Trop J Nat Prod Res, October 2023; 7(10):4256-4261 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antibacterial Properties of Leaves and Peels Extracts of *Citrus aurantifolia* cultivated in Algeria against Multi-Drug Resistant *Staphylococcus aureus* Originating from Raw Milk

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ARTICLE INFO

Article history:
Received 07 August 2023
Revised 10 September 2023
Accepted 07 October 2023
Published online 01 November 2023

ABSTRACT

Plants have an increased consumer preference and acceptability for the treatment of several diseases. Here, the antibacterial properties of organic extracts obtained from leaves and peels of *Citrus aurantifolia* from Algeria have been characterized. Two solvents, methanol, and ethanol, were employed to extract the bioactive components. Quantitative analysis of total phenols and flavonoids was conducted for the different extracts. The antibacterial activity was tested against multidrug-resistant *Staphylococcus aureus* strains originating from raw milk and reference strains including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 11778. The results revealed that leaves and peels extracts displayed significant antibacterial activity against the tested bacteria. The inhibition zone diameters observed ranged from 16.00 mm to 22.00 mm. The highest antimicrobial effect was observed with the ethanol extracts against the multidrug-resistant *S. aureus* strains, as indicated by a minimum inhibitory concentration of 1.56 mg/ml. The highest total phenolics and flavonoids contents were found to be 96 mg GAE/g and 54 mg QE/g in peels. Hence, the reported results unveil valuable insights into the antibacterial effects of *Citrus aurantifolia* extracts which have potential antimicrobial applications.

***Keywords*:** *Citrus aurantifolia*, antibacterial activity, *Staphylococcus aureus*, polyphenols, flavonoids, raw milk

Introduction

Staphylococcus aureus is widely observed in mastitis that causes mammary gland infections in cows.[1] *S. aureus* represents a significant and economic public health risk because it usually contaminates milk and enters the human food chain resulting in foodborne poisoning.[2] Specific toxin-mediated diseases, such as scalded staphylococcal food poisoning, skin syndrome, and toxic shock syndrome are usually observed.[3] Moreover, *S. aureus* is a principal cause of infective endocarditis, osteoarticular, and pulmonary diseases.[4] Studies on *S. aureus* have intrigued researchers around the world, particularly in relation to its global distribution.[5] Moreover, Methicillin resistance in *Staphylococcus aureus* (MRSA), has consistently been linked to significant mortality rates worldwide annually.[6]
Antimicrobial therapy is a well-recognized strategy used to treat dairy cow mastitis.[7,8] Nevertheless, its success is decreasing due to the emergence of resistant strains observed in bacterial populations, requiring the exploration of alternative sources.[9]

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Citation: Benredjem L. Antibacterial Properties of Leaves and Peels Extracts of *Citrus aurantifolia* cultivated in Algeria against Multi-Drug Resistant *Staphylococcus aureus* Originating from Raw Milk. Trop J Nat Prod Res. 2023; 7(10):4256-4261. http://www.doi.org/10.26538/tjnpr/v7i10.20.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Plants represent an inexhaustible source of novel molecules for antimicrobial discovery research.[10,11] In comparison to synthetic allopathic medications, antimicrobials derived from plants have little adverse effects and toxicity, while offering significant therapeutic potential for the treatment of various infections.[12]
Citrus plants members of the Rutaceae family are among the popular cultivated crops worldwide mostly in the tropical and sub-tropical regions. *Citrus reticulata* (mandarin), *Citrus sinensis* (orange), *Citrus paradisi* (grapefruits), *Citrus limon* (lemons), and *Citrus aurantifolia* (lime) are among the most significant commercially grown *Citrus* fruits in the world.[13] Based on the data reported by Food and Agriculture Organization Corporate Statistical Database (FAOSTAT),[14] its global output in 2020 amounted to 158,490,986 tons, up around 7.5% from 2017.[15] Citrus fruits are widely known as a promising source of diverse bioactive chemicals, including phenolic acids, carotenoids, vitamins, flavonoids, and essential oils.[16,17,18] The benefits of citrus plants for health have been the subject of several studies. These advantages consist of a variety of biological effects, including anti-inflammatory, antioxidant, anticancer, antimicrobial, antimalarial, and immunostimulatory properties.[15,19,20] Among the bioactive compounds identified, hesperidin and vitamin C from fruits have antiviral properties against SARS-CoV-2,[21] nobiletin is a potential drug against neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease,[22] and colon cancer.[23] In terms of antibacterial activity, *Citrus* metabolites have been effective against pathogenic bacteria responsible for human and animal diseases and food spoilage.[24-26] Recent reports demonstrated that essential oil and methanol: dimethyl sulfoxide (1:1) extract from *Citrus aurantifolia* fruit peels have antibacterial activity against MRSA.[27,28] Hence, this study aimed to assess the antibacterial activities of the methanol and ethanol extracts of leaves and peels of *Citrus aurantifolia* from Algeria against multi-drug resistant *Staphylococcus aureus* strains isolated from raw milk.

