PRODUCTION OF GREEN DETTOL AS BACTERIAL INHIBITOR AND DISINFECTANT

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ABSTRACT

The aim of this research is production of disinfection (Dettol) contains herbal formula and study the efficacy of it for inhibition of pathogenic bacteria as and comparing with traditional Dettol efficacy. In this research, a herbal disinfectant and antiseptic was prepared similarly to the industrial Dettol and in terms of composition, except for the active substance (chloroxylenol), which was replaced by a plant extract (thyme) and active compound (thymol) separately. total of 50 samples hand swabs were collected randomly from children and housewives and student at university of Baghdad. The following species were isolated from swabs and identified by microscopic morphology, morphological features on culture media, and Vital Index of Traditional Environmental Knowledge(VITEK2)System and that were *Staphylococcus aureus, Escherichia.coli, Bacillus cereus ,pseudomonas aeruginosa, Enterobacter cloacae*. The results of study showed that the highest antimicrobial activity was belonged to thymol. while a lowest antibacterial effect was belonged to thyme. Highest value of minimum inhibitor concentration (mic) was (0.09) of thymol against *staphylococcus aureus* while a lowest mic value was (6.25) of thyme against *Bacillus cereus* the result of minimum bactericidal concertation (mbc) showed that thymol and Dettol were the same value (1.56) against *Enterobacter cloacae.*

Key words: dettol, thymol, thyme, herbal disinfectant

انتاج الديتول الخضر كمثبط للبكتريا ومطهر

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المستخلص

الهدف من هذا البحث هو إنتاج مطهر (ديتول) يحتوي على تركيبة عشبية ودراسة مدى فعاليتها في تثبيط البكتيريا المسببة للأمراض ومقارنتها بفعالية الديتول التقليدي. في هذا البحث تم تحضير مطهر عشبي ومعقم مماثل لمادة الديتول الصناعية من حيث التركيب باستثناء المادة الفعالة (كلوروزايلينول) والتي تم استبدالها بمستخلص نباتي (الزعتر) والمركب الفعال (الثيمول) وبشكل منفصل. تم جمع 50 عينة كمسحات يدويا وبشكل عشوائي من الأطفال وربات البيوت والطلاب في جامعة بغداد. تم عزل الأنواع التالية من المسحات وتم تحديدها بوساطة المجهر الضوئي المظهري ، والسمات المظهرية على وسط الاستزراع ، والفهرس الحيوي لنظام المعارف البيئية التقليدية (VITEK 2) والتي كانت *Staphylococcus aureus*، *Escherichia. Coli* ، و *Bacillus cereus* ، و *pseudomonas aeruginosa* ، و *Enterobacter cloacae*. أظهرت نتائج الدراسة أن الثيمول كان أعلى نشاط مضاد للميكروبات. بينما كان أقل تأثير مضاد للجراثيم يعود إلى الزعتر. كانت أعلى قيمة للتركيز المثبط الأدنى هي (0.09) للثيمول ضد المكورات العنقودية الذهبية بينما كانت أقل قيمة للتركيز المثبط الأدنى هو (6.25) للزعتر ضد *Bacillus cereus* و. أظهرت نتيجة التركيز القاتل الأدنى (mbc) أن الثيمول والديتول كانت لهما نفس القيمة (1.56) ضد *Enterobacter cloacae*.

