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RESEARCH ARTICLE

Natural Polyphenol Oxidase Inhibitors from *Glochidion perakense*: LC-MS/MS Identification, *In Silico* Binding Analysis and Chitosan Microencapsulation for Food Preservation Applications

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Abstract

Plant-derived polyphenolic compounds offer natural alternatives for polyphenol oxidase (PPO) inhibition to prevent enzymatic browning in fresh-cut produce. However, their application is limited by poor stability and environmental sensitivity. This study screened nine Southeast Asian plant species for natural PPO inhibition and developed chitosan-based microencapsulation to enhance extract stability. Systematic evaluation revealed *Glochidion perakense* leaves exhibited exceptional antioxidant capacity (88.11% DPPH scavenging; IC_{50} = 3.04 ± 0.17 µg/mL) and superior PPO inhibitory activity (89.93% inhibition; IC_{50} = 278.00 ± 2.04 µg/mL), showing better enzyme inhibition than commercial kojic acid (IC_{50} = 311.58 ± 1.83 µg/mL). This represents the first comprehensive phytochemical characterization of *G. perakense* for food preservation applications. LC-MS/MS analysis identified 23 bioactive compounds, with molecular docking studies revealing (-)-amurensisin as the key inhibitor showing strongest PPO binding affinity (ΔG = –7.13 kcal/mol) compared to reference tropolone (ΔG = –5.20 kcal/mol). Chitosan microencapsulation at 1:1 chitosan-to-extract ratio (w/w) significantly improved extract stability, maintaining 4-fold higher phenolic content during 30-day storage compared to unencapsulated controls. Validation studies using fresh-cut potatoes demonstrated encapsulated extract achieved 2.5-fold superior PPO inhibition versus free extract. These findings establish *G. perakense* as a promising natural preservative and demonstrate microencapsulation as an effective delivery system for extending fresh-produce shelf life while addressing post-harvest losses in the food industry.

Keywords: Chitosan, *Glochidion perakense* (Man pu), Microencapsulation, Molecular docking, Polyphenol oxidase

Introduction

The growing demand for ready-to-eat fresh fruits and vegetables has brought to light significant challenges in maintaining product quality and extending shelf life. Enzymatic browning, catalyzed by polyphenol oxidase (PPO), causes substantial economic losses in the fresh-cut produce industry, estimated at several billion dollars annually [1]. PPO catalyzes the oxidation of phenolic compounds to quinones, which subsequently polymerize to form brown pigments, leading to undesirable color changes, reduced nutritional value,

and overall quality deterioration [2]. Although synthetic preservatives, especially sulfites, have traditionally been used to combat browning, their potential adverse health effects, including allergic reactions and respiratory issues, have led to regulatory restrictions and increasing consumer demand for natural alternatives [3,4].

Plant-based extracts have emerged as promising alternatives due to their favorable safety profiles, consumer acceptance and multifunctional properties. Southeast Asian traditional medicine has long utilized various plants for their preservative qualities, providing a rich yet largely unexplored source of potential natural food preservatives [5]. This study examines plant materials commonly found in Southeast Asia with documented traditional uses. Man pu (*Glochidion perakense*) has demonstrated significant antioxidant properties in traditional applications. Similarly, plant extracts like cashew leaves (*Anacardium occidentale*), sacha inchi (*Plukenetia volubilis* L.), kratom (*Mitragyna speciosa*), betel piper (*Piper betle*) and rambutan peel (*Nephelium lappaceum* L.) are rich in polyphenols with promising antioxidant activity that may contribute to food preservation applications [6-12]. Recent studies have revealed that plant-derived polyphenols can inhibit PPO through multiple mechanisms: Direct competitive inhibition at the enzyme active site, chelation of copper ions essential for PPO activity, and reduction of quinone intermediates [13]. The relationship between antioxidant activity and PPO inhibition is deeply interconnected, as many antioxidants can bind to copper ions in the catalytic site of PPO or reduce quinones back to phenols. For instance, ascorbic acid and flavonoid derivatives with high antioxidant capacity often exhibit high PPO inhibitory [14-16]. Molecular docking studies have identified specific interactions between plant polyphenols and the PPO binding pocket, highlighting the importance of hydrogen bonding networks and hydrophobic interactions [17-19]. However, the direct application of plant polyphenol extracts faces challenges including poor stability and sensitivity to environmental factors. Microencapsulation technology, particularly chitosan-based systems, offers promising solutions for preserving and delivering these bioactive compounds. The advantages of chitosan include biodegradability, biocompatibility and intrinsic antimicrobial properties [20]. Studies on various encapsulation materials have demonstrated significant enhancement in bioactive compound stability, with recent research showing that proper selection of encapsulation materials can increase stability by up to 2-fold during storage [21]. Additionally, the positive charge of chitosan enables the controlled release of active compounds and improves their interaction with bacterial cell membranes, further enhancing their antimicrobial potential [22].

