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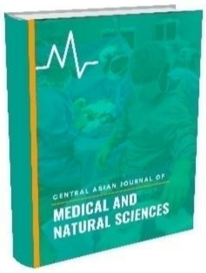

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A Study on the Physio-Therapeutic Effects of Myrtle (*Myrtus Communis* L.) Leaf- and *Eucalyptus* Leaf-Extracts on the Proliferation of Diarrhea-Inducing *Escherichia Coli*, *Shigella*, and *Salmonella* In Broilers Chickens.

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Abstract: This study was conducted to investigate the effectiveness of graded concentrations of myrtle (*Myrtus communis* L.) leaf- and Eucalyptus leaf-extracts (MLE and ELE, respectively) on the proliferation of diarrhea-inducing *Escherichia coli*, *Shigella*, and *Salmonella* in broilers chickens. The present study included the use of three bacterial isolates for each of the abovementioned bacteria obtained from the General Teaching Veterinary Hospital, Al-Diwaniyah City, Iraq, which previously were recovered from diarrheal cases of broiler chickens. These bacterial isolates were subjected to the leaf extracts (aqueous or alcoholic) in agar-gel diffusion tests at four concentrations (25, 50, 75, and 100mg/ml) as six plates for each concentration. Two control groups (water or ethanol) were used as six plates each. The results showed significant (p<0.05) effects of all concentrations for both extracts in inhibiting the growth of all bacteria compared with that from the controls. In a brief, significant (p<0.05) levels of bacterial inhibition were reported and were the highest from the aqueous MLE against the bacterial growth at 75 and 100mg/ml exceeding the effects of ampicillin and ciprofloxacin. At the second level, the aqueous and alcoholic ELE at 100mg/ml surpassed the impacts of ampicillin and ciprofloxacin against the bacterial growth. The alcoholic MLE came at the third place in its effectiveness against the tested pathogenic bacteria. The herein study findings suggest high effects of all myrtle leaf- and Eucalyptus leaf-extracts on the proliferation of diarrhea-inducing *Escherichia coli*, *Shigella*, and *Salmonella* in broilers chickens with the most impacts generated from the aqueous extract of myrtle leaves.

Keywords: Eucalyptus, herbal antibacterial agents, *Myrtus*

communis L.

Introduction

Throughout ancient history, humans have employed various natural resources, including plants and microorganisms, for medicinal purposes in order cure and manage diseases. Based on fossil records, it can be inferred that humans have been utilizing plants for medicinal purposes for a minimum of 60,000 years (1). The utilization of natural substances as therapeutic agents undoubtedly posed a significant obstacle for early human populations. There exists a strong likelihood that in their quest for sustenance, early Homo sapiens frequently ingested toxic plants, resulting in symptoms such as emesis, diarrhea, unconsciousness, or other adverse physiological responses, potentially culminating in fatality. Nevertheless, through this approach, early humans could acquire knowledge pertaining to consumable substances and medicinal resources (2).

Traditional medicines are derived from natural sources and hold significant value in healthcare practices. These forms of medicine have been widely practiced across the globe for several centuries, and in some cases, even centuries. Over time, they have evolved into well-structured and regulated systems of medical practice. Despite potential flaws in their different manifestations, these repositories remain a valuable source of human understanding (3–8).

