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Original Article
Özgün Araştırma

Antibacterial activity of thyme (*Thymus vulgaris L.*) on multidrug-resistant Escherichia coli strains

Oktay Genç[1], Bahar Güngör[2]

[1] OMU Kurupelit Campus Faculty of Veterinary Medicine, Department of Microbiology, Atakum, Samsun, Türkiye
[2] OMU Kurupelit Campus, Atakum, Samsun, Türkiye

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Abstract: Animal products contaminated with food-borne pathogens and/or resistant bacteria cause infections that are difficult to treat. However, with the invention of natural plant essential oils, thyme extracts have been replaced to ensure consumer safety, reduce losses and mitigate health risks in place of antibiotics, disinfectants and other chemicals. The objective of this study was to investigate the anti-bacterial effect of thyme extracts and determine the quantity of extraction yields. In the study, the anti-bacterial activity of thyme (*Thymus vulgaris* L.) extracts against multidrug-resistant *Escherichia coli (EC)* strains was investigated. The antibacterial activity of the extracts was investigated using the disc diffusion method by CLSI. The thyme extracts were prepared by methanol, ethanol, acetone, and water as solvents. The extraction yields of methanol and water solvents were higher (P=0.020) than ethanol and acetone solvents. However, anti-bacterial activities of ethanol, methanol, and acetone extracts were higher than water extract against EC1, EC3, EC4, and EC6 (P<0.001, P<0.001, P<0.001 and P=0.009, respectively). The results showed that methanol, ethanol, and acetone extracts of thyme have high anti-bacterial activity against multidrug-resistant *E. coli* strains. Methanol within the extracts can be preferred to other solvents for both yielding more product and higher anti-bacterial effect.

Keywords: Antimicrobial activity, *Escherichia coli,* poultry, thyme extracts, *Thymus vulgaris* L.

Kekik (*Thymus vulgaris L.*) ekstraktlarının çoklu dirençli Escherichia coli suşları üzerine antibakteriyel aktivitesi

Özet: Gıda kaynaklı patojen ve/veya dirençli bakterilerle kirlenmiş hayvansal ürünler, tedavisi zor enfeksiyonlara neden olur. Ancak doğal bitki esansiyel yağlarının icadıyla, tüketici güvenliğini sağlamak, kayıpları azaltmak ve sağlık risklerini hafifletmek için antibiyotikler, dezenfektanlar ve diğer kimyasalların yerine kekik özleri kullanılmaya başlanmıştır. Bu çalışmanın amacı, kekik özlerinin antibakteriyel etkisini araştırmak ve ekstraksiyon verimlerinin miktarını belirlemektir. Çalışmada, kekik (*Thymus vulgaris* L.) özütlerinin çoklu ilaca dirençli *Escherichia coli* (EC) suşlarına karşı antibakteriyel aktivitesi araştırılmıştır. Özütlerin antibakteriyel aktivitesi disk difüzyon yöntemi ile CLSI standartlarına göre araştırılmıştır. Kekik özleri çözücü olarak metanol, etanol, aseton ve su kullanılarak hazırlanmıştır. Metanol ve su çözücülerinin ekstraksiyon verimleri, etanol ve aseton çözücülerinden daha yüksek (P=0,020) bulunmuştur. Ancak, etanol, metanol ve aseton özütlerinin antibakteriyel aktiviteleri EC1, EC3, EC4 ve EC6'ya karşı su özütünden daha yüksekti (sırasıyla P<0.001, P<0.001, P<0.001 ve P=0.009). Sonuçlar, kekiğin metanol, etanol ve aseton özütlerinin çoklu ilaca dirençli *E. coli* suşlarına karşı yüksek antibakteriyel aktiviteye sahip olduğunu gösterdi. Özütlerdeki metanol, hem daha fazla ürün elde etmek hem de daha yüksek antibakteriyel etki sağlamak için diğer çözücülere tercih edilebilir.

Anahtar kelimeler: Antimikrobiyal aktivite, *Escherichia coli*, kanatlı, kekik ekstraktları, *Thymus vulgaris* L.