This study aims to screen selected Southeast Asian plant materials for their potential as natural PPO inhibitors and antioxidants, followed by developing an effective chitosan-based microencapsulation delivery system for the most promising extract to enhance its stability and application in fresh-cut potato preservation. This research contributes to the growing field of natural food preservatives while addressing the critical need for effective, safe, and sustainable solutions in the fresh-cut produce industry.

Materials and methods

Materials

Plant materials included young leaves of man pu (*Glochidion perakense*) and cashew (*Anacardium occidentale* L.); mature leaves of sacha inchi (*Plukenetia volubilis* L.), kratom (*Mitragyna speciosa* Korth.), betel piper (*Piper betle* L.), wild betel (*Piper sarmentosum* Roxb.), watercress (*Nasturtium officinale* R.Br.); seed pods and seed shells of sacha inchi; bulbs of garlic (*Allium sativum* L.); and peel of rambutan (*Nephelium lappaceum* L.). All materials were collected from Phatthalung province, Thailand and identified based on morphological characteristics using standard botanical references. Selection criteria prioritized tissues with documented high phenolic content and traditional use for antioxidant and preservative activities. Young leaves were preferentially selected for certain species based on higher bioactive compound concentrations reported in previous studies. Potatoes (*Solanum tuberosum* L.) were purchased from Papayom market, Phatthalung, Thailand.

Chemicals

Ethyl alcohol (95%), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphate-buffered saline (PBS), kojic acid, ascorbic acid, gallic acid, chitosan (molecular weight 50,000 - 190,000 Da, deacetylation degree ≥ 70%), polyphenol oxidase (tyrosinase from mushroom) and L-3,4-

dihydroxyphenylalanine (L-DOPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade.

Ultrasound-assisted extraction of plant materials

The plant parts were washed with distilled water, dried in a hot air oven (Memmert UF30, Germany) at 50 °C until constant weight, ground to powder using a mechanical grinder and stored in a desiccator until use. Final moisture content was maintained at 4 - 6 % (w/w), determined by a moisture analyzer. Each powder (10 g, dry weight basis) was mixed with 95% ethanol (100 mL, 1:10 w/v ratio) in glass vessels and extracted by ultrasound-assisted extraction (UAE) using an ultrasonic bath (Elmasonic S series, Germany). The mixture was sonicated at 200 W power and 40 kHz frequency for 1 h at room temperature (28 ± 2 °C) with periodic stirring every 15 min to ensure uniform extraction. After sonication, samples were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 40 °C, and dried to constant weight in a vacuum oven at 40 °C. All extracts were stored in a desiccator until use to maintain consistent moisture content. Yield percentage was calculated as

$$\% \text{ Yield} = (\text{mass of extract/mass of dry plant sample}) \times 100 \quad (1)$$

DPPH radical scavenging activity

Antioxidant activity was evaluated using the DPPH assay with modifications [23]. For initial screening, 100 µL of extract solution (200 µg/mL in 10% ethanol) was mixed with 150 µL of 0.2 mM DPPH solution in a 96-well microplate (final extract concentration: 80 µg/mL). The mixture was incubated in the dark at room temperature (28 ± 2 °C) for 30 min before measuring absorbance at 517 nm.

For IC_{50} determination, extracts showing significant activity were evaluated at multiple concentrations. Concentration ranges were individually optimized based on preliminary activities to ensure accurate IC_{50} calculation within the linear response range. Strong antioxidants were evaluated at 2 - 20 µg/mL final concentrations, while weaker extracts required 20 - 150 µg/mL. Assay volumes remained constant at 100 µL extract and 150 µL DPPH for all samples. Ethanol (10%) served as the negative control and ascorbic acid as the positive control. DPPH scavenging percentage was calculated as

$$\% \text{ DPPH scavenging} = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (2)$$

where $A_{control}$ represents the absorbance of DPPH solution with 10% ethanol and A_{sample} represents the absorbance of DPPH solution with the extract. IC_{50} values, representing the extract concentration required to achieve 50% DPPH radical scavenging, were determined using linear regression analysis.