الكلمات المفتاحية: ديتول,ثايمول,زعتر,مطهرعشبي

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INTRODUCTION

The Infectious diseases are a major cause of morbidity and mortality throughout the world. Nosocomial infections are illnesses that started or developed in a hospital or health-care facility. Nosocomial infections are on the rise and have become a major concern in hospital care, resulting in prolonged hospitalization, significant morbidity and mortality, and high expenditures, and excessive costs (9). The outbreak of the COVID-19 virus has affected countless people all over the world. Inadequate cleanliness and hygienic practices can lead to an increase in the infection rate during this outbreak (16) .Infection control procedures include the widespread use disinfectants in hospitals and medical centers to control the growth and elimination of microorganisms. Cleaning and sterilizing clinical equipment is also done with detergents and disinfectants. The antimicrobial activities of disinfectants are influenced by the disinfectant's composition, level of organic charge, temperature, dilution rate, and antimicrobial activity testing methodologies. The susceptibility of different types of microorganisms to antiseptics and disinfectants vary from one bacteria to another (10). Many pathogenic organisms had become resistant nowadays for many manufactured antibiotics(15). Traditionally, resistance refers to instances where the basis of increased tolerance is a genetic change, and where the biochemical basis is known (8). With the rising occurrence of medication resistance among common diseases, as well as the accompanying risk of chemotherapeutic medicines, finding an alternative to existing drugs has become critical. In ancient times, herbs bear been chronic within dense areas, together with nutrition, medicine, flavoring, beverages, cosmetics, etc...(14). Herbs with established pharmacological qualities may be the finest source of these alternative medications(18). In Ayurveda and homoeopathy, medicinal plants with a broad spectrum of biological activity are used as a home cure for a variety of human illnesses. (7) Thyme (Thymus vulgaris L.) is an aromatic plants of the Mediterranean flora commonly used as spices and for medicinal purposes (4). Thyme is possessing antimicrobial, antifungal, antioxidative and antiviral properties (25). Thymol is one of the most important components in thyme essential oil (17). Is an aromatic essence which is known as disinfectant in traditional medicine (24) . Thymol is a strong antimicrobial agent and is used as an antiseptic ingredient in some mouthwash's toothpastes on environment. There is a need for disinfection methods, which are rapidly effective, cost-effective, nontoxic, and that can be easily implemented (3). Most chemical disinfectants contain alcohol and usually brings adverse effects, which include allergic reactions and pathogens resistance (6) Global world is focusing now on use of herbal products because their considered are eco-friendly and more suitable to household applications than chemicals .(20) The aim of this study is to compare the efficacy of herbal disinfection using thymol and thyme in a green Dettol with commercially disinfectant Dettol.

MATERIAIS AND METHODS

Collections of samples : Total 50 skin swabs samples from the hands were collected randomly from children, housewives and student at university of Baghdad. the sample collected in Sterile moistened cotton swab sticks containing 5 ml of normal saline during the period from 1/9/2020 to 1/12/2020. To avoid drying, the cotton swabs were immediately moved to the laboratory (10).

Isolation and identification

Mac Conkey, mannitol, and blood agar were used to culture all of the samples. Subculture was done from each plate to separate and isolation colonies .The plates were incubated for 24h at 37 ℃ (13). These media used to distinguish and select the pure colonies according to (12).Several biochemical identification methods such as shape, Gram stain, Indole Test, Oxidase test, Catalase test, Citrate Utilization Test, Urease Test, Coagulase Test were conducted to identify the isolated bacteria (1, 11). Also, the diagnosis of the clinical isolates was confirmed by VITEK 2 compact. These identified cultures were transferred to nutrient agar slants for preservation and then stored in the refrigerator at 4°C (28)

Preparation of herbal disinfection: The preparations of thymol and thyme

disinfectant were conducted according to the Dettol procedure

1- ethanol 80 %(30 ml) was mixed with 30ml castor oil, and stir well until mixed

2-Dissolved the 3.95gm of NaOH in a of 9 ml water, after dissolving it add on the mixture of (ethanol and oil).

3- Stirred the ingredients well until the mixture becomes homogeneous, leaved it for two hours, where the oil can be dissolved in water without turbidity.

3-In a clean beaker mixed 5ml of the pine oil with 5 gm of the thymol and stirred well.

4- The second mixture added to the first (alcohol and castor oil) stirred well. The volume of mixture reached up to 100 ml by adding distilled water

5- Added the 0.21g of caramel and move it well, ph it must be at 8

Preparation of microorganism suspension

The microorganism species maintained in BHI (brain heart broth) – 20ºC. Cultures were kept for 24 h at 36ºC. After 24 h of incubation, adjust suspension to achieve turbidity equivalent to 0.5 McFarland turbidity.