The significance of mitigating the detrimental impact of microorganisms has gained prominence due to growing apprehensions regarding the sustainability of human existence. A diverse array of microorganisms coexists in a symbiotic relationship with the human body and its surrounding ecosystems. However, when the proliferation of microbes becomes unregulated and occurs at an accelerated rate, it can give rise to various hazardous complications (9–12). Antimicrobial agents serve as antibiotic medications for the purpose of managing infections within the body. However, it is important to note that these agents may induce various side effects, particularly by elevating the levels of reactive oxygen species (ROS) within the human body. Reactive oxygen species (ROS) pose significant hazards to health and overall well-being, contributing to the development of cancer. Additionally, they have the capacity to exacerbate health risks. Various botanical species are utilized as herbal substances in the context of medicinal plants. Several herbal materials have been found to exhibit medicinal properties, including antioxidant, anticancer, anti-inflammatory, and antimicrobial actions (3,13–15). Moreover, these botanical substances can assume a primary function in the synthesis and advancement of pharmaceutical compounds. The materials play a crucial role in various biological applications, including but not limited to cancer therapy, antimicrobial medicines, and treatment of cardiovascular disease. In certain developing nations, herbal medicines serve as the principal form of treatment for infections (16–19). The investigation of new compounds with potential antibacterial activity is an ongoing endeavor, as evidenced by the analysis of extracts from herbal materials. Numerous studies have demonstrated that various herbal medicines serve as reservoirs of a wide array of molecules, a significant proportion of which possess radical scavenging and antimicrobial properties. These properties enable the human body to combat pathogens and mitigate cellular oxidation reactions. Hence, the materials hold considerable importance in the process of synthesizing various forms of herbal medicine due to their demonstrated antimicrobial, antiviral, and antioxidant properties. These heterogeneous molecules possess the ability to regulate and impede the growth of pathogens while exhibiting minimal toxicity towards cells, thus rendering them potential candidates for further investigation in the field of antimicrobial medicine (20–22).

This study was conducted to investigate the effectiveness of graded concentrations of myrtle (*Myrtus communis* L.) leaf- and Eucalyptus leaf-extracts (MLE and ELE, respectively) on the proliferation of diarrhea-inducing *Escherichia coli*, *Shigella*, and *Salmonella* in broilers chickens.

Materials and methods

This study was conducted to investigate the effectiveness of graded concentrations of MLE and ELE on the proliferation of diarrhea-inducing *E. coli*, *Shigella*, and *Salmonella* in broilers chickens. These bacterial isolates were confirmed using tools described in (23,24)

The present study included the use of three bacterial isolates for each of the abovementioned bacteria obtained from the General Teaching Veterinary Hospital, Al-Diwaniyah City, Iraq, which previously were recovered from diarrheal cases of broiler chickens. These bacterial isolates were subjected to the leaf extracts (aqueous or alcoholic) in agar-gel diffusion tests at four concentrations (25, 50, 75, and 100mg/ml) as six plates for each concentration. Two control groups (water or ethanol) were used as six plates each.

Collection of plants

A specific quantity of leaves of myrtle and eucalyptus were collected during the month of August from the gardens of the University of Al-Qadisiyah, Al-Diwaniyah City. These leaves were clean-washed with tap water and dried in a ventilated room under (25-35°C). Then, they were ground using an electric grinder until a fine powder was obtained. The crushed samples were kept in plastic bags until extraction.

Plant extraction

Aqueous extraction

The leaf aqueous extract was performed using instructions from Anesini and Perez (25). In short, 15gm of the previously prepared dry matter powder were placed in a flask with 100ml of distilled water. The remaining methods were applied. The extract was placed in a fridge until the final tests.

Alcoholic extract

The leaf alcoholic extract was performed using instructions from Deshmuk and Borle (25). In short, 20gm of the previously prepared dry matter powder were placed together with 200-300ml of 95% ethanol. The remaining methods were applied. The extract was placed in a fridge until the final tests.

Preparation of bacterial suspension

The bacterial suspension was prepared using the following steps, in which a re-cultivation step was conducted on each bacterium from the original stock to the agar medium fed by the streaking method and then was placed in an incubator at 37°C for 24hrs. Later, a loop of the bacterial colony was transferred into tilted nutrient agar and incubated at 37°C for 24hrs. The bacterial growth was washed from the slant surface using 10ml of nutrient broth and then transferred to a clean test tube. The total number of bacteria was estimated by comparing the growth in the nutrient broth with the McFarland standards, which depends on the degree of turbidity of the suspension in estimating the number of bacterial cells in 1ml of the suspension, as compared with tube No. (2) which contains approximately 3×8^{10} cells/ml.

