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Original Article

Spectral characterization and biological evaluation of biomolecules from the peels of three orange fruits: a comparative study

Caracterização espectral e avaliação biológica de biomoléculas das cascas de três frutos de laranja: um estudo comparativo

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Abstract

The present work was designed to investigate the presence of bioactive chemicals in the reaction mixtures (RMs) of peels of Valencia, Mandarin, and African navel oranges, through GC-MS and FT-IR studies. Limonene, a unique compound, is present in the RMs of the three orange peels. Moreover, hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester was identified in the RMs of all the three-orange peels. The RM of Mandarin orange exhibited potent cytotoxic effect against MCF-7 ATCC human breast cancer cells (HBC). All the three RMs exhibited moderate antibacterial activity against the human pathogenic bacteria *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella choleraesis* (ATCC 10708), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus mirabilis* (ATCC 299).

Keywords: fruits, oranges, bioactive components, cytotoxicity, antibacterials.

Resumo

O presente trabalho almejou investigar a presença de produtos químicos bioativos nas misturas de reação (MRs) de cascas de Valência, Tangerina e laranja umbigo africana, por meio de estudos de GC-MS e FT-IR. Assim, constatou-se que limoneno, um composto único, está presente nos RMs das três cascas de laranja. Além disso, o éster 2-hidroxi-1-(hidroximetil) etílico do ácido hexadecanóico foi identificado nos RMs de todas as cascas de três laranjas. A RM da tangerina exibiu potente efeito citotóxico contra células de câncer de mama humano (HBC) MCF-7 ATCC. Todos os três RMs exibiram atividade antibacteriana moderada contra as bactérias patogênicas humanas dos seguintes tipos: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella choleraesis* (ATCC 10708), *Pseudomonas aeruginosa* (ATCC 27853) e *Proteus mirabilis* (ATCC 299).

Palavras-chave: frutas, laranjas, componentes bioativos, citotoxicidade, antibacterianos.

1. Introduction

Fruits are good sources of essential vitamins and minerals, as well as fiber. In addition, fruits contain a variety of antioxidants such as flavonoids and alkaloids which are beneficial to human health. It has been shown that eating diets rich in fruits and vegetables lowers the chances of acquiring heart disease and cancer, as well as inflammation and diabetes. Oranges are high in a variety of bioactive compounds that have therapeutic uses. Oranges

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promote cardiac health and reduce the risks of heart disease and cancers (Wang et al., 2021; Mas-Capdevila et al., 2020; Aune et al., 2018; Li and Schluesener, 2017). Due to the presence of antioxidants which neutralize free radicals that contribute to aging and several degenerative disorders, oranges are regarded as effective anti-aging fruits (Mohammed and Khan, 2022). This is the reason for large-scale use of oranges in the cosmetic industry. Valencia oranges are delicious summer fruits which are available in sizes ranging from medium to large, and their shapes range from round to oval. Valencia oranges are a great source of fiber, vitamin C, folate, and antioxidants that help to enhance the immune system (Saunt, 2000).

These oranges are exceptionally juicy, and they are the most utilized type of orange in the production of orange juice (Alfaro, 2022). In contrast, Mandarins are smaller and oblate in shape, as opposed to the spherical common oranges, and they have a sweeter flavor. Moreover, Mandarin oranges contain large amounts of juice; they are easy to peel, and they have an appealing deep orange-red color. Navel oranges are a type of winter orange that features a thick, vivid orange skin and sweet, juicy flesh. They are seedless, easy to peel, and are regarded as one of the best-tasting oranges in the world (Julie, 2018). The peel, which we normally discard, contains important elements such as fibre, vitamin C, folate, vitamin B6, calcium, and other necessary nutrients, as well as other nutrients. The peel of oranges includes a significant number of polyphenols, which are known to protect against a variety of ailments. It is believed that the presence of limonene, a naturally occurring molecule, confers anti-cancerous qualities on citrus peels. Furthermore, the essential oil contained within the peel has anti-inflammatory effects that help to enhance your immunity (TOI, 2019). The scheme of work is depicted in Figure 1 and the present study was focused on investigation of the biomolecules in the peels of the three different kinds of oranges which are locally available in markets, as well as their medicinal properties, to demonstrate the importance of consuming these peels of oranges as food supplements.

2. Materials and Methods

2.1. Materials

Methanol and other chemicals used in this study were analytical grade and purchased from Sigma Aldrich, USA. Fetal bovine serum, RPMI-1640, and methanol were products of Sigma Aldrich, while MCF-7 cancer cell line was obtained from ATCC, both in United States of America. The chemicals and bacteria culture media were supplied by Scharlau, Spain.

2.2. Collection and processing of oranges

Three different types of oranges namely Valencia, Mandarins and Navel were purchased from a local market. The oranges were thoroughly washed in pipe water to remove unwanted adherents. Furthermore, the oranges were washed with Millipore water, followed by peeling using orange plastic peelers to remove the skin layers. Cold maceration process was performed to extract the bioactive components from the orange peels. 25 g of peeled skin sample of each orange was sliced into small pieces and soaked in 50 mL of 100% v/v methanol. This formed the reaction mixture (RM). Each RM was placed on a hotplate and stirred with magnetic bead for 1 h at room temperature, after which it was placed in refrigerator for 24 h. Then, it was stirred again for 1 h using a magnetic stirrer. This protocol was followed for 7 days, after which the macerated liquid RM was centrifuged in a Sigma table-top centrifuge at 3000 × g for 10 min. The supernatant was taken up in a

