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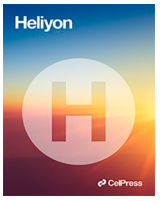

Research article

Validating the protective role of orange and tangerine peel extracts foramending food safety against microorganisms' contamination using molecular docking

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ABSTRACT

Latest studies indicated that agro-food wastes are considered renewable sources of bioactive compounds. This investigation aimed to utilize natural extracts of citrus peels as antimicrobial and anti-aflatoxigenic agents for food safety. The bioactivity of two citrus peels was assessed by total phenolic, flavonoids, and antioxidant activity. Nanoemulsions were manufactured using high-speed homogenization. The mean particle size of the nanoemulsions ranged from 29.41 to 66.41 nm with a polydispersity index of 0.11–0.16. The zeta potential values ranged from −14.27 to −26.74 mV, indicating stability between 81.44% and 99.26%. The orange peel extract showed the highest contents of total phenolic and flavonoids compared to the other extracts and nanoemulsions (39.54 mg GAE/g and 79.54 mg CE/100 g, respectively), which agreed with its potential antioxidant activity performed by DPPH free radical–scavenging and ABTS assays. Chlorogenic, caffeic, ferulic, and catechin were the dominant phenolic acids in the extracts and nanoemulsions, while quercitrin, rutin, and hesperidin were the most abundant flavonoids. Limonene was the major volatile component in both oils; however, it was reduced dramatically from 92.52% to 76.62% in orange peel oil and from 91.79 to 79.12% in tangerine peel oil. Consistent with the differences in phenolics, flavonoids, and volatiles between orange and tangerine peel extracts, the antibacterial properties of orange extracts had more potential than tangerine ones. Gram-positive bacteria were more affected by all the examined extracts than Gram-negative ones. The antifungal activity of orange extract and nanoemulsion on seven fungal strains from *Aspergillus* spp had more potential than tangerine extracts. Additionally, using a simulated media, the orange peel extract and its nanoemulsion had a more anti-aflatoxigenic influence. Molecular docking confirmed the high inhibitory action of flavonoids, especially hesperidin, on the polyketide synthase (−9.3 kcal/mol) and cytochrome P450 monooxygenase (−10.1 kcal/mol) key enzymes of the aflatoxin biosynthetic mechanism.

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1. Introduction

Fruits and vegetables are identified as key sources of a wide variety of non-digestible food components and phytochemicals, which, independently or in combination synergistically, function to contribute to nutritional and health benefits [1]. Furthermore, fiber consumption in a healthy dietary pattern, such as the Mediterranean Diet, has substantially affected health. Many polyphenols and carotenoids linked with the fiber matrix are transported through the human stomach by dietary fiber from fruits and vegetables. Despite these benefits and recommendations, plant food intake remains low, and most diets worldwide lack dietary fiber and antioxidant components [2].

The food-processing sector generates by-products with high antioxidant content and phytochemicals, sometimes representing a proportion more significant than the edible part. For example, citrus peels represent 30–50% of fruit weight during processing; these by-products are discarded and considered a vast environmental load [3]. Various plant food by-products have abundant fiber, essential oil, and polyphenolic chemicals. These components can play a pivot function, acting as antioxidant potency and antiradical power. These by-products can be used as food additive sources for their cheap valuable component [4]. Researchers have demonstrated that tangerine peel possesses high nutritional value due to phenolic, flavonoids, essential, and volatile compounds [5,6]. Pharmacological studies of citrus peels have revealed several bio-activities such as anti-inflammatory, antioxidant, antitumor, antimicrobial, and anti-atherosclerosis [7–9].Significant quantities of citrus peels are not used in proper applications, and little effort was made to use these residues as livestock feed [10]. The bioactive components extracted from the plant by-products were reported to possess antifungal activity, particularly against toxigenic fungi [11]. The significant risk is the capacity of these fungi to produce secondary harmful metabolites known as Mycotoxins [12,13].

Aflatoxins are a significant hazard that threatens food production, causing non-safety conditions for the final food product [14–16]. The presence of toxigenic fungi on food materials is the primary source of aflatoxin contamination. Non-traditional and essential oils are a source of active molecules having many therapeutic activities and play a key role against fungal infection. Applying bioactive components to fungal growth could reduce vegetative growth and metabolic-producing compounds [17]. Otherwise, polar extracts that contain phenolic compounds also participate in the antifungal impact of the plant extracts [18]. Phenolic acids were reported by efficiency toward the mycotoxin production in liquid media [13]. However, the volatile components present in essential oil may have other mechanisms of reduction impact [11]. The encapsulation could serve as a suitable application with control-releasing characteristics to preserve the activity of the bioactive molecule [19,20].

The study aimed to compare alcoholic and oil extracts activities of orange and tangerine peels as antioxidant, antimicrobial, and antifungal agents, particularly the toxigenic fungi. The bioactive components in extracts and oils under investigation were screened and identified using high-performance liquid chromatography and gas chromatography-mass spectrometry. Encapsulation was applied in the form of nanoemulsion by high-speed homogenization to preserve the bioactive components of the utilized extracts, achieve a controlled releasing impact, and decrease their hydrophobicity. A simulated media was used to evaluate the anti-aflatoxigenic effect of applied extracts to evaluate mycelial growth inhibition and aflatoxins reduction with an in-silico study through molecular docking against polyketide synthase and cytochrome P450 monooxygenase, which play a crucial role in AFB_1 biosynthesis via polyketide pathway.

2. Materials and methods

2.1. Chemicals, materials, and microorganisms

The chemicals and solvents of the analytical grade were utilized and obtained from Sigma-Aldrich® Solutions (Louis, USA), including the DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) and Folin-Ciocalteu were purchased from Sigma–Aldrich, Inc.). Citrus peels were acquired as fresh as possible under sanitary and hygienic conditions. The peels were cleaned, dried, ground, and kept in self-adhesive bags for evaluation. A knife was utilized for slicing the peel used for oil extraction, where the white pulp was excluded before extraction.

Microorganisms used for the antimicrobial assays were classified as Gram-positive bacteria (*Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 7644, *Enterobacter aerogenes* ATCC 13048); and Gram-negative bacteria (*Escherichia coli* NCIB 86, *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* NCIB 950, and *Klebsiella pneumonia* NCIB 418). These isolates were received from the DSMZ microbial collection (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), maintained on nutrient agar slants for 24 h/37 °C, and kept in the refrigerator (4 °C) until use. The toxigenic strains of *Aspergillus* fungi were *A. niger* ITEM 3856, *A. flavus* ITEM 698, *A. ochraceous* ITEM 828, *A.nomius* ITEM 303, *A. carbonarius* ITEM 5011, and *A. fumigatus* ATCC 16424. These strains were purchased from Agro-food microbial culture collection, Institute of Sciences of Food Production (ISPA), Italy.

2.2. Preparation of peel extracts

Isopropanol Extraction was done using the previous methodology [21]. In brief, the milled, dried powder from peels was sonicated in isopropanol solution (1:4; v/v) using an Ultrasonic probe (amplitude 45%, 80 kHz, duty 60%, time 40 min, 20 °C). The gained extract was then lyophilized by the same apparatus and kept dry for further evaluation.

Using Clevenger-type hydrodistillation method for the volatile components was carried out according to the British Pharmacopeia methodology described before [22]. The equipment was loaded with 200g of air-dried peel sample. The hydrodistillation was carried

out (4 h) in a circulatory Clevenger-type system until the volatile contained in the peels was exhausted.

2.3. Preparation of peel extract nanoemulsions

The peel extracts were transformed into a nanoemulsion using a modified version of the method described in a previous study [23]. To create the emulsion, 3% (w/v) carboxy-methylcellulose (CMC-food grade) was dissolved in double-distilled water and mechanically stirred for 4 h at 40 °C. The extract phase was then mixed with tween 20 (1:4; v:v) for 2 h to facilitate the formation of coarse emulsion. Next, the CMC solution was added during a high-speed homogenizer (Ultra-Turrax T25, IKA Janke & Kunkle, GmbH Co., Germany) at 18,000 rpm for 5 min. Finally, the emulsion was subjected to ultrasonic treatment using an ultrasonic probe (Q125 Qsonica Sonicator, Church Hill, Newtown 06470, USA).