Introduction

Avian pathogenic *Escherichia coli* (APEC) is the major cause of colibacillosis in poultry production (Sola-Gines *et al.*, 2015) and causes food-borne infections in humans. This disease causes considerable economic losses in the poultry industry due to high mortality rates, carcass condemnations, treatment, and preventive costs (Halfaoui *et al.*, 2017; Azam *et al.*, 2019). Antibiotics are extensively used to control APEC infections in poultry. Concerns related to the use of antibiotics with overdose and misuse have led to the development of resistance in the environment and microflora and, in that way, hindered the treatment and prevention of bacterial infections. Besides, it was led to transmission of resistant infectious agents to humans and caused public health problems (Yassin *et al,* 2007).

Yazışma adresi / Correspondence: *Genç Oktay, Ondokuz Mayıs Mayıs Üniversitesi Veteriner Fakültesi Mikrobiyoloji AD, Samsun, Türkiye e-posta: ogenc@omu.edu.tr*

ORCID IDs of the authors: *[1]0000-0003-0777-6824 • [2]0000-0001-7338-5878*

Medicinal plants have been used for the treatment, prevention of infections and contamination of dairy by-products since ancient times (Loziene *et al*, 2007). Thyme contains numerous molecular species with diverse therapeutic properties, which vary depending on their concentration in the extract. Among different thyme plants *Thymus vulgaris*, *T. vulgaris,* one of the most studied cultivars (Sokovic *et al*. 2010; Usai *et al.,* 2011). Extracts and oil include thymol and carvacrol with a huge amount of phenolic compounds (Köksal *et al.*, 2017). The antimicrobial effect of thyme extract was mainly affected by the type of solvent used in the extraction (Chidubem and Rapuruchukwu, 2020). Both water and alcoholic extracts are prepared due to strong antibacterial effect against most of the Gram-positive and Gram-negative bacteria (Fan and Chen, 2001; Al-Tarawneh, 2004; Bounatirou *et al.,* 2007; Ebrahimi *et al*., 2008; Gedikoğlu *et al.* ,2019).

Water, ethanol, methanol, and acetone extraction are commonly used ones because of the strong anti-bacterial effect of their extracts due to their high capacity to dissolve phenolic compounds in plants (Sun and Ho, 2005). Because antibacterial activity may vary depending on the content, researchers' preference usually includes both extraction methods. In the study of Nawaz *et al.* (2020) water content was found to be better for the retention of the phenolic compounds than methanol, ethanol, and acetone. In addition, Abdul Qadir *et al.* (2017) showed that methanol and ethanol were superior solvents to acetone and water for thyme extraction. Due to the variability of extraction output, in this study, antibacterial activity of ethanol, methanol, acetone, and water extracts of thyme was investigated against multiresistant *E. coli* strains.

Materials and Methods

Preparation of plant extracts

Thyme leaf was purchased in dried form from a local market in Samsun, Türkiye. Plant extracts were prepared by using four different solvents (methanol, ethanol, acetone, and water) according to the methods described by Yeo *et al.* (2014) with the addition of centrifugation to provide a more efficient filtration (Weerakkody *et al.*, 2010). Briefly, 2 g of dry powdered thyme was mixed with 20 ml of methanol, ethanol, acetone, or distilled water. The mixture was placed in a shaker at 150 rpm for 12 hours at room temperature. After the extraction period, the samples were centrifuged at 1000 g for 10 min. The supernatant was filtered through a filter paper and evaporated in a rotary evaporator to completely dry (at 40 °C for ethanol, methanol, and acetone extract, and 60 °C for aqueous extract). The dry extract was dissolved in 1 ml of its solvent.

Extraction yield

The extraction yield was calculated by the following equation (Gonelimali *et al.*, 2018);

Yield (%): (X1*100/X0)

X1: Weight of extract after evaporation

X0: Dry weight of plant powder before extraction

E. coli isolation and identification

In 2024, 14 cecal samples from a local broiler premise of Samsun were collected for bacteriological isolation. *E. coli* was isolated from 12 samples following the method developed by Lee *et al.* (2009). Briefly, after diluting 1 gram of cecal sample to 9 ml of sterile physiological saline (PBS), all were streaked onto a MacConkey agar (100205, Merck) for keeping at 37°C for 24 h incubation. The lactose-fermenters colonies were smeared and stained, and all gram-negative bacilli colonies were further processed. The isolates were subcultured onto blood agar and kept at 37°C for 24 h to isolate pure cultures. Biochemical identification for 12 *E. coli* suspicious isolates was based on oxidase, urea hydrolysis with Urea Agar (108492, Merck), and citrate utilization tests with Simmons' Citrate Agar (103855, Merck) in addition to the behavior of the isolates in triple sugar iron (TSI) agar (103915, Merck) butt and slant (MacFaddin, 2000).

