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COMPARATIVE STUDY OF QUERCETIN & LEMON PEEL EXTRACT ON MULTIPLE DRUG RESISTANT BACTERIA

Madhuri Patil[1], *Renu Jaisinghani**[1], *Ravindra Sharma*[2], *Rohini Patil*[2]

Address(es):
[1] Smt. Chandibai Himathmal Mansukhani College, Department of Microbiology, Railway Station Road, Ulhasnagar- 421003, Maharashtra.
[2] R.K.Talreja College, Department of Microbiology, Chatrapati Shivaji Maharaj Chowk, Ulhasnagar- 421003, Maharashtra.

*Corresponding author: renu.nj24@gmail.com

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ABSTRACT

The current investigation explores the antibacterial properties of Quercetin, a flavonoid found in plants, and lemon peel extract, renowned for its rich bioactive content, against multiple drug resistant bacteria. The study method involved the identification of multidrug-resistant bacteria through antimicrobial susceptibility testing (AST). Nine standard bacterial strains exhibited resistance to different antibiotics, with a MAR index exceeding 0.10, including Ampicillin, Vancomycin, Polymyxin B, Nitrofurantoin, Trimethoprim, and Penicillin. In the present study bioactive compounds from lemon peel were extracted using two solvents, methanol and water, and their efficacy was assessed against drug-resistant bacteria. Minimum Inhibitory Concentration (MIC) values, determined by using the well plate method, revealed higher MIC values for the water extract (LPWE) compared to the methanol extract (LPME) and quercetin. For instance, the MIC for LPWE against *E. hermanniensis* ATCC 700323 was 140 mg/ml, whereas it was 50 mg/ml for quercetin & 1 mg/ml for LPME. From the results, it can be interpreted that Methanol extract was most effective against drug-resistant bacteria when compared to water extract and quercetin.Furthermore, GC-MS analysis was performed to analyze the chemical entities present in each extract. Given the activity of LPME, LPWE, and Quercetin against both Gram-negative bacteria and Gram-positive bacteria, these extracts hold promise as broad-spectrum antibacterial agents applicable in various fields such as medicine, food, and cosmetics.

Keywords: Quercetin, Lemon Peel Extract, AST, MIC, MDR

INTRODUCTION

Quercetin is a plant flavone from the flavonoid group of polyphenols found in many fruits, vegetables, red onions etc. It is a yellow, crystalline, water insoluble substance found in citrus fruits. Different sources of Quercetin are red onion, apple, spinach, tea, cranberries etc. (**Sagi S.S., 2021**). It is known to have anti-carcinogenic,antioxidant, anti-inflammatory, antibacterial, antiviral&antifungal properties (**Azeem M.*et al.*, 2022**). Quercetin also has GRAS (Generally Recognized as Safe) status upto a level of 500mg/day assigned by U.S. Food & Drug Administration (USFDA) (**Mohtashami E. *et al.*, 2020**). Lemon, a Rutaceae family member, is a valuable medicinal plant recognised for its alkaloids, which has been reported to exhibit anticancer properties and antibacterial effects in various parts such as leaves, stem, root, and flower (**Naseer S. *et al.*, 2018**). *Citrus limon*, commonly known as lemon, ranks third among citrus species, following oranges, with global production during the 2001/2002 season exceeding 4.4 million tons. Lemon peel, a by-product obtained after processing of lemon juice, holds significant potential for utilization. It comprises two main tissues: the Flavedo, the outer colorful layer ranging from green to yellow, known for its abundance of essential oils (**Li H. *et al.*, 2019**), and the Albedo, the primary component beneath the Flavedo, characterized by its spongy and cellulosic nature (**Mhgub I. M. *et al.*, 2018**).

Lemon fruits are rich inwide variety of natural chemical substances, like phenolic compounds (mainly flavonoids) and other nutrients and non-nutrients (dietary fiber, minerals, carotenoids, essential oils, and vitamins) (**Belbase C.*et al.*, 2022**). Citrus flavonoids have a broad range of biological activity, which includes antifungal, antibacterial, antidiabetic, antiviral, and anticancer due to their naturally ocurring antioxidant characteristics (**Burt, 2004; Ortuno *et al.*, 2006**). Flavonoids have the potential to function as free radical scavengers and antioxidants with the ability to inhibit the activity of enzymes and inhibit cell proliferation (**Jucá M. M. *et al.*, 2020**). They seem to be playing a defensive role against invasive pathogens such as viruses, fungus and bacteria in plants. Preparations made from the peel, flowers, and leaves of orange (*Citrus aurantium* L) are frequently utilized to lessen central nervous system disorders (**Mejri H. *et al.*, 2022**). Despite citrus fruits being widely consumed globally as raw produce and juice, the peel is commonly disposed of as waste. However, the peel contains a diverse array of secondary components that has remarkable antioxidant activity, surpassing that of other parts of the fruit (**Russo C. *et al.*, 2021**). The global production of citrus fruit has seen a notable rise in recent years, reaching 166 million tons in the period of 2022-2023. Oranges, being the most commercially significant citrus fruit, contribute roughly around 48 million tons to this total, with about 41% utilized for production of juice, which results in approximately 44% peel as a by-product. Compounds extracted from fruit peel show potential within the food industry as an abundant reservoir of bioactive elements. Furthermore, the utilization of citrus peel would aid in mitigating pollution issues stemming from the improper disposal of such residues. (**Rafiq S. *et al.*, 2018**).

Resistance to antimicrobial agents, particularly antibiotics, is a significant worldwide concern. The challenges in treating resistant microbes pose a barrier to discovering new drugs that can effectively combat these resilient pathogens (**Li B. & Webster T.J., 2018**). Flavonoids have been found to possess a variety of biological functions and therefore have been the subject of medical research. These functions include antimicrobial, antitumor, vascular activity, antimalarial activity etc. they have also been used for treatment of diabetes mellitus & skin infection (**D'Arcy, M. S., 2022**). Thus, the current study evaluates the effect of methanolic and water lemon peel extract on multidrug resistant (MDR) bacteria. It involves determining the minimum inhibitory concentration (MIC) of Quercetin and lemon peel extract against standard bacterial strains, as well as conducting GC-MS analysis of the lemon peel extracts.