Macro dilution method

Microdilution and macrodilution methods have been used in the determination of MIC and MBC [19]. Usually, the determination of MIC and MBC by the serial Dilution. Macrodliution technique used in this study by serial two-fold dilutions of disinfectants were prepared in sterile MHB[29]. Label tubes from 1 to12 sterile test tubes numbered follows: #1 (25)mg/ml #2 (12.5)mg/ml #3 (6.25)mg/ml #4 (3.12)mg/ml; #5 (1.56)mg/ml #6 (0.78) mg/ml #7(0.39) mg/ml #8(0.19)mg/ml #9(0.09)mg/ml #10(0.04) mg/ml#11(negative control) #12(positive control) . by use micropipette, pipet 1 mL of muller huntion broth into all tubes . Than pipet 1 mL disinfection into tube #1. Mix gently by drawing the solution up and down 3 times (3X). Transfer 1 mL of solution from tube #1 into tube #2 and then Mix gently 3X. Continue to transfer and mix through 10 tube. Discard the last 1 mL from tube #10. After preparing serial dilutions, 100 µl (1.5 × 106 CFU/mL) of 0.5 McFarland bacteria was inoculated to each dilution and to positive control tube, add 1ml of disinfection in negative control, incubated at 37 °C for 24h. After, incubation, 150 µl of the resazurin indicator was added to each tube and incubated overnight [21] .This procedure was done for all disinfection (Dettol, thyme and thymol). All experiments were done in triplicates for each tested disinfectant and each bacterial strain. To determine the minimum bactericidal concentration of disinfectants, 5 µl of each tube was sub-cultured on the surface of MHA at 37 °C for 24 h and then colony count was done. No bacterial growth should be detected on the overnight NA plate [2].

RESUITS AND DISCUSSION

Total of 50 sample were collected from hand tested by microscopic examination, Colonial morphology, VITEK2 test and biochemical test. the test showed three types of bacterial isolates, which was (gram negative) *Escherichia coli*, *Enterobacter cloacae*, *pseudomonas aeruginosa* and two (gram positive) *Staphylococcus aureus and bacillus cereus* the percentages of microorganism got from hand skin swab as shown in (Fig. 1).

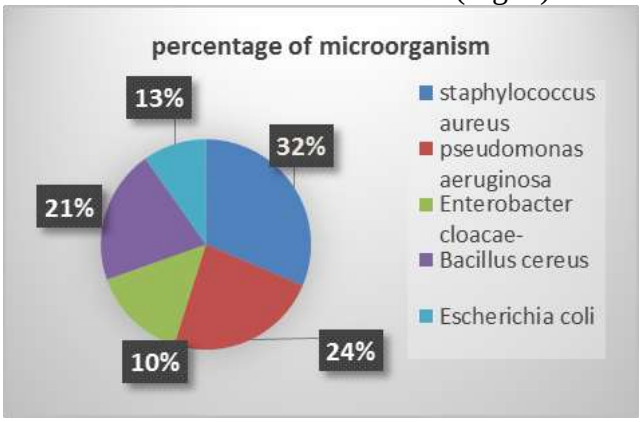

Figure 1. percentage of microorganism From skin swabs.

Determination of the Minimum Inhibitory Concentration (MIC). =

The bacteriostatic activity was evaluated by determining the minimum inhibitory concentration (MIC) (27). The MIC is recorded by observing the lowest concentration required to inhibit bacterial growth (26). The result of antibacterial activity of the disinfection against the bacterial isolates is shown in Table 1. The MIC assays showed that the highest antimicrobial activity was with thymol against *Staphylococcus aureus* (0.09) mg/ml as shown in (Fig2.).

Figure 2. mic of *Staphylococcus aureus.* A . Positive control(+) and negative control (-) B. Mic with Thymol .C. Mic with Dettol D. Mic with Thyme

While a lowest antibacterial effect was with thyme against *Bacillus cereus* (6.25) mg/ml. as shown in (Fig.3).