Preparation of cultivation plates to test the effectiveness of plant extracts

The following steps were applied; the cultivation agar was prepared after autoclaving it for 15mins at 121°C and 15pounds/inch^2. Then, 20ml of the medium was added to each sterile Petri dish and placed in a fridge for several minutes to help the medium solidify. Drops of the bacterial suspension was added into the medium and spread all over the plate. Later, five wells were performed in each plate, four of which were peripheral and one central. The solvents used to dissolve the plant extracts (distilled water, ethyl alcohol) were placed in the central well. As for the peripheral wells, the different concentrations of the plant extracts were placed in them at 0.1ml in each well. The mixtures in the plates were incubated at 37°C for 24hrs. The results were read by measuring the growth inhibition diameters around the wells.

Results

Effect of myrtle extract on bacterial growth

The results showed significant ($p<0.05$) effects of all concentrations for both extracts in inhibiting the growth of all bacteria compared with that from the controls. In a brief, significant ($p<0.05$) levels of bacterial inhibition were reported and were the highest from the aqueous MLE against the bacterial growth at 75 and 100mg/ml exceeding the effects of ampicillin and ciprofloxacin (Table 1) and (Figure 1A).

Table 1: Effects of aqueous and ethanolic extracts of myrtle leaves on bacterial growth.

Concentration (mg/ml)	**Growth inhibition (Mean±SEM)- (mm)**					
	Aqueous extract			**Alcoholic extract**		
	E. coli	***Shigella***	***Salmonella***	***E. coli***	***Shigella***	***Salmonella***
25	12±0.36	16.5±0.22	15.8±0.16	10.3±0.21	14.8±0.16	13.9±0.22
50	13.5±0.22	19± 0	18± 0	11.5±0.22	16.5±0.34	15.8±0.16
75	16± 0	21.6±0.21	22.3±0.21	13.6±0.21	18± 0	17.3±0.21
100	19.3±0.21	24.5±0.22	25.5±0.22	15± 0	20.5±0.22	20.1±0.30
Ampicillin (10µg/disc)	21.33±0.33	21±0	18.33±0.66	21.33±0.33	21±0	18.33±0.66
Ciprofloxacin (5µg/disc)	22	19	18	22	19	18

At the second level, the aqueous and alcoholic ELE at 100mg/ml surpassed the impacts of ampicillin and ciprofloxacin against the bacterial growth (Table 2) and (Figure 1B). The alcoholic MLE came at the third place in its effectiveness against the tested pathogenic bacteria.

Table 2: Effects of aqueous and ethanolic extracts of eucalyptus leaves on bacterial growth.

Concentration (mg/ml)	Growth inhibition (Mean±SEM)- (mm)					
	Aqueous extract			Alcoholic extract		
	E. coli	*Shigella*	*Salmonella*	*E. coli*	*Shigella*	*Salmonella*
25	12.6±0.21	16.6±0.21	15.3±0.21	13±0	17.6±0.21	16.1±0.16
50	15.5±0.34	19±0	18±0	15.3±0.21	20.5±0.22	18.5±0.22
75	17.6±0.21	19.5±0.22	19.5±0.22	17.8±0.16	23.3±0.42	21.6±0.21
100	18.5±0.22	23.6±0.21	21.6±.0.21	18.1±0.16	24±0	22.3±0.21
Ampicillin (10µg/disc)	21.33±0.33	21±0	18.33±0.66	21.33±0.33	21±0	18.33±0.66
Ciprofloxacin (5µg/disc)	22	19	18	22	19	18