Confirmation of *E. coli* Isolates by Genotypic Identification

DNAs were extracted using hot-boiling method. Briefly, 200 µL of the bacterial suspension in sterile normal saline was boiled at 100 C for 5 minutes. After centrifugation, supernatant was used as template DNA with 10 µL of proteinase K. Polymerase chain reaction (PCR) was adopted on the extracted DNA samples as recommended by Kong *et al.* (1999) with primers specific for *E. coli* housekeeping *phoA* gene sequence. The amplified product of 903 bp was electrophoresed in 1.8% agarose gel at an electric current of 1.5 V/cm of the agarose length and visualized with a Gel doc/UV transilluminator (WiseUV, WUV-M20, Daihan Scientific).

Antibiogram test

In the study, mostly applied antibiotics for the treatment of enteric diseases in poultry and at the premises of Samsun were selected. The antibiotic susceptibility of 12 *E.coli* strains to 6 antibiotic was determined using the Kirby Bauer disc diffusion method described by Bakht *et al*. (2011) according to CLSI (2023). First of all, the precultured *E. coli* strains were standardized to 0.5 McFarland. Sterile cotton swabs were used to transfer the bacterial strains aseptically and then cultured over Muller Hinton Agar (MHA, 103872, Merck). Whatman No. 1 filter paper discs (6 mm in diameter) were placed on the media with the help of sterile forceps, and 15 µl of each thyme extract was applied to the discs. Then, the petri dishes were placed in the incubator at 37 °C for 20-22 hours, and subsequently, the diameters of inhibition zones were determined and evaluated based on CLSI protocol (Wayne PA, 2023).

Statistical analysis

The experiments were performed in triplicate, and the mean values of the extraction yields and the diameter of inhibition zones were calculated. The results were expressed as mean ± standard deviation. All data were analyzed by one-way ANOVA using SPSS software version 17, and means were compared using Duncan's test. The level of significance was present at P<0.05.

Results

The yields of ethanol, methanol, acetone, and water extracts of thyme are shown in Table 1. In the study, methanol and water extracts had higher (P=0.020) extraction yields than ethanol and acetone extracts.

Antibiogram test was performed against the 6 antibiotics mentioned below with all strains of *E. coli*. Six out of 12 *E. coli* strains found resistant were used for the detection of the antibacterial effect of thyme extracts. Antibiotic resistance results were as follows. EC1 was resistant to lincomycin (2 µg/disc, L2), bacitracin (10 µg/disc, B10), amoxicillin (30 µg/disc, AMC30), ciprofloxacin (5 µg/disc, CIP5), sulfamethoxazole (25 µg/disc, SXT25), colistin (10 µg/disc, CT10), EC2 was resistant to L2, B10, AMC30, CIP5, SXT25, EC3 was resistant to L2, B10, AMC30, EC4 was resistant to L2, B10, AMC30, CIP5, SXT25, EC5 was resistant to L2, B10, AMC30, CIP5, SXT25, and EC6 was resistant to L2, B10, CIP5 (Gungor, 2023).

The antibacterial activity of thyme extracts against multidrug-resistant *E. coli* strains is given in Table 2. In the study, ethanol, methanol, and acetone extracts showed higher antibacterial activity than water extract against EC1, EC3, EC4, and EC6 (P<0.001, P<0.001, P<0.001 and P=0.009, respectively), while water extracts had no inhibition zone against EC1-4. Methanol extract showed the highest (P<0.001) zone against EC2 compared to other extracts. Similarly, methanol and ethanol extracts had the highest (P<0.001) antimicrobial activity on EC5 compared to other extracts. Water extracts had lower inhibition zones to EC5 and EC6 strains than the other extracts (P<0.001 and P=0.009, respectively).

Table 1. *Extraction yields of ethanol, methanol, acetone, and water extracts of thyme*

Extraction Methods	Extraction Yield (%)
Ethanol	$7.62^{b} \pm 2.96$
Methanol	$11.52^{a} \pm 1.10$
Acetone	$7.00^{b} \pm 1.93$
Water	$12.30^{a} \pm 0.96$
P-value	0.020

All values represents mean ± standard deviation (n=3).

