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Study of antimicrobial activity of *Thymus vulgaris* extract Against bacterial acne vulgaris.

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Abstract: *One of the most significant species in the Lamiaceae family, thymus vulgaris is endemic to the Mediterranean region and is used widely for both medicinal and culinary purposes. this study aims to evaluate the use of natural antimicrobials as an alternative therapy because the long-term use of antibiotics in the treatment of acne has resulted in the formation of resistance. It may be inferred that Thymus vulgaris (T. vulgaris) extracts have the ability to suppress the test bacteria in the plan, indicating that they may be used as antimicrobial agents against pathogenic bacteria.*

Keyword: *thymus vulgaris, acne vulgaris, extract, alcoholic, aqueous*

1. INTRODUCTION

One of the most prevalent inflammatory chronic skin conditions is acne vulgaris. Acne affects around 85% of people at some point in their lives [1]. Patients with acne experience severe morbidity and physical and psychological effects [2]. Having acne is defined by mostly on the, face, arms, nick, back, and upper body, developing inflammatory and non-inflammatory lesions [3]. The etiology of acne vulgaris is complex, involving multiple contributing elements such as follicular hyperkeratosis, immunological inflammatory responses, alterations in skin microbiota, and hormonally-induced excess sebum production (seborrhea) [4].

Seborrhea and enhanced keratinization of the follicular epithelium provide an environment that is conducive to Cutibacterium acnes (formerly known as Propionibacterium acnes) expansion over Staphylococcus epidermidis and Staphylococcus aureus. These factors interact with one another in this way. These microbes' metabolites cause inflammation in the follicles and the area surrounding them, particularly when chemotactic chemicals are released [5]. The majority of acne treatments involve using antibiotics like Clindamycin and erythromycin [6]. However, these antibiotics have the potential to trigger the growth of bacteria resistant to them as well as have negative side effects on the normal microbiota of the skin. In an effort to combat the growth of antibiotic resistance, the potential effectiveness of alternative natural antimicrobial agents in the treatment of acne has been studied. Plants are living chemical factories that produce a vast variety of secondary metabolites. In fact, these metabolites are the building blocks of many pharmaceutical products that are sold

commercially and herbal treatments that are made from medicinal plants [7]. One of the most significant species in the Lamiaceae family, thymus vulgaris is endemic to the Mediterranean region and is used widely for both medicinal and culinary purposes [8]. Many people have considered thyme to be antiseptic, antibacterial, astringent, anthelmintic, carminative, disinfectant, therapeutic medicine, and tonic. When treating a variety of intestinal diseases and infestations, including Candida albicans, gram-positive and gram-negative bacteria, as well as fungus and yeasts, thyme is an invaluable remedy. Thymol, one of its active ingredients, has the ability to combat both enterobacteria and cocci bacteria. Thyme may also stimulate appetite and enhance liver function. Urinary tract, bronchial, and cartilaginous tube infections will all be treated with it.
Thyme can be gargled to assist relieve inflammation and laryngitis. Enterobacteria and cocci bacteria are susceptible to the active ingredient in thyme volatile oil, thymol. Thymus vulgaris is applied to skin conditions, dermatitis, greasy skin, sciatica, acne, and insect bites [9].

Aim of study

The purpose of this study was to evaluate the efficacy of thymus vulgaris extract in treating acne vulgaris as well as its antibacterial qualities, which are widely utilized in the Mediterranean region.

2. MATERIAL AND METHOD

Preparation of the herbal powder: The herbal plants (*Thymus vulgaris)* were collected from the local market of Baghdad.

Preparation of the extraction: 500 grams of herbal powder were mixed on a magnetic stirrer for 12 hours with 1500 milliliters of either ethanol for an alcoholic extraction or water for an aqueous extraction. The mixture was then filtered using Whatman filter paper No 1. Supernante was gathered. The filtrates from the alcoholic extraction were then allowed to dry at 55 °C. The alcoholic extract's herbal material was combined with 1.5 milliliters of DMSO, and the mixture was filtered through a Millipore filter 0.22 microfilter before being stored as stoke solution at 4C until needed. Meanwhile, the aqueous extraction was lyophilized to create a powdered formulation [10].

Investigation of Secondary Metabolites

1. Tannin detection tests

To the extract, a few drops of 1% lead acetate solution were added. The formation of a white

or gelatinous precipitate suggested the presence of tannins.

2. finding polysaccharides

One milliliter of the extract and two milliliters of the Benedict reagent were combined, brought to a boil for five minutes, and then allowed to cool. Poly saccharides were present, as shown by the red deposit.

