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Chemical Profiling and Selective Antiproliferative Activity of Orange Peel (*Citrus sinensis*) Extract Against MCF-7 and HepG2 Cells

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Abstract

The results of the analysis conducted using the GC-MS demonstrate that the orange peel's alcoholic extract is composed of multiple different compounds, including flavonoids and terpenoids. 1-Methyl-4-(1-methylethinyl)cyclohexene represents the largest portion of these multiple components at 38.89%, while the hesperidin derivative makes up 21.19% of the composition as well. The cytotoxicity analysis revealed a clear concentration-dependent cytotoxic effect on MCF-7 breast cancer cells; with an IC50 value of approximately 3 µM at 24 h, compared to 1.74 µM for doxorubicin, that indicates a strong antiproliferative activity. As the extract was tested with no significant effect found at IC_{50} of greater than 50µM on HepG2 cells, it does not appear to have much potential as an inhibitor. Meanwhile, there was no apparent impact from the extract based on measured parameters at all levels (IC_{50}>50>100) in terms of inhibitory activities against HepG2, with an IC_{50} approximately equal to 3.2% (tube) and 2.6% (beaker) at their maximum concentration yielding an inhibition rate near 99% suggests that orange peels are very high in the number of phenolic antioxidant compounds. These results reinforce the possibility of considering orange peel as a promising source of plant compounds with antioxidant and selective antiproliferative effects.

Keywords: *Citrus sinensis;* GC MS; MCF-7; HepG2; DPPH

1. Introduction

The peel of the orange fruit (*Citrus sinensis*) is one of the segments of the orange plant that holds various essential qualities and several wellness advantages due to a plentiful reservoir of several constituents, most notably phenols, terpenoids, and flavonoids which display varied biological actions, like antioxidant and antimicrobial characteristics (Mojo *et al*., 2024).

The possible function of flavonoids in combating cancers is frequent in orange rind due to their capacity to encourage apoptosis routes and adjust cellular communication linked with cell division stoppage (Rahmani, 2023). Conversely, chemical assessments employing GC-MS technology revealed a fluctuation in the proportion of volatile to non-volatile elements in orange rind, with flavonoids (such as hesperidin) and monoterpenes prevailing in the organic isolates. This somewhat clarifies the difference in bioactivity trends between water-based and alcohol-based extracts (Salman *et al*., 2026; Ashraf *et al*., 2024). Observed inhibitory effect of alcoholic extracts was noted in cancer cell lines such as MCF-7, while liver cell cultures like HepG2 exhibited a lesser response or relative immunity under parallel conditions. This is due to the nature of the metabolic differences occurring in hepatocytes and protozoa, as well as the abundance of excretory ABC transporters, which leads to the accumulation and reduction of intracellular active compounds (El Hachlafi, 2024; Guo et al., 2011). On the other hand, DPPH tests for antioxidant activity indicated that the alcoholic extract showed similar activity at high concentrations to known standards, which supports the fact that the electron-donating properties of flavonoids contribute to their antioxidant effects, and may help in the death of cancer cells through oxidative stress (Ashraf *et al*., 2024). The configuration of observed selective compounds and their combined

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properties within the botanical extract can clarify and offer a foundation for developing standardized and uniform plant constituents or boosting the effectiveness of conventional medications (Rahmani, 2023; El Hachlafi, 2024).

According to the above, This present investigation sought to pinpoint and describe the primary elements of the examined alcoholic concentrate via GC-MS analysis, assess its selective cell-killing effect toward MCF-7 and HepG2 lines, and employ the DPPH test to gauge its free-radical scavenging ability..

2. Materials and Methods

2.1. Preparation of the Extract

The modified soaking method described by Evans (2009) was used to prepare the alcoholic extract of orange peels, in which orange peels were collected, washed, dried, and ground into a fine powder. Use 100 ml of 75% ethanol and place it in a glass container. Mix it with 100 grams of powder and leave the mixture for 72 hours at room temperature, shaking it twice a day, and then, the extract was filtered employing filter paper, and the resulting liquid was concentrated using a rotary evaporator under diminished pressure at 40 °C until the solvent vanished. The residual extract was dried to a steady mass, then kept at 4°C until needed.