2.4. Estimation for the nanoemulsion characteristics

The characteristics of nanoemulsion for particle size, zeta potential, polydispersion index (PDI), and emulsion stability were evaluated by the same methodology described by Ref. [24]. The Malvern apparatus (Nano-S90, Zetasizer, Malvern Panalytical Ltd, Enigma Business Park, Grove Wood Road, United Kingdom) was used to determine these key parameters. The emulsion stability was calculated using the following equation

$$\% EE = \frac{VT - VS}{VT} \times 100$$

Where; VT: is the total volume of nanoemulsion and VS: is the separated solution volume.

2.5. Gas chromatography-mass spectrometry (GC-MS)

The impact of nanoencapsulation was studied with GC-MS analysis. 2 mL of the nanoemulsion and 4 mL of diethyl ether were mixed using a vortex mixer. After settling and drying with sodium sulfate anhydrous, the supernatant was transferred to a screw-cap vial and wrapped with aluminum foil at 20 °C until analysis [25]. The extraction process was repeated three times. The components of the supernatant and hydrodistilled peel oils were analyzed by GC/MS apparatus. The mixture was separated using a Trace GC Ultra Chromatography system (Thermo Scientific, USA) with an ISQ-mass spectrometer and a TG-5MS capillary column (60 m × 0.25 mm × 0.25 μm-thick; Thermo Scientific, USA). The column separation was programmed from 50 °C to 260 °C in a total run time of 52 min. The identification of compounds was conducting by matching them with the MS computer NIST library, comparing them with authentic compounds, and published data. The relative percentage of the volatiles was calculated from the GC peak areas. Kovat's index was estimated for each compound by the retention times of a homologous series of C6–C26 n-alkanes and matching them with the literature [26].

2.6. Determination of phenolic fraction in peels extract

The investigation used an Acquity H class UPLC apparatus and a Waters Acquity PDA detector (Waters, USA). The condition and column features were the same as previously mentioned [27]. The phenolic content was calculated by comparing the retention durations of analyte peaks with the injected references at 280 and 320 nm. The quantification limit was set at 10 ng/g material, and the findings were calculated in triplicate.

2.7. Determination of physicochemical parameters of peels and volatile oil

2.7.1. Determination of total phenolic content

To determine the total phenolic contents, the Folin-Ciocalteu technique was utilized following the method described by Ref. [28]. A test tube was used to transfer the extract (500 μL), which was subsequently oxidized with 250 μL of Folin-Ciocalteu reagent. After 5 min, the solution was neutralized using 1.25 mL of aqueous Na_2CO_3 solution (20%). The absorbance was measured against a solution blank (at 725 nm) after 40 min. The total phenolic contents were calculated based on a Gallic acid calibration curve and expressed as mg of Gallic acid equivalent (GAE) per gram of sample.

2.7.2. Determination of total flavonoids contents

The aluminum chloride ($AlCl_3$) colorimetric test was used to evaluate the total flavonoid concentration according to Ref. [29] methodology. In a nutshell, 300 μL of 5% sodium nitrite ($NaNO_2$) was combined with 100 L μL extract and rested for 5 min. Then, 300 μL of $AlCl_3$ solution (10%) was added, and the volume was adjusted to 2.5 mL with distilled water. After 7 min, 1.5 mL NaOH (1 M) was added to the mixture and centrifuged (5000×g/10 min). The absorbance of the supernatant was measured (510 nm) compared to the blank. The total flavonoid content was calculated using a catechin calibration curve and represented as milligrams of catechin equivalent per gram of sample (mg CE/g).

2.7.3. Determination of antioxidant activity

The materials extracted were evaluated for their antioxidant activity using two methods: the DPPH* and ABTS. To determine the free radical scavenging capacity of the extracts, the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH*) was used, following the procedure by Ref. [30]. The final concentration used for DPPH* was 200 μM, while the final reaction volume was 3.0 mL. After incubation in a dark environment for 60 min, absorbance was measured at 517 nm against a blank of pure methanol. The following equation calculated the percent inhibition of the DPPH free radical:

$$\%\ \text{inhibition} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \text{x} 100$$

Where: Ac: is the absorbance of the control reaction (without sample), as: is the absorbance with the test compound.

The 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt radical cation (ABTS+) is the second assay applied for the antioxidant activity measurement. The absorbance was measured at 700 nm using a Shimatzo spectrophotometer for the reducing power evaluation [31], where ascorbic acid was utilized as a positive control and deionized water functioned as a blank.

2.8. Determination of the peel antimicrobial activities

The antimicrobial potentials of extracts from peels were evaluated by paper disc diffusion assay. The antimicrobial activities of citrus peel nanoemulsion were determined against the Gram-negative and Gram-positive bacteria. One milliliter of bacterial strains suspension was inoculated in the nutrient agar medium. After solidifying these media, filter paper discs (Whatman No. 4) containing 100 μL of peel extract were put on the media surface for the disk-diffusion assay.

Six toxigenic fungi strains were investigated to determine the inhibition impact of nanoemulsion extracts. The fungal spores were prepared in a tween-water solution [32], where the spore-count was adjusted before being inoculated. The peel extracts were tested at a concentration of 250 mg loaded for the applied disk. The results were expressed as millimeters of zone inhibition (mm), whereas a more zone inhibition area showed a more sensitive strain against the peel extract. The preliminary studies highlight the applied concentrations as the minimal inhibitory and fungicidal concentrations.

2.9. Determination of aflatoxin reduction in liquid media

A fungal strain of *Aspergillus flavus* ITEM 698 was inoculated to flasks at a 1.31 x 10^4 spore/mL concentration. This strain was tested for aflatoxin production, and it was able to produce the four aflatoxins (AFs) types in liquid media growth. Aflatoxins produced were present aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). The flasks contain a broth media of Yeast extract sucrose (YES). In contrast, the negative control flasks have fungal spores, and the positive control contains the inoculated spores and the treatment (citrus peel or citrus oil extracts). Flasks were incubated (28 °C/10 days), and the mycelia were filtrated and dried in a hot air oven. The constant weight and the reduction in mycelia growth were calculated compared to the negative control growth (fungi spore without treatments).

The peel extracts' impact on reducing mycotoxin secretion was also evaluated in the media filtrate for the simulated experiment. The AFs were extracted and estimated by the AOAC-approved technique used for the extraction step [33]. The culture broth (10 mL) was mixed twice with 10 mL chloroform, shaking vigorously for about 10 min, and then separated using a separating funnel. The lower phase was dried over anhydrous extra-pure sodium sulfate, evaporating the chloroform in nitrogen. HPLC-grade acetonitrile was used to dissolve the dry film. One milliliter of the solution was combined with 10 mL of distilled water before being placed in an Afla-test® immune affinity column and washed twice with 10 mL of distilled water (flow rate: 2 mL/min). Two milliliters of methanol (flow rate: 0.5 mL/min) were used to elute AFs. The quantitative analyses were performed using Agilent 1100 apparatus under the condition mentioned before [13].