Material and Methods

Plant Material Collection

Citrus aurantifolia fresh leaves and fruits (Figure 1) were collected in March 2023 from Annaba City (36.85508, 7.70647), Algeria. The plant material was identified by Dr. Azzeddine Zeraib, Department of Agronomy, University Abbes Laghrour Khenchela, Algeria, and stored under the voucher specimen number BLCIT-001-1-2023. After harvesting, leaves and fruits were washed. Then, *Citrus aurantifolia* peels and leaves were air-dried for five days. The dried samples were ground into a fine powder and used immediately.

Preparation of crude extracts

Methanol (MeOH) (80% v/v) (absolute, Sigma-Aldrich) and ethanol (EtOH) (70% v/v) (absolute, Sigma-Aldrich) were used for the extraction at room temperature by maceration. 10 g of powder biomass were resuspended in 200 mL of each solvent and allowed to stand for 72 hours. Then, the extracts were filtered through a Whatman No. 1 filter paper (GE Healthcare Life Sciences). The filtrates were dried under reduced pressure at 37−40ºC (Buchi R-210 Rotary Evaporator) and kept at -20ºC for further studies.

Bacterial strains

The antibacterial activity of *Citrus aurantifolia* extracts was determined against seven bacterial strains, including reference strains obtained from the National Center for Biotechnology Research (Constantine, Algeria): *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, and tree *Staphylococcus aureus* strains: SaS1, SaS2, SaS3 isolated from raw cow milk from dairy farms located in Remila (Khenchela). Staphylococcal isolates were identified by API STAPH test strips (bioMerieux, France).

Antibiotic Susceptibility pattern testing

The antimicrobial drug susceptibility of the staphylococci strains was assessed and interpreted in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines.[29] Six antibiotic agents (Thermo Scientific, France) that belong to four categories were used: penicillin, tetracycline, neomycin, kanamycin, erythromycin, and oxacillin.

Antibacterial activity evaluation

The antibacterial activities of extracts derived from *C. aurantifolia* were evaluated using the agar well diffusion test.[29] Bacterial suspensions were prepared in sterile physiological solution (0.9% of NaCl) from overnight cultures, and turbidity was adjusted to 0.5 McFarland's index. Bacterial suspensions were spread on Petri plates containing Muller-Hinton medium (MH, Sigma-Aldrich) using sterile swabs. The extracts were prepared using a solution of 4% dimethyl sulfoxide (DMSO, Sigma Aldrich, UK) at a concentration of 200 mg/mL and loaded in the wells of 6 mm (40µL/well). DMSO and ampicillin (10 µg/disc) were utilized as negative and positive controls, respectively. After pre-diffusion at 4°C for 3 hours, the plates were incubated (BF 115, Binder, Germany) at 37°C for 24 hours. Subsequently, the diameters of the inhibition zones were recorded in millimeters.

Figure 1: *Citrus aurantifolia* leaves and fruits

Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) was determined for each extract against all tested bacterial strains using the microdilution broth assay following the National Committee for Clinical Laboratory Standards (NCCLS) recommendations.[30] Bacterial cultures incubated for 24 hours in nutrient broth were used to prepare inoculums corresponding to 10^6 CFU/mL. Stock solutions of leaves and peel extracts were prepared at 200 mg/ml in 10% (v/v) DMSO. 100 µL of each bacterial inoculum and 100 µL of each extract were loaded in 96 well plates (Thermo Scientific, UK). Subsequently, serial dilutions ranging from 200 to 0.097 mg/mL were performed. The plates were subjected to incubation at a temperature of 37 ºC for 18 to 24 hours. The MICs were recorded as the minimal concentration at which no observable growth was detected.

