigenous knowledge that has been passed down through generations. An indication of how important these plants are, indeed, not only for the conservation of the biological diversity of the region, but especially for the preservation of the local culture and the knowledge held in it (8).

In Balochistan, the older women are highly knowledgeable in combination of herbs for medicinal purposes. They have a treasure of unmatched expertise and competencies to heal diseases using plants indigenous to their respective regions. Their many years of dealing with alternative-based plants have made them sufficiently able to proffer cures for many kinds of diseases (9). The Thymus genus, belonging the Lamiaceae family, is a genus where around 400 species of aromatic perennial plants are located. They are annuals or sub-shrubs that are known to be locals of the Mediterranean region, naturally integrated into the dense and diverse flora of that particular province (10).

Thymus vulgaris, or thyme scientifically, has remained throughout the years an indispensable ingredient in both cooking and healing as it its phenol monoterpene derivatives, such as cymene, thymol, and carvacrol. Thyme is a diversified protein with unique chemical compounds that brings out its continued significance in the industries (11).

Thyme oil produced from *Thymus vulgaris* is among the list of the 10 best essential oils across the world. The multifaceted thyme oils not only lend the concoctions an exhilarating appeal and rejuvenating impact, but they also stand as an essential preservative in the food industry (12). *Thymus vulgaris* has a traditional medical background with its use to treat different disorder by the olden day people. In the same manner it was used to address some respiratory conditions like whooping cough, bronchitis and asthma. People have utilized thyme in various forms, including tea, ointment, tincture, syrup, and even through steam inhalation, showcasing its versatility in providing therapeutic benefits for respiratory health over the years (13).

MATERIALS AND METHODS

COLLECTION OF PLANT *THYMUS VULGARIS*

The plant was collected from Hanna Valley in Quetta and the plant specimen was identified by Chairperson Pharmacognosy Department, UoB, Quetta. Plant was allotted voucher specimen no1684/FOPHS/18 and deposited in Pharmacognosy department.

PREPARATION OF METHANOLIC EXTRACT

To prepare the *Thymus vulgaris* extract, fresh leaves or stems were collected, ensuring there were no contaminants and that the plant material was accurately identified. The collected material was then cleaned and air-dried in the shade. After drying, the material was ground to increase its surface area. The finely ground material was immersed in an 80% methanol solution for 15 days at 25°C, with intermittent shaking to facilitate extraction. The mixture was then filtered using Whatman paper No. 4 to separate the liquid from the solid. The solvent was removed using a rotary evaporator, concentrating the extract. The concentrated methanolic extract was preserved in a sealed dark glass container to protect it from light and air.

PHYTOCHEMICAL TEST

To test for alkaloids, nitrogen-containing compounds known for their pharmacological effects, a 1 ml solution of the extract was used. A few drops of Mayer's reagent were added to the solution, and the formation of off-white or pale precipitate confirmed the presence of alkaloids in the sample (14).

To investigate the presence of saponins, 1 gram of the extract was mixed with 10 ml of purified water. The mixture was gently shaken for 2 minutes to avoid interference with mitochondrial isolation. The presence of saponins was indicated by the formation of froth, which persisted at a height of at least 1 cm for 30 minutes after adding the sample (15).

For the detection of cardiac glycosides, 2 ml of thyme extract was combined with 3 drops of lead acetate, and the mixture was filtered. The filtrate was then stirred with 5 ml of chloroform, producing a chloroform coating that faded. The remaining fraction was dissolved in glacial acetic acid, and a few drops of ferric chloride were added. The addition of 1 ml of concentrated H_2SO_4 resulted in the formation of a brown ring at the interface, signifying the presence of deoxy-sugar cardenolides. Occasionally, a purple ring emerged beneath the surface, indicating the presence of other components (16).

To test for terpenoids, a 5 ml portion of methanolic extract was mixed with 2 ml of chloroform, followed by the addition of 3 ml of concentrated sulfuric acid. The formation of a reddish-brown color at the interface between the methanolic extract and chloroform layers indicated the presence of terpenoids (17).