PPO inhibition assay

PPO inhibition was tested using L-DOPA as substrate [24]. For initial screening, 20 µL of extract solution (5 mg/mL in 10% ethanol) was mixed with 40 µL of L-DOPA substrate (2 mM) and 100 µL of 0.1 M phosphate buffer (pH 6.8), then pre-incubated at 37 °C for 10 min. PPO solution (40 µL, 1,000 Unit/mL) was added to initiate the reaction (final extract concentration: 500 µg/mL in 200 µL total volume), followed by incubation at 37 °C for 15 min. Dopachrome formation was measured at 475 nm using a microplate reader.

For IC_{50} determination, *G. perakense* extract and kojic acid were evaluated at multiple concentrations ranging from 100 to 1000 µg/mL with assay volumes maintained constant. Ethanol (10%) served as the negative control and kojic acid as the positive control. Inhibition percentage was calculated as

$$\% \text{ PPO inhibition} = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (3)$$

where $A_{control}$ represents the absorbance of the reaction mixture with 10% ethanol and A_{sample} represents the absorbance of the reaction mixture with extract. Linear regression analysis was used to calculate IC_{50} values, which represent the concentration required for 50% inhibition of PPO activity.

Liquid chromatography-mass spectrometry (LC-MS) identification of *G. perakense* extract

G. perakense extract (10 mg) was dissolved in LC-grade ethanol (1 mL), sonicated in an ultrasonic bath for 30 min at room temperature, centrifuged at 10,000 rpm for 5 min at 4 °C, and filtered through a 0.22 µm nylon

membrane filter before analysis. LC-MS/MS analysis was performed using an Agilent 1,290 Infinity II UHPLC system coupled to a 6,545 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 Rapid Resolution HD column (100×2.1 mm^2, 1.8 µm particle size) maintained at 40 °C. The mobile phase consisted of 0.1 % (v/v) formic acid in water (A) and 0.1 % (v/v) formic acid in acetonitrile:water (80:20, v/v) (B) at a flow rate of 0.2 mL/min. Injection volume was 2 µL. The gradient elution program was applied as follows: 0 min, 100% A/0% B; 45 min, 0% A/100% B; 50 min, 0% A/100% B; 60 min, 100% A/0% B; 65 min, 100% A/0% B. Total analysis time was 65 min. Mass spectrometric detection was performed in negative electrospray ionization (ESI^-) mode with the following parameters: Gas temperature 325 °C, drying gas flow 13 L/min, nebulizer pressure 35 psi, sheath gas temperature 275 °C, sheath gas flow 12 L/min, capillary voltage 4,000 V, nozzle voltage 2,000 V, fragmentor voltage 175 V, skimmer voltage 65 V and octopole RF voltage 750 V. Data acquisition was performed in full scan mode over the mass range m/z 50 - 1,200. Compound identification was performed using MassHunter Qualitative Analysis software (version B.08.00, Agilent Technologies) with spectral library matching against METLIN, NIST and MassBank databases. Compounds were tentatively identified based on accurate mass measurements, retention time and available MS/MS fragmentation patterns.

In silico binding analysis

Computational binding interactions between *G. perakense* bioactive compounds and polyphenol oxidase (PPO) were investigated using the crystal structure of tyrosinase from *Agaricus bisporus* (mushroom) obtained from the Protein Data Bank (PDB ID: 2Y9X, resolution: 2.30 Å). The enzyme structure was prepared by removing water molecules, heteroatoms, and adding hydrogen atoms using AutoDock Tools. Compound structures were constructed with GaussView 5.0 and geometrically optimized using Hartree-Fock/6-31G basis set calculations in Gaussian09 [25].

The active site was defined by setting up a grid box (60×60×60 Å) centered around the copper-binding site (coordinates: x = –9.527, y = 6.098, z = 22.171). Docking simulations targeted the enzyme active site with 100 independent runs per compound using AutoDock 4.2 (The Scripps Research Institute, La Jolla, CA, USA). Results were analyzed based on binding energies (ΔG, kcal/mol) and conformational cluster analysis with active site residues. Tropolone, a known PPO inhibitor, was used as a reference compound for comparison. Binding poses were visualized and analyzed using PyMOL 2.5.0 (Schrödinger, LLC).