2.2. GC-MS analysis

An Agilent Technologies gas chromatography-mass spectrometry (GC-MS) apparatus (fitted with a mass spectrometer sensor) was employed to ascertain the constituents of the citrus peel alcoholic infusion and to contrast the mass spectra with (NIST) and calculate their proportions relying on the magnitude of the chromatographic peaks (Watson and Sparkman, 2012).

2.3. Cytotoxicity assay

The MTT colorimetric test was employed to assess the cytotoxic effects of an alcoholic extract from orange peel against both breast carcinoma cells (MCF-7) and hepatic cancer cells (HepG2) (Mossman, 1983). The cells were grown in altered DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, then kept in a moist incubator at 37°C and 5% CO2. (Freshney, 2016). Subsequently, a 0.5 mg/ml MTT solution was introduced to each well, and the plates were incubated for 4 hours (Mosman, 1983). The culture medium was then decanted, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the resulting crystals. A microplate reader was utilizing at 570 nm to determine the light absorption. Cells were treated with different concentrations (0.125, 0.25, 0.5, 1, 5, 10, 25, and 50 µg/ml) in three independent replicates (n = 3).

Cell viability was determined using the following formula:

$$\% \text{ Cell V.} = (\text{Treated internalization} / \text{Control internalization}) \times 100.$$

2.4. Evaluation of Antioxidant Activity

The measurement of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was utilized to evaluate the antioxidant effectiveness of the alcoholic extract, following the method outlined by (Brand-Williams *et al*., 1995). DPPH was readied in methanol, combined with the extract and left for thirty minutes to react at ambient temperature. The spectral absorption decrease at 517 nm was noted using UV-Vis spectroscopy, with a sample-free DPPH liquid employed as a standard. The inhibition percentage was computed using the subsequent equation: Inhibition (%) = [(control sample Abs. - sample Abs.) / control sample Abs.] × 100

The IC_{50} value was extracted from the inhibition concentration curve. Assessments were performed three times from the inhibition concentration curve. Measurements were taken three times, and results are presented as mean SD± standard deviation.

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.0. (ANOVA) analysis followed by Tukey's multiple comparison test was used to determine inter-group differences. Differences were considered statistically significant when the p-value was less than 0.05 ($P < 0.05$) (Motulsky, 2022). The value of IC_{50} was ascertained using nonlinear regression analysis of concentration-response plots following the variable-slope dose-with GraphPad Prism program according to (Sebaugh, 2011).

3. Results and Discussion

3.1. GC–MS analysis

Table (1) of the GC-MS chemical analysis data shows that the alcoholic extract of orange peel contains a diverse range of chemically active compounds. The most prominent of these compounds are terpenoids, flavonoids, fatty acid esters, and phenolic derivatives. The largest relative amount of 1-methyl-4-(1-methylethinyl)cyclohexene was observed at 38.89%, succeeded by the flavonoid hesperidin, trimethylsilyl ether at 21.19%, suggesting the prevalence of terpenoids and flavonoids in the alcoholic extract. These findings show the effectiveness of ethanol extraction in retrieving semipolar substances, particularly flavonoids and their silica derivatives following derivatization, which aligns with the outcomes of research on the chemical makeup of citrus rinds and their function as a plentiful source of active agents (Goulas and Manganaris, 2012).

Table 1 Major Compounds identified by GC–MS Analysis

RT (min)	Compound Name	Library Score	TIC %
4.802	Phenacylidene diacetate	78.34	2.7578
11.621	Nonane, 4,5-dimethyl-	66.72	0.4132
19.261	Succinic acid, diphenyl ester	48.79	2.1603
19.433	Myo-Inositol, 4-C-methyl-	51.46	2.5862
19.584	Hesperidin, trimethylsilyl ether	66.64	21.1977
22.731	Hexadecanoic acid, ethyl ester	48.77	0.4064
27.522	1-Methyl-4-(1-methylethenyl) cyclohexene	91.09	38.8984
31.126	1-(4-Trimethylsilyloxyphenyl)-2,3-diphenyl-prop-2-en-1-one	37.63	0.5234
31.611	N-[4-(1H-Benzoimidazol-2-yl)-phenyl]-acetamide	36.8	0.6892
32.089	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene (2TMS derivative)	63.02	0.6786
32.908	4-Butylbenzoic acid, 3-methylbut-2-yl ester	51.03	3.5134
35.641	[Terphenyl]-4-carbonitrile derivative	29.18	1.2119
38.336	Pyrrolo[2,1-b]benzothiazole, 2-phenyl-	32.84	3.5926
42.074	Butane, 1,4-bis(dicyclopentylphosphino)-	30.57	5.0743
47.454	Benzenesulfonamide derivative	37.74	3.4805