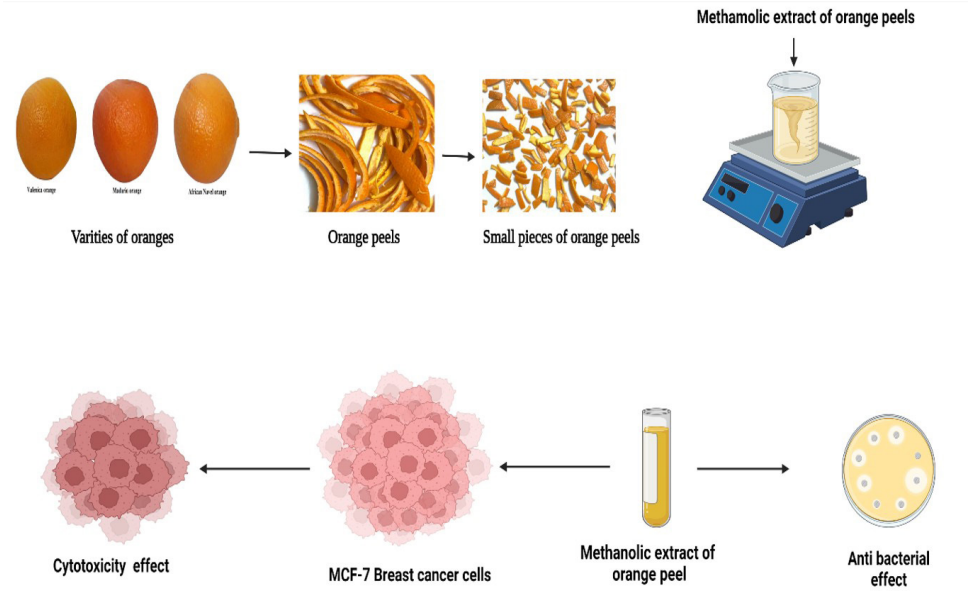

Figure 1. Schematic representation of experimental protocol.

separate container, filtered through a Whatman no. 1 filter paper, and kept in refrigerator prior to analysis.

2.3. GC-MS analysis

The essential phytochemical components in RM of individual orange peels were evaluated using GC-MS with AS3000 autosampler and ISQ detector from Thermo Scientific, USA. The carrier gas used was helium, and it was passed through the column at a flow rate of 1.2 mL/min. The mass spectrophotometer was operated, and spectral analysis was performed using Xcalibur software. The mass spectra were interpreted using the MAINLIB and NIST libraries (Moni et al., 2023).

2.4. FT-IR analysis

The FT-IR analyses of RMs were carried out using the pressed pellet method at λ values spanning 400-4000 cm^{-1} (resolution = 4 cm^{-1}), with KBr pellet as standard (Makeen et al., 2020).

2.5. Antibacterial studies

The bacterial strains used in the study were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella choleraesuis* (ATCC 10708), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus mirabilis* (ATCC 2990). In this process, 24-h cultures were prepared in nutrient broth from stock bacterial cultures prior to the antimicrobial experimentation. Bacterial sensitivity test was carried out using the procedure of Moni et al. (2018). Muller Hinton (MH) agar plates were created, and agar well diffusion method was used, while disc diffusion was used for standard ciprofloxacin discs (5 mcg/disc). A sterile cotton swab was dipped into the standardized culture (CFU/mL) and streaked on MH agar plate while turning the petri dish to spread the culture equally. The plates were allowed to dry for approximately ten minutes prior to administering the analytes. The agar well diffusion procedure was carried out by punching holes in inoculated MH agar plates with a sterile stainless borer. 100 µL of 100% v/v concentrated RM and 100% v/v of methanol (Solvent control) were placed in the respective wells of MH agar plate to determine the antibacterial effect. After 24 h incubation at 37°C, the antibacterial spectrum was determined in terms of establishment of inhibitory zones.

2.6. Cytotoxic effect of RP on MCF-7 HBC

Cytotoxic study was carried out in accordance with the methodology reported by Salam et al. (2022) and Sultan et al. (2020). This technique used MCF-7 HBC that had been cultured at 37 °C in RPMI-1640 in sodium bicarbonate buffer system (2.0 g/L), pH 7.4. The medium was supplemented with 10% fetal bovine serum (FBS), 100 units of penicillin per milliliter, and streptomycin (100 micrograms per milliliter) in a 5% CO_2 atmosphere, and humidity of 90%. Cells were treated with varied doses of RP dissolved in DMSO in 96-well plates and cultured for 2 days. Thereafter, 20 µL of MTT solution (5 mg/mL) was added to each well, followed by further incubation away from light for 4 h. Then, the medium was discarded, and the resultant formazan crystals in each well were solubilized in DMSO (0.1 mL). The absorbance of each formazan solution was read at 490 nm in a microplate reader. After taking all appropriate controls into consideration, the % cellular viability was calculated. The experiment was repeated three times, and the % suppression of cell multiplication was measured as indicated below (Equation 1):

$$\text{Suppression of cell viability } (\%) = \left(Abs_{control} - Abs_{test}\right) \times 100 \quad (1)$$

Where Abs is absorbance.

2.7. Statistical analysis

Data processing was done with GraphPad Prism 9. Comparison amongst groups was done with ANOVA and Tukey's post hoc test. Statistical significance was fixed at $p < 0.05$.