3. Alkaloids are found using the Dragangroff test.

Approximately dissolved Bismuth sub-nitrate in 0.2 milliliters of HCL (solution A). 600 mg of potassium iodide are present in 1 ml of distill water in solution B. Alkaloids can be detected in the extract by adding the combined solution [A+B], which turns orange to brown.

4. Saponin detection

Shaking the extract solution well will be the next step in the detecting process. The extract's top layer of foam will show that saponins are present.

5. identification of flavonoids

Alkaline reagent test: a brilliant yellow color indicates the presence of flavonoids when sodium hydroxide solution is combined with a little amount of extract solution and left.

6. Polyphenolic compound detection

A brown deposition occurred when a few drops of a 3% ferric chloride solution were added to the extract solution.

Isolation and identification of bacteria: four samples of bacteria were collected from patients has acne vulgaris. All samples were cultured on blood and MacConkey agar, then Gram stain, Urease test, Catalase test and other media like mannitol salt agar, nutrient agar was done for further isolation and identification of pathogenic bacteria.

Antibiotic resistance: A common microbiological approach for assessing antibiotic susceptibility is the Kirby-Bauer method, often known as the disk diffusion method. This procedure involves covering agar plates with disks that have been impregnated with antibiotics after they have been infected with a standardized bacterial solution. The zone of inhibition that forms around the disks is then measured and compared to interpretative criteria in order to determine the microorganism's sensitivity or resistance to the tested antimicrobial medicines.

The antibacterial activity of the ethanol and aqueous herbal extracts:

Antibacterial activity was investigated using the Mueller-Hinton agar disc diffusion method [11].

A sterile cork borer was used to create three wells on a Muller Hinton agar plate that had previously been covered with bacterial culture.

Three wells were used: one held alcoholic extract, one had aqueous extract, and the third held simply DMSO as a control. After 24 hours of 37°C incubation, the diameter of the inhibitory zones was measured on the plates.

Determination of minimal inhibitory concentration (MIC):

microplate method based-Resazurin

Establishing the Minimum Inhibitory Concentration (MIC) Using Sterile 96-well micro titer plates, the MIC was ascertained in compliance with the Clinical and Laboratory Standards Institute (CLSI) Guidelines [12].

1. Fill each microliter plate completely with 100 microliter of the broth medium.
2. Fill the first well with 100 microliters of extract at a concentration of mg/ml400.
3. Transfer 100 microliters from the first well of the container containing the prior additions to the second well. Additionally, transfer 100 microliters from the second well to the third well. Finally, take 100 microliters from the last well, wasting it.
4. Adding 10 microns of activated bacteria to every well in the previous row, with the exception of the final two (one containing only broth and extract, and the other containing simply broth as a control), 24 hours after comparing it with McCafferland.
5. Keep the incubator in use for 18-24 hours
6. Making Resazurin dye at a 0.015% concentration

The findings will be displayed by adding 0.015g in 100 D.W., adding 20 Microtiterplate Solution to every well without fail, and re-incubating the microtiter plate in the incubator for two hours. A shift in hue from blue to red or purple was thought to indicate the presence of bacteria [13].

3. RESULTS AND DISSECTION

Investigation of secondary metabolites

Research on the phytochemistry of thymus vulgaris have shown that their secondary metabolites—such as lignin, coumarins, phenolic acids, flavonoids, and terpenoids—are important therapeutic agents for gastrointestinal, circulatory, neurological, and other conditions [14,15,16]. Furthermore, polyphenols and flavonoids exhibit superior antibacterial, antioxidant, and anticancer properties [17,18]. In this study we found flavonoids + and polyphenols ++ in alcoholic and aqueous extracts shown below in table 1 and figure 1,2 These results are good for the use of thymus vulgaris extract as an antibacterial.

Table1: Investigation of secondary metabolites

Sample	flavonoides	saponins	polyphenols	alkaloids	tannins	glycosides
Alcoholic thymus vulgaris	+	+++	++	**-ve**	++	++
Sample	flavonoides	saponins	polyphenols	alkaloids	tannins	glycosides
Aqueous thymus vulgaris	+	**_ve**	++	+	+++	++