3.2. The toxic effect of the extract on MCF-7 cells

The results in Table 2 and Figure 1 indicate that treatment with the alcoholic orange peel extract after (24 h) resulted in a dose-proportional decrease in MCF-7 cell viability, As well as, a marked, progressive inhibition was observed at high concentrations (10–50 μM), with cell survival decreasing to 9–11%, while viability gradually increased at low concentrations (≤1 μM). The approximate IC_{50} value of the extract was ≈ 3 μM, suggesting potent inhibiting efficacy against breast cancer cells. Regarding doxorubicin, the IC_{50} value was recorded at 1.74 μg/ml. The similarity of these values suggests that the studied alcoholic extract exhibited a significant antiproliferative effect, which may be attributed to the presence of flavonoids and terpenes among its main components. These results are consistent with those of Minotti et al. (2004), who linked the sensitivity of cancer cells to doxorubicin to low concentrations of the drug, and with the ability of phenols to inhibit apoptosis pathways and modulate BAX/BCL-2 balance.

Table 2 The toxic effect of ethanolic extract on MCF-7 cells

Concentration (µg/mL)	Cell Viability (%)	Cell Viability (%)- DOX
50	9 ± 0.25	5 ± 0.5
25	11 ± 0.25	7 ± 0.6
10	10 ± 0.11	12 ± 0.7
5	26 ± 1.91	28 ± 1.4
1	74 ± 1.48	55 ± 1.9
0.5	77 ± 3.21	72 ± 2.2
0.25	80 ± 3.77	84 ± 2.9
0.125	84 ± 1.41	91 ± 3.2

Data are expressed as mean ± SD (n = 4).

IC_{50} (24 h) = ≈ 3 µM

IC_{50} (24 h) = ≈ 1.74 µM for DOX

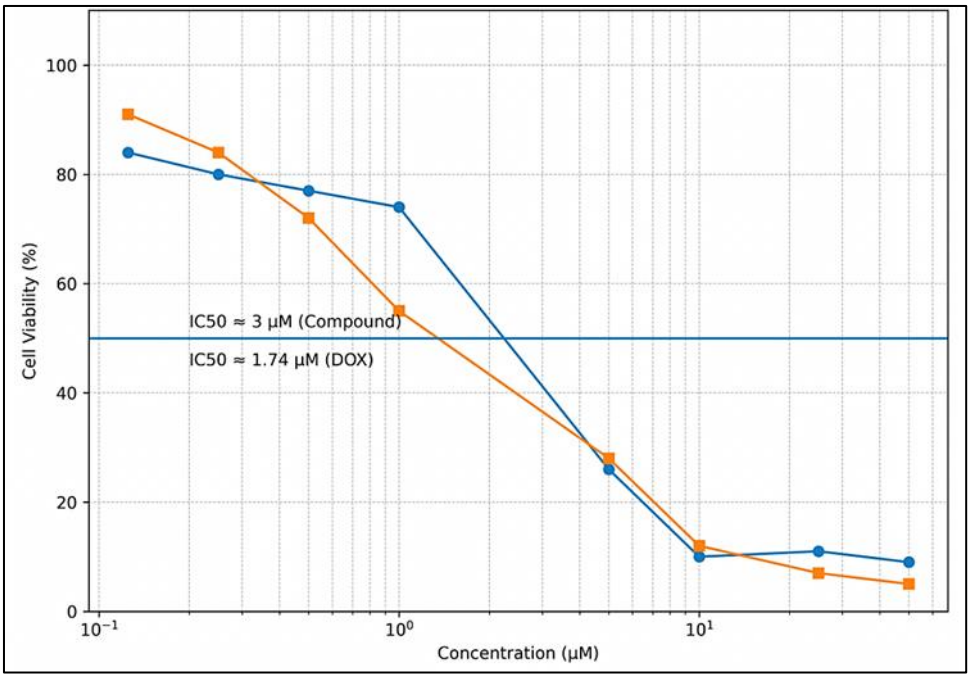

Figure 1 Dose – Response curve (MCF7)