3. Results and Discussion

Figure 2 shows the morphological features of the oranges which were screened for biologically active components with a view to demonstrating their medicinal properties. The GC–MS results for RMs revealed the presence of numerous bioactive compounds, as depicted in the chromatogram displayed in Figures 3A-3C. The bioactive compounds in the RMs are presented in Tables 1-3, while the structures of these compounds are shown in Figures 4-6. Limonene is the chief constituent of the RM of each of the three oranges. Figure 3A shows the GC-MS chromatogram of RM prepared from the skin peels of Valencia orange. The unique peak representing limonene appeared at

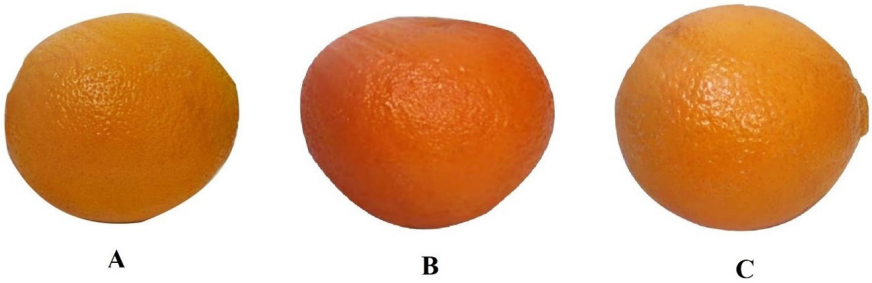

Figure 2. The morphology of orange fruits. (A) Valenica orange; (B) Madarin orange; (C) African Navel orange.

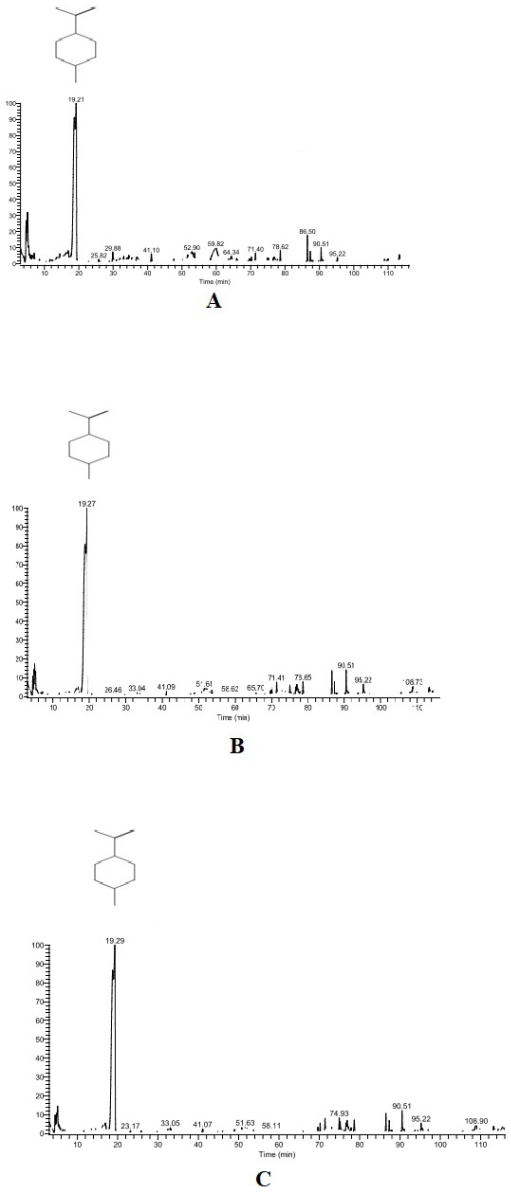

Figure 3. GC-MS chromatogram of the RMs of orange peels. (A) RM of Valencia orange; (B) RM of Mandarin orange; (C) RM of African Navel orange.

retention time (RT) of 19.22 min, and it occupied 32.87% of the curve, with a probability index of 25.93. Figure 3B shows the GC-MS chromatogram of RM prepared from the skin peels of Mandarin orange. The unique peak representing limonene appeared at RT of 19.27 min, and it occupied 39.06% of the curve, with a probability index of 19.98. Figure 3B shows the GC-MS chromatogram of RM prepared from the skin peels of Mandarin orange. The unique peak representing limonene appeared at RT of 19.27 min, and it occupied 39.06% of the curve, with a probability index of 19.98. In the present study, the GC-MS chromatogram of RM pre-pared from the skin peels of African Navel orange showed a unique peak representing limonene, with RT of 19.29 min, and it occupied 39.06% of the curve, with a probability index of 19.98. Limonene is a cyclic monoterpene with a human metabolite role that has been detected as a prominent component in the RM of all tree oranges examined in this study. A recent study reported that limonene was not only the primary constituent of essential oils from the leaves of Valencia orange (accounting for 11.1-12.6%), but also the primary constituent of essential oils from the peel, accounting for 70.3-71.5% (Khalid et al., 2021). Interestingly, the highest concentration of limonene (82.1%) was derived from Valencia oranges from the southern location of Egypt (Khalid and Ahmed, 2020). The oil extracted from the peel of Mandarin orange (*C. tangerina*) was previously reported to be dominated by the presence of limonene which accounted for 83.57% (Erman et al., 2016). A previous study found that the primary component of the essential oils from Mandarin orange peel was limonene which accounted for 74.7% of the oil constituents (Viuda-Martos et al., 2009). Moreover, Yang et al. reported that limonene was the predominant constituent (74.6%) of the essential oils from Gannan Navel orange peel (Yang et al., 2017). Previously, limonene was identified as a significant component of the essential oils from Washington navel peels (Njoroge et al., 2005). According to the findings of a recent study, citrus limonene may aid in weight loss by reducing blood sugar and cholesterol levels which are associated with metabolic syndrome (Jing et al., 2013). Limonene may contribute to the prevention of peptic ulcers and other inflammatory gastrointestinal diseases (Moraes et al., 2009).