For the detection of flavonoids, 1 ml of methanolic extract was combined with a few drops of concentrated hydrochloric acid (HCl) and magnesium (Mg). The appearance of a pink or magenta-red color confirmed the presence of flavonoids (18).

To test for tannins, a 5 ml extract solution was subjected to two tests. In the first test, a few drops of ferric chloride were added, causing the solution to change color to purple, green, black, or blue, indicating the presence of tannins. In the second test, 5 ml of the extract was added to 10% lead acetate, and the observation of precipitation further confirmed the presence of tannins (15).

DPPH RADICAL SCAVENGING ACTIVITY

Solutions with various concentrations, ranging from 1 mg/mL to 0.0625 mg/mL, were prepared from the stock solution. The required amount of DPPH (approximately 0.0039432 g) was accurately measured using a balance. To prepare a 0.1 mM DPPH solution, 0.0039432 g of DPPH was dissolved in 100 ml of absolute ethanol. Then, 0.5 ml of the 0.1 mM DPPH solution was mixed with 50 µl of the prepared plant extract. Additionally, blanks (ethanol), standards (ascorbic acid), and controls (DPPH solution without the plant extract) were prepared. The reaction mixture was shaken and incubated in the dark at room temperature for 30 minutes. Ascorbic acid was used as the standard for comparison. The degradation of DPPH was determined by measuring the decrease in absorbance at a wavelength of 517 nm using a spectrophotometer. Ethanol, ascorbic acid, and DPPH without plant extracts were used as the blank, standard, and control, respectively (19).

The percentage inhibition of DPPH was calculated using the following equation:

$$\% \text{ inhibition of DPPH} = AC - AS / AC \times 100$$

BRINE SHRIMP LETHALITY BIOASSAY

Firstly, distribute 50 mg of brine shrimp eggs into a rectangular hatching tank (22x32 cm) filled halfway with accurately filtered brine solution. Create varying concentrations (10, 100, 500, and 1000 µg/ml) in four vials from a stock solution, ensuring homogeneity through overnight solvent evaporation. Incubate these vials at a controlled temperature of 25-27°C for 24 hours under continuous illumination and aeration to allow egg hatching. Introduce 30 shrimp larvae into each vial after a 2-day hatching period, adjusting the volume to 5 mL with seawater.

Extra vials were set up for standard drug Etoposide, a cytotoxic drug, and a solvent control. These act as benchmarks for comparison, serving as positive and negative controls. Evaluate survival by macroscopic counting of surviving brine shrimp larvae in each vial, using a magnifying glass against a lit background (20).

The following formula was used to calculate the percentage survival for each concentration.

$$\% \text{ Survival} = (\text{No. of survivors} / \text{No. of shrimps}) \times 100$$

And following formula was used to calculate the percentage mortality for each concentration.

$$\% \text{ Mortality} = (\text{Number of Deaths} / \text{Total Number of Shrimps}) \times 100$$

MICROBIAL STRAINS AND REFERENCE DRUGS

Microbial strains and reference drugs Different bacterial strains *Staphylococcus aureus* (NCTC 6571), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella typhi* (ATCC14028) and *Bacillus subtilis* (ATCC 5230), were identified and obtained from CASVAB, UoB, Quetta.

ANTIBACTERIAL ACTIVITY

Prepared strong solutions of Thymus plant extracts (CME) at 300 µg/ml using methanol and then made weaker solutions at 25, 50, and 75 µg/ml. Similar dilutions were done for reference drugs. To test the antibacterial activity of *Thymus vulgaris* L., we used the Disc diffusion method. First, we made fresh Muller Hinton Agar, poured it into plates, and checked for contamination after letting it cool. We spread bacteria evenly on the agar plates using a swab, and then placed antibiotic disks with ofloxacin and *Thymus vulgaris* L. extracts. These disks were refrigerated before use. After incubating the plates at 37°C for 24 hours, let the bacteria grow and see if there were any clear areas (inhibition zones) around the discs. We used DMSO as a control, repeating the process three times for reliability. After incubation, we measured the clear zones with a millimeter ruler to understand how well the substances inhibited bacterial growth (21).