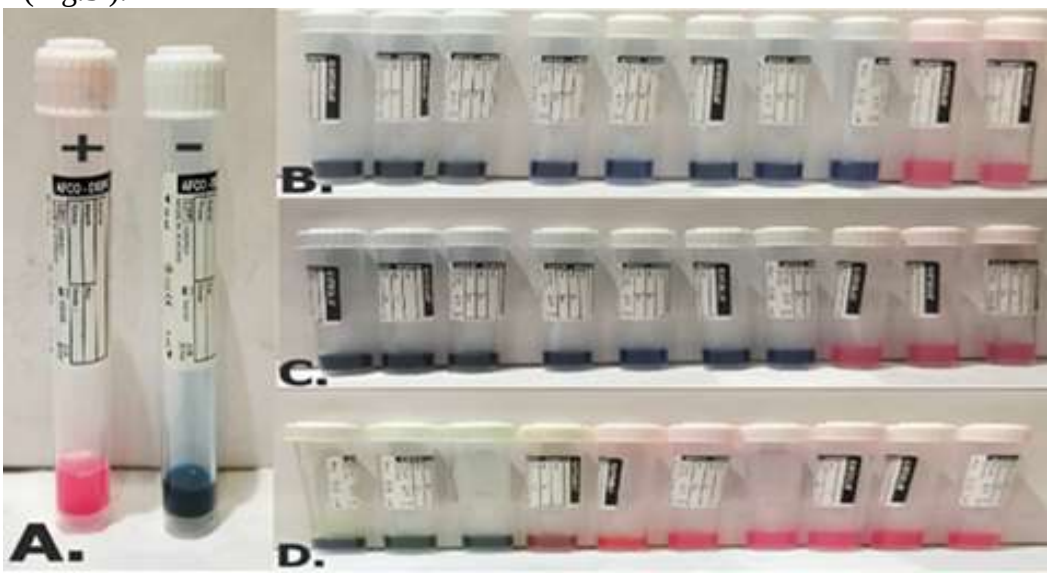

Figure 3. mic of *Bacillus cereus* .A . positive control (+)and negative control (-). B. Mic with Thymol .C. Mic with Dettol D. Mic with Thyme

Thymol exhibited a common antimicrobial activity comparison with Dettol and thyme the antimicrobial activity of Dettol was less than that of Thymol for each of the *Escherichia. coli, Enterobacter. cloacae* with MIC (0.78) mg/ml and (0.39) mg/ml However, both Thyme and Dettol possess the same MIC (1.56) mg/ml present a against *pseudomonas aeruginosa* .thyme show lower antimicrobial activity to other bacteria.

Table 1. minimum inhibition concentration of (Thymol, Dettol,Thyme)

Isolation	Mic value of Disinfection		
	Dettol	Thymol	Thyme crude
Escherichia coli	0.78	0.39	3.12
Enterobacter cloacae	0.78	0.39	3.12
staphylococcus aureus	0. 39	0.09	3.12
bacillus cereus	0.39	0.19	6.25
Pseudomonas aeruginosa	1.56	0.19	1.56

The minimal bactericidal concentration (MBC).: The result of mbc for antibacterial activity of the green Dettol against the bacterial isolates are shown in Table 2. The result in table show the highest bactericidal Microorganism disinfection was with thymol against *staphylococcus aureus* as shown in (Fig.4).

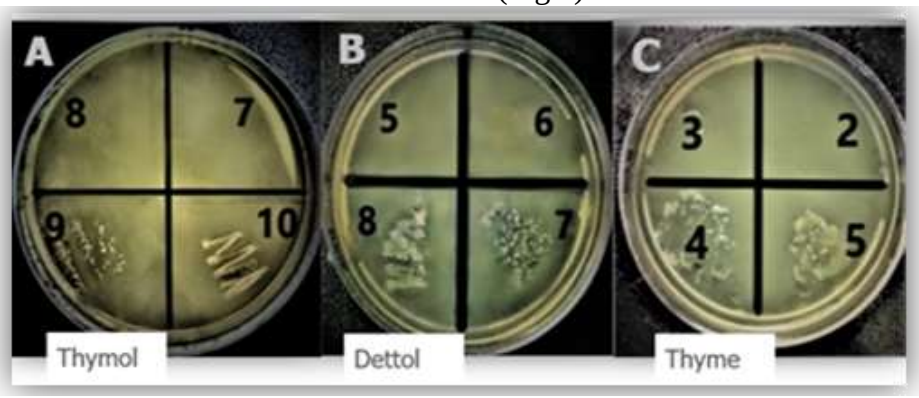

Figure 4. Mbc of *Staphylococcus aureus*. A. Mbc with Thymol. B Mbc with Dettol .C. Mbc with Thyme

While lowest bactericidal concentration was with thyme against bacillus cereus as shown in (Fig.5)..