The compound 9-octadecenamide (Z)-, also known as oleamide, was identified in the RM of Valencia orange peels (Akanmu et al., 2007). However, it was not identified in the other two oranges studied using GC-MS. An earlier study suggested that oleamide induces sleep and decreased body temperature in rats (Huitrón-Reséndiz et al., 2001). In the GC-MS analysis, hexadecanoic acid (also known as palmitic acid) was identified in the RM of Mandarin orange peels at RT of 71.41 min, with a probability index of 78.31. Hexadecanoic acid 2-OH-1-(OH-CH3) ethyl ester, a derivative of hexadecanoic acid, otherwise called 2-palmitoylglycerol, was identified in the RM of each of the/ three orange peels. 2-Palmitoylglycerol is a fatty acid ester that improves the capacity of 2-arachidonoylglycerol to bind to cannabinoid receptors on CB1 and CB2 endocannabinoid receptors. It is also a vital compound for modulation of pain sensitivity (Walker et al., 2002). It is a polyunsaturated omega-6 fatty acid often used as weight loss agent (Benjamin et al., 2015). α-Sitosterol was identified in the RMs of Valencia and African Navel oranges. α-Sitosterol belongs to the class of organic compounds known as stigmastane and derivatives.

9,12-Octadecadienoic acid (Z, Z)-methyl ester (otherwise known as linoleic acid) was identified in the RM of African Navel orange peel. Linoleic acid, the most prevalent omega-6 fatty acid in diet and an important structural component of cell membranes, influences membrane function. It is also a precursor of eicosanoids, which are hormones that regulate renal and pulmonary function, vascular tone, and inflammation responses (Mori and Hodgson, 2013). The RM of Mandarin orange peels exhibit

Table 1. GC-MS detection of possible bioactive compounds in the RM of Valencia orange peel.

S.no	Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
1	Limonene	$C_{10}H_{16}$	136	19.21	25.93	32.87
2	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	86.50	87.91	1.75
3	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	$C_{19}H_{38}O_4$	330	90.51	64.76	0.84
4	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	$C_{21}H_{42}O_4$	358	95.22	71.90	0.31
5	Tetradecanamide	$C_{14}H_{29}NO$	227	78.62	37.17	0.78
6	Hexadecanamide	$C_{16}H_{33}NO$	255	71.40	29.96	0.78
7	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	67.33	16.08	0.58
8	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	59.82	39.88	0.32
9	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210	52.90	10.78	0.78
10	α-Sitosterol	$C_{29}H_{50}O$	414	114.34	38.33	0.1
11	Vitamin E	$C_{29}H_{50}O_2$	430	108.02	66.17	0.1
12	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-	$C_{20}H_{20}O_7$	372	109.57	79.05	0.1

Table 2. GC-MS detection of possible bioactive compounds in the RM of Mandarin orange peel.

S.no	Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
1	Limonene	$C_{10}H_{16}$	136	19.27	16.70	36.31
2	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	$C_{21}H_{42}O_4$	358	95.22	55.79	0.78
3	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	$C_{19}H_{38}O_4$	330	90.51	63.90	1.5
4	Tetradecanamide	$C_{14}H_{29}NO$	227	78.65	47.55	0.92
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	71.41	78.31	0.74
6	α-D-Mannofuranoside, 1-O-(10-undecenyl)-	$C_{17}H_{32}O_6$	332	65.70	27.79	0.31
7	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162	58.62	65.30	0.30
8	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	51.26	17.77	0.8
9	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150	41.09	58.27	0.32
10	Decanal	$C_{10}H_{20}O$	156	33.04	42.10	0.12
11	Vitamin E	$C_{29}H_{50}O_2$	430	108.01	53.86	0.08
12	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	107.77	35.34	0.02

the presence of palmitic acid, which is potent bioactive molecule. An earlier report showed that palmitic acid exhibited an anti-inflammatory effect (Aparna et al., 2012. Moreover, it exerted potent antibacterial effect against biofilm forming bacteria (Bakar et al., 2017). According to a recent study, palmitic acid produced significant anti-cancer effects against HT–29 colon cancer cells (Bharath et al., 2021). A study conducted by Sangpairoj et al. (2022) found that an extract of *Halymenia durvillei* with high content of palmitic acid stimulated apoptosis and autophagy in

Table 3. GC-MS detection of possible bioactive compounds in the RM of African Navel orange peel.

S.no	Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
1	Limonene	$C_{10}H_{16}$	136	19.29	19.98	39.06
2	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	$C_{19}H_{38}O_4$	330	90.51	65.93	1.25
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	74.93	31.57	0.64
4	Terephthalic acid, di(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	95.60	35.24	0.14
5	α-Sitosterol	$C_{29}H_{50}O$	414	114.33	40.06	0.15
6	α-D-Glucopyranose, 4-O-α-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	51.90	11.86	0.12
7	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	41.07	66.82	0.27
8	Eugenol	$C_{10}H_{12}O_2$	164	44.52	27.97	0.14
9	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	76.88	18.68	1.00
10	Decanal	$C_{10}H_{20}O$	156	33.05	49.18	0.14
11	Vitamin E	$C_{29}H_{50}O_2$	420	108.02	48.07	0.16
12	Dichloroxylenol	$C_8H_8C_{12}O$	190	46.67	53.36	0.12