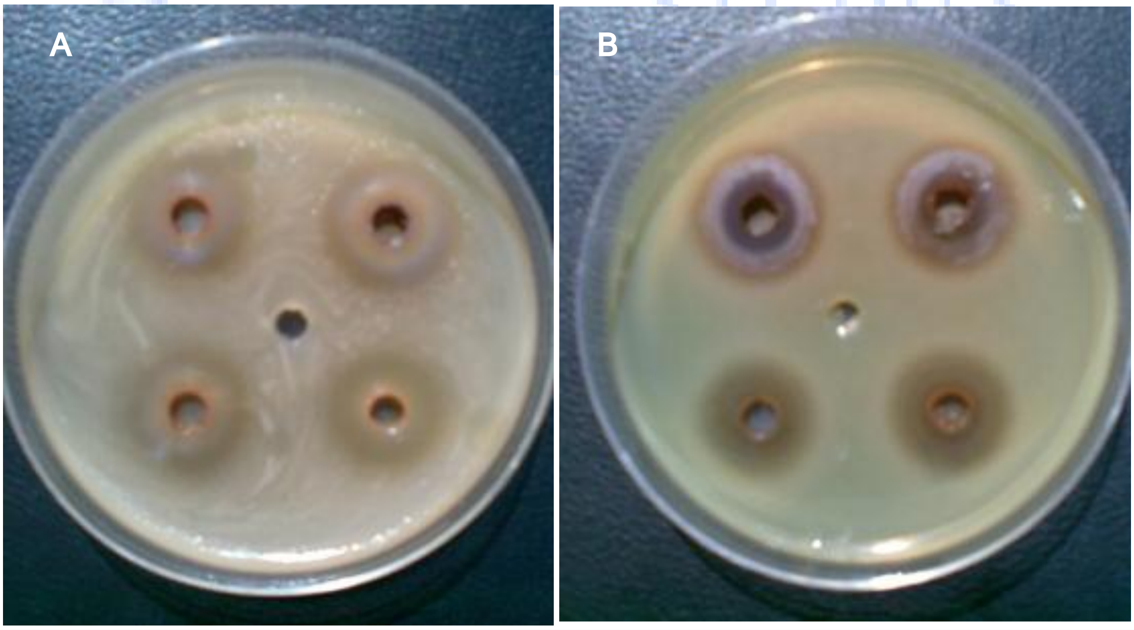

Figure 1: Effect of aqueous extracts on the growth of Shigella bacteria for concentrations (25, 50, 75, and 100mg/ml). A. Marytle. B. Eucalyptus.

Discussion

The study found that the aqueous and alcoholic ethanolic extracts of myrtle and eucalyptus leaves demonstrated significant medical effectiveness. These extracts effectively inhibited the growth of the pathogenic bacteria, specifically *E. coli*, *Salmonella, and shigella*, which are known to cause enteritis in broiler chickens. The inhibitory effect was observed across all concentrations of the herbal extracts utilized in the study. In order to inhibit the growth of germs in culture dishes, we created holes in the culture media and introduced concentrations of two plant extracts. These were compared with control groups consisting of distilled water, ethyl alcohol, and two commonly used antibiotics (ampicillin and ciprofloxacin). These antibiotics are typically employed in the management of gastrointestinal infections triggered by these bacteria in broiler breeding (26).

Tables (1 and 2) present the findings of the study, which examined the impact of various concentrations of aqueous and alcoholic extracts from two plants on the growth of different bacteria. The inhibitory impact of these extracts was found to increase with higher concentrations. Statistical analysis confirmed the existence of substantial variances. Statistically, there is a significant difference in the diameters of inhibition of growth rates across various concentrations. This may be primarily attributed to the greater concentration of bioactive compounds that inhibit bacterial growth. The aqueous MLE demonstrated significant inhibitory effects on the growth of *E. coli*, *Shigella*, and *Salmonella* bacteria. The inhibitory effect was observed at concentrations as low as 25 mg/ml, with diameters of growth inhibition ranging from 12 ± 0.36 mm to 16.5 ± 0.22 mm. At the highest concentration of 100 mg/ml, the diameters of growth inhibition elevated to 19.3 ± 0.21 mm, 24.5 ± 0.22 mm, and 25.5 ± 0.22 mm for E. coli, Shigella, and Salmonella, respectively. Earlier investigations have also reported the effectiveness of various myrtle plant extracts in inhibiting the growth of both Gram-positive and Gram-negative bacteria. Additionally, it has been noted that myrtle plant extracts have a stronger effect on gram-negative bacteria compared to gram-positive bacteria. Al-Mahna conducted a study on nutmeg and myrtle plants, which supports the current findings. At a concentration of 100 mg/ml, it demonstrated notable superiority over the antibiotic gentamicin in inhibiting the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, which are responsible for skin infections in both humans and animals (26–28). The nutmeg plant demonstrated inhibitory effects on the growth of pathogenic microorganisms responsible for skin infections. The effectiveness of this inhibition was found to be directly correlated with the concentration of nutmeg extract used (29–32). It was discovered that the aqueous extract of myrtle exhibited significant inhibitory effects on the growth of *P. aeruginosa* bacteria isolated from burn wounds. The extract's germicidal efficacy can be attributed to its chemical composition (33). The bioactive ingredients in the plant, such as flavonoids and glycosides (specifically glycosides, saponins, tannins, and resins), have been found to be effective against pathogenic germs. The abundance of phenolic compounds and polyphenols in the plant and its extracts contribute to this sensitivity. Tannins found in the myrtle plant have been shown to inhibit the growth of Gram-positive bacteria for 7 days. The researcher attributed the potent antimicrobial properties of this plant to its abundance of medicinal compound (2-III) and perpns (1-III), which were extracted from myrtle leaves. These compounds have demonstrated efficacy against antibiotic-resistant germs (34).