^{a,b}Means followed by different letter vary significantly (P<0.05)

Table 2. *Inhibition zones of thyme extracts against multidrug-resistant E. coli strains*

Extraction Methods	***E. coli* strains**					
	EC1	EC2	EC3	EC4	EC5	EC6
Ethanol	$11.33^{a} \pm 1.53$	$10.33^{b} \pm 0.58$	$11.00^{a} \pm 0.00$	$11.00^{a} \pm 1.00$	$11.50^{ab} \pm 0.50$	$10.67^{a} \pm 0.58$
Methanol	$12.67^{a} \pm 2.08$	$11.83^{a} \pm 1.04$	$11.17^{a} \pm 1.04$	$12.33^{a} \pm 2.08$	$13.17^{a} \pm 1.89$	$12.50^{a} \pm 2.29$
Acetone	$11.17^{a} \pm 0.76$	$9.67^{b} \pm 0.58$	$11.17^{a} \pm 1.61$	$11.67^{a} \pm 0.58$	$11.00^{b} \pm 0.00$	$11.83^{a} \pm 2.36$
Water	$6.00^{b} \pm 0.00$	$6.00^{c} \pm 0.00$	$6.00^{b} \pm 0.00$	$6.00^{b} \pm 0.00$	$6.33^{c} \pm 0.58$	$6.33^{b} \pm 0.58$
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.009

All values represents mean ± standard deviation (n=3).

^{a,b,c}Means followed by different letter vary significantly (P<0.05)

Discussion and Conclusion

Thyme preference depends on its therapeutic properties, such as anti-oxidative, anti-inflammatory, anti-fungal, anti-parasitic, anti-viral, anti-tumoral and immune modulatory characteristics (Roby *et al*., 2013; Thamer *et al*., 2021; Vassiliou *et al.,* 2023). Therefore, studies have focused on the optimization of yield productivity and stability. Since solvent polarity has been significantly affecting the extraction yield and antimicrobial activity of phenolic compounds in plant material (Roby *et al.,* 2013; Nawaz *et al*., 2020; Alam *et al*., 2022) both alcoholic and aqueous extraction methods were used in the study. In the present study, similar to Nawaz *et al.* (2020), the extraction yield of water was higher than those of ethanol and acetone (Table I). Similarly, Parzhanova *et al*. (2018) reported that aqueous extract had a higher yield than 70% of ethanol. In this study parallel results have been obtained with Martins *et al*. (2015) who have reported water yield was higher than methanol in thyme extracts. Different results between the studies can also be due to the differences in the temperatures of the aqueous extract prepared with distilled water (Alam *et al*., 2022). In this study, contrary to ethanol and acetone, a higher yield was obtained with methanol following water.

Water as a polar solvent is better for retaining the phenolic compounds of plant materials than with lower polarity, such as methanol, ethanol, and acetone (Nawaz *et al*., 2020). However, several authors have reported that ethanol and methanol are more efficient solvents than water for extracting antimicrobials, including various groups of flavonoids (Zhishen *et al,* 1999; Moure *et al*., 2000; Siddhuraju and Becker, 2003). Although the extraction yield of water was higher than ethanol and acetone, the antimicrobial activity of ethanol, methanol, and acetone extracts was higher than aqueous extract in these studies. Similarly, Parzhanova *et al.* (2018) reported that the aqueous extraction yield was higher than that of 50% ethanol, although total phenol and flavonoids were lower in aqueous extract than in 50% ethanol. The phenolic compounds in the aqueous extract may have a lower antimicrobial effect against studied *E. coli* strains compared to other extracts (Sola-Gines *et al*., 2015; Azam *et al*., 2019; Chidubem *et al*., 2020). In the present study, the aqueous extract was concentrated at 60 °C by evaporation, but other extracts were processed at 40 °C. In the study, higher temperatures may have caused a loss of some phenolic compounds in aqueous extracts, which can be another reason for the lower antimicrobial activity of aqueous extracts. Similar to this study, Abdul Qadir *et al.* (2017) reported that methanol and ethanol extract of thyme leaf showed antimicrobial activity against *E. coli,* while it was not observed in aqueous extract (prepared at 70 °C). In contrast, it has been reported that acetone extract of thyme (Abdul Qadir *et al*., 2017), acetone and ethanol extract of lemon thyme (*Thymus pulegioides* L., (Loziene *et al*., 2007) showed no antimicrobial activity against *E. coli*. This difference comes from the changes in thyme variety, extraction methods, or *E. coli* strains between the studies.