Determination of total phenolic and flavonoids contents

The total phenolic contents in the various extracts were determined using the Folin-Ciocalteu method described by Arruda *et al.*[31] The results were expressed in milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g). The quantification of flavonoid contents was conducted using the aluminum chloride ($AlCl_3$) colorimetric assay.[32] Flavonoid contents were expressed as milligrams of quercetin equivalents per gram of sample in dry weight (mg QE/ g).

Statistical analysis

For each data point, at least three independent measurements were performed. The results were presented in mean and standard deviation. The obtained data were treated using GraphPad Prism® 7.01 program Software Inc., San Diego, CA, USA. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer post-hoc test for multiple comparisons. P values < 0.05 were considered significant.

Results and discussion

Antibiotic susceptibility of S. aureus strains

Table 1 shows the results for antibiotic susceptibility against a set of six antibiotic agents. Two strains, *S. aureus* SaS1 and *SaS2*, exhibited resistance to all antibiotics that were subjected to testing. On the other hand, the third strain *S. aureus* SaS3 presented an intermediate sensitivity to kanamycin and neomycin. According to the European Centre for Disease Prevention and Control (ECDC), which defines multidrug resistance (MDR) as the lack of susceptibility to three or more antimicrobial categories,[33] isolated *S. aureus* strains are recorded as MDR. Wendlandt *et al.*[34] indicated that staphylococci originating in animals often have more than 40 antimicrobial resistance genes. More importantly, antimicrobial resistance is considered a major global health issue; this phenomenon is responsible for approximately 700,000 deaths per year worldwide.[35]

Antibacterial activity

The antimicrobial activity of different *C. aurantifolia* extracts against three *S. aureus* strains isolated from raw milk and reference strains was investigated by well diffusion method. The results obtained are represented in Table 2. All strains were sensitive to the different leaves and peels extracts, and statistically significant differences between the different treatments were observed for each strain. For the methanol extract of the leaves, the highest inhibition effect was found against *Bacillus cereus* ATCC 11778, with a diameter zone inhibition of 22 ± 0.8 mm. It was found that this extract acts better on *B. cereus* than ampicillin. The lowest inhibition zone diameter, 16 mm, was recorded against *Pseudomonas aeruginosa* ATCC 27853. For the ethanol extract of the leaves, Gram-positive strains, namely *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *S. aureus* SaS1, *S. aureus* SaS2, and *S. aureus* SaS3, exhibited the highest inhibition zone diameters in comparison with Gram-negative strains, where the inhibition zone diameters registered were 14 ± 1.25 mm for *Escherichia coli* ATCC 25922 and 16 ± 0.5 mm for *Pseudomonas aeruginosa* ATCC 27853. Concerning the peel extracts, the highest inhibition zone diameters were observed against *Staphylococcus aureus* ATCC 25923 for the methanol (21 ± 0.8 mm) and the ethanol (22 ± 0.5 mm) extracts, while the lowest inhibition zone was 16 ± 0.8 for the ethanol extract against *Escherichia coli* ATCC 25922.

According to our study, previous results with *C. aurantifolia* indicated that peels and leaves extracts obtained with methanol and ethanol exhibit antibacterial activity against pathogenic bacteria.[36] For instance, Phattayakorn and Wanchaitanawong[37] reported the antimicrobial properties of ethanol extract of fruit peel from Thailand against *Bacillus licheniformis*; however, the obtained results, between 11.00 and 14.00 mm, were less than those observed in our study with the species *Bacillus cereus*. The result of Shakya *et al.*[38] with the absolute ethanol extract from peel fruit from Nepal against *Bacillus* sp. (20.33 ± 1.527 mm) was very close to the results observed in our study for the strain *Bacillus cereus*. However, the same extract was found to have weak activity against *S. aureus* ATCC 25923 (12 ±1 mm), unlike our findings. Another study made with methanol extract from the leaves of *Citrus aurantifolia* from Sudan showed positive antibacterial effect against *Bacillus cereus* ATCC 10876. Nevertheless, the same extract demonstrated insufficient ineffectiveness towards various multi-drug resistant clinical strains.[39] It was reported that bacterial sensitivity to bioactive compounds is strain-dependent.[40,41] Overall, Gram-negative bacteria demonstrate a notable level of resistance to plant bioactive metabolites due to the presence of the outer complex membrane, which is connected to the cell wall. This particular structural characteristic serves to obstruct the flow of chemical substances.[42]