MATERIAL AND METHODS

Materials

The lemon peels were obtained from a fruit juice center located in Ambernath, Mumbai, Maharashtra. Nutrient Agar (M001) and Mueller Hinton Agar (M173), Antibiotic discs were purchased from HiMedia, Mumbai while the Methanol and Quercetin Dihydrate was purchased from Sigma Aldrich, Mumbai. Pure standard ATCC cultures of *Enterococcus hermanniensis* ATCC 700323, *Staphylococcus aureus* ATCC BAA 1026, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Stenotrophomonas maltophilia* ATCC 17666, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538, that were used in the study were provided by Department of Microbiology, Smt. C.H.M. College, Dist. Thane, Maharashtra.

Methods

Culture Maintenance

Pure standard ATCC cultures of test organisms were subcultured on sterile nutrient agar slants and then incubated for 24 hrs at 37°C. After the incubation period, the subcultured organisms were grown on nutrient agar plates. Subsequently, cultures were inoculated in Trypticase soy broth for their enrichment. (**Fouda A. *et al.*, 2021**).

Lemon peel extract preparation

Citrus fruit peel samples were prepared into extracts using the method outlined by **Irkin *et al.*** (**2015**), with slight modification.The peels were meticulously rinsed under running water and then washed with sterile purified water. Subsequently, they were air-dried for two days at 40°C, grounded into a fine powder using a mixer grinder which was sterilized, and stored in storage containers (air-tight). For preparation of lemon peel extract, dried lemon peel powder was mixed in solvents (Methanol, Distilled Water separately) to make methanol, water extracts respectively. Lemon peel powder and solvent were mixed in 1:10 proportion and kept for 48 hours at room temperature on the shaker. Water extract was prepared by boiling the lemon peel powder till the volume was reduced to ¼ th, filtrated and kept at 500C for drying. For the solvent extracts, the extracts were filtered by using Whatman filter paper No.1 to remove unwanted particles and were concentrated under vacuum using a rotary vacuum evaporator (Trident Labortek, India) below 40°C. The resulting dried extracts after exposure to the UV rays for 2 hrs was assessed for sterility on nutrient agar media plates. Subsequently, they were stored in Labelled sterile bottles at 4°C until further use.

Antimicrobial Susceptibility testing (AST) by disc diffusion Method

The clinical microbiology laboratory's proficiency in conducting AST is crucial for determining susceptibility to selected empirical antimicrobial agents and then identifying resistance in individual bacterial isolates. The primary objective of such test is to detect potential resistance to drug in common pathogens and confirm susceptibility to drugs selected for specific infections. The disk diffusion susceptibility method, being straightforward and pragmatic, has been thoroughly standardized. In this study, the disk diffusion susceptibility method was employed. The test involved applying a bacterial inoculum, prepared from a 24-hour-old culture suspension of approximately 1×10^8 cfu/ml, onto the surface of Muller-Hinton (MH) agar plates using a swab. Subsequently, paper antibiotic disks were positioned on the MH agar plate with sterile forceps, and the plates were then incubated for 24 hours at 37°C. Following incubation, the zones of growth inhibition surrounding each antibiotic disk were measured. (**Sakarikou C. *et al.*, 2018**). The zone diameters of each drug were elucidated by using the criteria published by CLSI or Kirby- Bauer's chart (**Iqubal *et al.*, 2020**). The MAR (Multiple antibiotic resistances) index of each organism was calculated by using the below formula (Kusuma SAF et al., 2021). MAR index (A/B) = Number of antibiotics resistant (A) ÷ Number of antibiotics used (B).

Minimum inhibitory concentration (MIC) determination

For MIC determination Agar well diffusion method was used for all the pure standard ATCC organisms mentioned in the materials section. The concentrations of extracts i.e. Methanolic extract (LPME), Water extract (LPWE) and Quercetin dilutions were prepared in the range of 1mg/ml to 100mg/ml, 20mg/ml to 200mg/ml and 10mg/ml to 100mg/ml respectively, by using 25% DMSO (Dimethyl sulphoxide) as diluent (**Bush A.A. *et al.*, 2020**). Concentration of stock solutions was prepared ranging from 1mg/ml to 200mg/ml by using 25% DMSO (Dimethyl sulphoxide) as diluent. Highest concentration used for LPME & Quercetin was 10mg/ml and that of for LPWE was 200mg/ml ranging from 20mg/ml to 200mg/ml of the extract. The agar well diffusion test was conducted using Mueller and Hinton (MH) agar media. The inoculum was prepared using a 24 hrs-old bacterial culture, and the suspension was made with sterile peptone water. The suspension's turbidity was adjusted using a spectrophotometer at 530 nm to match the concentration of 0.5 McFarland standards. 20 milliliters of MH agar butt were inoculated with 1 ml of the test organism suspension and poured into empty sterile petri plates, allowing them to cool down. Once the medium solidified, four wells, with diameter of 10 mm each, were made in the agar using a sterile cork borer. Next, 100 µl of each concentration of the antibacterial substance, such as lemon peel extracts and quercetin, were added into each well. The plates were then incubated at 37°C for 24 hours. After the incubation period, the zone of inhibition was measured in millimeters. Controls were performed concurrently, and these tests were carried out in triplicate. (**Malhotra-Kumar S. *et al.*, 2018**).

GC-MS analysis of Lemon peel extracts

The samples of Lemon peel extract i.e. (Methanolic and water extract) were subjected to gas chromatography-mass spectrometer analysis for identification of compounds present in the extracts. The extracts were processed for GC-MS by dissolving it in 60% methanol. To ensure the removal of any debris, filtration was carried out twice for each sample. The column was washed with 60% methanol before the samples were subjected to analysis.The analysis was carried out on AccuTOF GCV JMS-T100GCV(Agilent Technologies, Santa Clara, CA) present at SAIF laboratory, IIT Bombay. Compounds were separated on an HP5 column of dimensions 30m×0.2mm× 2mm, temperature programmed at 80°C to 250°C with a hold time of 30-40 min. Helium was utilized as the carrier gas at a flow rate of 1 milliliter per minute. The temperature of the injection port was set to 250°C, and under manual injection mode. Mass spectrometric detection parameters were configured as mentioned below, ionization method Electron Impact (EI), electron energy set at 70 electron volts (eV), and the ion source temperature maintained at 230°C. Identification was performed by comparing the mass spectra with reported libraries (**Ramu R. *et al.*, 2021**).