RESULTS

PHYTOCHEMICAL TEST

This study presents a comprehensive investigation into the phytochemical composition and bioactive properties of the methanolic extract derived from Thymus vulgaris L. The study reveals the absence of glycosides in the extract, while detecting the presence of Alkaloid, Saponins, Terpenoids, Flavonoids, and Tannins, shown in (Table I.), highlighting the diverse nature of its chemical constituents.

Table I. Phytochemical test of *Thymus Vulgaris* L. crude extract

Phytochemical Group	Detection
Alkaloid	Detected
Saponins	Detected
Glycosides	Not Detected
Terpenoids	Detected
Flavonoids	Detected
Tannins	Detected

DPPH RADICAL SCAVENGING ACTIVITY

At varying concentrations, *Thymus vulgaris* displayed inhibition percentages ranging from 26.30% to 86.18%, with the lowest concentration at 0.0625 mg/ml. In comparison, Ascorbic Acid consistently exhibited higher inhibition percentages, ranging from 37.45% to 95.50%. Thymus vulgaris L. demonstrated promising antioxidant properties across all concentrations tested, despite Ascorbic Acid outperforming it. The calculated IC50 for *Thymus vulgaris* is 158.2±1.13 µg/ml (Table II). Despite the higher inhibition demonstrated by Ascorbic Acid, *Thymus vulgaris* L. exhibits promising antioxidant properties across all concentrations tested. The IC50 of *Thymus vulgaris* calculated is 158.2±1.13 µg/ml (Table III).

Table II. For % inhibition for *Thymus vulgaris* and ascorbic acid

Conc. (mg/ml)	%inhibition by *Thymus vulgaris* ±SD	%inhibition by Ascorbic Acid ±SD
1	86.18±1.15	95.50±1.70
0.5	80.15±1.70	90.63±1.38
0.25	73.27±1.13	82.19±1.80
0.125	59.41±1.38	64.24±1.47
0.0625	26.30±1.50	37.45±1.13

All values in table are mean±SEM n=3 where*show Significant results (P<0.05) and**show highly significant Results (P<0.01)

Table III. IC50 of methanolic extracts of *Thymus vulgaris* L. and ascorbic acid

Compound	DPPH Assay IC50 (mg/ml) ± SEM
Thymus vulgaris L.	158.2±1.13 µg/ml.
Ascorbic acid	39.45 ± 1.18 µg/ml

BRINE SHRIMP LETHALITY BIOASSAY

At 10µg/ml concentration, the extract showed low mortality (3.34%) compared to the standard drug Etoposide, which had a 70% mortality rate at a concentration of 7.46µg/ml. At 100µg/ml concentration, the TV extract mortality rate increased to 13.34%. At 500µg/ml, the *Thymus vulgaris* extract exhibited a comparable level of lethality (73.34%) when contrasted with Etoposide (Table IV). This suggests that at this concentration, the extract has a similar impact on Artemia salina as the standard drug. At the highest concentration (1000µg/ml), the result of this bioassay results indicate a concentration-dependent response of *Thymus vulgaris* methanolic crude extract on brine shrimp (*Artemia salina*), with significant lethality observed at higher concentrations.