Many factors can also affect the biological activity of plant extracts including the plant parts used, extraction process, environmental conditions, and differences in geographical locations.[43] Furthermore, during the extraction process, each solvent has a unique potential to dissolve particular phytochemical compounds, depending on the structure and the polarity of the substance.[44] Pasaribu *et al.*[45] investigated the antibacterial effect of citrus peel extracts against two bacterial species involved in periodontitis, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. Among the three solvents tested, the ethanol extract was more effective than the n-hexane and ethyl acetate solvents with an inhibition zone diameter of 16.05 mm. Similarly, the ethanol peel extract of *Citrus aurantifolia* (Christm.) Swingle was more efficient against *Vibrio parahaemolyticus* than hexane and dichloromethane extracts.[46] A similar study conducted by Munawaroh *et al.*[47] on the antibacterial properties of lime peel extracts using two solvents, ethyl acetate, and ethanol, found that the ethyl acetate was more efficient in the extraction of compounds from *C. aurantifolia* peels than ethanol. However, among three extracts made with methanol, ethanol, and acetone of fruit peels of three different species of *Citrus aurantifolia*, used against pathogenic bacteria, the methanol extract showed inhibitory effect against *Pseudomonas* sp.[48] In addition, the efficacy of the methanol extract derived from the leaves was found to be superior to that of the aqueous and ethanol extracts when tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Trichophyton rubrum*.[49]

Minimum inhibitory concentration (MIC)

Leaves and peels extracts showed MIC values between 12.5 and 1.56 mg/mL (Figure 2). The best results (1.56 mg/mL) are shown by the ethanol extracts of peels and leaves on *S. aureus* multidrug-resistant strains. According to our study,[50] Zage *et al.* observed the lowest MIC value with the ethanol leaves extract (3.125mg/mL) against *Shigella* sp. Ugwu *et al.*[49] also observed the lowest MIC value, 0.76 mg/mL, for *Staphylococcus aureus* and *Pseudomonas aeruginosa* ATCC 27853 with the same solvent and leaves extract.

Total phenols and flavonoids contents

Total phenolics and flavonoids contents values were higher in peels than leaves and varied between 81 and 96 GAE mg/g, and 48 and 54 QE/g, respectively (Table 3). It has been confirmed that citrus peels and leaves are rich in phenols and flavonoids,[49,50,51] which have been linked to potent antibacterial properties.[52] These chemicals can alter the membrane permeability, impact enzymes involved in intracellular functions, interact with the cell membrane resulting in compromised structural integrity, and inhibit DNA and ATP synthesis.[53] Flavonoids, can also inhibit energy metabolism and DNA replication, modify intracellular pH, and disrupt ATP production pathways.[54]

Table 1: Antibiotic susceptibility of *S. aureus* strains according to CLSI guidelines

Chemical class	Antibiotics (concentration)	Sensitive (S)/ Intermediate (I)/ Resistant (R)		
		***S. aureus* SaS1**	***S. aureus* SaS2**	***S. aureus* SaS3**
β-lactam	Oxacillin (5 µg)	R	R	R
Macrolide	Erythromycin (15 µg)	R	R	R
Aminoglycoside	Kanamycin (30 µg)	R	R	Intermediate
Aminoglycoside	Neomycin (30 µg)	R	R	Intermediate
Tetracyclines	Tetracycline (30 µg)	R	R	R
β-lactam	Penicillin-G (10 µg)	R	R	R

Table 2: Mean inhibition zone diameters of *Citrus aurantifolia* extracts on the test bacteria

	Zone of inhibition (mm)*				
Bacterial strains	**Ampicillin**	**Leaves extracts**		**Peels extracts**	
Gram-negative bacteria	10 µg/disc	MeOH 80%	EtOH 70%	MeOH 80%	EtOH 70%
Escherichia coli ATCC 25922	17 ± 0.0 ^{a}	18 ± 0.0 ^{a}	14 ± 1.25 ^{b}	18 ± 0.5 $^{a\,c}$	16 ± 0.8 $^{a\,b}$
Pseudomonas aeruginosa ATCC 27853	17 ± 0.0 ^{a}	16 ± 0.0 ^{b}	16 ± 0.5 ^{c}	17 ± 0.5 ^{a}	20 ± 0.0 ^{c}
Gram-positive bacteria					
Staphylococcus aureus ATCC 25923	27 ± 0.5 ^{a}	19 ± 0.8 ^{b}	22 ± 0.5 ^{c}	21 ± 0.8 $^{b\,c}$	22 ± 0.5 ^{c}
Bacillus cereus ATCC 11778	11 ± 0.0 ^{a}	22 ± 0.8 ^{b}	21 ± 0.5 $^{b\,c}$	17 ± 0.5 ^{d}	19 ± 0.9 $^{c\,d}$
S. aureus SaS1	19 ± 0.0 ^{a}	20 ± 0.0 ^{a}	22 ± 0.5 ^{b}	18 ± 0.8 ^{ac}	19 ± 0.5 ^{a}
S. aureus SaS2	19 ± 0.5 ^{a}	21 ± 0.0 ^{b}	22 ± 0.0 ^{b}	19 ± 0.0 ^{a}	19 ± 0.9 ^{a}
S. aureus SaS3	20 ± 0.5 ^{a}	19 ± 0.5 $^{a\,b}$	21 ± 0.9 a	17 ± 0.0 ^{c}	18 ± 0.5 $^{b\,c}$