RESULTS AND DISCUSSION

Antimicrobial susceptibility test (AST)

Antimicrobial Susceptibility Test (AST) of the selected organism was done by Disc diffusion method using the Kirby Bauer technique (**Segawa I. *et.al.*, 2020**) and as per recommendation of NCCLS all test was performed on Muller- Hinton agar. The antibiotic susceptibility pattern of each organism was studied using different antibiotics for different organisms as shown in Table 1.
All the isolates exhibited resistance to more than 60% of antibiotics used. All the nine organisms were selected for further study.

Minimum Inhibitory Concentration (MIC) determination

Two different lemon peel extracts (methanol extract and water extract) were used for minimum inhibitory concentration against nine different drug resistant organisms including *Enterococcus hermanniensis* ATCC 700323, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC BAA 1026, *Staphylococcus aureus* ATCC 29213, *Stenotrophomonas maltophilia* ATCC 17666, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 27853.
Lowest concentration showing inhibition was considered as MIC (minimum inhibitory concentration) of that antimicrobial agent here extracts. The antimicrobial activity assessments were conducted in triplicate, and the outcomes are reported as the average of three measurements.

MIC of Lemon Peel Methanol Extract (LPME)

The methanol extract concentration used to study MIC ranges from 1 mg/ml to 100mg/ml. The inhibitory action of methanol extract was exhibited at 1mg/ml yeilding an inhibition zone of 15 ± 0.8 mm against *Enterococcus hermanniensis*. Notably, the extarct exhibited its highest inhibition against *Enterococcus hermanniensis* at 1 mg/ml concentration followed by *S. aureus* up to concentration of 50 mg/ml and other organisms were inhibited between 60 mg/ml to 100mg/ml concentrations. Against the methanolic extract *Escherichia coli* exhibited the lowest inhibition at the concentration of 100 mg/ml. Furthermore, Minimum Inhibitory Concentration (MIC) assessments were conducted to provide quantitative insights into the efficacy of the studied extracts, corroborating the earlier findings. MIC values of the methanolic extract against various isolates are detailed in Table 2. Findings reported by Saleem, and his coworker demonstrate that the methanol extract derived from lemon peel exhibits greater efficacy as an antimicrobial agent against gram-negative bacteria when compared to gram-positive (**Saleem M. & Saeed M.T., 2020**). Researchers conducting antimicrobial activity studies using methanol solvent extracts have reported similar results for *Pseudomonas aeruginosa*, *E. coli*, and *E. faecalis* bacteria. (**Liya S.J. & Siddique R., 2018**).

Table 1 Antibiotic susceptibility testing (AST) of bacterial strains

Antibiotics Used	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 6538	*E. faecalis* ATCC 29212	*E. hermanniensis* ATCC 700323	*S. aureus* ATCC 29213	*S. aureus* ATCC BAA1026	*S. maltophilia* ATCC 17666	*S. aureus* ATCC 25923
Amikacin	S	S	S	-	-	S	R	S	S
Ampicillin	R	S	R	R	R	R	R	-	R
Chloramphenicol	S	-	S	S	S	S	S	S	S
Nalidixic acid	S	-	-	-	-	-	-	S	-
Ciprofloxacin	S	S	S	I	S	S	R	S	S
Tetracycline	S	-	S	S	S	S	S	S	S
Erythromycin	-	-	S	I	R	S	R	-	S
Streptomycin	S	-	S	-	-	S	S	-	S
Rifampicin	-	S	S	S	R	S	S	-	S
Vancomycin	R	-	S	I	S	S	S	-	S
Polymyxin B	R	R	-	-	-	-	-	R	-
Ofloxacin	I	S	S	-	-	S	R	S	S
Trimethoprim	S	-	S	R	R	S	S	R	S
Nitrofurantoin	S	R	S	I	S	S	R	-	S
Amoxicillin	I	R	-	-	-	-	-	-	-
Gentamicin	S	S	S	-	-	R	R	S	S
Kanamycin	S	-	S	S	S	S	R	-	S
Tobramycin	S	S	S	-	-	S	R	-	S
Azithromycin	-	S	S	-	-	S	R	-	S
Penicillin	-	R	R	S	S	R	R	-	R
Oxacillin	-	-	R	-	-	R	R	-	R
Clindamycin	-	-	S	-	-	S	R	-	S
Bacitracin	-	-	S	-	-	S	S	-	S
MAR INDEX	0.18	0.33	0.15	0.18	0.36	0.20	0.65	0.22	0.15

Legend: S – susceptible, R – resistance, I – intermidiate, - –not used

Table 2 Antibacterial activity of Lemon Peel Methanol extract against drug Resistant Organisms

Concentration of Methanol Extract (mg/ml)	*Zone of Inhibition (mm)**								
	E. coli ATCC25922	*P. aeruginosa* ATCC 27853	*E. faecalis* ATCC 29212	*E. hermanniensis* ATCC 700323	*S. aureus* ATCC 29213	*S. aureus* ATCC 6538	*S. aureus* ATCC 25293	*S. maltophilia* ATCC 17666	*S. aureus* ATCC BAA1026
1	-	-	-	15 ± 0.8	-	-	-	-	-
2.5	-	-	-	16 ± 1.7	-	-	-	-	-
5	-	-	-	16 ± 0.1	-	-	-	-	-
7.5	-	-	-	17 ± 0.7	-	-	-	-	-
10	-	-	-	17 ± 0.5	-	-	-	-	-
20	-	-	-	18 ± 0.9	-	-	-	-	-
30	-	-	-	19 ± 1.4	-	-	-	-	-
40	-	-	-	20 ± 0.8	-	-	-	-	-
50	-	-	15 ± 0.3	20 ± 0.5	-	-	12 ± 1.1	-	12 ± 0.9
60	-	16 ± 0.1	15 ± 0.2	21 ± 0.7	13 ± 1.0	-	11 ± 1.9	-	13 ± 0.3
70	-	17 ± 0.4	15 ± 0.1	21 ± 0.2	13 ± 0.4	12 ± 1.1	13 ± 0.3	-	14 ± 0.6
80	-	18 ± 0.5	17 ± 0.1	22 ± 0.4	14 ± 0.3	13 ± 0.9	12 ± 0.5	12 ± 0.3	14 ± 0.5
90	-	18 ± 0.6	17 ± 0.7	20 ± 2.7	15 ± 0.3	13 ± 0.2	12 ± 0.5	12 ± 0.3	15 ± 0.2
100	13 ± 0.9	17 ± 1.0	16 ± 0.2	21 ± 1.5	17 ± 0.5	14 ± 0.3	13 ± 0.5	13 ± 0.3	16 ± 0.3

Legend: - – No Inhibition (0mm)
* Mean value ± SD, n = 3.