Table IV. Brine shrimp (Artemia salina) % survival & mortality against *Thymus vulgaris* L. methanolic extract

Conc. (µg/ml)	No. of shrimps	%Survival	% mortality	STD drug	% mortality
10µg/ml	30	96.67	3.33	Etoposide	70%
100µg/ml	30	86.67	13.33		
500µg/ml	30	26.67	73.33		
1000µg/ml	30	0	100		

All values in table are mean±SEM n=3 where*show Significant results (P<0.05) and**show highly significant results (P<0.01)

ANTIBACTERIAL ACTIVITY

Thymus vulgaris L. was also tested for its antibacterial activity along with standard drug Ofloxacin against different strains of bacteria at various concentrations 25, 50 and 75µg/ml. The *Thymus vulgaris* extract demonstrated notable activity against a range of pathogens. At high concentrations, it exhibited the highest inhibitory effect against *Staphylococcus aureus* (26.67), *Bacillus salitus* (23.50), *Pseudomonas aeruginosa* (21.02), and *Escherichia coli* (20.67). However, it showed relatively less activity against *Salmonella Typhi* (17.15) at elevated concentrations as shown in (Table VI). The results in (Table V) indicate variable potency of *Thymus vulgaris* extract against different pathogens, with its strongest impact on certain bacterial strains.

Table V. The results of LC50 in µg/ml for *Thymus vulgaris* L. and Etoposide

Compound	Conc. (µg/ml)	LC50 (µg/ml)	Toxicity class (Meyer)
Thymus vulgaris L.	10-1000µg/ml	268µg/ml	Toxic
Etoposide	1-10.23 µg/mL	5.62 µg/ml	Toxic/highly toxic

All values in table are mean±SEM n=3 where*show Significant results (P<0.05) and**show highly significant Results (P<0.01)

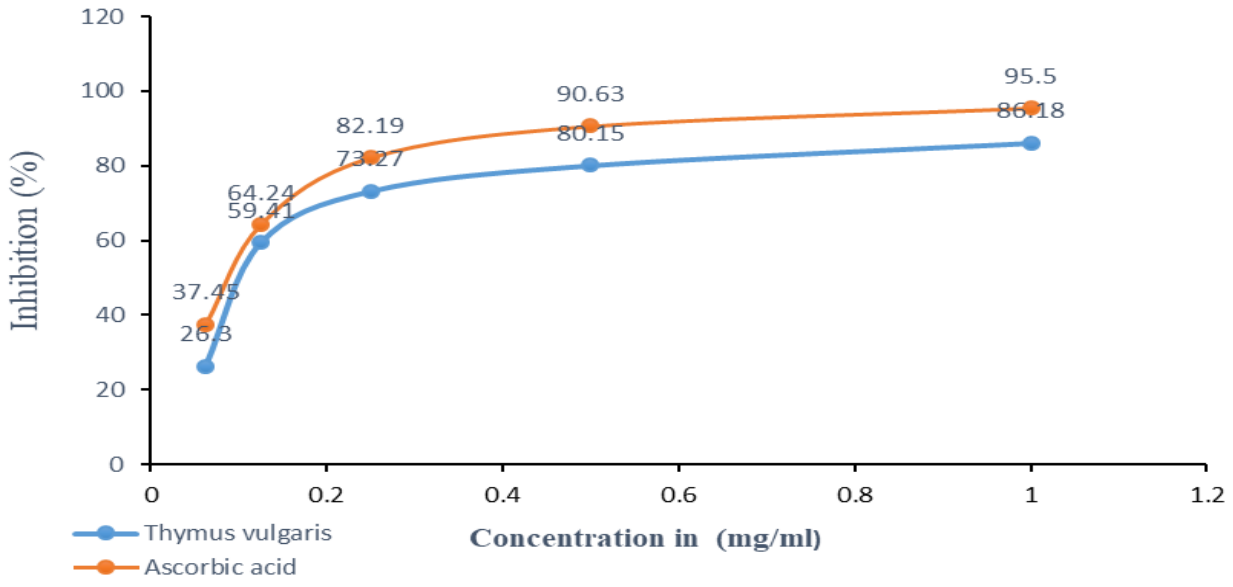

Fig. 1. % inhibition for *Thymus vulgaris* and ascorbic acid

Table VI. For Antibacterial activity of *Thymus vulgaris* L. and drug Ofloxacin against target pathogens in millimeters (mm)