Figure1: alcoholic thymus extract

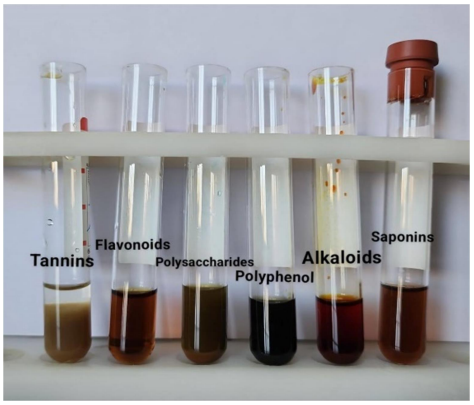

figure2: aqueous thymus extract

Antibiotics sensitivity result

Five types of antibiotic were used to detect the sensitivity of some microbes isolated from acne vulgaris such as *Staph.epidermids* and *Staph. Aureus* and the zone of inhibition ranged between 9 mm to18mm, this result mean most of isolates sensitive to antibiotics used except sample 2 resistant to amikacin and ceftriaxone. While sample 1, 3 and 4 resistants to doxycycline. In the current study, antibiotic susceptibility patterns showed that most of isolates were resistant to doxycycline in acne group. The data show that since this antibiotic was once used to treat acne, widespread antibiotic use can result in major issues with antimicrobial resistance. [19] The severity of the acne, cost-effectiveness, benefit-risk ratios, and the possibility of resistance development should all be considered when selecting an antibiotic. [20] Acne treatment choices are far from perfect [21], therefore a logical therapy to effectively treat this skin disease should result from our growing understanding of acne pathophysiology.

Table2: Antibiotics sensitivity result

Bacteria	LEV	AMK	RA	Do	CRO
1	12mm	15mm	12mm	-	14mm
2	14mm	-	12mm	9mm	-
3	17mm	13mm	10mm	-	14mm
4	18mm	13mm	11mm	-	13mm

Do: doxycycline, RA: rifampin, LEV: levofloxacin, AMK: amikacin, CRO: ceftriaxone,

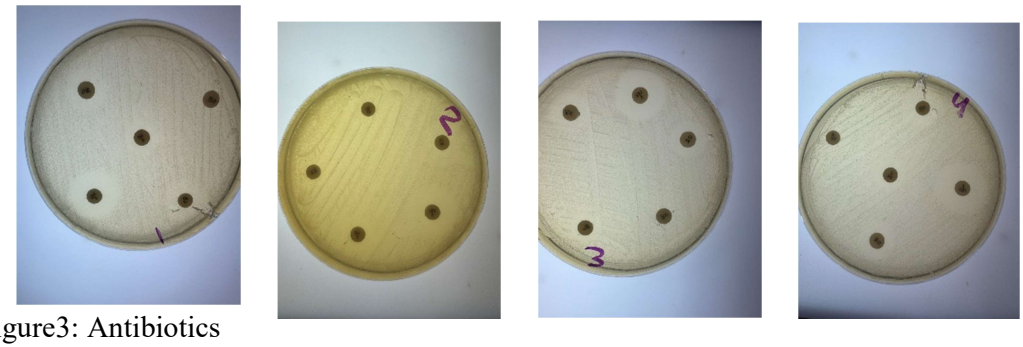

Figure3: Antibiotics sensitivity result

Antibacterial activity of *thymus vulgaris* extract

The antibacterial impact of alcoholic and aqueous *thymus vulgaris* extract was detected using the same bacteria that were used to determine antibiotic sensitivity the results showed a decrease in the isolate's growth rate as a result of the extract's action. , the observation of antibacterial activity of the extract on pathogenic bacteria using agar well diffusion method showed that the alcoholic extract has maximum zone of inhibition 18mm against sample 3,4 and has minimum zone of inhibition 14 mm against sample 2 while the aqueous extract has maximum zone of inhibition19mm against sample 4 and has minimum zone of inhibition 12mm against sample 1, Studies on the phytochemistry of species of thyme have revealed that their secondary metabolites such polyphenols and flavonoids exhibit superior antibacterial, antioxidant, and anticancer properties [17,18]. In this study we found flavonoids + and polyphenols ++ in alcoholic and aqueous extracts that explain why alcoholic and aqueous extract has similar antibacterial effect on bacteria isolate from *acne vulgaris.*

Table 3**:** Effect of *thymus vulgaris* extract on bacteria

No. sample	Control (DMSO)	Alcoholic extract	Aqueous extract
1	zero	15mm	13mm
2	zero	14mm	14mm
3	zero	18mm	17mm
4	zero	18mm	19mm