2.10. Molecular docking

The crystal structures of cytochrome P450 monooxygenase (A0A1R3RGJ7) and polyketide synthase (A0A1R3RGK0) were both obtained from UniProt (accessed on February 3, 2022). The Pymol program (Schrödinger, Inc., Mannheim, Germany, Ver. 2.5.1) removed the co-crystallized ligands and ions and then protonated the material to make it a receptor. Avogadro Software (Version 1.2.0) employed the MMFF94 force field to optimize the 3d structures of the ligands that were obtained from the PubChem database (accessed on 3–5 February 2022 and January 3, 2023) [34]. Blind docking was carried out using a web-based program called CB-Dock2, which may be accessed starting on January 4, 2023, at (https://cadd.labshare.cn/cb-dock2/php/index.php).Upon submission; CB-Dock2 analyzed the input files and transformed them into pdbqt-formatted files utilizing MGL Tools and Open Babel. Subsequently, CB-Dock2 computed the top N (n = 5 by default) centers and diameters of the protein cavities predicted. Each center, size, and pdbqt file was forwarded to Auto Dock Vina for docking. The final outcomes were displayed after N cycles of processing. Liu et al.'s benchmarks [35] demonstrated that top-ranking postures with RMSDs less than 2 Å from their X-ray crystal structure position had high success rates. In the interface and visualization profiles for the best-docked complexes, CB-Dock2 surpassed other blind docking systems, as verified by Discovery Studio software Ver. 21.1.0.20298 [36].

2.11. Statistical analysis

The tests were conducted three times, and the results were presented as averages with standard deviations (SD). Statistical analysis was performed using social science software (SPSS V.16). The ANOVA method was utilized to determine significant differences in mean values, and Duncan's multiple range test was conducted with a significance level of $p = 0.05$.

3. Results and discussion

3.1. Nanoemulsion characteristics

To ensure that nanoemulsion is of high quality, it is crucial to analyze the particle size. The data in **Table (1)** confirms that the mean particle size of the nanoemulsion is indeed ultra-fine (<100 nm). Additionally, the polydispersity index (PDI<0.2) and the zeta potential range from −14.27 to −26.74 mV. These values indicate the excellent stability of the emulsion against creaming and sedimentation phenomena [37]. The high efficiency found at 81.44–99.26% (Table 1) further confirms the superior quality of the nanoemulsion.In this study, the nanoemulsion's size distribution was found to be finer than those formulated by Ref. [36] for *Citrus sinensis* L. peels oil, indicating that the particle size increased due to micelles swelling to accommodate the solubilized load of extracts. The emulsion's average capsule diameter and size distribution depend on several factors such as surfactants' nature and quantity, viscosity of the aqueous and oil phase, oil phase composition, and manufacturing formulation circumstances. The zeta potential is crucial in ensuring the physical stability of emulsions, with a higher zeta potential (positive or negative) indicating more stable emulsions. In this regard, the orange oil nanoemulsion exhibited the highest potential with the smallest particle size and PDI, as well as increased stability (Table 1).

In emulsion formulation, polydispersity implies the capability to create stable solutions. The influence of polydispersity on emulsion characteristics has been studied for a long time, and its relevance can be demonstrated across academic fields, from fundamental solution research to practical applications. A sample's polydispersity may result from its natural size distribution or from the sample's agglomeration or aggregation during isolation or analysis. The polydispersity index (PDI) quantifies the size-based heterogeneity of a sample. The PDI may be measured experimentally or calculated from electron micrographs. The PDI 0.05 are more typical of mono-disperse samples, whereas values > 0.7 are more typical of a wide size range of particles, as determined by international standards organizations.

Interestingly, the larger particle size of the peels nanoemulsion and their lower stability compared to oils nanoemulsion is owed to Ostwald ripening phenomena associated with the nature of phenolic extract, which has both hydrophilic and hydrophobic compounds. Ostwald ripening, the primaryinstability mechanism for such nanoemulsion is a process in which molecules from the dispersed phase permeate through the continuous phase due to changes in particle curvature, expanding larger droplets and contracting smaller ones. When there is an Ostwald ripening, a concentration gradient appears because the dispersed phase solubility in large droplets (slight curvature) is lower than in tiny droplets (large curvature).

The study showed that the nanoemulsion with orange oil had the smallest particle size observed. When particles are smaller, they may be able to penetrate the cell walls of microorganisms more easily, disrupting their essential functions and causing the disintegration and dispersal of their contents [38]. This oil nanoemulsion had the highest stability value, with most values being attributed to the zeta value. These values increase the effectiveness of the emulsion in reducing toxins by influencing fungal metabolites [39].

3.2. Characterization of phenolic acids and flavonoids using HPLC

Identification of the phenolic compounds in the orange and tangerine peels in **Table (2)** revealed that ferulic acid is the major phenolic acid in the orange peel extract (131.71 μg/g), followed by caffeic acid (131.4 μg/g), then chlorogenic acid (125.3 μg/g),and catechin (124.31μ g/g). On the other hand, ferulic acid (69.77 μg/g), gallic acid (57.24 μg/g), and catechin (47.32 μg/g) were predominant in tangerine peel extract (Table 2). Minor phenolic acids composed of less than 10 μg/g were identified in orange peel extract or not detected in tangerine peel extract like rosmarinic, vanillic, and syringic acids. Flavonoids were also identified in orange and tangerine peel extracts, and the results indicated that quercitrin was the major flavonoid in orange peel extract (226.41 μg/g), followed by rutin (78.93 μg/g) and luteolin (59.83 μg/g). Hesperidin (92.94 μg/g) was the predominant flavonoid in the tangerine peels extract followed by quercetin (23.71 μg/g), kaempferol (16.85 μg/g), and luteolin (16.51 μg/g), while naringin was the lowest detected with 1.97 μg/g (Table 2).

Table 1
Characteristics of the orange and tangerine peel extracts and oils nanoemulsion.

Sample	Particle size	Zeta potential (mV)	PDI	Encapsulation stability (%)
	droplet size (nm)			
Orange Peel Nanoemulsion	34.28 ± 0.21	−14.27 ± 3.17	0.15 ± 0.08	81.44
Tangerine Peel Nanoemulsion	66.41 ± 0.28	−17.05 ± 1.79	0.16 ± 0.09	84.16
Orange Oil Nanoemulsion	29.41 ± 0.05	−26.74 ± 3.54	0.11 ± 0.04	99.26
Tangerine Oil Nanoemulsion	34.21 ± 0.08	−16.31 ± 1.84	0.14 ± 0.05	98.34

Results are expressed in means ± SD (SD: standard division; n = 3; p = 0.05).

Table 2
Phenolic acids and flavonoids in orange and tangerine peel extracts and their nanoemulsion.

Phenolic compound	OP	OPN	TP	TPN	Flavonoids Compound	OP	OPN	TP	TPN
	μg/g	μg/g	μg/g	μg/g		μg/g	μg/g	μg/g	μg/g
Gallic acid	31.21 ± 1.27	32.57 ± 1.71	57.24 ± 1.36	59.17 ± 1.55	Luteolin	59.83 ± 0.71	61.28 ± 0.34	16.51 ± 0.88	18.11 ± 0.38
Chlorogenic	125.3 ± 3.56	117.1 ± 2.55	22.56 ± 1.27	24.67 ± 1.88	Rutin	78.93 ± 1.03	79.55 ± 0.84	5.16 ± 0.73	6.22 ± 0.49
Hydroxybenzoic	27.1 ± 1.34	28.41 ± 2.05	ND	ND	Naringin	57.12 ± 0.82	55.24 ± 1.77	14.33 ± 0.81	15.79 ± 0.28
Catechin	124.31 ± 4.21	127.27 ± 1.31	47.32 ± 1.34	43.05 ± 4.56	Quercitrin	226.41 ± 2.22	229.08 ± 3.14	ND	ND
Epicatechin	62.14 ± 1.84	67.45 ± 1.08	20.54 ± 0.88	21.74 ± 0.37	Hesperidin	ND	ND	92.94 ± 1.23	90.55 ± 1.71
Syringic acid	2.77 ± 0.41	3.49 ± 1.23	ND	ND	Kaempferol	37.6 ± 1.23	37.91 ± 1.09	16.85 ± 0.41	18.05 ± 0.94
Caffeic acid	131.4 ± 1.05	139.61 ± 1.02	2.57 ± 0.67	ND	Quercetin	14.03 ± 0.02	14.66 ± 0.14	23.71 ± 0.5	27.11 ± 0.37
Vanillic	2.74 ± 0.37	3.49 ± 0.21	ND	ND	Naringin	7.34 ± 0.51	10.34 ± 0.81	1.97 ± 0.37	2.18 ± 0.05
Rosmarinic	8.66 ± 0.51	10.07 ± 0.73	ND	ND					
Ferulic	133.71 ± 5.14	135.05 ± 2.38	69.77 ± 1.47	70.92 ± 1.08					
***p*-Coumaric**	34.94 ± 2.26	36.18 ± 1.59	29.81 ± 0.74	32.01 ± 0.24					

Results are expressed in means ± SD (SD: standard division; n = 3; p = 0.05). OP: orange peel extract; OPN: orange peel nanoemulsion; TP: tangerine peel extract; TPN: tangerine peel nanoemulsion.