*Values are presented as the mean of triplicates ± standard deviation of 3 independent experiments. $^{a,b,c,\,d}$ in the lines indicated significant differences between treatments according to Tukey test ($p<0.05$).

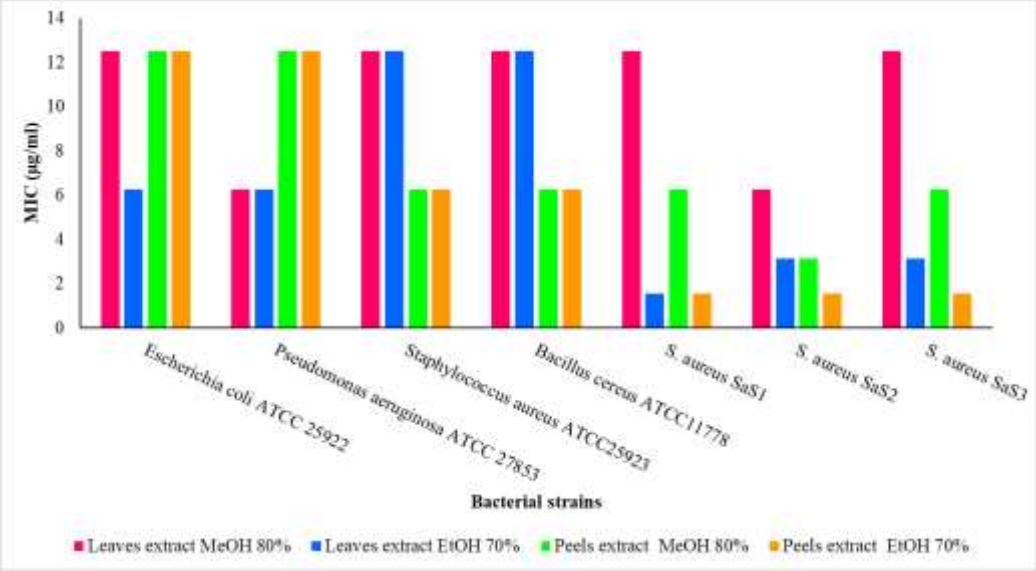

Figure 2: Minimum inhibitory concentrations of *C. autantifolia* extracts

Table 3: Phenolic and flavonoids contents of *Citrus aurantifolia* extracts

	Phenolic contents (GAE mg/g)		Flavonoids contents (QE mg/ g)	
Plant part	**MeOH 80%**	**EtOH 70%**	**MeOH 80%**	**EtOH 70%**
Leaves	75	74	42	37
Peels	96	81	54	48

Conclusion

In this study, methanol and ethanol extracts of *Citrus aurantifolia* leaves and peels showed important antimicrobial activity against three multi-resistant *Staphylococcus aureus* strains originating from raw milk, and pathogenic strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 11778. Based on the MIC values, the ethanol extracts exhibited the highest level of effectiveness. This suggests that *C. aurantifolia* could be a valuable source for discovering antimicrobial products. Moreover, additional investigation is required to elucidate the structure of bioactive compounds.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The author is grateful to Dr. Boussa Abdelhalim for providing reference strains.