Encapsulation and characterization of *G. perakense* extract with chitosan

Chitosan-based microcapsules containing *G. perakense* extract were prepared using an emulsion-based self-assembly technique [26]. Five formulations were prepared with different chitosan-to-extract ratios (w/w, dry basis): C1MP0.5 (1:0.5 w/w), C1MP1 (1:1 w/w), MP0.5 (extract only, 0:0.5 w/w), MP1 (extract only, 0:1 w/w) and C1 (chitosan only, 1:0 w/w). Chitosan (2% w/v) was dissolved in 1% acetic acid under continuous magnetic stirring at room temperature until complete dissolution, while extract was solubilized in ethanol:water (1:1 v/v) with 2% Tween 80 as an emulsifying agent. For encapsulated formulations, extract solution was added dropwise to the chitosan solution. The self-assembly process occurred through electrostatic interactions between protonated amino groups (NH_3^+) of chitosan and negatively charged phenolic hydroxyl groups, while Tween 80 facilitated emulsion stability through micellar encapsulation of hydrophobic bioactive compounds. All formulations were homogenized using a high-speed homogenizer at 10,000 rpm for 15 min. To remove unencapsulated materials and ensure product quality, microcapsule suspensions were subjected to gentle centrifugation (3,000 rpm, 5 min) followed by careful supernatant collection.

The morphological characteristics were examined using optical microscopy (Olympus CX23, Japan) and scanning transmission electron microscopy (STEM). For STEM analysis, the C1MP1 sample was diluted, placed on a copper grid, air-dried and imaged at 60,000× magnification (30 kV). Particle size, polydispersity index (PDI) and zeta potential were determined using a Zetasizer Nano ZS (Malvern Instruments, UK). The samples were diluted with deionized water (1:100, v/v)

and measured at 25 °C using dynamic light scattering. All measurements were performed in triplicate.

Microcapsule application on fresh-cut potatoes

Fresh potatoes were sanitized, peeled and cut into 1-cm^3 pieces, then immersed in diluted microcapsule solutions (1:10 v/v) for 20 min. Samples were drained for 3 min, sealed in plastic bags and stored at room temperature. PPO activity was assessed at 30 min, 2 and 18 h by homogenizing treated samples with PBS, centrifuging (4,000 rpm, 2 min, 4 °C), and measuring the increase in absorbance at 475 nm using L-DOPA as substrate. One unit was defined as 0.001 absorbance increase per min. The enzyme activity (mUnit/mL) was calculated as Eq. (4)

$$\text{PPO activity} = (\Delta A475/\text{min} \times 1000) / V \quad (4)$$

where ΔA475/min is the change in absorbance at 475 nm per min, 1,000 is the conversion factor to milliunits and V is the volume of enzyme extract (supernatant) used in the reaction (mL).

Storage stability study

Stability of microcapsules was evaluated at 4 ± 2 °C, room temperature (28 ± 2 °C), and elevated temperature (45 ± 2 °C) for 30 days in sealed amber containers. Antioxidant activity was measured using the DPPH method as described previously. Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid standard. Briefly, 30 µL of sample was mixed with 110 µL of diluted Folin-Ciocalteu reagent and 110 µL of sodium carbonate (7.5% w/v), then incubated at 37 °C for 20 min before measuring absorbance at 765 nm. The total phenolic content was calculated using a gallic acid calibration curve and expressed as micrograms of gallic acid equivalents per milliliter (µg GAE/mL).

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD). Statistical differences among treatments were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SPSS statistics software.

Results and discussion

Plant extracts rich in antioxidant compounds have been employed as safe natural PPO inhibitors. This study evaluated various botanical extracts for their PPO inhibitory and antioxidant activity through spectroscopic analyses and computational simulations. Based on the screening results, the most effective extract was selected for chitosan-based encapsulation. The stability and PPO inhibitory effects were then compared between free and encapsulated forms, with particular emphasis on their application in preventing enzymatic browning in potato samples. This research provides valuable insights into developing natural preservation systems for preventing browning in fresh-cut fruits and vegetables.

Screening of antioxidant activity and PPO inhibitory effects of *G. perakense* and selected plant extracts

The extraction yields varied significantly among the tested plant materials (**Table 1**). The highest yield was obtained from *G. perakense* leaves (32.2 ± 1.49%), followed by *P. betle* L. leaves (26.78 ± 2.08%) and *A. occidentale* L. leaves (25.98 ± 0.33%). Moderate yields were observed from *M. speciosa* leaves (20.51 ± 0.21%) and *N. officinale* leaves (22.63 ± 0.12%). The lowest yields were obtained from *A. sativum* (13.71 ± 0.4%), *P. volubilis* leaves (11.12 ± 0.16%) and *N. lappaceum* peel (9.83 ± 0.89%). These variations were attributed to differences in plant matrix composition and solvent-solute interactions during the extraction process [27]. The ultrasound-assisted extraction method employed in this study significantly enhanced the recovery of bioactive compounds from *G. perakense* leaves, consistent with findings by Núñez *et al*. [28]. who demonstrated that this technique yields higher polyphenol concentrations while requiring less processing time and solvent compared to conventional Soxhlet extraction [28].