Diameter of zone of inhibition against target pathogens in millimeters(mm)						
Samples	**Conc.(µg/ml)**	***S. aureus***	***P. aeruginosa***	***E.coli***	***S.Typhi***	***B. subtilis***
Thymus vulgaris L	25	18.35	13.88	15.35	14.32	18.45
	50	21.45	17.67	17.45	15.9	21.13
	75	26.67	21.02	20.67	17.15	23.50
Ofloxacin	25	32.45	12.41	28.45	22.15	22.5
	50	34.67	21.45	30.67	25.38	25.59
	75	39.26	29.50	35.20	30.7	29.93

All values in table are mean±SEM n=3 where*show Significant results (P<0.05) and**show highly significant Results (P<0.01)

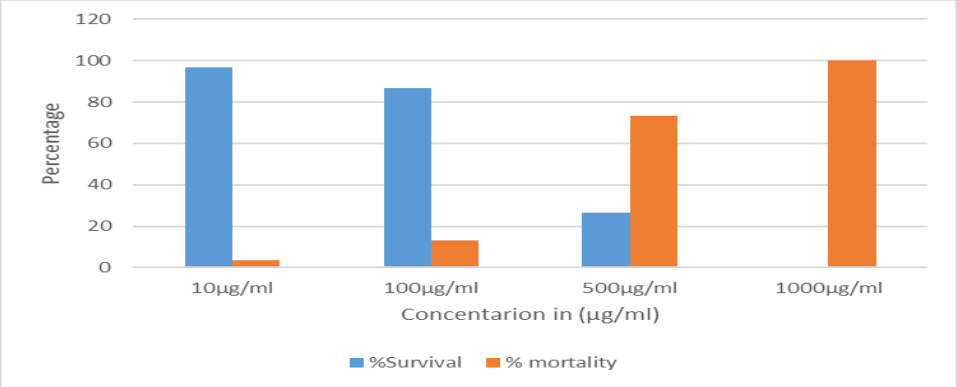

Fig. 2. For Brine shrimp (*Artemia salina*) %Survival & Mortality against *Thymus vulgaris* L. methanolic extract

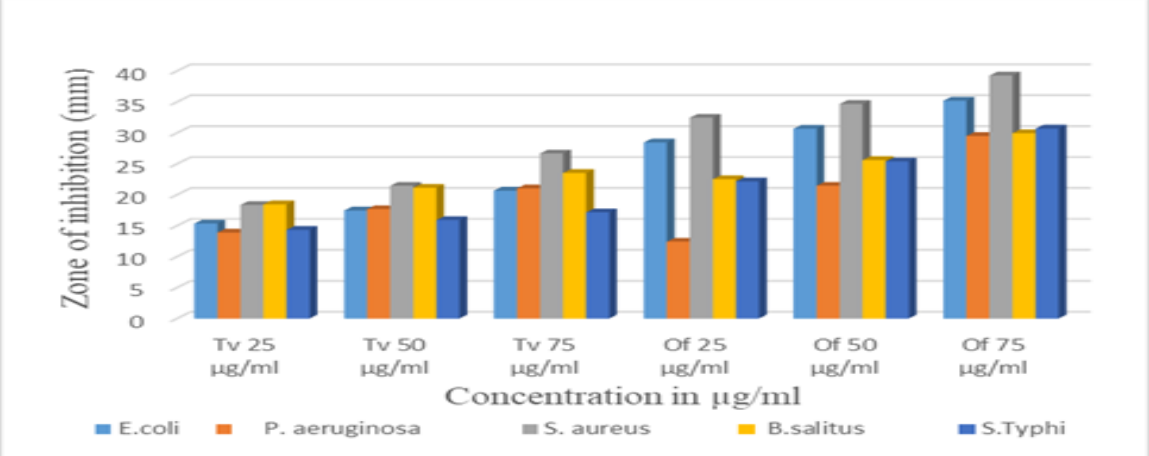

Fig. 3. Antibacterial activity of *Thymus vulgaris* L. and drug Ofloxacin against target pathogens in millimeters (mm)

DISCUSSION

Plants are like nature's medicine cabinets. People have known this for a long time. We've figured out that plants can make us feel better. Back in the day, we used leaves and roots from plants as medicine, and now we keep exploring. Plants have special things inside them that are good for our health. Scientists are like detectives trying to find and understand these special things, called bioactive compounds, in each plant. It's like a treasure hunt to learn how these compounds can keep us healthy. (22).