3.3. Selective Cytotoxicity Against HepG2 Cells

Regarding HepG2 liver cells treated with the ethanolic extract for 24 hours, the results in Table 2 and Figure 2 did not show any clear inhibitory effect, as cell viability remained high (>58%) even at the high concentration of 50 µM. Furthermore, the IC_{50} value did not reach the studied range of IC_{50} > 50 µM, reflecting the weak response of HepG2 cells to the extract components compared to MCF-7 cells.

Conversely, the results in the same table indicated that doxorubicin exhibited strong inhibitory activity against HepG2 cells, with an IC_{50} value of 3 µM, reflecting the expected result for a typical broad-spectrum drug. The high metabolic properties of the studied plant extract may be the reason for the low response of HepG2 cells to it, including its ability to initiate clearance pathways as well as promote the emergence of drug removal transporters. This difference between

the two types of cells studied may be due to molecular and metabolic differences between them (Guo *et al*., 2011; Al-Hassnawi and Al-Burki, 2023).

Table 3 Cytotoxic Effect of the Ethanolic Orange Peel Extract on HepG2 Cells

Concentration (µg/mL)	Cell Viability (%)	Cell Viability (%)- DOX
50	58.4 ± 3.2	4 ± 0.6
25	82.1 ± 2.8	6 ± 0.7
10	89.6 ± 2.5	15 ± 0.9
5	92.6 ± 2.2	32 ± 1.7
1	96.4 ± 1.8	68 ± 2.2
0.5	97.8 ± 2.4	82 ± 2.8
0.25	98.2 ± 3.1	92 ± 2.1
0.125	98.7 ± 2.7	96 ± 3.3

IC_{50} (24 h) = > 50 µM

IC_{50} (24 h) = ≈ 3.0 µM for DOX

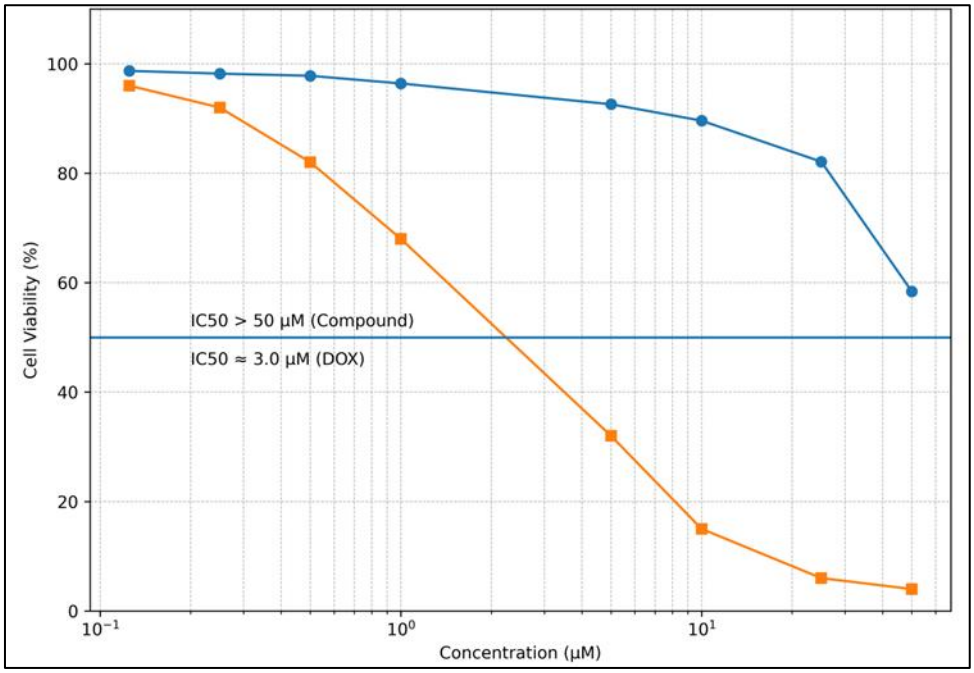

Figure 2 Dose–Response Curve (HepG2, 24 h)