The above findings agreed with [40], where many phenolic acids were predominant in citrus peels, including gallic, caffeic, chlorogenic, protocatechuic, hydroxybenzoic, syringic acid, *p*-coumaric, acid, and vanillic acids. In the same line, ferulic, chlorogenic, caffeic acids, rutin, and hesperidin were among the characteristic constituents of tangerine extracts [41]. The quantitative differences observed compared to the above references could be due to the extraction techniques and solvents used in addition to the endogenous and exogenous factors such as genetic variability, differences in growing sites, agronomical practices, and environmental conditions, that were stated as the chief reasons affecting the chemical composition of the plant. Notably, the wide variation in the flavonoids and phenolic compounds and the difference in their concentrations are responsible for the variation in bioactivity.

3.3. Volatile constituents of the HD oils and their nanoemulsion

GC-MS characterized the orange peel oil's chemical constituents (Table 3). Five components were identified, representing 99.99%,

Table 3
Identification of the volatile constituents of peels oils and their nanoemulsion using GC-MS.

S/N	Compound	RI[a]	LRI[b]	Area%				Identification method[c]
				Orange peel oil	Orange oil nanoemulsion	Tangerine peel oil	Tangerine oil nanoemulsion	
1	α- Pinene	937	939	1.83	1.7	1.47	1.14	RI, MS, STD
2	Sabinene	978	976	–	0.95	1.81	0.68	RI, MS, STD
3	α- Myrcene	986	991	3.39	4.25	3.41	1.68	RI, MS, STD
4	Limonene	1034	1031	92.52	76.62	91.79	79.12	RI, MS, STD
5	γ- Terpinene	1070	1062	1.06	0.76	0.31	0.29	RI, MS, STD
6	Linalool	1100	1098	1.19	0.61	0.3	1.8	RI, MS
7	Limonene oxide	1139	1130	–	1.32	–	0.61	RI, MS
8	Decanal	1209	1204	–	1.01	–	0.35	RI, MS
9	*trans* - Carveol	1220	1217	–	1.74	–	–	RI, MS
10	Carvone	1239	1243	–	3.99	–	–	RI, MS, STD
11	Limonene aldehyde	1325	1328	–	1.29	–	–	RI, MS
12	*Z*- Citral	1381	1383	–	–	–	5.83	RI, MS
13	*E*- Citral	1392	1391	–	–	–	7.95	RI, MS
14	Dodecanal	1412	1407	–	1.37	–	–	RI, MS
–	**Total**	–	–	**99.99**	**95.61**	**99.09**	**99.45**	–

[a] RI: retention indices calculated on DB-5 column using alkanes standards.
[b] LRI: retention indices according to literature.
[c] Confirmed by comparison with the retention indices, the mass spectrum of the authentic compounds, and the NIST mass spectra library data.

of the total oil. Limonene (92.52%), α-myrcene (3.39%), α-pinene (1.83%), and γ-terpinene (1.47%) were the dominant compounds in the peels oil. Similar results were reported by Ref. [36]. On the other hand, six compounds were detected in tangerine peels oil, accounting for 99.09%. Again, limonene (91.79%), α-myrcene (3.41%), sabinene (1.81%), and α-pinene (1.47%) were the major compounds in the tangerine peel oil (Table 3), which agreed with [42,43].

The chemical analysis of the nanoemulsion by GC–MS showed a significant difference from that of the essential oils, which supports previous reports by Ref. [36]. Twelve and ten constituents were identified in orange and tangerine oils nanoemulsion, representing 95.61% and 99.45% of the total nanoemulsion oil, respectively (Table 3). Like tangerine peel oil, limonene was predominant in both nanoemulsions but with different quantities (76.62 and 79.12%), as shown in Table 3. Despite their absence in oils, some of the oxygenated terpenes, e.g., limonene oxide, *trans*-carveol, carvone, limonene aldehyde, and citral isomers, were generated in both nanoemulsions (Table 3). A few studies have suggested that the emulsion's Ostwald ripening, flocculation, or coalescence may change its physical stability and biological activity [36]. Examples of energy-intensive processes that cause the disintegration of the active components of essential oils and the buildup of others include high-pressure homogenization, high-intensity ultrasound, and high-shear homogenization. Interestingly, non-oxygenated terpene concentrations in the crucial oil decreased as oxygenated terpene concentrations increased in the oil nanoemulsion (Table 3). As a result, more research can be done to determine the stability of various fragrances and volatile chemicals during microencapsulation procedures, particularly under harsh conditions, and to learn how these volatile compounds are transferred to other substances.

3.4. Phenolic and flavonoid contents and the antioxidant activity

In the present study, isopropanol extracts, HD oil contents, and their nanoemulsion (fororange and tangerine) were used to determine total phenols (Table 4). Data indicate that the tangerine hadthe highest total phenolic content (41.9 mg GAE/g) compared to orange peels, which had 39.54 mg GAE/g. Compared to the literature, results were consistently found to depend on the solvent used during the extraction procedure. For example, despite the solvent used, the above findings agreed with [44], where the mandarin orange peel methanolic extract had higher total phenol content than the orange peel extract. In contrast, fresh orange peel contained higher total phenolic content than mandarin and lemon. According to Ref. [45], the effectiveness of total phenolic extract from citrus peels was based on the solvent type in the order of ethanol > methanol > acetone > water > petroleum ether > hexane which established the effectiveness of polar solvents in comparison with to organic non-polar solvents.

The amount of total flavonoids acquired from the peels ranged from 79.54 to 30.2 mg catechol (CE)/100 g dry weight for orange and mandarin isopropanol extracts to 0.84 and 0.09 mg catechol/100 g for the HD oils (Table 4). The highest total flavonoid for the orange peel extract was in agreement with [32], where orange peel ethanolic extract was the highest among many citrus peels. Again, the extraction of flavonoids depended on the type of solvent used, in the order of ethanol > acetone > water and methanol > petroleum ether > hexane. Generally, avoiding aggressive intensive-energy extraction steps and using environment-friendly solvents can offer an economic and safe source of bioactive constituents like phenolic and flavonoids from citrus fruit wastes that could be applied for food processing and preservation.

The antioxidant capacities of the investigated samples were determined by DPPH and ABTS radical scavenging assays (Table 4). Based on the DPPH assay, the most excellent antioxidant capacity was obtained for the extract of tangerine peel (IC_{50} 11.53 mg/ml). In contrast, the extract of orange peel had the lowest antioxidant capacity (IC_{50}23.47 mg/ml). Both tangerine and orange peel oils had higher antioxidant efficiency than orange peel extract; IC_{50} 15.8 and 18.21 mg/ml. The above findings agreed with [44]. According to the correlation analysis performed by [46], the essential factor in determining the antioxidant activity potency of phenolic and flavonoids is their molecular structure rather than their content. Therefore, the total contents of phenolic and flavonoids cannot be a critical factor in predicting antioxidant efficiency. The potential antioxidant activities of the oils were owed to the presence of limonene as a significant component in both oils and its combination with caryophyllene which may enhance the bioactivity of these oils [47].