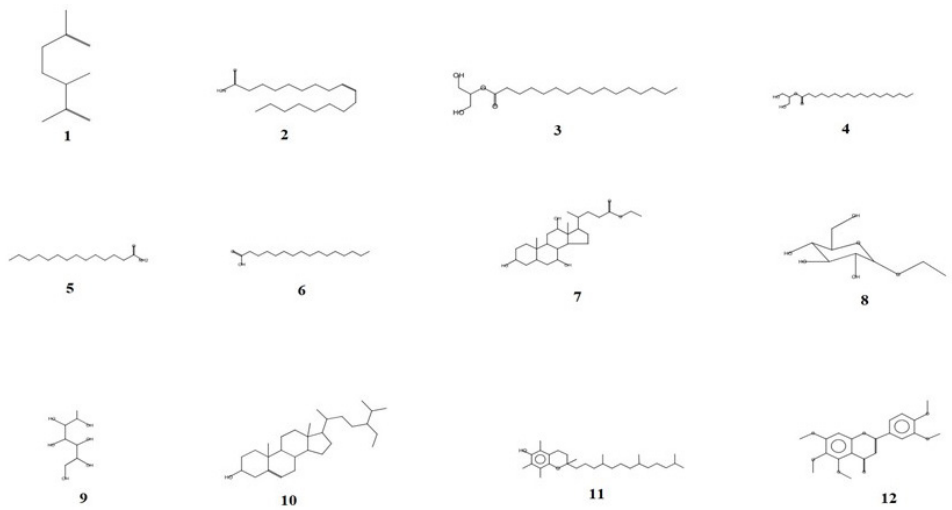

Figure 4. The structure of bioactive compounds of RM of Valencia orange peel (1) Limonene; (2) 9-Octadecenamide, (Z)-; (3) Hexadecanoic acid, 2- hy-droxy-1-(hydroxymethyl)ethyl; (4) Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl; (5) Tetradecanamide; (6) Hexadecanamide; (7) Ethyl iso-allocholate; (8) Ethyl α-d-glucopyranoside; (9) d-Glycero-d-galacto-heptose; (10) α-Sitosterol; (11) Vitamin E; (12) 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-.

human triple-negative breast cancer cells (Sangpairoj et al., 2022). Thus, the extract may be investigated further as a potential anticancer agent (Sangpairoj et al., 2022). It had been reported that the presence of palmitic acid in the peels of bitter orange peel, *Citrus aurantium* L led to reduced antioxidant effect (Radan et al., 2018). In contrast, a recent study showed that dietary palmitic acid enhanced cancer spread and induced aggressive memory in tumor cells. The dietary metabolite (palmitic acid) produced long-lasting transcriptional and chromatin alterations that promoted metastasis associated with a pro-regenerative state in tumor-activated Schwann cells (Pascual et al., 2021). In this study, desulphosinigrin was found exclusively in Mandarin orange peels. It was previously demonstrated that desulphosinigrin produced anticancer action via cyclin-dependent kinases (Krishnaveni, 2015). Vitamin E was identified in the RMs of all the oranges that were screened.

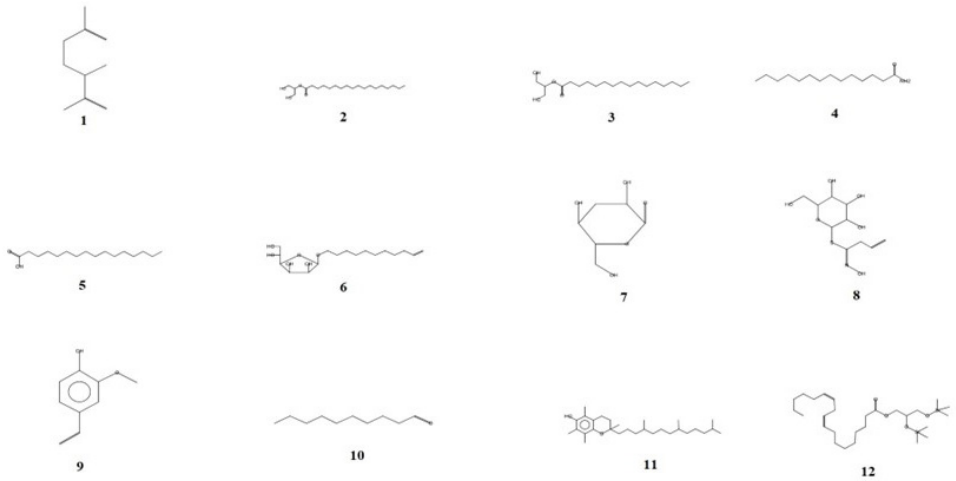

Figure 5. The structure of bioactive compounds of RM of Mandarin orange peel (1) Lim-onene; (2) Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl; (3) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl; (4) Tetradecanamide; (5) n-Hexadecanoic acid; (6) α-D-Mannofuranoside, 1-O-(10-undecenyl)-; (7) 3-Deoxy-d-mannoic lactone; (8) Desulpho-sinigrin; (9) 2-Methoxy-4-vinylphenol; (10) Decanal; (11) Vitamin E; (12) 1-Monolinoleoylglycerol trimethylsilyl ether.