3.4. DPPH Radical Scavenging Activity

The data from Table (4) and Figure (3) regarding the DPPH test show that the inhibitory effect of the alcoholic extract of orange peel is highly concentration-dependent in both the tube and beaker tests. At 100% concentration, the inhibition rate was approximately 99%, with a gradual decrease at lower concentrations,confirming a clear dose-response trend. The IC_{50} values for the tube were 3.2% and for the beaker 2.6%, while the IC_{50} value for vitamin C (as a standard antioxidant) was less than 1.625%, meaning it is more effective. The abundance of phenolic and flavonoid compounds in orange peel does suggest they are effective at donating electrons and neutralising stable free radical species like DPPH. Numerous studies results have shown that orange peels contain a greater quantity of compounds with high antioxidant activity, esepecially hesperidin and phenolic derivatives (Goulas and Manganaris, 2012).

Table 4 DPPH Radical Scavenging Activity of the Ethanolic Orange Peel Extract

Concentration (%)	% Inhibition (Tube)	% Inhibition (beaker)	Ascorbic Acid
100	99.48 ± SD	98.45 ± SD	99.12 ± SD
50	90.74 ± SD	96.70 ± SD	94.98 ± SD
25	84.97 ± SD	93.51 ± SD	87.96 ± SD
12.5	81.27 ± SD	84.97 ± SD	78.19 ± SD
6.25	69.50 ± SD	66.66 ± SD	75.06 ± SD
3.12	49.53 ± SD	58.62 ± SD	67.16 ± SD
1.625	31.36 ± SD	34.27 ± SD	59.14 ± SD

IC_{50}:

Tube extract = ≈ 3.2%

beaker extract = 2.6%

Ascorbic acid = < 1.625%

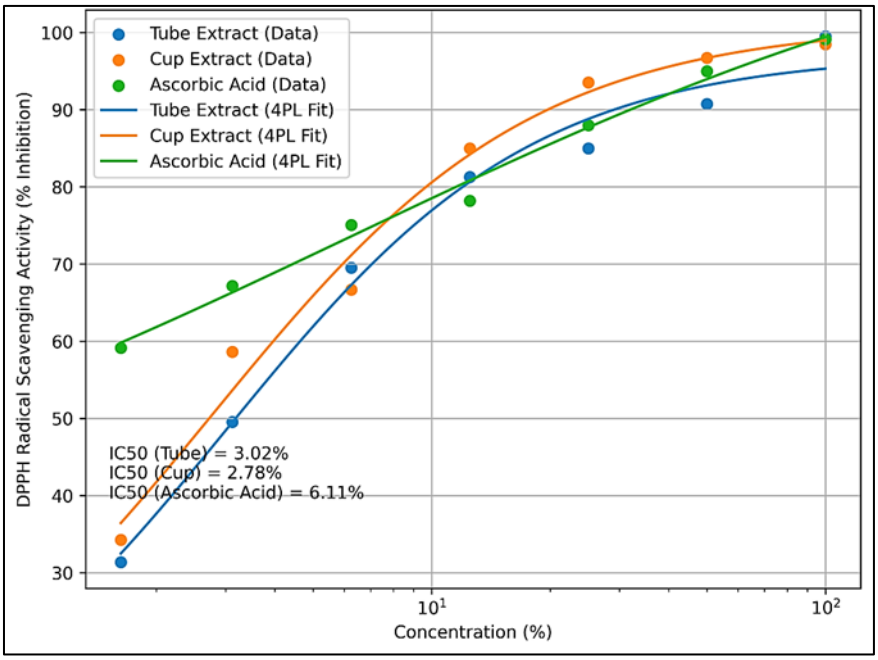

Figure 3 DPPH Radical Scavenging Activity (4PL Nonlinear Regression)

4. Conclusion

The ethanolic orange peel (*Citrus sinensis*) extract has an active chemical composition and high concentration of flavonoids and terpenoids that GC-MS revealed are responsible for very high levels of antioxidant activity and selectively inhibit MCF-7 breast cancer cells (IC_{50} ≈ 3 μM) by approximately 50%, In contrast, its effect on HepG2 cells was weak, supporting its potential as a promising source of anti-cancer compounds that warrant further in-depth studies.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declares no conflict of interest.

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