Table 4
Total phenolic and flavonoid contents and the antioxidant activities of orange and tangerine peels extracts, oils, and their nanoemulsion.

Variables	Total phenolic (mg GAE/g)	Total flavonoid (mg CE/100 g)	Antioxidant activity (mg Asc./100 mL)		DPPH IC_{50} (mg/mL)
			DPPH	ABTS	
Orange peel	39.54 ± 1.88	79.54 ± 0.95	71.39 ± 0.55	66.1 ± 0.67	20.47 ± 0.57
Tangerine peel	41.9 ± 0.37	30.2 ± 0.36	55.1 ± 0.34	53.2 ± 0.64	11.53 ± 0.15
Orange nanoemulsion	31.11 ± 2.08	40.664 ± 1.87	43.55 ± 1.64	34.19 ± 2.05	21.91 ± 2.17
Tangerine nanoemulsion	32.77 ± 1.54	27.98 ± 2.18	37.34 ± 1.74	36.55 ± 2.37	14.82 ± 2.11
Orange oil	2.11 ± 0.31	0.84 ± 0.05	43.27 ± 0.3	47.3 ± 0.34	10.21 ± 1.08
Tangerine oil	0.42 ± 0.54	0.09 ± 0.02	31.02 ± 0.44	33.4 ± 0.77	9.18 ± 0.94
Orange oil nanoemulsion	2.89 ± 00.54	0.91 ± 0.02	41.57 ± 1.56	32.18 ± 1.66	11.74 ± 1.87
Tangerine oil nanoemulsion	0.74 ± 0.54	0.1 ± 0.12	28.51 ± 0.94	25.41 ± 0.37	10.08 ± 0.74
Ascorbic acid	–	–	–	–	3.11 ± 0.22

Results are expressed in means ± SD (SD: standard division; n = 3; p = 0.05).mg GAE: milligrams of Gallic acid equivalent; Asc: ascorbic acid; mg CE: milligrams of catechol.

3.5. Antibacterial activity

The antibacterial properties of orange and tangerine extract revealed that Gram-positive bacteria were more affected by the extract than Gram-negative ones. **Table (5)** results indicate that *Listeria monocytogenes* were the most affected strain with orange peel extract, followed by *Staphylococcus aureus*, *Enterobacter aerogenes*, and finally, *Bacillus cereus*. While for the Gram-negative bacteria, *Salmonella typhi* was the most affected by orange peel extra as the maximum zone inhibition was recorded at 16.14 ± 0.87 mm followed by E coli 15.71 ± 1.05 mm and *Pseudomonas aeruginosa*11.25 ± 1.54 mm while *Klebsiella pneumoniae* was the least affected with zone inhibition of 10.97 ± 1.66 mm (Table 5).

A different trend was recorded for tangerine peel extract, as there was no significant difference in the response of Gram (–ve) and Gram (+ve) bacteria. The most affected strain was the Gram (+ve) *Staphylococcus aureus,* followed by the Gram (–ve) *Pseudomonas aeruginosa*, and the least affected was Gram (-ve)*E coli* and *Salmonella typhi,* and the overall inhibitory effect of orange peel extract was higher than tangerine peel extract.

According to our findings, the essential oils derived from mandarin, Hallabong, Cheonhyehyang, Redhyang, and orange contain limonene, γ-terpinene, linalool, β-myrcene, β-ocimene, octanal, and sabinene as their main components. These essential oils have exhibited antibacterial properties against certain food-borne pathogens. Based on this information, it is suggested that Citrus peel essential oil can be utilized for its antibacterial benefits [48–50].

Natural extracts can have antibacterial effects against Gram-negative, Gram-positive, and pathogenic bacteria. These antibacterial activities can be observed as well in plant extracts. Secondary metabolites are naturally occurring chemicals that many plants produce, and these metabolites often exhibit antibacterial activity. These substances function as plant defense mechanisms, combating illnesses caused by microorganisms. It is essential to be aware that even though natural extracts can exhibit antibacterial activities, these effects' intensity and efficacy may vary depending on variables such as the particular source extracted, the extraction process utilized, and the bacteria being targeted. In addition, natural extracts could be evaluated as possible adjuncts to treating bacterial infections with other preserving technology.

3.6. Antifungal activity

Assessment of The antifungal activities of orange and tangerine peel extracts was assessed on seven fungal strains from *Aspergillus*spp (Table 6); it revealed that *A. carbonarius* was the most sensitive to orange peel extract, followed by *A. ochraceous* and *A. fumigatus* then *A. parasiticus* and *A. niger* showed the minor zone inhibition this trend differed for tangerine peel extract as *A. fumigatus* was the most affected by tangerine peel extract then *A. ochraceous* and *A. nomius* respectively followed by *Aspergillus parasiticus* while both *A. niger* and *A. carbonarius* showed a minor sensitivity to tangerine peel extract.

Natural extracts' antifungal properties and nanoemulsions have been shown against various toxigenic fungal strains; Plant-based chemicals may have antifungal effects in addition to their antibacterial products. The activity of Natural extracts and essential oils against microorganism infection may increase if transformed into nanoemulsion solutions. Benefits of nanoemulsions that may contribute to their antifungal efficacy include more stability, solubility, and bioavailability of the active components. Improved antifungal activity against several fungal infections, including *Candida* species, has been established in nanoemulsions containing tea tree oil. Coconut oil also has antifungal qualities thanks to fatty acids like lauric acid. Several types of fungi, including *Candida albicans* and *Aspergillus* species, are inhibited by nanoemulsions containing coconut oil. Citrus polyphenols and citric acid are natural components of grapefruit seed extract with antifungal properties. Grapefruit seed extract nanoemulsions have shown effective antifungal activity against various fungal infections, including *Candida* species [48].

The previous investigations indicated that a nanoemulsion including two antifungal components derived from citrus successfully exhibited remarkable stability and showed exceptional antifungal efficacy against *P. italicum*. The possible antifungal mechanism was linked to the suppression of fungal spore germination and mycelial development and the disruption of cell membrane integrity and permeability [51]**.** A further investigation by Ref. [52] used nanoemulsion application to restrict fungal growth and mycotoxin synthesis. The antifungal mechanism was associated with the suppression of fungal spore germination and cell membrane disruption, resulting in the release of internal proteins and nucleic acids [53]. conducted further research to examine the effects of nanoemulsions

Table 5
Antibacterial activity of orange and tangerine by-products determined as inhibition zone diameter for peels and oil extracts.

	Orange extract	Tangerine extract	Orange oil	Tangerine oil
Gram-positive strains				
Staphylococcus aureus	19.84 ± 1.0	11.17 ± 0.34	22.33 ± 1.04	11.67 ± 0.76
Bacillus cereus	18.27 ± 0.88	9.64 ± 0.55	15.90 ± 0.79	9.35 ± 0.51
Listeria monocytogenes	21.15 ± 1.57	8.91 ± 0.54	20.81 ± 1.54	12.24 ± 1.02
Enterobacter aerogenes	18.56 ± 1.14	8.77 ± 0.84	18.21 ± 1.34	10.71 ± 1.22
Gram-negative strains				
E. coli	15.71 ± 1.05	7.3 ± 0.61	18.25 ± 1.02	3.34 ± 0.11
Salmonella typhi	16.14 ± 0.87	7.28 ± 1.04	14.35 ± 0.73	5.47 ± 0.27
Pseudomonas aeruginosa	11.25 ± 1.54	10.15 ± 1.37	13.28 ± 0.54	4.81 ± 0.54
Klebsiella pneumoniae	10.97 ± 1.66	9.84 ± 1.22	14.88 ± 2.14	5.57 ± 1.76

Results are expressed in means ± SD (SD: standard division; n = 3; p = 0.05).