The results showed that methanol, ethanol, and acetone extracts of thyme have a high potential for bacterial inhibition against multidrug-resistant *E. coli* strains. Methanol extraction of thyme leaves yielded better antibacterial activity than the other solvents in the study.

The study shows that further detailed studies need to be conducted to verify antimicrobial spectrum of clinical strains with purified thyme constituent. Future perspectives should be concentrated on the antimicrobial mechanisms with pharmacokinetic and dynamic properties of the constituent to enrich the product range and create alternatives in terms of suitability for applications.

Conflict of Interest: The authors declared that there is no conflict of interest.

Ethics committee approval: This study is not related any experimental animal study and design, thus, is not required approval of Animal Experiments Local Ethiques Committee.

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References

Abdul Qadir M, Shahzadi SK, Bashir A, Munir A, Shahzad S, (2017). Evaluation of phenolic compounds and antioxidant and antimicrobial activities of some common herbs. *Int J Anal Chem*.1-6.

Alam T, Jilani G, Chaudhry AN, Ahmad MS, Aziz R, Ahmad R, (2022). Terpenes and phenolics in alcoholic extracts of pine needles exhibit biocontrol of weeds (*Melilotus albus* and *Asphodelus tenuifolius*) and insect-pest (*Plutella xylostella*). J *King Saud Univ Sci*. 34,101913.

Al-Tarawneh AA, (2004). Study on *Pseudomonas aeruginosa* isolated from infected patients: copper uptak, hematological findings and effect of some medicinal plants. M.Sc. Thesis, Sudan University for Science and Technology, Sudan.

Azam M, Mohsin M, Saleemi MK, (2019). Virulence-associated genes and antimicrobial resistance among avian pathogenic

Escherichia coli from colibacillosis affected broilers in Pakistan. *Trop Anim Health Pro.* 51,1259-1265.

Bakht J, Ali H, Khan MA, Khan A, Saeed M, Shafi M, Islam A, Tayyab M, (2011). Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion method. *Afr J Biotechnol.* 10,19825-19835.

Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, Costa MM, Figueiredo AC, Barroso JG, Pedro LG, (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus*. *Food Chem.*, 105, 146-155.

Burt S, (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *Int J Food Microbiol.* 94, 223-253.

Chidubem ON, Rapuruchukwu LO, (2020). The antimicrobial effects of *Thymus vulgaris* on *Staphylococcus aureus* and *Escherichia coli*. *International Network Organization for Scientific Research* 6, 32-43.

Dahiya P, Purkayastha S, (2012).. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian J Pharm Sci.* 74, 443.

Ebrahimi SN, Hadian J, Mirjalili MH, Sonboli A, Yousefzadi M, (2008). Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. *Food Chem*., 110, 927931.

Fan M, Chen J, (2001). Studies on antimicrobial activity of extracts from thyme. *Wei Sheng Wu Xue Bao.*, 41, 499-504.

Gedikoğlu A, Sökmen M, Çivit A, (2019). Evaluation of *Thymus vulgaris* and *Thymbra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition.* **7**, 1704-1714.

Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR, (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol.* 9,1639.

Gungor B, (2023). Antibiyotiğe dirençli *Escherichia coli* suşlarına spesifik litik bakteriyofaj izolasyonu ve litik spektrumlarının belirlenmesi. MSc Thesis, Institute of Graduate Studies, Department of Veterinary Microbiology. 43.

Halfaoui Z, Menoueri NM, Bendali LM, (2017). Serogrouping and antibiotic resistance of *Escherichia coli* isolated from broiler chicken with colibacillosis in center of Algeria. *Vet World* 10, 830.

Kong RYC, So CL, Law WF and Wu, RSS, (1999). A sensitive and versatile multiplex PCR system for the rapid detection of enterotoxigenic (ETEC) enterohemorrhagic (EHEC) and enteropathogenic (EPEC) strains of Escherichia coli. *Marine Pollution Bulletin* 38 (12): 1207 - 1215.