Figure 5. Mbc of *Bacillus cereus*. A. Mbc with Thymol. B Mbc with Dettol .C. Mbc with Thyme

On other hand, minimal bactericidal of thymol and Dettol for *Enterobacter cloacae* was the same value (1.56) mg/ml.

Table 2. Minimum bactericidal concentration of (Thymol, Dettol ,Thyme)

Isolation	MBC value of Disinfection		
	Dettol	Thymol	Thyme crude
Escherichia coli	1.56	0.78	6.25
Enterobacter cloacae	1.56	1.56	6.25
staphylococcus aureus	0. 78	0. 19	6.25
bacillus cereus	0.78	0.39	12.5
Pseudomonas aeruginosa	3.12	0.39	3.12

Statistical analysis

The results of this study were analyzed by one-way Anova test to find out if there were any significant effect of Thymol ,Dettol and Thyme extract. Moreover, to test whether the difference in means is statistically significant and if the ANOVA F-test shows there is a significant difference between the groups . One-way analysis of variance (abbreviated one-way ANOVA) is a statistical technique forMoreover, to test whether the difference in means is statistically significant and if the ANOVA F-test shows there is a significant difference between the groups . One-way analysis of variance (abbreviated one-way ANOVA) is a statistical technique for comparing the means of two or more samples (using the F distribution). This can only be used with numerical data. The

values of F and p define the significance of the parameters, and the higher the value of F and the lower the value of p, the more significant [22]. In all the ANOVA statistical tests, the level of significance was $p \leq 0.05$ or $p \leq 0.01$ that suggesting that the test is considered to be statistically significant. According to the table (3)and(4) P- value was 0.000 for the significant differences among ability of (Dettol Thymol Thyme extract) This mean there is significant differences between, Dettol Thymol Thyme extract because P-value is more than 0.05 while, Statistical analysis for MBC of Isolation Pathogenic Bacteria in table (5) show that P- value was 0.916 and Statistical analysis for MIC of Isolation Pathogenic Bacteria in table (6)Show that P- value was 0.903 This mean there is no significant differences between Pathogenic Bacteria.

Table 3. Statistical analysis for MIC of Dettol, Thymol, Thyme

Group	Number of Subject	Mean	Standard Deviation	F-Value	P-Value
Dettol	**5**	**0.78**	**0.42722**	**17.158**	**0.000**
Thymol	**5**	**0.25**	**0.12**		
Thyme extract	**5**	**3.434**	**1.53215**		

At: P ≤ 0.05

Table: 4 Statistical analysis for MBC of Isolation Pathogenic Bacteria

Group	Number of Subject	Mean	Standard Deviation	F-Value	P-Value
Dettol	**5**	**1.56**	**0.85444**	**16.336**	**0.000**
Thymol	**5**	**0.662**	**0.48807**		
Thyme extract	**5**	**6.874**	**3.06308**		

At: P ≤ 0.05

Table 5. Statistical analysis for MIC of Isolation Pathogenic Bacteria

Group	Number of Subject	Mean	Standard Deviation	F-Value	P-Value
E.Coli	**3**	**1.43**	**1.20557**	**0.229**	**0.916**
E.Cloacae	**3**	**1.43**	**1.20557**		
S.aureus	**3**	**1.2**	**1.36315**		
B.cereus	**3**	**2.24333**	**2.83578**		
P.aeruginsa	**3**	**1.10333**	**0.64582**		