The alcoholic MLE also exhibited inhibitory effects against the tested germs, although to a lesser extent than the aqueous MLE, as indicated in (Table 1). The aqueous and alcoholic

ELE exhibited comparable effects on the growth of the evaluated bacteria, as shown in (Table 2). They ranked second in terms of their impact. The aqueous MLE, at a concentration of 100 mg/ml, demonstrated superior efficacy compared to the ampicillin and ciprofloxacin. Two isolates of *E. coli*, a standard isolate, and a urine isolate, were used to test the effectiveness of essential oils from *Rosmarinus officinalis* and *Eucalyptus globules* (35–37). The study found that the volatile oil from the eucalyptus plant exhibited antibacterial activity against a group of pathogenic bacteria, including *E. coli* ATCC25922, *P. aeruginosa*, and *Salmonella typhimurium*. The antibacterial activity of the eucalyptus oil was compared to the antibiotics Piperacillin and Ampicillin. The efficiency has improved. It was discovered that the eucalyptus plant extract has a potent inhibitory effect on the bacteria responsible for respiratory tract infections in humans, such as *Staphylococcus aureus* and Streptococcus pneumoniae. Chemical analysis of eucalyptus leaf extract revealed the presence of tannins, saponins, cardenolides, and anthraquinones. These compounds are known for their antibacterial properties, which contribute to the antibacterial activity of eucalyptus leaves (38–41).

Conclusion

The herein study findings suggest high effects of all myrtle leaf- and Eucalyptus leaf-extracts on the proliferation of diarrhea-inducing *Escherichia coli*, *Shigella*, and *Salmonella* in broilers chickens with the most impacts generated from the aqueous extract of myrtle leaves.

References

1. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001 Mar;109(Suppl 1):69–75.
2. QingWen S, LiGeng L, ChangHong H, ManLi Z, YuFang W. Study on natural medicinal chemistry and new drug development. Zhongcaoyao Chin Tradit Herb Drugs. 2010;41(10):1583–9.
3. Aferu T, Mamenie Y, Mulugeta M, Feyisa D, Shafi M, Regassa T, et al. Attitude and practice toward traditional medicine among hypertensive patients on follow-up at Mizan–Tepi University Teaching Hospital, Southwest Ethiopia. SAGE Open Med. 2022 Mar 15;10:20503121221083210.
4. Chali BU, Hasho A, Koricha NB. Preference and Practice of Traditional Medicine and Associated Factors in Jimma Town, Southwest Ethiopia. Evid-Based Complement Altern Med ECAM. 2021 May 29;2021:9962892.
5. Hailu F, Cherie A, Gebreyohannis T, Hailu R. Determinants of traditional medicine utilization for children: a parental level study in Tole District, Oromia, Ethiopia. BMC Complement Med Ther [Internet]. 2020 [cited 2023 Jul 14];20. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7178580/
6. Rizvi SAA, Einstein GP, Tulp OL, Sainvil F, Branly R. Introduction to Traditional Medicine and Their Role in Prevention and Treatment of Emerging and Re-Emerging Diseases. Biomolecules. 2022 Oct 9;12(10):1442.
7. Thorsen RS, Pouliot M. Traditional medicine for the rich and knowledgeable: challenging assumptions about treatment-seeking behaviour in rural and peri-urban Nepal. Health Policy Plan. 2016 Apr;31(3):314–24.