Table 6
Antifungal activity of orange and tangerine by-product determined as inhibition zone diameter for raw and nanoemulsion extracts.

Raw extracts of orange and tangerine				
	OP	**TP**	**OO**	**TO**
A. niger	9.71 ± 1.02	8.41 ± 0.54	14.24 ± 0.93	12.42 ± 1.05
A. parasiticus	12.64 ± 0.54	9.33 ± 0.42	17.52 ± 1.51	11.74 ± 0.97
A. ochraceous	13.73 ± 0.74	11.41 ± 0.37	14.21 ± 1.34	11.31 ± 1.22
A. nomius	11.12 ± 0.51	10.05 ± 0.66	15.24 ± 0.96	14.81 ± 1.47
A. carbonarius	15.57 ± 1.84	8.74 ± 0.28	14.38 ± 1.05	11.88 ± 1.74
A. fumigatus	13.74 ± 0.67	12.14 ± 0.94	18.41 ± 1.68	12.15 ± 2.05
Nanoemulsion of orange and tangerine extracts				
	NOP	**NTP**	**NOO**	**NTO**
A. niger	3.987 ± 0.54	1.802 ± 0.112	2.108 ± 0.174	0.927 ± 0.054
A. parasiticus	4.087 ± 0.37	1.804 ± 0.174	2.314 ± 0.105	0.915 ± 0.037
A. ochraceous	4.112 ± 0.28	1.902 ± 0.012	2.288 ± 0.389	0.925 ± 0.028
A. nomius	4.088 ± 0.67	1.805 ± 0.155	2.117 ± 0.264	0.972 ± 0.067
A. carbonarius	4.102 ± 0.59	1.907 ± 0.187	2.251 ± 0.228	0.914 ± 0.059
A. fumigatus	3.994 ± 0.71	1.901 ± 0.171	2.371 ± 0.307	0.901 ± 0.071

Results are expressed in means ± SD (SD: standard division; n = 3; p = 0.05).OP: orange peel extract; TP: tangerine peel extract; OO: orange oil extract; TO: tangerine oil extract.NOP: orange peel nanoemulsion; NTP: tangerine peel nanoemulsion; NOO: orange oil nanoemulsion; NTO: tangerine oil nanoemulsion.

on fungi and their potential as an antifungal treatment. These methods include the disruption of fungal cell membranes, mitochondrial membrane potential, and ergosterol production. Also [53], found that changes in cell membrane ergosterol content due to the presence of nanoemulsion with fungal media were nominated to be the main reason for fungal inhibition by the emulsion application.

3.7. Anti-aflatoxigenic activity for extracts (raw and nanoemulsion)

Using a simulated media, the orange peel extract existed by a more anti-aflatoxigenic influence (Fig. 1). This effect was clearly shown for orange extract and orange nanoemulsion. By utilizing a nanoemulsion solution in simulated media of fungal growth, it was noticed that the reduction effect was recorded more significantly for AFB_1, AFB_2, AFG_1, and AFG_2 concentrations. For raw extracts application and compared to the control, the aflatoxin reduction could order ascending as tangerine oil < orange oil < tangerine extract < orange extract. The exact order can be shown for their nanoemulsion solutions impacting aflatoxin reduction with more efficiency. The applied strain of *A. flavus* for the simulated media was capable of secreting aflatoxins amount as 546.2 ± 5.34, 377.3 ± 4.57, 488.2 ± 5.17, and 274.5 ± 5.22 ng/mL for AFB_1, AFB_2, AFG_1, and AFG_2, respectively as the control. The most effective aflatoxins reduction for raw byproduct-extracts was recorded for the orange peel extract, where values are 254.1 ± 8.4, 157.5 ± 4.22, 202.4 ± 7.41, and 139.2 ± 7.05 ng/mL for AFB_1, AFB_2, AFG_1, and AFG_2, respectively. Otherwise, orange peel nanoemulsion recorded reduced aflatoxin concentration to be 191.37 ± 3.67, 106.77 ± 4.31, 179.58 ± 6.02, 96.15 ± 4.32 ng/mL for AFB_1, AFB_2, AFG_1, and AFG_2, respectively.

Recent studies investigated whether nanoemulsion from citrus peel could decrease aflatoxin impacts and exhibit anti-aflatoxigenic properties [54,55]. Limonene, flavonoids, and phenolic acids are some bioactive components in peels associated with antibacterial and antifungal activities. Orange peel extract has several uses in both the culinary and medical industries. The brain's oxidative stress is reduced because ofthe protective properties of peel extract and phenolic acids. The anti-Alzheimer's influence was linked to treating rats with orange peel extract, with its gallic acid content [**55**]. Nanoemulsions, which consist of extracts or essential oil that are just a few nanometers in size and suspended in water, have increased stability and bioavailability.

Previous research showed the antifungal and anti-aflatoxigenic properties of lemon peel extract. It effectively prevented tissue and biochemical changes in remedy rats with aflatoxicosis by administering a nanoemulsion containing lemon peel extract [54]. By transforming the extract into a nanoemulsion, its activity was ameliorated. The overall health of treated rats was improved, and the risk of toxicity from exposure was reduced; this was connected to lemon peel treatment that positively impacted the immune system.

To decrease aflatoxin production, nanoemulsion could enhance the penetration and interaction of the bioactive substances with fungal cells [56]. Nanoemulsions extracted from citrus peel have been shown to have anti-aflatoxigenic properties. For instance, peel and essential oil nanoemulsions have been found to efficiently limit aflatoxin formation in contaminated processed foods by inhibiting the development of aflatoxigenic fungi [36]. Nanoemulsion formulations may be sprayed on food or inserted in food packaging to stop fungal and bacteria growth and avoid aflatoxin contamination, preventthe development of fungal, bacterial, and avoid aflatoxin contamination.

3.8. Molecular docking analysis

The best poses discovered through molecular docking analyses are shown in Fig. 2A and B, expressed as binding free energies (ΔG) for the non-volatiles (phenolic acids and flavonoids) and volatiles of orange and tangerine wastes docked at polyketide synthase (PKS) and cytochrome P450 monooxygenase (C450) receptors. Generally, volatiles have lower affinities (from −4.8 to −6.8 kcal/mol) for the