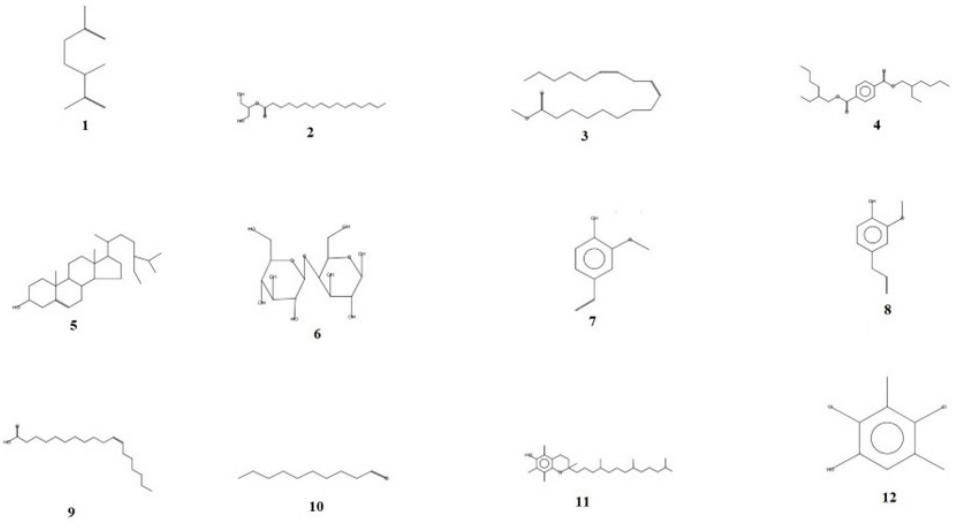

Figure 6. The structure of bioactive compounds of RM of African Navel orange peel (1) Limonene; (2) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl; (3) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; (4) Terephthalic acid, di(2-ethylhexyl) ester; (5) α-Sitosterol; (6) α-D-Glucopyranose, 4-O-α-D-galactopyranosyl-; (7) 2-Methoxy-4-vinylphenol; (8) Eugenol; (9) cis-Vaccenic acid; (10) De-canal; (11) Vitamin E; (12) Dichloroxylenol.

Results from FT-IR spectroscopy revealed multiple peaks for RMs of all the three oranges in several fingerprint regions which are depicted in Figures 7-9. The FT-IR spectroscopy study reported the presence of various bioactive components in the RM of all three orange peels which correspond to the biomolecules detected in GC-MS analysis. The FT-IR spectrum analysis of RM Valencia revealed various fingerprint regions at 3327.23, 2946, 2834, 1649, 1450, 1320, 1110 and 1017 cm^{-1} representing the presence of various bioactive components such as glycosides, flavonoids, saponins, polysaccharides, limonene, beta-sitosterol, and fatty acids (Table 4). Interestingly, Yaradoddi et al. (2022) reported that chloroform extract of Citrus sinensis orange peels exhibited FT-IR spectroscopy at 3352.82, 2919.84, 1691.92, 1604.83, 1398.99, 1051.75 and 636.94 cm^{-1}. These frequencies corresponded with polymeric OH, carboxylic acids, alkenes, aromatic groups, amino acids, carbohydrates and beta-d-galacturonic acid, respectively.

The FT-IR spectroscopy of RMs of Mandarin and African Navel oranges are tabulated in Tables 5-6. These results show the unique compounds in the various frequencies. The FT-IR spectrum analysis of RM Mandarin orange revealed various fingerprint regions at 333.56, 2944.83, 2833.10, 1654.53, 1449.56, 1115.34 and 1018.63 cm^{-1} representing the

presence of various bioactive components such as Eugenol, Vitamin E, Glycosides, Flavonoids, Saponins, Limonene, beta-Sitosterol, Fatty amides, and Steroidal glycosides (Table 5). The RM of African Navel orange revealed the presence of bio-active compounds through FT-IR spectral study. The fingerprint region at 333.81, 2945.02, 2832.98, 1658.61, 1449.72, 1111.45, and 1019.52 cm^{-1} showed the presence of Vitamin E, Glycosides, Flavonoids, Saponins, beta-Sitosterol, Limonene, and glycosides (Table 6).

The results presented in Figure 10 indicate that RM of Mandarin orange inhibited the proliferation of MCF-7 breast cancer cells. The IC_{50} for RM of Mandarin orange was 2 ± 0.04 µg / mL after 48 h incubation, which was highly significant. The IC_{50} value for the RM of Valencia orange was 38 ± 1.5 µg / mL. However, the RM of African Navel or-ange peels exhibited an IC_{50} value above 50 µg / mL. Therefore, the RM of Mandarin orange produced a unique spectrum of activity against MCF-7 breast cancer cells. In this study, the RM of Mandarin orange peels demonstrated the potent cytotoxicity effect against

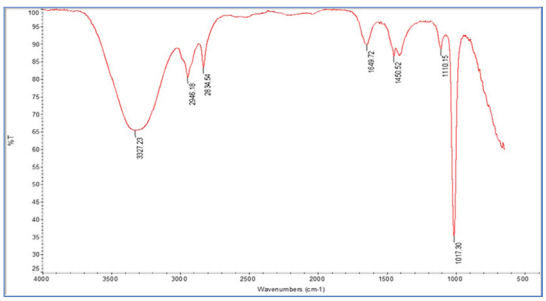

Figure 7. FT-IR spectroscopy of RM of Valencia orange peel.

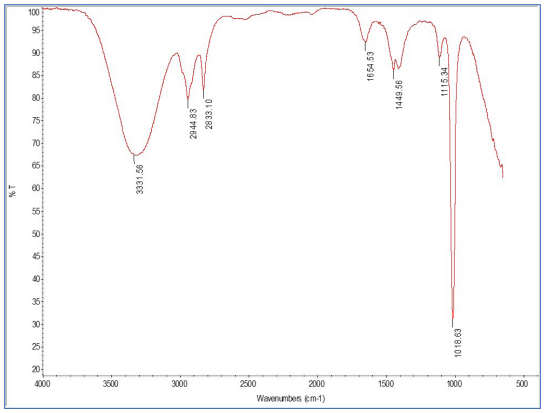

Figure 8. FT-IR spectroscopy of RM of Mandarin orange peel.