MIC of Lemon Peel Water Extract (LPWE)

The Lemon peel water extract concentration used to study MIC of drug resistant isolates ranged from 20mg/ml to 200mg/ml.Water extract exhibited highest inhibition against *Pseudomonas aeruginosa* and *Enterococcus hermanniensis* at concentrations of 60 mg/ml and140 mg/ml respectively. Most of the organisms were inhibited at the higher concentration ranging from 140-200mg/mL. Water extract is less effective as compared to that of methanolic extract, therefore the concentration required to inhibit organism by water extract is high when compared to methanol extract. The lowest inhibition of the organism was seen against *S.aureus* BAA1026 at concentration of 200mg/ml. It should be emphasized that the extracts exhibit effectiveness against almost all of the studied gram-negative bacteria and gram-positive bacteria. But they demonstarte minimal efficacy against *S. aureus* ATCC BAA1026 and *S. aureus* ATCC 29213.These results exhibit that both the extracts were effective against organisms but at different concentrations, MIC values of water extract against different isolates are mentioned in Table 3. In a study by, Mehmood T. and coworkers they reported that the stage of ripening can also impact the results (**Mehmood T. *et al., 2018***). They found that when compared to water extract of unripe lemon the water extract of ripe lemon exhibited higher zone of inhibition. In this study when compared to methanol extract and quercetin water extract exhibited a higher zone of Inhibition. Similiarly in a study conducted by Saleem M. and his team they also reported same results for antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli,* and based on methanol solvent extract *(***Saleem M. & Saeed M.T., 2020***)*.

MIC of Quercetin

The quercetin concentration used to evaluate their antibacterial activity against drug resistant bacteria ranged from 10mg/ml to 100mg/ml. At the concentration of 50mg/ml Quercetin exhibited highest inhibition against *S. aureus* 6538 and *E. Hermanniensis* ATCC 700323 and the lowest inhibition was observed against *S.aureus* ATCC 29213 at the concentration of 90mg/ml. No antibacterial activity was observed against *S. aureus* BAA1026 and *E. coli* ATCC25922 by Quercetin. Concentrations required to inhibit the growth of other organisms ranged from 60mg/ml to 100mg/ml. Compared to all extracts used in this study the MIC value of Quercetin is less. This may be due to the fact that the antibiotic compound is pure and more refined compared to the extracts used in this study. MIC values of quercetin against different isolates are mentioned in Table 4. In a study done by M.G. Shehata and coworkers they reported that when phenol is used as a solvent in lemon peel extraction the yield obtained was highest and the extract exhibited strong antibacterial activity in lemon peel (**Shehata *et al.*, 2021**). But in this study we found that the highest antibacterial activity was exhibited by LWPE. This difference could possibly be attributed to the extraction method, the difference in phytochemical composition in different parts of the plant, and genotypes differences of the citrus plants used in the research.

Table 3 Antibacterial activity of Lemon Peel Water extract against drug Resistant Organisms

Concentration of Water Extract (mg/ml)	*Zone of Inhibition (mm)**								
	E. coli ATCC25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 6538	*E. faecalis* ATCC 29212	*E. hermanniensis* ATCC 700323	*S. aureus* ATCC 29213	*S. aureus* ATCC 25293	*S. aureus* ATCC BAA1026	*S. maltophilia* ATCC 17666
20	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
60	-	11 ± 0.3	-	-	-	-	-	-	-
80	-	13 ± 0.3	-	-	-	-	-	-	-
100	-	13 ± 0.4	-	-	-	-	-	-	12 ± 0.3
120	-	15 ± 0.5	-	-	-	-	-	-	13 ± 0.4
140	13 ± 0.5	16 ± 0.2	-	-	12 ± 0.7	-	-	-	13 ± 0.4
160	15 ± 0.4	18 ± 0.3	-	11 ± 0.5	12 ± 0.8	-	14 ± 0.4	-	13 ± 0.3
180	16 ±0.5	18 ± 0.5	14 ± 0.6	11 ± 0.8	14 ± 0.9	13 ± 0.3	15 ± 0.2	-	14 ± 0.3
200	16 ±0.5	18 ± 0.4	14 ± 0.3	13 ± 0.3	15 ± 0.3	15 ± 0.3	15 ± 0.2	12 ± 0.5	14 ± 0.7

Legend: - – No Inhibition (0mm) * Mean value ± SD, n = 3.

Table 4 Antibacterial activity of Quercetin against Drug Resistant Organisms

Concentration of Quercetin (mg/ml)	*Zone of Inhibition (mm)**								
	*E. coliATCC*25922	*E. faecalis* ATCC 29212	*P. aeruginosa* ATCC 27853	*E. hermanniensis* ATCC 700323	*S. aureus* ATCC 29213	*S. aureus* ATCC 6538	*S. aureus* ATCC 25293	*S. aureus* ATCC BAA1026	*S. maltophilia* ATCC 17666
10	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-
50	-	-	-	11 ± 0.3	-	13 ± 0.8	-	-	-
60	-	-	14 ± 0.8	11 ± 0.2	-	14 ± 0.8	-	-	-
70	-	-	14 ± 0.3	12 ± 0.8	-	14 ± 0.7	-	-	12 ± 0.3
80	-	10 ± 0.3	15 ± 0.4	13 ± 0.3	-	14 ± 1.0	12 ± 0.4	-	12 ± 0.5
90	-	12 ± 1.1	15 ± 0.6	11 ± 0.4	14 ± 0.8	13 ± 0.6	13 ± 0.7	-	14 ± 1.2
100	-	12 ± 1.4	16 ± 0.8	13 ± 0.6	15 ± 1.1	14 ± 0.3	13 ± 1.3	-	15 ± 0.8

Legend: - – No Inhibition (0mm) * Mean value ± SD, n = 3.

From the above data, it can be interpretated that against all the organisms the methanol extract was more effective than water extract and quercetin. The results also revealed that the peel extracts were potent in inhibiting microbial growth. While water extract was effective against organisms only at higher concentrations the LPME extract was more effective at concentration of 100mg/ml exhiobiting inhibition against all organisms. Quercetin was more effective against *Enterococcus* when compared with water extract. Out of these 9 organisms *Enterococcus hermanniensis* and *Pseudomonas aeruginosa* were more sensitive to all the extracts as well as to Quercetin.