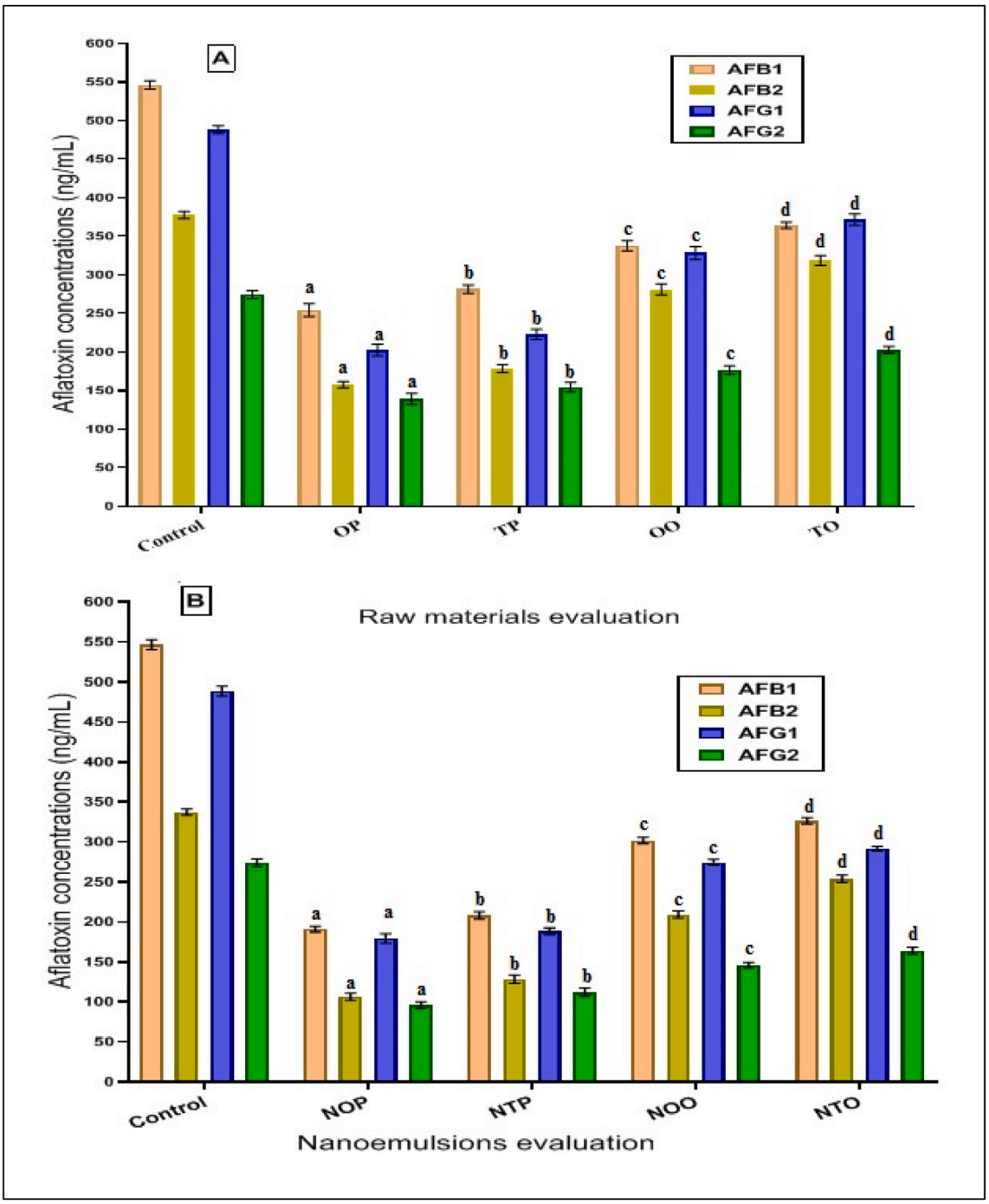

Fig. 1. Aflatoxins reduction in simulated growth media of toxigenic fungi contained raw or nanoemulsion extracts of orange and tangerine.

investigated receptors than non-volatiles (from −5.9 to −10.1 kcal/mol). With decreasing ΔG, the significance of the connection between the receptor and the ligand with potential activity grows. Particularly for hesperidin (−9.3 kcal/mol) and rutin (−9.2 kcal/mol) at PKS and for naringin (−10.1 kcal/mol) and hesperidin (−9.7 kcal/mol) at C450, flavonoids showed higher binding affinities with high docking scores. The highest affinity for both receptors among phenolic acids was demonstrated by rosmarinic acid, which had values of −8.0 and −9.1 kcal/mol, and chlorogenic acid, which had values of −7.8 and −8.8 kcal/mol for PKS and C450, respectively (Fig. 2A). The previous results are agreed with [57], where flavonoids showed higher affinities at PKS and non-ribosomal peptide synthetase compared to phenolic acids. As shown in Fig. 2B, limonene aldehyde recorded the highest binding free energies among the investigated volatiles at PKS (−6.4 kcal/mol) and C450 (−6.8 kcal/mol).The above findings agree with the

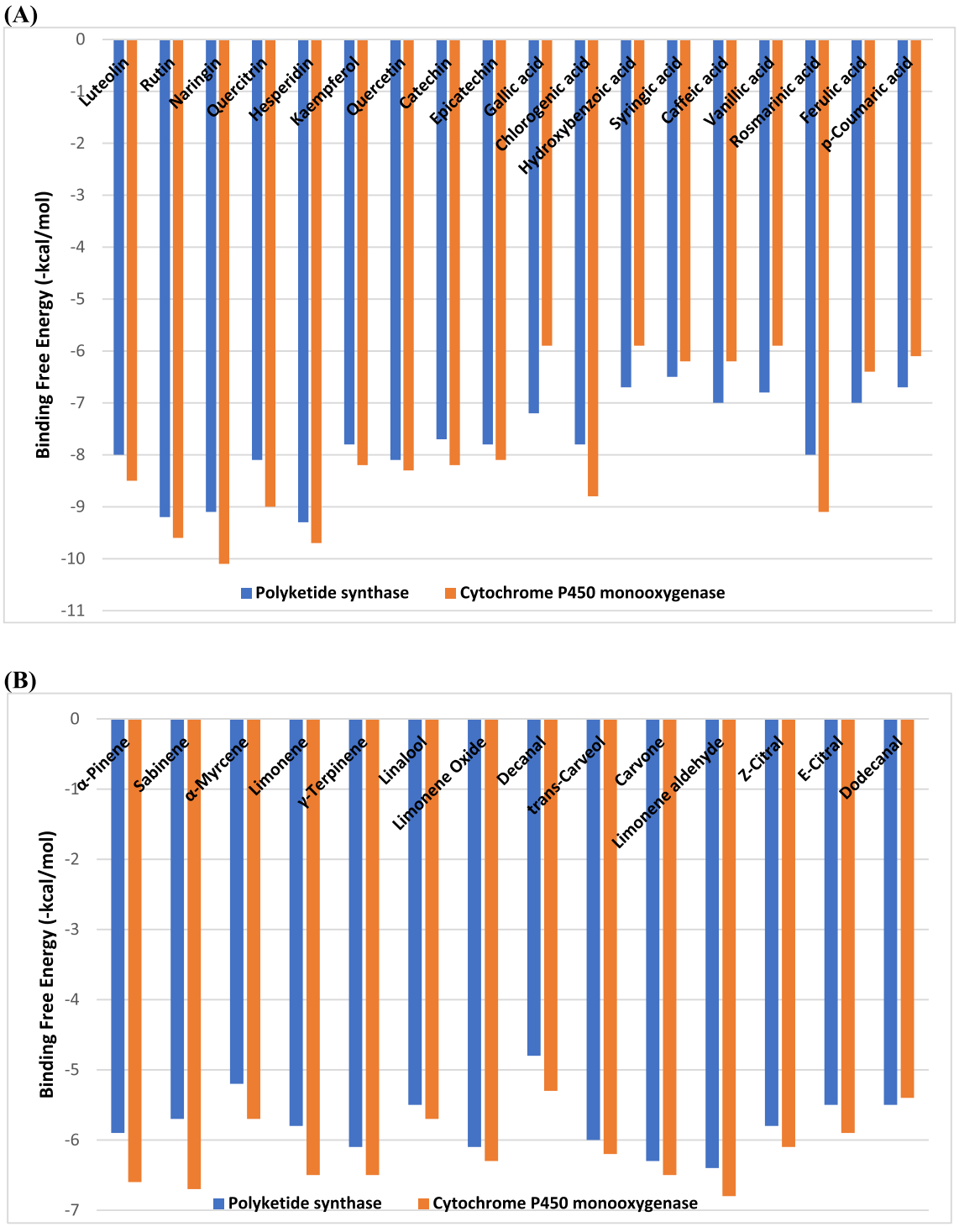

Fig. 2. Binding free energy values are calculated by docking (**A**) phenolic acids and flavonoidsand (**B**) volatiles extracted from orange and tangerine wastes against receptors.

anti-aflatoxigenic activity results, where the extracts showed potential activities compared to the volatiles. It is noteworthy that the quantitative differences, especially in flavonoids, revealed the higher activity of the orange extract and its nanoemulsion than the tangerine extract or its nanoemulsion.There is a possibility to use plant-based metabolites as an alternative to combat the production of harmful aflatoxins. Plants produce various chemicals naturally in response to stress or infections, and some can inhibit aflatoxin production. Many bioactive chemicals such as terpenes, phenolics, phenylpropanoids, and nitrogen-containing compounds have been

found in herbal treatments that inhibit AF biosynthesis. Phenylpropanoid molecules such as dillapiol and apiol have been identified as potent inhibitors of AFG1 production by A. parasiticus. These molecules are believed to prevent AFG_1 production by inhibiting CypA, a cytochrome P450-dependent monooxygenase involved in the AF biosynthetic pathway's conversion of O-methyl sterigmatocystin to AFG_1. Certain compounds, such as quercetin, catechin, and polyphenols, have been found to inhibit fungal growth and the production of AFB1. Two possible explanations for this observed suppression are modification of cell membranes, which directly impacts cytosolic polyketide synthase and specific contact with hydrophobic domains of AF pathway enzymes [58].