References

1. El-Deeb W, Fayez M, Alhumam N, Elsohaby I, Quadri SA, Mkrtchyan H. The effect of staphylococcal mastitis including resistant strains on serum procalcitonin, neopterin, acute phase response and stress biomarkers in Holstein dairy cows. PeerJ. 2021; 9. doi: 10.7717/peerj.11511.
2. Liu H, Dong L, Zhao Y, Meng L, Wang J, Wang C, Zheng N. Antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from different raw milk samples in China. Front Microbiol. 2022; 13: 840670. doi: 10.3389/fmicb.2022.840670.
3. Becker K, Skov RL, von Eiff C. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. In: Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al. (Eds.). Manual of Clinical Microbiology. Washington, DC: ASM Press; 2015. 354–382 p.
4. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28(3): 603-61. doi: 10.1128/CMR.00134-14.
5. Rai A, Khairnar K. Overview of the risks of *Staphylococcus aureus* infections and their control by bacteriophages and bacteriophage-encoded products. Braz J Microbiol. 2021; 52(4): 2031-2042. doi: 10.1007/s42770-021-00566-4.
6. Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. *PLoS One*. 2017; 12:e0189621. doi: 10.1371/journal.pone.0189621.
7. Bengtsson B, Unnerstad HE, Ekman T, Artursson K, Nilsson-Öst M, Waller KP. Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. Vet Microbiol. 2009; 136(1-2): 142-149. doi: 10.1016/j.vetmic.2008.10.024.
8. Ruegg PL, Oliveira L, Jin W, Okwumabua O. Phenotypic antimicrobial susceptibility and occurrence of selected resistance genes in gram-positive mastitis pathogens isolated from Wisconsin dairy cows. J Dairy Sci. 2015; 98(7): 4521-4534. doi: 10.3168/jds.2014-9137.
9. Kovačević Z, Samardžija M, Horvat O, Tomanić D, Radinović M, Bijelić K, Vukomanović AG, Kladar N. Is there a relationship between antimicrobial use and antibiotic resistance of the most common mastitis pathogens in dairy cows? Antibiotics. 2023; 12(1). doi: 10.3390/antibiotics12010003.
10. Rajput M, Kumar N. Medicinal plants: A potential source of novel bioactive compounds showing antimicrobial efficacy