Results of antibacterial activity of lemon peel extracts suggested that *E. coli* was most resistant strain to plant extracts and as well as to Quercetin followed by *Stenotrophomonas* and *S. aureus* BAA1026 while *Pseudomonas* and *Enterococcus* were the most susceptible strains against the LPWE and LPME (Table 5.). Higher concentration (upto 200mg/ml) of LPWE may be required to be effective against drug resistant organisms.

Table 5 MIC of LPME, LPWE and Quercetin against selected organisms

Organisms	MIC (mg/ml)		
	LPWE	*LPME*	*Quercetin*
*E. coliATCC*25922	140	100	-
E. faecalis ATCC 29212	160	50	80
P. aeruginosa ATCC 27853	60	60	60
E. hermanniensis ATCC 700323	140	1	50
S. aureus ATCC 29213	180	60	90
S. aureus ATCC 6538	180	70	50
S. aureus ATCC 25293	160	50	80
S. aureus ATCC BAA1026	200	50	-
S. maltophilia ATCC 17666	100	80	70

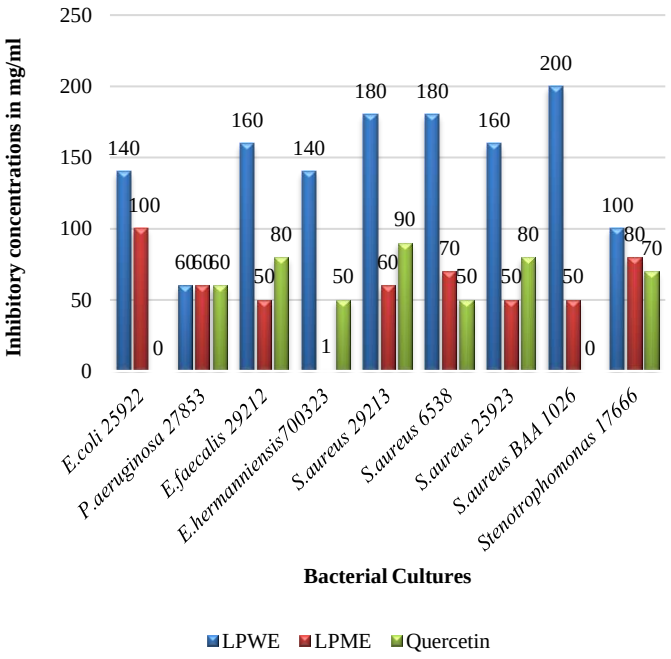
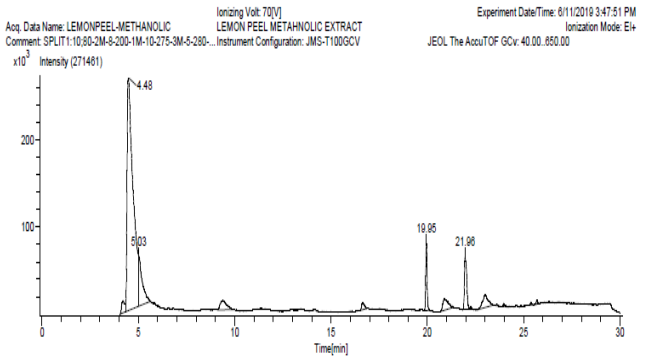


Figure 1 MIC of Lemon peel Methanol extract, Water extract and Quercetin against selected organisms

GC-MS analysis of Lemon Peel Extracts

Analysis of Lemon Peel Methanolic Extract

The lemon peel methanolic extracts GC-MS analysis exhibited the presence of 14 different compounds. The below chromatogram presents the chemical compounds identified in methanolic extract.



Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	4.20	BV	0.2275	158083.99	13993.20		4.06	61	4.35	2719
2	4.48	VV	0.3301	5789347.54	267505.60		4.35	2719	5.03	9087
3	5.03	VB	0.1654	688358.90	57324.98		5.03	9087	5.63	14652
4	9.38	BB	0.3783	250717.98	10837.03		9.09	4764	9.97	5153
5	16.64	BB	0.1796	77888.49	8017.93		16.46	3761	16.86	6462
6	19.95	BB	0.0750	384966.19	77683.83		19.84	3713	20.12	4070
7	20.88	BB	0.3843	242206.48	12986.75		20.68	3161	21.34	7782
8	21.96	BV	0.1352	532646.69	61393.51		21.83	4742	22.21	4800
9	22.27	VB	0.0833	21192.76	4004.76		22.21	4600	22.41	4523
10	23.01	BB	0.3022	286415.82	14494.74		22.63	5088	23.47	10628
11	23.61	BB	0.0645	7272.48	1693.55		23.55	9754	23.70	9145
12	23.97	BB	0.0649	14217.96	3528.62		23.88	8427	24.04	8953
13	25.37	BB	0.0970	19654.02	3475.08		25.30	9963	25.50	10357
14	25.68	BB	0.0746	23920.32	4834.66		25.60	11092	25.80	11771

Figure 2 Chromatogram of Lemon Peel Methanol Extract

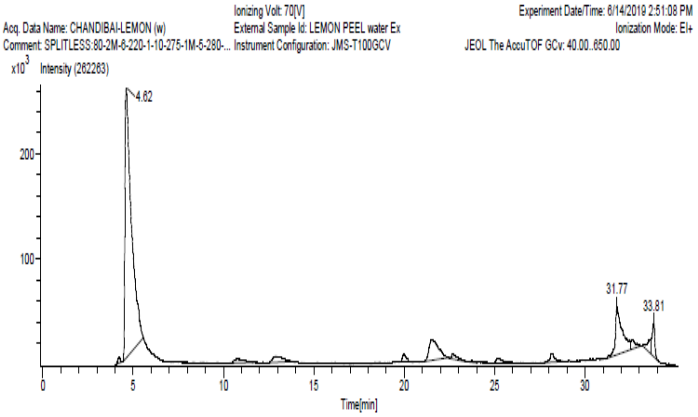
Table 6 Various constituents present in LPME extract after GC-MS Analysis