At: P≤ 0.05

Table 6. Statistical analyses for MIC of Isolation Pathogenic Bacteria

Group	Number of Subject	Mean	Standard Deviation	F-Value	P-Value
E.Coli	**3**	**2.86333**	**2.41581**	**0.250**	**0.903**
E.Cloacae	**3**	**3.12333**	**2.21088**		
S.aureus	**3**	**2.40666**	**2.72830**		
B.cereus	**3**	**4.55666**	**5.61904**		
P.aeruginsa	**3**	**2.21**	**1.28693**		

At: P ≤ 0.05

Conclusions

From result obtained in this is study, it can be concluded that thymol have the broad activity against pathogenic microorganism and When used within commercial disinfectant (Dettol) ingredients as an antimicrobial agent instead of chloroxylenol and comparison with the results concluded that they were much for efficient than the chemicals compound (chloroxylenol) in the same tested concentration .The composition of thymol is natural and is less toxic than chloroxylenol, could be important motivators towards the pursuit of developing such products, the production of disinfection from natural compounds as an alternative to harmful chemical compounds, which could, eventually, safeguard and promote the public health.

REFERENCES

1. Abdulkadir, M. and S.Waliyu. 2012. Screening and isolation of the soil bacteria for Ability to produce antibiotics, european Journal of Applied Sciences, 4, 211-215

2. Al-Beshari, K. A. Y., T. Abdullah and A.Safirah, 2018 The impact of diluted detergents on *escherichia coli* K12 (JM109). International Journal of Sciences: Basic and

Applied Research (IJSBAR) Volume 39, No 1, pp 184-193

3 .Anand, P. S., S. Athira ,S. Chandramohan, K.Ranjith V. Veena Raj, and V. D. Manjula 2016. Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. Journal of International Society of Preventive & Community Dentistry, 6(1), 22

4.Anwar, H. M., & Baker, I. A. 2022. Effect of ethanolic extract of Thyme and olive leave in quality characteristic of lamb patties during storage. Iraqi Journal of Agricultural Science, 53(1), 219-229. https://doi.org/10.36103/ijas.v53i1.1528

5.Azelee, N. I. W., H. El-Enshasy, D.J.Dailin, N.H.A.Manas, R.Abd Malek,M.A. Mokhter, ... and R.A.Rahman. 2020. AJAB. Asian J Agric & Biol, 8(3), 348-367

6.Burke, J. P. 2003. Infection control-a problem for patient safety. New England Journal of Medicine. 348(7), 651-656

7.Chundran, N. K., I. R. Husen, and I.Rubianti. 2015. Effect of Neem leaves extract *(Azadirachta indica*) on wound healing. Althea Medical Journal, 2(2), 199-203

8.Cloete, T. E. (2003). Resistance mechanisms of bacteria to antimicrobial compounds. International Biodeterioration & Biodegradation, 51(4), 277-282

9.Dancer, S. J. 2009. The role of environmental cleaning in the control of hospital-acquired infection. Journal of hospital Infection, 73(4), 378-385

10.Gondar, E. (2015). Isolation and characterization of bacteria from mobile phones of students and employees at University of Gondar, Ethiopia. Bulletin of Pharmaceutical Research, 5(3), 96-100

11.Holding A. J. and J. G.Collee. 1971 Routine biochemical tests, In Methods in Microbiology, (Norris JR and Ribbons DW, eds). Academic Press Inc. Ltd, London, 1, 32

12.Holt, R. M. 1994. Effects of coring on petrophysical measurements. In International Symposium of the Society of Core Analysts, Stavanger, Norway. Society of Core Analysts, Fredericton, Canada (Vol. 77: 86).