Köksal E, Bursal E, Gülçin İ, Korkmaz M, Çağlayan C, Gören AC, Alwasel SH, (2017). Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry. *Int J Food Prop.* 20 (3), 514–525.

Lee GY, Jang HI, Hwang IG, Rhee MS, (2009). Prevalence and classification of pathogenic Escherichia coli isolated from fresh beef, poultry, and pork in Korea. *Int. J. Food Microbiol.,* 134, pp. 196-200.

Loziene K, Venskutonis PR, Sipailiene A, Labokas J, (2007). Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. *Food Chem.* 103, 546-559.

MacFaddin JF, (2000). Biochemical Tests for Identification of Medical Bacteria (3rd Edition), Lippincott Williams & Wilkins, Philadelphia, PA, p. 912.

Martins N, Barros L, Santos-Buelga C, Silva S, Henriques M, Ferreira IC, (2015). Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. *Food Chem.* 167, 131-137.

Moure A, Franco D, Sineiro J, Domínguez H, Nunez MJ, Lema JM, (2000). Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *J Agri and Food Chem.* 48, 3890-3897.

Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N, (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Braz J Pharm Sci.* 56.

Omonijo FA, Ni L, Gong J, Wang Q, Lahaye L, Yang C, (2018). Essential oils as alternatives to antibiotics in swine production. *Anim Nutr.* 4, 126-136.

Parzhanova AB, Petkova NT, Ivanov IG, Ivanova SD, (2018). Evaluation of biologically active substance and antioxidant potential of medicinal plants extracts for food and cosmetic purposes. *J Pharm Sci Res.* 10, 1804-1809.

Roby MHH, Sarhan MA, Selim KA-H, Khalel KI, (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind Crops Prod.* 43, 827-831.

Siddhuraju P, Becker K, (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agri Food Chem.* 51, 2144-2155.

Sienkiewicz M, Lysakowska M, Denys P, Kowalczyk E, (2012). The antimicrobial activity of thyme essential oil against multidrug resistant clinical bacterial strains. *Microbial Drug Resistance.* 18, 137-148.

Sokovic M, Glamoclija J, Marin PD, Brkic D, Van Griensven LJ, (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* 15, 7532-7546.

Sola-Gines M, Cameron-Veas K, Badiola I, Dolz R, Majo N, Dahbi G, Viso S, Mora A., Blanco J, Piedra-Carrasco N, (2015). Diversity of multi-drug resistant avian pathogenic *Escherichia coli* (APEC) causing outbreaks of colibacillosis in broilers during 2012 in Spain. *PLoS One.* 10, e0143191.

Sun T, Ho C-T, (2005). Antioxidant activities of buckwheat extracts. *Food Chem.*90, 743-749.

Thamer NA, Hammadi AH, Yaseen MM, (2021). Evaluation of antioxidant activity and cytotoxic potential of *Thymus vulgaris* leaf extracts. *Tropical Journal of Natural Product Research (TJNPR).* 5, 1389-1396.

Usai M, Marchetti M, Foddai M, Del Caro A, Desogus R, Sanna I, Piga A, (2011). Influence of different stabilizing operations and storage time on the composition of essential oil of thyme (*Thymus officinalis* L.) and rosemary (Rosmarinus officinalis L.). LWT - *Food Sci Tec*, 44 (1), 244–249.

Vassiliou E, Awoleye O, Davis A, Mishra S, (2023). Anti-inflammatory and antimicrobial properties of thyme oil and its main constituents. *Int J Mol Sci.* 24(8), 6936.

Wayne PA, (2023). *The Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing.* 30th ed. CLSI supplement M100 (ISBN 978-1-68440-067-6).

Weerakkody NS, Caffin N, Turner MS, Dykes GA (2010). *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control*, 21(10), 1408-1414.

Yassin AK, Gong J, Kelly P, Lu G, Guardabassi L, Wei L, Han X, Qiu H, Price S, Cheng D, (2017). Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. *PloS One.* 12, e0185326.

Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS, (2014). Effectiveness of maceration periods with different extraction solvents on *in-vitro* antimicrobial activity from Fruit of *Momordica charantia* L. *J Appl Pharm Sci.* 4, 016-023.

Zhishen J, Mengcheng T, Jianming W, (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555-559.