8. Alves RR, Rosa IM. Biodiversity, traditional medicine and public health: where do they meet? J Ethnobiol Ethnomedicine. 2007 Mar 21;3:14.
9. Stubbe DE. Complementary and Alternative Medicine: If You Don't Ask, They Won't Tell. Focus J Life Long Learn Psychiatry. 2018 Jan;16(1):60–2.
10. Street R, Falkenberg T, Sundberg T, Balakrishna Y, Abrams A, Kredo T. Participation of traditional, complementary and alternative health practitioners in conventional health systems in low- and middle- income countries. Cochrane Database Syst Rev. 2019 Aug 7;2019(8):CD013391.
11. Lee EL, Richards N, Harrison J, Barnes J. Prevalence of Use of Traditional, Complementary and Alternative Medicine by the General Population: A Systematic Review of National Studies Published from 2010 to 2019. Drug Saf. 2022;45(7):713.
12. James PB, Wardle J, Steel A, Adams J. Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. BMJ Glob Health [Internet]. 2018 [cited 2023 Jul 14];3(5). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6231111/
13. Adegbola P, Aderibigbe I, Hammed W, Omotayo T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. Am J Cardiovasc Dis. 2017 Apr 15;7(2):19–32.
14. Chandran R, Abrahamse H. Identifying Plant-Based Natural Medicine against Oxidative Stress and Neurodegenerative Disorders. Oxid Med Cell Longev. 2020 Sep 15;2020:8648742.
15. Hahn D, Shin SH, Bae JS. Natural Antioxidant and Anti-Inflammatory Compounds in Foodstuff or Medicinal Herbs Inducing Heme Oxygenase-1 Expression. Antioxidants. 2020 Nov 27;9(12):1191.
16. Park MN, Rahman MdA, Rahman MdH, Kim JW, Choi M, Kim JW, et al. Potential Therapeutic Implication of Herbal Medicine in Mitochondria-Mediated Oxidative Stress-Related Liver Diseases. Antioxidants. 2022 Oct 17;11(10):2041.
17. Talib WH, Al-Ataby IA, Mahmod AI, Jawarneh S, Al Kury LT, Al-Yasari IH. The Impact of Herbal Infusion Consumption on Oxidative Stress and Cancer: The Good, the Bad, the Misunderstood. Mol Basel Switz. 2020 Sep 14;25(18):4207.
18. Zielinska-Blizniewska H, Sitarek P, Merecz-Sadowska A, Malinowska K, Zajdel K, Jablonska M, et al. Plant Extracts and Reactive Oxygen Species as Two Counteracting Agents with Anti- and Pro-Obesity Properties. Int J Mol Sci. 2019 Sep 14;20(18):4556.
19. Targeting Reactive Oxygen Species in Cancer via Chinese Herbal Medicine - PMC [Internet]. [cited 2023 Jul 14]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6754955/
20. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants. 2020 Dec 21;9(12):1309.
21. Liang J, Huang X, Ma G. Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. RSC Adv. 2022 Oct 10;12(45):29197.
22. Bittner Fialová S, Rendeková K, Mučaji P, Nagy M, Slobodníková L. Antibacterial Activity of Medicinal Plants and Their Constituents in the Context of Skin and Wound Infections, Considering European Legislation and Folk Medicine—A Review. Int J Mol Sci. 2021 Oct 4;22(19):10746.