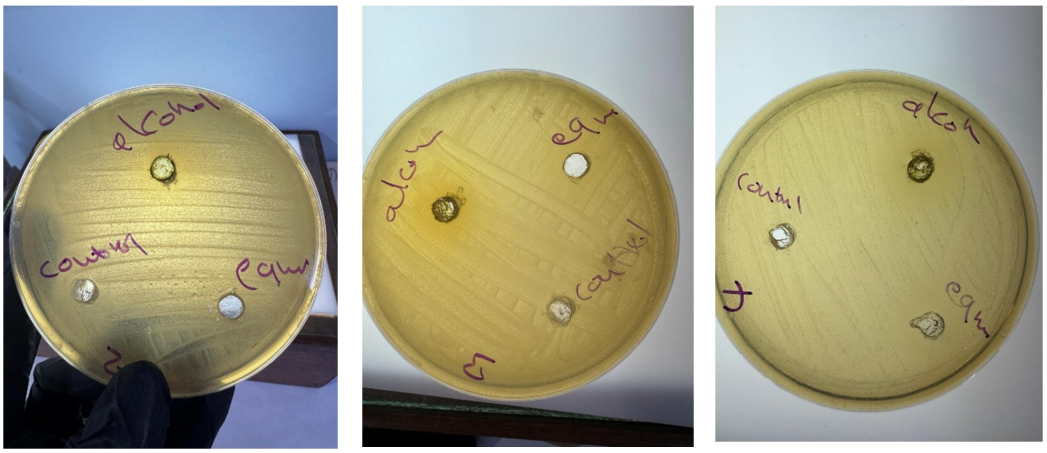

Figure4: Effect of *thymus vulgaris* extract on bacteria

Minimum Inhibitory Concentration (MIC) of thymus *vulgaris* Extract

The MIC of alcoholic and aqueous *thymus vulgaris* extract on tested bacteria it 50 mg/ml as shown in figure5. Sandasi (2008) showed that the T. vulgaris extract exhibited inhibitory activity against the planktonic forms of Candida albicans, Lactobacillus monocytogenes, and Candida aeruginosa. Alcoholic extract had a greater inhibitory impact than aqueous extract. This study shown that T. vulgaris extract, at the same dose in alcoholic and aqueous extract, could suppress the development of bacterium isolates from acne vulgaris. It is known that T. vulgaris has antibacterial activity and that extracts from it are efficient against pathogenic microorganisms based on the results of this inquiry as well as earlier studies that have examined different species of the plant. Therefore, additional study is necessary to identify the active components in these extracts, refine them, and understand how they affect the formation of biofilms in order to create a powerful antibacterial agent that kill harmful microbes.

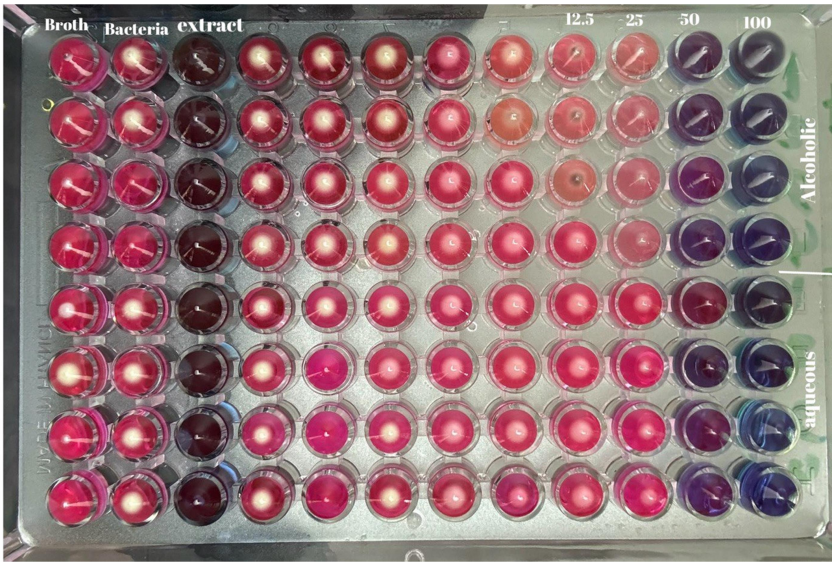

Figure 5: Minimum Inhibitory Concentration (MIC) of thymus *vulgaris* Extract

4. CONCLUSION

Extracts of Thymus vulgaris (T. vulgaris) have the ability to suppress the test bacteria in the plan, which suggests that they can be used as antimicrobial agents against pathogenic bacteria.

REFERENCES

15029.

2006, 8, 2195–2205.

CLCI (2011) Performance standards for antimicrobial suscep-tibility testing. Wyne. PA: CLC

Dermatol. Online J. **2011**, 17, 1.

Dunn, L.K.; O'Neill, J.L.; Feldman, S.R. Acne in adolescents: Quality of life, self-esteem, mood and psychological disorders.

Fournière, M.; Latire, T.; Souak, D.; Feuilloley, M.G.J.; Bedoux, G. Staphylococcus epidermidis and Cutibacterium acnes: Two major

induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. Microbes Infect.