In current study photochemical test are performed The presence of flavonoids was detected in the methanolic *Thymus vulgaris* L. extract, highlighting the potential antioxidant and other biological activities associated with this group of compounds According to another study flavonoids, found abundantly in vegetables and fruits, are the biggest bunch of phytochemicals. They're like the main constituents of polyphenols. These compounds are great at stopping harmful things in our bodies because they act as antioxidants. They do this by grabbing and neutralizing free radicals and ROS (reactive oxygen species). They protect our cells by neutralizing harmful substances like free radicals and ROS. They also shield against metal damage and keep our heart healthy by preventing LDL oxidation (23).

The outcomes of our study disclosed a diverse array of phytochemicals in the *Thymus vulgaris* extract, encompassing flavonoids, Alkaloids, Phenolic compounds, Essential oils, Tannins, and Saponins. The demonstrated capacity of the methanolic extract to scavenge DPPH radicals signifies its notable antioxidant potential in our research, this results is coherent with another study in which they observed a noteworthy antioxidant capacity in the *Thymus vulgaris* (*T. vulgaris*) essential oil. This heightened antioxidant property is likely attributed to the abundance of carvacrol, a prominent phenolic compound within the oil. Consequently, the essential oil rich in carvacrol, as identified in this study, emerges as a promising alternative source of natural antioxidants. This finding underscores the potential value of *T. vulgaris* essential oil as a beneficial and natural antioxidant resource (24).

The changes in antioxidant activities may be explained by the different levels of phenolic compounds and flavonoids due to variances in extracts. Numerous studies have shown that the antioxidative activity of a particular extract directly depends on the contents of phenols and flavonoids. This implies that what distinguishes antioxidants from the others is composed of more of the components that are essential to them in contrast to the extracts. *T. vulgaris* essential oil has antioxidant activity in level of strength. The presence of a large amount of carvacrol is probably the reason for this, being a major chemical compound with well-known antioxidant effects. Thus, the oil of *T. vulgaris* containing carvacrol among its principal components brings up the issue of using nature for creating antioxidants of synthetic origin (25).

The *Thymus vulgaris* L. have strong anti-oxidant activity which may lead to our research results deviating from others' findings. In the present experiment, *Thymus vulgaris* was observed to produce an inhibition percentage of 86.18% ± 1.15, at a concentration of 1 mg/ml, while ascorbic acid revealed a higher inhibition of 95.50% ± 1.70. The variation inhibition percentages (antioxidant activity) with other studies is likely to be due to the quality of the extracted compounds, influenced by factors like the plant's collection location, time of harvesting and the extraction conditions (including temperature, extraction duration, and solvent polarity) (26).

In current study we use *Thymus vulgaris* L. plant extract to assess brine shrimp lethality at 10,100,500 and 1000µg/ml. The result of this bioassay shown in table IV indicate a concentration-dependent response of *Thymus vulgaris* methanolic crude extract on brine shrimp (*Artemia salina*), with significant lethality observed at higher concentrations. According to another study the brine shrimp lethality test (BSLT) has been demonstrated to show a reliable correlation with the cytotoxic effects against certain human solid tumors. This study is simple, easy and valid procedure to assess the cytotoxic activity of plants. Researchers have applied the BSLT to assess the cytotoxic properties of various essential oils. While the BSLT effectively identifies adequate anticancer activity in tested compounds, its drawback lies in its limited sensitivity to differentiate between strong to moderate and weak anticancer potentials. Consequently, the BSLT serves as a preliminary screening tool for potential cytotoxins, but for a more refined assessment of anticancer activity, further testing on human cancer cells is necessary (27).