against pathogens infecting hair and scalp. Gene Rep. 2020; 21: 100879. doi:10.1016/j.genrep.2020.100879.
11. Reghu R, Sahadevan P, Sugathan S. Antimicrobial Agents from Plants. In: Sugathan S, Pradeep N, Abdulhameed S (Eds.). Bioresources and Bioprocess in Biotechnology. Springer, Singapore; 2017. 271-290 p. doi:10.1007/978-981-10-4284-3_11.
12. Gadisa E, Weldearegay G, Desta K, Tsegaye G, Hailu S, Jote K, Takele A. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. BMC Complement. Altern. Med. 2019; 19(24): 1-9.
13. Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN, Aladedunye FA. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. JAOCS. 2008; 85(4): 321-330. doi:10.1007/s11746-008-1204-3.
14. Food and Agriculture Organization of the United Nations (FAO) FAOSTAT. Crops and livestock products. [Online]. 2020 [Cited 2023 July 13] Available from: https://www.fao.org/faostat/en/#data/QCL [Cited 2023 July 13].
15. Andrade MA, Barbosa CH, Shah MA, Ahmad N, Vilarinho F, Khwaldia K, Silva AS, Ramos F. Citrus by-products: valuable source of bioactive compounds for food applications. Antioxidants. 2023; 12(38). doi: 10.3390/antiox12010038.
16. Mahato N, Sinha M, Sharma K, Koteswararao R, Cho MH. Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. Foods. 2019; 8: 523. doi:10.3390/foods8110523.
17. Panwar D, Saini A, Panesar PS, Chopra HK. Unraveling the scientific perspectives of citrus by-products utilization: Progress towards circular economy. Trends Food Sci Technol. 2021; 111: 549-562. doi:10.1016/j.tifs.2021.03.018.
18. Abayomi OJ, Oyewole AO, Olagunju JA, Akinola OO, Omotosho OE. Curative Effect of Aqueous Seed Extract of *Citrus paradisi* against Carbon Tetrachloride-Induced Nephrotoxicity in Wistar Rats. TJNPR. 2021; 5(4): 749–752. doi.org/10.26538/tjnpr/v5i4.25.
19. Laksemi DA, Tunas K, A Damayanti PA, Sudarmaja IM, Widyadharma IPE, D Wiryanthini IA, Linawati NM. Evaluation of antimalarial activity of combination extract of *Citrus aurantifolia* and honey against *Plasmodium berghei*–İnfected mice. TJNPR. 2023; 7(1): 2168–2171. doi: 10.26538/tjnpr/v7i1.13.
20. Shorinwa OA, Otu VS. Immunomodulatory activity of the aqueous extract of the pith of *Citrus limon* L. (Rutaceae) using cyclophosphamide induced myelosuppression. TJNPR. 2023; 7(3): 2660–2664. doi: 10.26538/tjnpr/v7i3.29.
21. Bellavite P, Donzelli A. Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. *Antioxidants*. 2020; 9(8):742. https://doi.org/10.3390/antiox9080742
22. Nakajima A, Ohizumi Y. Potential benefits of nobiletin, a citrus flavonoid, against Alzheimer's disease and Parkinson's disease. *Int J Mol Sci*. 2019; 20(14): 3380. https://doi.org/10.3390/ijms20143380
23. Goh JXH, Tan LT-H, Goh JK, Chan KG, Pusparajah P, Lee L-H, Goh B-H. Nobiletin and Derivatives: Functional compounds from citrus fruit peel for colon cancer chemoprevention. *Cancers*. 2019; 11(6): 867. https://doi.org/10.3390/cancers11060867
24. Lemes RS, Alves CCF, Estevam EBB, Santiago MB, Martins CHG, Santos TCLD, Crotti AEM, Miranda MLD. Chemical composition and antibacterial activity of essential oils from *Citrus aurantifolia* leaves and fruit peel against oral pathogenic bacteria. An Acad Bras Cienc. 2018; 90(2): 1285-1292. doi: 10.1590/0001-3765201820170847.
25. El-Desoukey RMA, Saleh ASB, Alhowamil HF. The phytochemical and antimicrobial effect of *Citrus sinensis* (Orange) peel powder extracts on some animal pathogens as eco-friendly. EC Microbiol. 2018; 14(6): 312-318.
26. Bambeni T, Tayengwa T, Chikwanha OC, Manley M, Gouws PA, Marais J, Fawole OA, Mapiye C. Biopreservative efficacy of grape (*Vitis vinifera*) and clementine mandarin orange (*Citrus reticulata*) by-product extracts in raw ground beef patties. Meat Sci. 2021; 181: 108609. doi: 10.1016/j.meatsci.2021.108609.
27. Torimiro N, Adegun B, Abioye O, Omole R. Antibacterial activity of essential oil from *Citrus aurantifolia* (Christm.) Swingle peels against multidrug-resistant bacterial isolates. AIM. 2020; 10: 214-223. doi: 10.4236/aim.2020.105017.
28. Evy Ratnasari Ekawati, Windarmanto, Sri Puji Astuti Wahyuningsih. Study of lime peel flavonoid as effectively antibacterial against Methicillin-resistant *Staphylococcus aureus*. RJPT. 2022; 15(7): 3002-8. doi: 10.52711/0974-360X.2022.00501
29. CLSI, 2020. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI Supplement M100. Wayne, PA: Clinical Laboratory Standards Institute; 2020.
30. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial disc susceptibility tests. Approved standard. NCCLS document M2-A5. Wayne, Pa: National Committee for Clinical Laboratory Standards; 1993.
31. Arruda HS, Pereira GA, Pastore GM. Optimization of extraction parameters of total phenolics from *Annona crassiflora* Mart. (Araticum) fruits using response surface methodology. Food Anal Methods. 2017; 10(1): 100-110. doi:10.1007/s12161-016-0554-y.
32. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and Bark extracts of *Zanthoxylum armatum* DC. Sci World J. 2020; 2020. doi: 10.1155/2020/8780704.
33. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3): 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.
34. Wendlandt S, Feßler AT, Monecke S, Ehricht R, Schwarz S, Kadlec K. The diversity of antimicrobial resistance genes among staphylococci of animal origin. Int J Med Microbiol. 2013; 303(6-7): 338-349. doi: 10.1016/j.ijmm.2013.02.006.
35. Mestrovic T, Robles Aguilar G, Swetschinski LR, *et al*. The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic analysis. Lancet Public Health. 2022; 7(11): e897-e913. doi:10.1016/S2468-2667(22)00225-0.
36. Lee JH, Cho S, Paik HD, Choi CW, Nam KT, Hwang SG, Kim SK. Investigation on antibacterial and antioxidant activities, phenolic and flavonoid contents of some Thai edible plants as an alternative for antibiotics. Asian-Australas J Anim Sci. 2014; 27(10): 1461-1468. doi: 10.5713/ajas.2013.13629.
37. Phattayakorn K, Wanchaitanawong P. Antimicrobial activity of Thai herb extracts against coconut milk spoilage microorganisms. Nat Sci. 2009; 43: 752-759.
38. Shakya A, Luitel B, Kumari P, Devkota R, Dahal PR, Chaudhary R. Comparative study of antibacterial activity of juice and peel extract of Citrus fruits. TUJM. 2019; 6: 82-88. doi:10.3126/tujm.v6i0.26589.
39. Abdallah EM. Preliminary phytochemical and antibacterial screening of methanolic leaf extract of *Citrus aurantifolia*. Pharm Biotechnol Curr Res. 2016; 1:1.
40. Rawat S, Jugran AK, Bahukhandi A, Bahuguna A, Bhatt ID, Rawal RS, Dhar U. Anti-oxidant and anti-microbial properties of some ethno-therapeutically important medicinal