Sr. No.	Retention Time(min)	Name of Compound	Molecular Formula	Molecular Weight
1	4.20	Cyclopropyl carbinol	C_4H_8O	72
2	4.48	2,5-Furandione, dihydro-3-methylene	$C_5H_4O_3$	112
3	5.03	Proline,3-4-didehydro	$C_5H_7NO_2$	113
4	9.38	Glycidol	$C_3H_6O_2$	74
5	16.64	Esculetin	$C_9H_6O_4$	178
6	19.95	Pentadecanoicacid, 14-methyl, methyl ester	$C17H_{34}O_2$	270
7	20.88	n-decanoic acid	$C_{10}H_{20}O_2$	172
8	21.96	1-Tridecyne	$C_{13}H_{24}$	180
9	22.27	Pentanoic acid,4-methyl	$C_{16}H_{12}O_2$	116
10	23.01	Anthracene,9-butyltetradecanhydro	$C_{18}H_{32}$	248
11	23.61	9-Hexadecanoicacid, hexadecyl ester	$C_{32}H_{62}O_2$	478
12	23.97	Kryptogenin 2,4-dinitrophenyl hydrazone	$C_{39}H_{50}$	790
13	25.37	2(1,2-dihydroxyethyl)-9-(b-d-ribofuranosyl) hypoxanthine	$C_{12}H_{16}N_4O_7$	328
14	25.68	6,9,12-octadecatrienoic acid	$C_{25}H_{36}O_2$	368

The major constituents present in Methanol Extract are tabulated in Table 6. CyclopropylCarbinol serves as an intermediate in agrochemicals, pharmaceuticals, and various organic synthesis. Additionally, it finds applications polymer additives, electronics chemicals, adhesives, coatings, surfactants, and other diverse fields (**Ntushelo K., 2016**). **Pinski *et al*.**, (**2021**) has reported that 3,4-Dehydro-L-proline serves as both a substrate and inhibitor of various enzymes. It acts as an alternate substrate for the amino acid oxidase (NikD) which inhibits collagen secretion by chondrocytes. On the other hand, glycidol an epoxide functions as a chemical intermediate in manufacturing of glycidyl urethanes, pharmaceuticals, functional epoxides, and other goods. Additionally, it is employed as a reactive diluent in epoxy resin systems and as a sterilant (**Cespi *et al*., 2016**). Esculetin has been used extensively not only as an antitussive agent and expectorant but also as a therapeutic option for antioxidant, antibacterial, anti-inflammatory, and antitumour purposes (**Liang *et al.*, 2017**). Pentadecanoic acid, 14-methyl ester, a member of the fatty acid family exhibits antifungal and antibacterial activity. Moreover, it also serves as a biomarker for rheumatoid arthritis detection (**Beschi *et al*., 2021**). Duffy and his team (**2018**) have reported that n-decanoic acid serves various roles, including as an anti-inflammatory agent, an antibacterial agent, a component of volatile oils, a human metabolite, and metabolites in both plants and algae. Hence, from the Chromatogram we can conclude that the methanol extract of lemon peel contains compounds which have medicinal applications and hence can be used as a cheaper alternative.

Analysis of Lemon Peel Water Extract

GC-MS analysis of lemon peel water extract exhibited the presence of 15 different compounds. The below chromatogram presents the chemical compounds identified in water extract.



Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	4.23	BB	0.1468	46093.52	4960.73		4.08	271	4.46	2518
2	4.62	BB	0.3831	6317811.43	256497.29		4.46	2518	5.57	25116
3	10.75	BB	0.7073	154963.49	3873.74		10.34	813	11.77	1460
4	12.93	BB	0.7103	231407.22	5862.29		12.53	1151	13.77	2329
5	19.99	BB	0.2553	118720.92	7295.85		19.79	1788	20.41	1704
6	21.48	BB	0.6369	708972.21	19668.62		20.99	1949	22.43	6297
7	22.79	BB	0.2710	107961.97	4714.55		22.51	5396	23.38	2575
8	23.74	BB	0.1172	13341.73	1509.01		23.61	2046	24.01	1380
9	25.19	BB	0.3979	108767.41	4299.91		24.93	1083	26.08	1078
10	28.17	BB	0.2465	155088.38	8117.80		27.66	1083	28.78	2561
11	31.32	BV	0.1320	15133.46	1978.88		31.14	5056	31.39	6815
12	31.77	VV	0.3896	1182837.35	45945.70		31.39	6815	32.53	13632
13	32.63	VB	0.2826	150510.53	9356.22		32.53	13632	33.06	16914
14	33.66	BV	0.4796	183991.17	14148.86		33.18	17522	33.66	9423
15	33.81	VB	0.2035	335679.24	31991.34		33.66	9423	34.08	2336

Figure 3 Chromatogram of Lemon Peel Water Extract

The major constituents present in Water Extract are tabulated in Table 7. 2H-1-Benzopyran-2-one-4,7-dihydroxy has not been quantified, but detected in, several different foods, such as green vegetables, and teas like black tea, green tea, and herbal tea. This compound could potentially serve as a biomarker for the consumption of these foods (**The Metabolics Innovation Centre, 2019**). Floxuridine is used for treating gastrointestinal (GI) tract cancer (specifically stomach or intestinal cancer) that has metastasized to the liver. Classified as antimetabolite, floxuridine operates by inhibiting the growth of cancer cells in the body either by slowing their growth or halting it altogether (**Society of Health-System Pharmacists, Inc., 2012).**

Table 7 Various constituents present in LPWE extract after GC-MS Analysis

Sr. No.	Retention Time(min)	Name of Compound	Molecular Formula	Molecular Weight
1	4.23	Phosphine imide,p,p,p-tris(P-chlorophenyl)-N-phenyl	$C_{24}H_{17}C_{l3}NP$	455
2	4.62	2,4-Furandione, dihydro-3-methylene	$C_5H_4O_3$	112
3	10.75	Oxiranemethanol	$C_3H_6O_2$	74
4	12.93	4-Ethyl-2-hydroxycyclopent-2-en-one	$C_7H_{10}O_2$	126
5	19.99	2H-1-Benzopyran-2-one-4,7-dihydroxy	$C_9H_6O_4$	178
6	21.48	Methyl-4-o-acetyl-2,3,6-trio-o-ethyl,d-galactopyranoside	$C_{15}H_{28}O_7$	320
7	22.70	1,2,4-trioxolane,3,5-dipropyl	$C_8H_{16}O_3$	160
8	23.74	Acetamide,N-(4-hydroxy-2-benzothiazolyo)	$C_9H_8N_2O_2S$	208
9	25.19	2,3-Epoxyhexanol	$C_6H_{12}O_2$	116
10	28.17	3,3-Diphenyl-5-methyl-3H-pyrazole	$C_{16}H_{14}N_2$	234
11	31.32	1-Hepten-4-ol	$C_7H_{14}O$	114
12	31.77	Hexanoic acid,decyl ester	$C_{16}H_{32}O_2$	256
13	32.63	Floxuridine	$C_9H_{11}FN_2O_5$	246
14	33.66	1H-Indene,1-hexadecyl-2,3-dihydro	$C_{25}H_{42}$	342
15	33.81	2-Formyl-4-methylpentanoic acid,ethyl ester	$C_9H_{16}0_3$	172