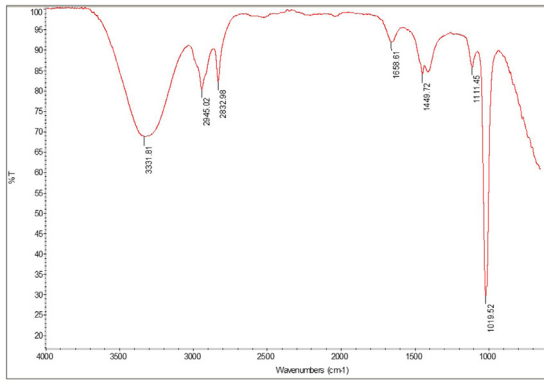

Figure 9. FT-IR spectroscopy of RM of African Navel orange peel.

Table 4. FT-IR spectroscopy of bioactive compounds in the RM of Valencia orange peel.

Wave number (cm^{-1})	Intensity Estimation	Functional Groups/Class	Compounds
3327	Strong	O-H str. (hydroxyl)	Glycosides, Flavonoids, Saponins, Polysaccharides, Lignin
2946	Strong	C-H str. (Alkene) C-H str. (Aldehyde/ketones)	Limonene, beta-Sitosterol, Lignin, Aliphatic chains present in the lignocellulose,
2834	Strong	CH_2 str. (Symmetrical)	Aliphatic compounds, beta-Sitosterol Saponins, Fatty acids
1649	Strong	C=O str (Carboxylic group) C=C str (Aromatic)	Fatty amides (Hexadecanamide, 9-Octadecenamide, (Z) etc.), Pectin, Lignin, Flavonoids, Proteins, Fatty acids
		C=C str (Alkenes)	Sitosterol, Limonene
1450	Medium	C-H (Asymmetrical bending)	Flavonoids, Limonene
1320	Weak	CH_2 bend (Alkane, Symmetrical)	Steroidal glycosides, Flavonoids, Saponins, hexose sugars
1110	Strong	C-O-C str (Ether, Ester)	Glycosides, Pectin, Polysaccharides, Lignin, Cellulose, Flavonoids
1017	Strong	C-O str.	Glycogens

Str: Stretching vibration.

Table 5. FT-IR spectroscopy of bioactive compounds in the RM of Mandarin orange peel.

Wave number (cm^{-1})	Intensity Estimation	Functional Groups/Class	Compounds
3331	S	O-H str. (hydroxyl)	Eugenol, Vitamin E, Glycosides, Flavonoids, Saponins, Lignin, Polysaccharides, Glucopyranose, Dichloroxylenol
2944	S	C-H str. (Alkene) C-H str. (Aldehyde/ketones)	Limonene, beta-Sitosterol, Lignin, Aliphatic chains present in the lignocellulose, Vitamin E
2833	S	CH_2 str. (Symmetrical)	Aliphatic compounds, beta-Sitosterol, Saponins, Fatty acids, Vitamin E
1654	S	C=O str (Carboxylic & Aldehyde group) C=C str (Aromatic) C=C str (Alkenes)	Fatty amides (Hexadecanamide, 9-Octadecenamide, (Z), Vaccenic acid etc.), Decanal, Pectin, Lignin, Flavonoids, Sitosterol, Limonene, Proteins, Fatty acids, Esters, Eugenol
1449	M	C-H (Asymmetrical bending)	Limonene, Flavonoids
1300	W	CH_2 bend (Alkane, Symmetrical)	Steroidal glycosides, Saponins, hexose sugars, Flavonoids
1115	S	C-O-C str	Glycosides, Polysaccharides, Lignin, Cellulose, Flavonoids, Tocopherol, Glucopyranose, Pectin
1018	S	C-O str	Eugenol, Glycogens

S: Strong; M: Medium; W: Weak; Str: Stretching vibration.

Table 6. FT-IR spectroscopy of bioactive compounds in the RM of Navel orange peel.

Wave number (cm^{-1})	Intensity Estimation	Functional Groups/Class	Compounds
3331	S	O-H str. (hydroxyl)	Vitamin E, Glycosides, Flavonoids, Saponins, Lignin, Polysaccharides
2945	S	C-H str. (Alkene) C-H str. (Aldehyde/ketones)	beta-Sitosterol, Limonene, Lignin, Aliphatic chains present in the lignocellulose, Vitamin E
2832	S	CH_2 str. (Symmetrical)	Aliphatic compounds, beta-Sitosterol, Saponins, Fatty acids, Vitamin E
1658	S	C=O str (Carboxylic group) C=C str (Aromatic) C=C str (Alkenes)	Fatty amides (Hexadecanamide, 9-Octadecenamide, (Z) etc.), Pectin, Lignin, Flavonoids, Sitosterol, Limonene, Proteins, Fatty acids
1449	M	C-H (Asymmetrical bending)	Limonene, Flavonoids
1310	W	CH_2 bend (Alkane, Symmetrical)	Flavonoids, Steroidal glycosides, Saponins, hexose sugars
1111	S	C-O-C str	Pectin, Glycosides, Polysaccharides, Lignin, Cellulose, Flavonoids, Tocopherol
1019	S	C-O str.	Glycogens

S: Strong; M: Medium; W: Weak; str: Stretching vibration.