13.Hussein, N. N., & A. H. Muslim, 2019. Detection of the antibacterial activity of AgNPs biosynthesized by *Pseudomonas aeruginosa*. Iraqi Journal of Agricultural Sciences, 50(2), 617-625. https://doi.org/10.36103/ijas.v2i50.661

14.Ibraheem, R. M., A. A Mhawesh and K. W Abood 2018. Estimation of the whole flavonoid, antioxidant, anti bacterial challenge concerning viola odorata (banafsha) methanolic extract. Iraqi Journal of Agricultural Sciences, 49(4),655-662. https://doi.org/10.36103/ijas.v49i4.75

15.Karm, I. F. A. 2019. Investigation of active compound in clove (*Syzygium aromaticum)* Extrac AND compared with inhibitors of growth of some types of bacteria causing food poisoning. Iraqi Journal of Agricultural Sciences, 5(6): 1645-1651 https://doi.org/10.36103/ijas.v50i6.855

16.Maurya, D., M. K.Gohil , U. Sonawane, D. Kumar, A. Awasthi, A.K.Prajapati,... and A.K .Agarwal,2020.Development of autonomous advanced disinfection tunnel to tackle external surface disinfection of COVID-19 virus in public places. Transactions of the Indian National Academy of Engineering, 5(2), 281-287

17.Majda, B., A., Barchan, A., Aarab, M., Bakkali, A., Arakrak, and A. Laglaoui, 2022. Evaluation Of The Antibactrial Activity Of Essential Oils Against E. COLI isolated From Rabbits. Iraqi Journal of Agricultural Sciences, 53(4), 802-809**.** **https://doi.org/10.36103/ijas.v53i4.1592**

18. Mustafa, M. M. 2022. Effect of different levels of oleobote® on production performance and egg quality traits In Japanese Quail. Iraqi Journal of Agricultural Sciences, 53(3), 578-583. **https://doi.org/10.36103/ijas.v53i3.1566**

19. Owuama, C. I. 2017. Determinationof minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. African Journal of Microbiology Research, 11(23), 977-980

20. Pandya, U. A. Doshi, and N. S. Sahay. 2017. Development of herbal disinfectants formulation for mopping households and its antibacterial activity. Natural product research, 31(22), 2665-2668

21.Patil, S. S., S. T. Mohite, S. A. Kulkarni, and U. S. Udgaonkar. 2014. Resazurin tube method: rapid, simple, and inexpensive

method for detection of drug resistance in the clinical isolates of mycobacterium tuberculosis. Journal of Qlobal Infectious Diseases, 6(4), 151

22. Ravikumar, K., S. Ramalingam ,S. Krishnan and K. Balu 2006. Application of response surface methodology to optimize the process variables for Reactive Red and Acid Brown dye removal using a novel adsorbent. Dyes and Pigments. 70(1): 18–26

23.Rahman, J. K., Jaff, D. M., and Dastan, D. 2020. Prangos platychlaena boiss essential oils: a novel study on its toxicity, a ntibacterial activity and chemical compositions effect on burn rats. Iraqi Journal of Agricultural Science, 51(2), 519-529. https://doi.org/10.36103/ijas.v51i2.978

24.Santoyo, S., S.Cavero, L .Jaime, E.Ibanez, F.J.Senorans, and G.Reglero. 2006. Supercritical carbon dioxide extraction of compounds with antimicrobial activity from Origanum vulgare L.: determination of optimal extraction parameters. Journal of Food protection, 69(2), 369-375.

25. Soliman, K. M., and R. I. Badeaa 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology, 40(11), 1669-1675

26.Sellon, D. C. and M.Long. 2013. Equine infectious diseases E-book. Elsevier Health Sciences

27.Suhail, R. N., Mustafa, S. A., & E. E. Al-Obodi, 2022. Efficiency of silver nanoparticales as antibacterial against Aeromonas hydrophila isolated from infected common carp. Iraqi Journal of Agricultural Sciences, 53(3), 589-597. https://doi.org/10.36103/ijas.v53i3.1568

28.Tawfiq, S.M., 2018. Bacteriologigal and genetic study of *Pseudomonas aeruginosa* isolates. Iraqi Journal of Agricultural Sciences, 49(1),:27-35. https://doi.org/10.36103/ijas.v49i1.201

29.Tapouk, F. A., R.Nabizadeh, , N.Mirzaei, , N. H., Jazani, M. Yousefi, and M. A. V. Hasanloei 2020. Comparative efficacy of hospital disinfectants against nosocomial infection pathogens. Antimicrobial Resistance & Infection Control, 9(1), 1-7.