CONCLUSION

The present study aimed to investigate the antibacterial activity of LPWE, LPME and quercetin against MDR organisms. The test organisms were resistant to several drugs including Ampicillin, Vancomycin, Polymyxin B, Nitrofurantoin, Trimethoprim and penicillins with MAR index greater than 0.10. In the present study, these bioactive compounds were extracted using two different solvents (Methanol and Water) and their efficiency was checked against drug resistant organisms. MIC values were high in case of LPWE (water extract) when compared to LPME and Quercetin. From the results it can be interpreted that Methanol extract was most effective against drug resistant bacteria.

Methanol extract exhibited highest inhibition activity against all organisms when compared to water extract and Quercetin. As all three compounds (LPME, LPWE and Quercetin) were active against both Gram negative and Gram-positive organisms hence they can be considered as broad spectrum whereas these extracts can be used in various creams as anti-infectious agent in treatment of various diseases. Peel extracts are rich in phytocemicals which exhibit properties like antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory etc. The extracts may be used in treatment of skin diseases as well as can be used as an ingredient in sunscreen to protect skin from harmful UV radiations. The fruit peel extracts rich in phenolic compounds possess excellent properties to reduce the oxidative damage. Thus, Lemon peel extracts and Quercetin can find applications in medicine, cosmetics, food industry.

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REFERENCES

AHFS Patient Medication Information [Internet]. Bethesda (MD): American Society of Health-System Pharmacists, Inc. (2012). Floxuridine. Retrieved December 15, 2012 from https://medlineplus.gov/druginfo/meds/a682006.html#:~:text=Floxuridine%20is%20used%20to%20treat,cancer%20cells%20in%20your%20body.

Alam, F., Mohammadin, K., Shafique, Z., Amjad, S. T., & Asad, M. H. H. B. (2022). Citrus flavonoids as potential therapeutic agents: A review. Phytotherapy Research, 36(4), 1417-1441. https://doi.org/10.1002/ptr.7261.

Azeem, M., Hanif, M., Mahmood, K., Ameer, N., Chughtai, F. R. S., & Abid, U. (2022). An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. Polymer Bulletin, 1-22. https://doi.org/10.1007/s00289-022-04091-8.

Belbase, C. (2022). EFFECT OF EVAPORATION TEMPERATURE ON BIOACTIVE COMPONENT AND SENSORY QUALITY OF LEMON JUICE CONCENTRATE (Doctoral dissertation, Department of Food Technology Central Campus of Technology Institute of science and Technology Tribhuvan University, Nepal 2022). http://202.45.146.37:8080/jspui/handle/123456789/232.

Beschi, D. A., Appavoo, M. R., & Wilsy, J. I. (2021). GC-MS analysis, collected from Kavalkinaru area, Tirunelveli District, Tamil Nadu, India. European Journal of Molecular & Clinical Medicine, 8(11), 2021.

Cespi, D., Cucciniello, R., Ricciardi, M., Capacchione, C., Vassura, I., Passarini, F., & Proto, A. (2016). A simplified early stage assessment of process intensification: glycidol as a value-added product from epichlorohydrin industry wastes. Green Chemistry, 18(16), 4559–4570. https://doi.org/10.1039/C6GC00882H.

D'Arcy, M. S. (2022). A review of biologically active flavonoids as inducers of autophagy and apoptosis in neoplastic cells and as cytoprotective agents in non-neoplastic cells. Cell Biology International. https://doi.org/10.1002/cbin.11813.

Duffy, E., Albero, G., & Morrin, A. (2018). Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry Analysis of Scent Profiles from Human Skin. Cosmetics, 5(4), 62. https://doi.org/10.3390/cosmetics5040062.

Fouda, A., Awad, M. A., & Kabadaia, M. M. (2021). The Insecticidal Activity of Entomopathogenic Bacteria Isolated from Musca domestica, Chrysomya albiceps and Lucilia sericata against the Mosquito Larvae of Culex pipiens. Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology, 13(1), 23-28. https://dx.doi.org/10.21608/eajbse.2021.177955.

Idelevich, E. A., & Becker, K. (2019). How to accelerate antimicrobial susceptibility testing. Clinical Microbiology and Infection, 25(11), 1347-1355. https://doi.org/10.1016/j.cmi.2019.04.025.

Irkin, R., Dogan, S., Degirmencioglu, N., Diken, M. E., & Guldas, M. (2015). Phenolic content, antioxidant activities and stimulatory roles of citrus fruits on some lactic acid bacteria. *Archives of Biological Sciences*, *67*(4), 1313-1321.https://doi.org/10.2298/ABS140909108I

Jucá, M. M., Cysne Filho, F. M. S., de Almeida, J. C., Mesquita, D. D. S., Barriga, J. R. D. M., Dias, K. C. F., ... & Vasconcelos, S. M. M. (2020). Flavonoids: biological activities and therapeutic potential. Natural product research, 34(5), 692-705. https://doi.org/10.1080/14786419.2018.1493588.

Karak, P. (2019). Biological activities of flavonoids: an overview. Int. J. Pharm. Sci. Res, 10(4), 1567-1574. https://doi.org/10.13040/IJPSR.0975-8232.10(4).1567-74.

Kusuma, S. A. F., Rostinawati, T., Hendriani, R., Budiman, M. F., & Parwati, I. (2021). Effect of water reservoirs types on the prevalence and antibiotic resistance profiles of Pseudomonas aeruginosa isolated from bathroom water in hospitals. Journal of advanced pharmaceutical technology & research, 12(1), https://doi.org/52.10.4103/japtr.JAPTR_103_20.

Li, B., & Webster, T. J. (2018). Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research®, 36(1), 22-32. https://doi.org/10.1002/jor.23656.

Li, H. (2019). Citrus Plant Botanic Characteristics and Its Abiotic and Biotic Stress. In Handbook of Plant and Crop Stress, Fourth Edition (pp. 755-772). CRC Press. https://doi.org/10.1201/9781351104609.