23. Colle J, Fraser A, Marmion B, Simmons A. Practical Medical Microbiology. 14th ed. New York: Churchil Livivingstone;
24. Baron E, Paterson L, Finegold S. Baily and Scotts. Diagnostic Microbiology . 9th ed. USA: Mosby; 1994.
25. Anesini C, Perez C. Screening of plants used in Argentine folk medicine for antimicrobial activity. J Ethnopharmacol. 1993 Jun;39(2):119–28.
26. Timbermont L, Lanckriet A, Dewulf J, Nollet N, Schwarzer K, Haesebrouck F, et al. Control of Clostridium perfringens-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids and essential oils. Avian Pathol J WVPA. 2010 Apr;39(2):117–21.
27. Peng L, He Z, Chen W, Holzman IR, Lin J. Effects of Butyrate on Intestinal Barrier Function in a Caco-2 Cell Monolayer Model of Intestinal Barrier. Pediatr Res. 2007 Jan;61(1):37–41.
28. Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E, Botsoglou NA, Spais AB. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with eimeria tenella. Arch Anim Nutr. 2003 Apr 1;57(2):99–106.
29. Protective effect of Artemisia campestris extract against aspirin-induced gastric lesions and oxidative stress in rat - RSC Advances (RSC Publishing) [Internet]. [cited 2023 Jul 14]. Available from: https://pubs.rsc.org/en/content/articlelanding/2014/ra/c4ra08564g/unauth
30. Jabri MA, Rtibi K, Tounsi H, Hosni K, Souli A, El-Benna J, et al. Myrtle berry seed aqueous extract inhibits human neutrophil myeloperoxidase in vitro and attenuates acetic acid-induced ulcerative colitis in rats. RSC Adv. 2015 Jul 29;5(80):64865–77.
31. Hosseinzadeh H, Khoshdel M, Ghorbani M. Antinociceptive, anti-inflammatory effects and acute toxicity of aqueous and ethanolic extracts of Myrtus communis L. Aerial parts in mice. J Acupunct Meridian Stud. 2011 Dec;4(4):242–7.
32. Alem G, Mekonnen Y, Tiruneh M, Mulu A. Invitro antibacterial activity of crude preparation of myrtle (Myrtus communis) on common human pathogens. Ethiop Med J. 2008 Jan;46(1):63–9.
33. Tumen I, Senol FS, Orhan IE. Inhibitory potential of the leaves and berries of Myrtus communis L. (myrtle) against enzymes linked to neurodegenerative diseases and their antioxidant actions. Int J Food Sci Nutr. 2012 Jun;63(4):387–92.
34. Serce S, Ercisli S, Sengul M, Gunduz K, Orhan E. Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits. Pharmacogn Mag. 2010 Jan;6(21):9–12.
35. Yu H, Lin ZX, Xiang WL, Huang M, Tang J, Lu Y, et al. Antifungal activity and mechanism of d-limonene against foodborne opportunistic pathogen Candida tropicalis. LWT. 2022 Apr 1;159:113144.
36. Pan J, Zhu Y, Abdel-Samie MA, Li C, Cui H, Lin L. Biological properties of essential oil emphasized on the feasibility as antibiotic substitute in feedstuff. Grain Oil Sci Technol. 2023 Mar 1;6(1):10–23.
37. Applegate TJ, Klose V, Steiner T, Ganner A, Schatzmayr G. Probiotics and phytogenics for poultry: Myth or reality?1. J Appl Poult Res. 2010 Jul 1;19(2):194–210.
38. Sabo VA, Knezevic P. Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: A review. Ind Crops Prod. 2019 Jun;132:413.

39. Park JW, Wendt M, Heo GJ. Antimicrobial activity of essential oil of Eucalyptus globulus against fish pathogenic bacteria. Lab Anim Res. 2016 Jun;32(2):87.
40. Panahi Y, Sattari M, Pour Babaie A, Beiraghdar F, Ranjbar R, Hedaiat joo A, et al. The Essential Oils Activity of Eucalyptus polycarpa, E. largiflorence, E. malliodora and E. camaldulensis on Staphylococcus aureus. Iran J Pharm Res IJPR. 2011;10(1):43–8.
41. Mieres-Castro D, Ahmar S, Shabbir R, Mora-Poblete F. Antiviral Activities of Eucalyptus Essential Oils: Their Effectiveness as Therapeutic Targets against Human Viruses. Pharmaceuticals [Internet]. 2021 Dec [cited 2023 Jul 14];14(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8706319/