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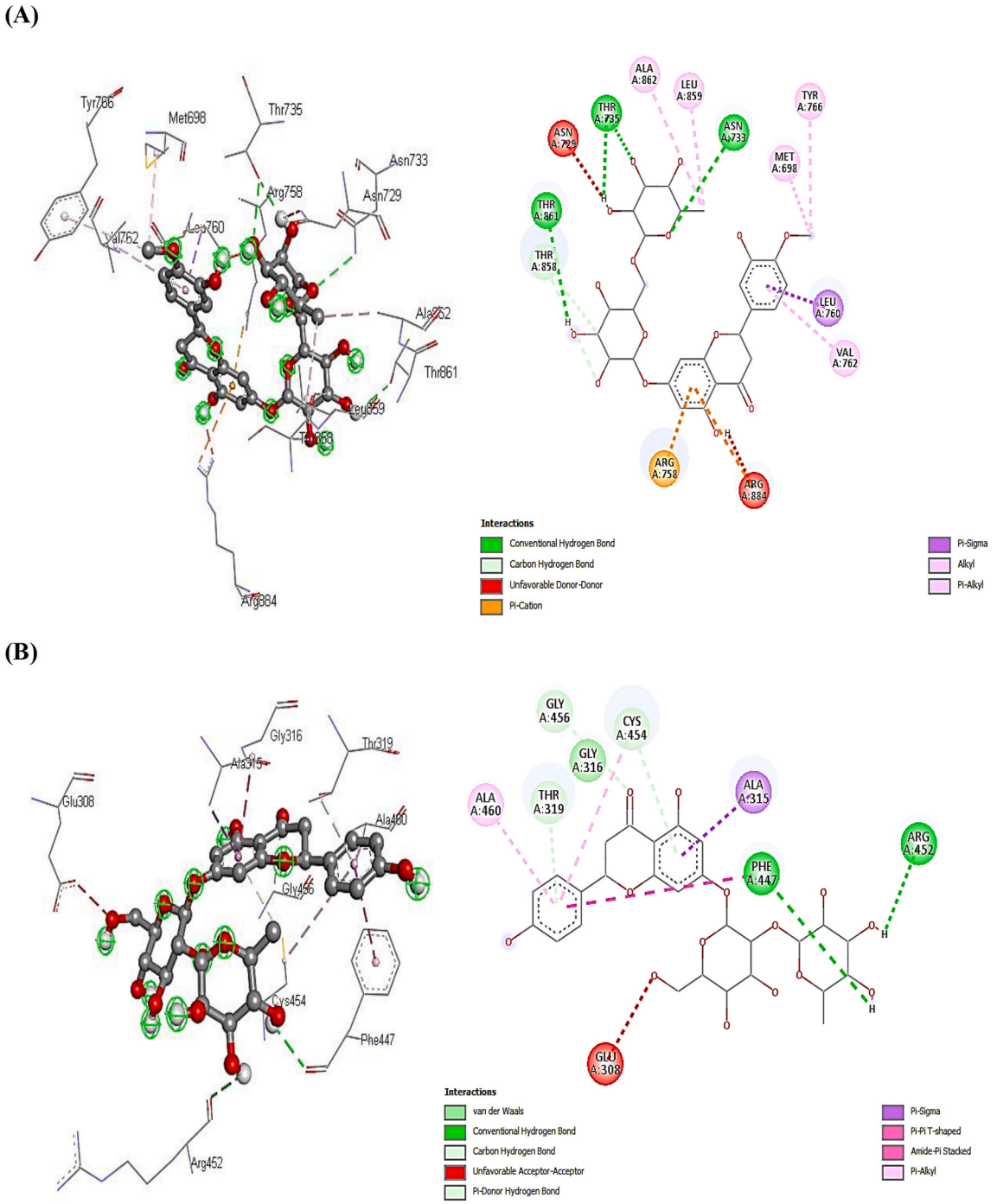

Fig. 3. Visualization for the docked (**A**)hesperidinwithpolyketide synthase and (**B**) naringin with cytochrome P450 monooxygenase.

polyketide synthase and specific contact with hydrophobic domains of AF pathway enzymes [58].

Fig. 3A and B illustrates the docking details of hesperidin and naringin towards PKS and C450. The highest receptor-ligand binding position scores reveal that hesperidin has a higher binding affinity with PKS (−9.3 kcal/mol). This is due to the formation of conventional hydrogen bonding and carbon-hydrogen interaction with THR A: 861, ASN A: 733, THR A: 735, and THR A:858. Additionally, hydrophobic interactions such as alkyl, π-cation, π-donor, π-σ, and π-alkyl were observed with ALA A: 862, LEU A: 859, ARG A: 884, ARG A: 758, LEU A: 760, VAL A: 762, and TYR A: 766. It is worth noting that hydrophobic contacts are the dominant interactions in protein-ligand complexes [59]. In this category, interactions are commonly initiated by carbon in the receptor and carbon in the legend, with the former being aliphatic and the latter being aromatic. This indicates that small molecule inhibitors typically contain aromatic rings. The most prevalent aromatic ring is the benzene ring system, found in 76% of commercially available drugs. Extensive research has been conducted on the role of π-cation interactions in protein structures. According to a study by Ref. [60], it is uncommon for π-cation interactions to be buried, and arginine side chains are more likely to form these connections than lysine. In the case of ARG A: 758, the cation interaction may also be considered a hydrogen-bonded system, as electron-deficient alkyl substituents often make direct contact instead of the cationic center, as depicted in Fig. 3A by Ref. [57]. Lastly, two undesirable donor-donor interactions were identified between hesperidin and ARG A: 884 and ASN A:729, which decrease the stability of the protein-ligand complex due to the repulsion that occurs between two molecules or atoms, according to Ref. [61].

Similar interactions could be observed between naringin and C450 residues but with more hydrophobic interactions and lower unfavorable bonds than the hesperidin-PKS complex, which explains the exact value of binding affinity (−10.1 kcal/mol). For example, conventional hydrogen bonds, van der Waals, π-donor hydrogen, and carbon-hydrogen interaction were noticed with the moieties: ARG A:452, PHE A:447, CYS A:454, THR A:316, GLY A:456, and GLY A:316 (Fig. 3B). Again, other hydrophobic interactions were detected, such as π -alkyl with ALA A:460, π-π stacked with PHE A:447, π-σ with ALA A:315, and amide π-stacked with GLY A:316. In contrast, only one unfavorable acceptor-acceptor interaction with GLU A: 308 were shown (Fig. 3B).

4. Conclusion

The result obtained in the current study reinforced previous report on the treasure of bioactive components abundant in the citrus peel (orange and tangerine) and shed the light on the fact that this bio-waste could easily be a source of wealth in both pharmaceutical and food industrial sectors rather than considered an environmental problem as it can be used to limit pathogenic microorganisms which in turn could be reflected on food security and food safety and trails should increase on its bioactivity in several pharmaceutical aspects.

CRediT authorship contribution statement

Bassem A. Sabry: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Ahmed Noah Badr:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Dina Mostafa Mohammed:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Marwa A. Desoukey:** Investigation, Validation, Writing – original draft, Writing – review & editing. **Amr Farouk:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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