Liang, C., Ju, W., Pei, S., Tang, Y., & Xiao, Y. (2017). Pharmacological Activities and Synthesis of Esculetin and Its Derivatives: A Mini-Review. Molecules, 22(3), 387. https://doi.org/10.3390/molecules22030387.

Liya, S. J., & Siddique, R. (2018). Determination of antimicrobial activity of some commercial fruit (apple, papaya, lemon and strawberry) against bacteria causing urinary tract infection. European Journal of Microbiology and Immunology, 8(3), 95–99. https://doi.org/10.1556/1886.2018.00014

MAQBUL, M. S., BOKHARI, Y. A., GUMAAN, S., Basalib, S. N., Omar, B. M., KHAN, A. A., ... & MOHAMMED, T. (2020). A comparative study of different types of thyme essential oils against Streptococcus pyogenes to determine their biochemical and antimicrobial properties. Orient J Chem, 36(2). http://dx.doi.org/10.13005/ojc/360202.

Mehmood, T., Afzal, A., Anwar, F., Iqbal, M., Afzal, M., & Qadir, R. (2019). Variations in the Composition, Antibacterial and Haemolytic Activities of Peel Essential Oils from Unripe and Ripened Citrus limon (L.) Osbeck Fruit. Journal of

Essential Oil-Bearing Plants, 22(1), 159–168. https://doi.org/10.1080/0972060X.2019.1588172

Mejri, H., Khetatfa, T., Aidi Wannes, W., Smaoui, A., & Saidani Tounsi, M. (2022). Histochemistry, chemical composition and antioxidant activity of Citrus aurantium L. essential oil during leaf development. Journal of Essential Oil Research, 1-10. https://doi.org/10.1080/10412905.2022.2067255.

Mhgub, I. M., Hefnawy, H. T., Gomaa, A. M., & Badr, H. A. (2018). Chemical composition, antioxidant activity and structure of pectin and extracts from lemon and orange peels. Zagazig Journal of Agricultural Research, 45(4), 1395-1404. https://dx.doi.org/10.21608/zjar.2018.48589.

Mohammed, S. A., & Omer, A. F. (2019). Assessment of antimicrobial activity of methanolic extract of lemon peels against clinical bacterial isolates. African Journal of Medical Sciences, 4(11), 1-5.

Mohtashami, E., Shafaei-Bajestani, N., Mollazadeh, H., Mousavi, S. H., Jalili-Nik, M., Sahebkar, A., & Afshari, A.R. (2020). The current state of potential therapeutic modalities for gioblastoma multiforme: a clinical review. Current drug Metabolism, 21 (8), 564-578. https://doi.org/10.2174/1389200221666200714101038.

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). The phytochemistry and medicinal value of Psidium guajava (guava). Clinical Phytoscience, 4(1), 1-8. https://doi.org/10.1186/s40816-018-0093-8.

Ntushelo, K. (2016). Chlorinated organic compounds produced by Fusarium graminearum. Acta Biologica Hungarica, 67(2), 220–224. https://doi.org/10.1556/018.67.2016.2.10.

Patil, S. M., Martiz, R. M., Ramu, R., Shirahatti, P. S., Prakash, A., Kumar, B. P., & Kumar, N. (2021). Evaluation of flavonoids from banana pseudostem and flower (quercetin and catechin) as potent inhibitors of α-glucosidase: An in silico perspective. Journal of Biomolecular Structure and Dynamics, 1-15. https://doi.org/10.1080/07391102.2021.1971561.

Pinski, A., Betekhtin, A., Kwasniewska, J., Chajec, L., Wolny, E., & Hasterok, R. (2021). 3,4-Dehydro-L-proline Induces Programmed Cell Death in the Roots of Brachypodium distachyon. International Journal of Molecular Sciences, 22(14), 7548. https://doi.org/10.3390/ijms22147548.

Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F., & Nayik, G. A. (2018). Citrus peel as a source of functional ingredient: A review. Journal of the Saudi Society of Agricultural Sciences, 17(4), 351-358. https://doi.org/10.1016/j.jssas.2016.07.006.

Russo, C., Maugeri, A., Lombardo, G. E., Musumeci, L., Barreca, D., Rapisarda, A., ... & Navarra, M. (2021). The second life of Citrus fruit waste: A valuable source of bioactive compounds. Molecules, 26(19), 5991. https://doi.org/10.3390/molecules26195991.

Sagi, S. S. (2021). Quercetin: A Potential Flavonol with Multiple Health Benefits. Arch Food Sci Nutr Res, 1(1), 1002.

Sakarikou, C., Altieri, A., Bossa, M. C., Minelli, S., Dolfa, C., Piperno, M., & Favalli, C. (2018). Rapid and cost-effective identification and antimicrobial susceptibility testing in patients with Gram-negative bacteremia directly from blood-culture fluid. Journal of microbiological methods, 146, 7-12. https://doi.org/10.1016/j.mimet.2018.01.012.

Segawa, I., Ssebambulidde, K., Kiiza, D., & Mukonzo, J. (2020). Antimicrobial Sensitivity Testing Using the Kirby-Bauer Disk Diffusion Method; Limited Utility in Ugandan Hospitals. https://doi.org/10.31730/osf.io/jh96e.

Shehata, M. G., Awad, T. S., Asker, D., El Sohaimy, S. A., Abd El- Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. Current Research in Food Science, 4(May), 326–335. https://doi.org/10.1016/j.crfs.2021.05.001.

Spitsin, A. I., Bush, A. A., & Kamentsev, K. E. (2020). Piezoelectric and dielectric properties of Bi3TiNbO9 prepared by hot pressing from powders activated using the serial dilution method. Scientific Reports, 10(1), 1-13. https://doi.org/10.1038/s41598-020-78826-w.

The Metabolics Innovation Centre (2019). FOODB Compounds for PID FDB010937, 4,7-Dihydroxy-2H-1-benzopyran-2-one. Retrieved November 26, 2019 from https://foodb.ca/compounds/FDB010937.

Turlej-Rogacka, A., Xavier, B. B., Janssens, L., Lammens, C., Zarkotou, O., Pournaras, S., ... & Malhotra-Kumar, S. (2018). Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. European Journal of Clinical Microbiology & Infectious Diseases, 37(2), 345-353. https://doi.org/10.1007/s10096-017-3140-3.