Certain essential oils (EOs) from *Thymus vulgaris* L. have garnered attention as promising agents in the fight against cancer, undergoing scrutiny for their cytotoxic and antiproliferative effects in cancer cell lines and experimental animals. Various mechanisms contribute to the cytotoxic impact of EOs or their constituents, encompassing apoptosis and/or necrosis-induced cell death, antimutagenic and antiproliferative properties, antioxidant attributes, cell cycle arrest, and disruption of vital organelle functions. Notably, research findings highlight the anticancer potential of essential oils against a spectrum of cancers such as those affecting the mouth, breast, lung, prostate, liver, colon, brain, and leukemia. Furthermore, studies suggest that specific components within essential oils enhance the cytotoxic activity of chemotherapy drugs like docetaxel, paclitaxel, and 5-fluorouracil across various cell lines, potentially enabling a reduction in drug dosage while maintaining efficacy (28).

We evaluated the antibacterial ability of *T. vulgaris* plant extracts against both gram-positive and gram-negative bacteria. The results, as shown in Table VI, revealed substantial antibacterial effects across the tested bacteria. The highest efficacy was observed against S. aureus (26.67 mm), while the least activity was noted against *S. typhi* (17.15 mm). Notably, the plant extracts also exhibited antibacterial activity against

Staphylococcus aureus. Research on the chemical compounds found in *T. vulgaris* (thyme) extracts has revealed the presence of potent antimicrobial elements (29).

These results antibacterial activity provide information about the effectiveness of *Thymus vulgaris* L. at different concentrations against the specified bacteria. The values represent the diameter of clear zones (inhibition zones) around the discs, indicating the extent to which bacterial growth is inhibited at 75 µg/ml, the inhibition zones was observed for *S. aureus* (26.67 mm), *P. aeruginosa* (21.02 mm), *E. coli* (20.67 mm), S. *typhi* (17.15 mm), and *B. salitus* (23.50 mm). According to another study the zones of inhibition less than 10 mm are categorized as weak, those ranging from 10 to 16 mm are considered moderate, and those exceeding 16 mm are classified as active (30).

CONCLUSION

This study extensively analyzed the phytochemical composition of *Thymus vulgaris* methanolic extract. It showed significant antioxidant potential by scavenging DPPH radicals and initial indications of cytotoxicity, mainly at higher concentrations. At lower doses, it posed no harm, suggesting potential safety. These findings provide valuable understandings into *Thymus vulgaris* and its therapeutic potential. Further research could focus on isolating bioactive compounds, understanding their mechanisms, and exploring pharmaceutical or nutraceutical applications.

References:

1. Singh JS. The biodiversity crisis: a multifaceted review. Current Science. 2002;638–47.
2. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 2011;8(1).
3. Westh H, Zinn CS, Rosdahl VT, Sarisa Study Group. An International Multicenter Study of Antimicrobial Consumption and Resistance in *Staphylococcus aureus* Isolates from 15 Hospitals in 14 Countries. Microbial Drug Resistance. 2004;10(2):169–76.
4. Jack DB. One hundred years of aspirin. The Lancet. 1997;350(9075):437–9.
5. Zhang BB, Moller DE. New approaches in the treatment of type 2 diabetes. Current opinion in chemical biology. 2000;4(4):461–7.
6. Ahmed M, Khan MA, Zafar M, Sultana S. Treatment of common ailments by plant-based remedies among the people of district Attock (Punjab) of Northern Pakistan. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(1):112–20.
7. Tareen RB, Bibi T, Khan MA, Ahmad M, Zafar M, Hina S. Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan, Pakistan. Pak J Bot. 2010;42(3):1465–85.
8. Turner NJ. The Ethnobotany of Southern Balochistan, Pakistan, with Particular Reference to Medicinal Plants, by Steven M. Goodman and Abdul Ghafoor. Journal of Ethnobiology. 1995;15:70-86.
9. Dashti N. The cultural context of health: a Baloch perspective: an exploration of the cultural context and consequences of perceptions of illness and health-seeking behaviour of the Baloch. Balochi Academy; 2008.
10. Martino LD, Bruno M, Formisano C, Feo VD, Napolitano F, Rosselli S. Chemical composition and antimicrobial activity of the essential oils from two species of Thymus growing wild in southern Italy. Molecules. 2009;14(11):4614–24.
11. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MDM, Segura-Carretero A. Thymol, thyme, and other plant sources: Health and potential uses. Phytotherapy Research. 2018;32(9):1688–706.
12. Stahl-Biskup E. The Chemical Composition of *Thymus* Oils: A Review of the Literature 1960–1989. Journal of Essential Oil Research. 1991;3(2):61–82.
13. Javed H, Erum S, Tabassum S, Ameen F. An overview on medicinal importance of Thymus vulgaris. Journal of Asian Scientific Research. 2013;3(10):974.
14. Vasanthi P, Ganapathy M, Evanjelene VK, Ayyavuv N, Angamuthu J. Phytochemical screening and antioxidant activity of extracts of the leaf and bark of Albizzia lebbeck (Benth). Acad J Med Plant. 2014;2:026–31.
15. Kaur L, Joseph L, George M. Phytochemical analysis of leaf extract of Aesculus indica. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(5):232–4.

16. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). InVeterinary research forum: an international quarterly journal 2014;5(2):95.
17. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical journal of pharmaceutical research. 2008;7(3):1019–24.
18. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia. 1978;41(3):234–46.
19. Koleva II, Van Beek TA, Linssen JPH, Groot AD, Evstatieva LN. Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochemical Analysis. 2002;13(1):8–17.
20. Moshi MJ, Mbwambo ZH. Some pharmacological properties of extracts of Terminalia sericea roots. Journal of Ethnopharmacology. 2005;97(1):43–7.
21. Jo CR, Park BJ, Chung SH, Kim CB, Cha BS, Byun MW. Antibacterial and anti-fungal activity of citrus (Citrus unshiu) essential oil extracted from peel by-products. Food science and Biotechnology. 2004;13(3):384–6.
22. Vanitha C, Kathiravan M. Importance and scope of medicinal plants. Agric Herbal Visi. 2006;15:30–1.
23. Hassanpour SH, Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna Journal of Phytomedicine. 2023;13(4):354.
24. Núñez V, Monagas M, Gomez-Cordovés MC, Bartolomé B. Vitis vinifera L. cv. Graciano grapes characterized by its anthocyanin profile. Postharvest Biology and Technology. 2004;31(1):69–79.
25. Lamien-Meda A, Lamien CE, Compaoré MM, Meda RN, Kiendrebeogo M, Zeba B. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules. 2008;13(3):581–94.
26. Hossain MA, Alabri THA, Al-Musalami AHS, Akhtar MS, Said S. Evaluation of in vitro antioxidant potential of different polarities stem crude extracts by different extraction methods of Adenium obesum. Journal of Coastal Life Medicine. 2014;2(9):699–703.
27. McLaughlin JL, Rogers LL, Anderson JE. The Use of Biological Assays to Evaluate Botanicals. Drug Information J. 1998;32(2):513–24.
28. Blowman K, Magalhães M, Lemos MF, Cabral C, Pires IM. Anticancer properties of essential oils and other natural products. Evidence-Based Complementary and Alternative Medicine. 2018;2018(1):3149362.
29. Aminzare M, Hashemi M, Abbasi Z, Mohseni M, Amiri E. Vibriosis phytotherapy: A review on the most important world medicinal plants effective on Vibrio spp. Journal of Applied Pharmaceutical Science. 2018;8(01):170–7.
30. Irshad A, Khan N, Farina Y, Baloch N, Ali A, Mun LK. Synthesis, spectroscopic characterization, X-ray diffraction studies and in-vitro antibacterial activities of diorganotin (IV) derivatives with N-methyl-4-bromobenzohydroxamic acid. Inorganica Chimica Acta. 2018;469:280–7.