Kim, Y.-S.; Lee, S.-J.; Hwang, J.-W.; Kim, E.-K.; Kim, S.-E.; Kim, E.-H.; Moon, S.-H.; Jeon, B.-T.; Park, P.-J. In vitro protective effects of Thymus quinquecostatus Celak

extracts on t-BHP-induced cell damage through antioxidant activity. Food Chem. Toxicol 2021, 91,153673. [CrossRef]

Lomholt HB, Kilian M. Is acne caused by colonization with the "wrong" strain of Propionibacterium acnes? A review of the role of Propion

management of acne vulgaris. J. Am. Acad. Dermatol. **2016**, 74, 945–973.

Massimo, N.; Oliviero, M.; Beatrice, M.M.; Giovanna, N.; Romilde, I.; Consuelo, A.; Giorgio, S.; Filippo, M. Thyme extract increases mucociliary-beating frequency in primary cell lines from chronic obstructive pulmonary disease patients. Biomed. Pharmacother. 2018, 105, 1248.

MORALES, R., 2002. The history, botany and taxonomy of the genus Thymus. In: E. STAHL-BISKUP and F. SÁEZ, eds. Thyme: the genus thymus. London: Taylor and Francis, pp. 1-43.

Nagy, I.; Pivarcsi, A.; Kis, K.; Koreck, A.; Bodai, L.; McDowell, A.; Kemény, L. Propionibacterium acnes and lipopolysaccharide

Nikoli, M.; Glamoclija, J.; Ferreira, I.; Calhelha, R.C.; Fernandes, A.; Markovi´c, T.; Markovi´c, D.; Giweli, A.; Sokovi´c, M. Chemical composition, antimicrobial, antioxidant and antitumor activity of Thymus serpyllum L., Thymus algeriensis Boiss. and Reut and Thymus vulgaris L. essential oils. Ind. Crops Prod. 2014, 52, 183–190. [CrossRef]

Parisa Seyedemadi*a*, Mehdi Rahnema**c*, Mohammad Reza Bigdeli*b* in The Neuroprotective Effect of Rosemary Hydro-alcoholic Extract on Cerebral Ischemic Tolerance in Experimental Stroke,2016.

Patel M, Bowe WP, Heughebaert C, Shalita AR. The development of antimicrobial resistance due to the antibiotic treatment of acne vulgaris: A review. J Drugs Dermatol 2010;9:655-64.

patients. J. Dermatol. **2018**, 45, 1166–1171.

Patil, S.M.; Ramu, R.; Shirahatti, P.S.; Shivamallu, C.; Amachawadi, R.G. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. Heliyon 2021, 7, e07054. [CrossRef] [PubMed]

Prasanth Reddy V1*, Ravi Vital Kandisa1, Varsha PV1 and Satyam S2, Review on Thymus vulgaris Traditional Uses and Pharmacological.

Salehi, B.; Mishra, A.P.; Shukla, I.; Sharifi-Rad, M.; Contreras, M.D.M.; Segura-Carretero, A.; Fathi, H.; Nasrabadi, N.N.; Kobarfard, F.; Sharifi-Rad, J. Thymol, thyme, and other plant sources: Health and potential uses. Phytother. Res. 2018, 32, 1688–1706. [CrossRef] [PubMed]

Sarker SD, Nahar L,Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application inthe in vitro antibacterial screening of phytochemicals. Methods (San Diego, Calif.). 2007; 42: 321-4

secondary metabolites in medicinal plants. Plant Physiology and Biochemistry, vol. 148, pp. 80-89. PMid:31951944.

sentinels of skin microbiota and the influence of cosmetics. Microorganisms **2020**, 8, 1752.

Tan HH. Antibacterial therapy for acne: A guide to selection and use of systemic agents. Am J Clin Dermatol 2003; 4:307-14.

Tuchayi, S.; Makrantonaki, E.; Ganceviciene, R.; Feldman, S.R.; Zouboulis, C.C. Acne vulgaris. Nat. Rev. Dis. Primers **2015**, 1,

Winn, Jr., W., et al. 2006. Konemann's color atlas and diagnostic text of microbiology, 6th ed., p. 945–1021. Lippencott Williams & Wilkins Publishers, Philadelphia, PA.

Yan, H.M.; Zhao, H.J.; Guo, D.Y.; Zhu, P.Q.; Zhang, C.L.; Jiang, W. Gut microbiota alterations in moderate to severe acne vulgaris

Zaenglein, A.L.; Pathy, A.L.; Schlosser, B.J.; Alikhan, A.; Baldwin, H.E.; Berson, D.S.; Bhushan, R. Guidelines of care for the