plants of Indian Himalayan Region. 3 Biotech. 2016; 6:154. doi: 10.1007/s13205-016-0470-2. Epub 2016 Jul 18.
41. Ouerghemmi I, Bettaieb Rebey I, Rahali FZ, Bourgou S, Pistelli L, Ksouri R, Marzouk B, Tounsi MS. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. J Food Drug Anal. 2017; 25(2): 350-359. doi: 10.1016/j.jfda.2016.04.001.
42. Inouye S, Yamaguchi H, Takizawa T, Inouye S, Yamaguchi · H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother. 2001; 7: 251-254. doi: 10.1007/s101560170022.
43. Al Ubeed HMS, Bhuyan DJ, Alsherbiny MA, Basu A, Vuong Q V. A comprehensive review on the techniques for extraction of bioactive compounds from medicinal Cannabis. Molecules. 2022; 27: 604. doi:10.3390/molecules27030604.
44. Akinmoladun AC, Falaiye OE, Ojo OB, Adeoti A, Amoo ZA, Olaleye MT. Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of *Chrysophyllum albidum* G. Don (African Star Apple). Bull Natl Res Cent. 2022; 46(1). doi:10.1186/s42269-022-00718-y.
45. Pasaribu F, Ervina I, Suryanto D. The effectiveness antimicrobial activity test of citrus peel extract on some periodontal pathogenic bacteria (*In vitro*). Int J Appl Dent Sci. 2018; 4(3): 146-150.
46. Singhapol C, Tinrat S. Virulence genes analysis of *Vibrio parahaemolyticus* and anti-*Vibrio* activity of the citrus extracts. Curr Microbiol. 2020; 77(8): 1390-1398. doi:10.1007/s00284-020-01941-4.
47. Munawaroh R, Yani JA, Pos T, Kartasura P. Optimum conditions for extraction of antibacterial compounds from *Citrus aurantifolia* fruit peel waste. PHA. 2017; 18: 34-38.
48. Rumzum Bhuiyan F, Hasan M, Abdus Shukur Imran M, Rashel Ahmed S, Shukur Imran A, Shanzana P. Antimicrobial activity screening for three Citrus pulp extracts and phytochemical constituency profiling. J Pharmacogn Phytochem. 2019; 8(4): 157-161.
49. Ugwu CC, Mbah-Omeje KN, Ezeugwu RI, Onuorah SC, Agbo MC. Antimicrobial activities and phytochemical screening of *Citrus aurantifoila* (Lime) leaf extracts and fruit juice on some microorganisms. IJIRD. 2018; 7(3). doi:10.24940/ijird/2018/v7/i3/mar18031.
50. Zage AZ, Tajo S, Ali M. Antibacterial activity of *Citrus aurantifolia* leaves extracts against some enteric bacteria of public health importance. MAMS. 2018; 1(2): 33-38. doi:10.32474/mams.2018.01.000107.
51. Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Ahmad Nayik G. Citrus peel as a source of functional ingredient: A review. J Saudi Soc Agric Sci. 2018; 17(4): 351-358. doi:10.1016/j.jssas.2016.07.006.
52. Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, Degraeve P, Bordes C. Antibacterial properties of polyphenols: Characterization and QSAR (Quantitative structure-activity relationship) models. Front Microbiol. 2019; 10: 829. doi:10.3389/fmicb.2019.00829.
53. Efenberger-Szmechtyk M, Nowak A, Czyzowska A. Plant extracts rich in polyphenols: antibacterial agents and natural preservatives for meat and meat products. Crit Rev Food Sci Nutr. 2021; 61(1): 149-178. doi: 10.1080/10408398.2020.1722060.
54. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem. 2014; 22(1): 132-149. doi: 10.2174/0929867321666140916113443.