MCF-7 ATCC human breast cancer cells. This might be due to the presence of the biomolecules hexadecanoic acid and desulphosinigrin. In a previous investigation, hexadecenoic acid was found to be cytotoxic to human leukemia cells at concentrations ranging from 12.5 to 50 µg / mL, but it was not cytotoxic to normal human dermal fibroblasts (Al-Wahaibi et al., 2020). It has been reported that the cytotoxicity of bornyl acetate (IC50 = 85.6 µg / mL) on MCF-7 cells was superior to that of cisplatin (Karan et al., 2018). A study which employed docking and simulation methods, with desulphosinigrin as a ligand, found that it inhibited cyclin dependent kinase (Krishnaveni, 2015). In a recent study, orange peel was shown to be effective against doxorubicin-treated esophageal cancer stem cells (Tajaldini et al., 2020). Indeed, it has been found that the flavonoid portion of orange peels may be a more effective cancer treatment and may be considered as a better alternative for treating cancer than any other strategy (Shirisha et al., 2019). On other hand, the RM of African navel orange showed better

Table 7. Antibacterial study.

Organisms	Concentration CFU[$]/mL	Zone of Inhibition (mm)			
		Test 1	Test 2	Test 3	Ciprofloxacin (5 μg/Disc)
Staphylococcus aureus ATCC 25923	2×10^{-5}	12.33 ± 1.15	11 ± 1	10.6 ± 0.47	33.6 ± 1.25
Staphylococcus epidermidis ATCC 12228	3×10^{-5}	10.3 ± 0.55	10.6 ± 0.57	11 ± 1.4	33.3 ± 1
Enterococcus faecalis ATCC 29212	2×10^{-4}	10.6 ± 0.8	10.3 ± 0.6	11.3 ± 1.2	25.3 ± 0.5
Escherichia coli ATCC 25922	4×10^{-6}	12.3 ± 2.08	10.6 ± 0.57	12.3 ± 1.6	35.6 ± 0.94
Klebsiella pneumoniae ATCC 700603	2×10^{-3}	12.6 ± 1.15	10.6 ± 0.7	10.3 ± 0.5	26 ± 1.14
Salmonella choleraesuis ATCC 10708	2×10^{-5}	10.3 ± 0.57	10.3 ± 0.57	12.3 ± 0.47	33.23 ± 1.24
Pseudomonas aeruginosa ATCC 27853	2×10^{-5}	13.3 ± 0.5	10.7 ± 2.5	11.6 ± 0.5	33 ± 0.81
Proteus mirabilis ATCC 299	3×10^{-5}	12.3 ± 1.5	12.6 ± 0.57	12.6 ± 0.48	23.6 ± 1.25

Each value is the mean of 3 batches with standard deviation. All the values are compared to standard ciprofloxacin disc by performing Tukey Kramer test (post hoc). All the test values are significantly lesser than standard ciprofloxacin disc at $p< 0.05$. Test 1: RM of Valencia orange; Test 2: RM of Mandarin orange; Test 3: RM of African navel orange. [$]CFU: Colony forming unit.

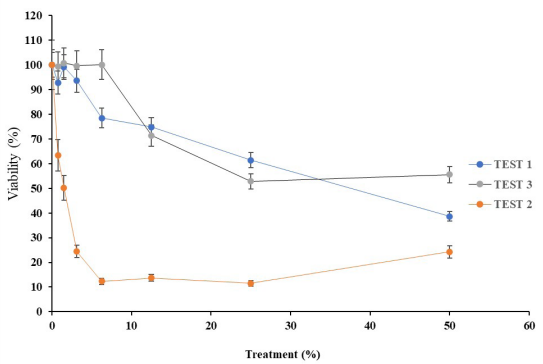

Figure 10. The dose-response curve of RM of orange peels against MCF-7 cells.

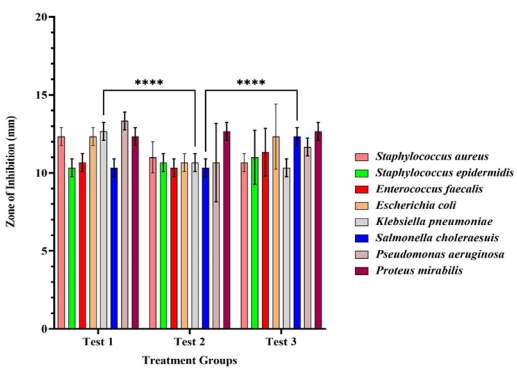

Figure 11. The efficacy of RM of orange peels against human pathogenic bacteria. ****Extremely significant among compared groups at p < 0.05 level. Test 1: RM of Valen-cia orange; Test 2: RM of Mandarin orange; Test 3: RM of African navel orange.

antibacterial effect against Salmonella choleraesuis than the RM of Mandarin orange ($p < 0.001$) (Mehmood et al., 2015). The RMs exerted moderate activities against the screened bacteria (Table 7). The antibacterial effect of RM of Valencia orange against *Klebsiella pneumoniae* was significantly higher than that of RM of Mandarin orange ($p < 0.001$; Figure 11). An earlier study reported that orange peel extract was effective against *Klebsiella pneumoniae* (Evrendilek, 2015). An earlier study demonstrated the antibacterial efficacies of the peel extracts of *Citrus sinensis* and *Citrus aurantium* (Madhuri et al., 2014). The study showed that *Citrus sinensis* peel extract had better activity than that of *Citrus aurantium*. Among all screened bacteria, antibacterial activity was very high against *Klebsiella pneumoniae* and *Bacillus cereus*.

4. Conclusion

The presence of different biomolecules in the RMs of Valencia, Mandarin, and African navel oranges was demonstrated in this study. The study found that the RMs evaluated had modest antibacterial impacts against human pathogenic bacteria, with comparable spectrum of activity. The RM of Mandarin orange, on the other hand, had a strong cytotoxic effect on MCF-7 ATCC breast cancer cells. The findings in this study indicate that the RMs have promising potential for use in the development of new antibacterial and anti-cancer therapeutic compounds. The development of new antibacterial and anticancer agents will be beneficial to mankind in the quest for enhanced health care.

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