The DPPH assay revealed varying levels of antioxidant activity among the extracts. *G. perakense* leaves demonstrated the highest DPPH inhibition (88.11 ± 0.34%), followed by *N. lappaceum* (86.89 ± 0.46%) and *A. occidentale* (81.56 ± 0.22%). In terms of IC_{50} values, which provide a more precise measure of antioxidant activity, *G. perakense* leaves exhibited exceptional activity with the lowest IC_{50} value (3.04 ±

0.17 µg/mL), comparable to the ascorbic acid control (2.84 ± 0.02 µg/mL). *N. lappaceum* peel also showed strong antioxidant activity with an IC_{50} of 5.67 ± 0.47 µg/mL, which could be attributed to concentrated phenolic compounds such as ellagic acid and geraniin. *A. occidentale* leaves and *P. betle* leaves demonstrated moderate antioxidant potency with IC_{50} values of 9.45 ± 2.97 µg/mL and 14.16 ± 0.04 µg/mL, respectively. *P. volubilis* (seed-coated) and *M. speciosa* leaves showed relatively weaker antioxidant activity with IC_{50} values of 44.31 ± 0.25 µg/mL and 49.41 ± 1.36 µg/mL, respectively. These findings were consistent with previous studies on indigenous vegetables from Southern Thailand, where *G. perakense* leaves demonstrated superior total polyphenol content and antioxidant properties among 15 traditional plant extracts, with young *A. occidentale* leaves ranking as the second most potent extract [12].

In the PPO inhibition screening, *G. perakense* leaves exhibited remarkable activity (89.93 ± 0.61%) with an IC_{50} value of 278.00 ± 2.04 µg/mL, superior to the commercial PPO inhibitor kojic acid (IC_{50} = 311.58 ± 1.83 µg/mL). Other extracts showed substantially lower PPO inhibition, with *N. lappaceum* peel (17.96 ± 2.97%), *P. betle* leaves (17.60 ± 0.93%) and *A. occidentale* leaves (7.13 ± 0.12%) showing moderate to weak activity. Several samples, including all *P. volubilis* preparations and *N. officinale* leaves, showed no detectable PPO inhibition. Although *P. volubilis* exhibited potent antioxidant activity, it showed no PPO inhibition. This disparity was attributed to different molecular mechanisms, where antioxidant activity relies on electron donation from lipophilic compounds abundant in *P. volubilis*, while PPO inhibition requires specific structural features for active site binding [29]. The relationship between antioxidant activity and PPO inhibition has been previously demonstrated in other plant species, such as *Alnus cordata* stem bark extract, which showed both potent antioxidant activity (DPPH IC_{50} 5.83 µg/mL) and tyrosinase inhibition (IC_{50} 77.44 µg/mL), attributed to its rich phenolic composition including hydroxycinnamic acids and flavanones [30]. Furthermore, the moderate to weak PPO inhibition observed in several extracts could be attributed to the presence of compounds exhibiting dual functionality, where some molecules may act as enzyme activators while others serve as inhibitors, depending on their chemical structures and reaction conditions [31].

Compared to Sae-leaw and Benjakul (2019), who reported higher PPO inhibition in *A. occidentale* leaves compared to *G. perakense* leaves, our findings showed different results [32]. This discrepancy might be attributed to the extraction methods employed, as their study utilized water extraction with chlorophyll removal, which likely resulted in different bioactive compound profiles. Our findings suggested that ethanol successfully extracted potent antioxidant and PPO inhibitory compounds from *G. perakense* leaves, possibly phenolics and flavonoids, making it a suitable solvent for obtaining bioactive extracts.

The dual functionality of *G. perakense* leaves in both antioxidant and PPO inhibition assays was particularly noteworthy. This bifunctional activity suggested the presence of compounds capable of both electron donation and enzyme inhibition, a valuable combination rarely found in natural extracts. The distinct patterns of antioxidant and PPO inhibitory activities observed across the extracts could be explained by the different structural features required at the molecular level for these biological activities. The exceptional performance of *G. perakense* leaves in both assays positioned it as a promising candidate for applications in natural food preservation and cosmetic formulations, where both antioxidant and anti-browning properties are desired. These findings warranted further investigation of its chemical constituents to identify the specific compounds responsible for these dual antioxidant and PPO inhibitory properties.

Characterization of bioactive compounds from *G. perakense* leaves by LC-MS/MS analysis

This study represents the first metabolic profiling investigation of *G. perakense* leaves using LC-MS/MS analysis. LC-MS/MS identified twenty-three compounds in *G. perakense* leaves extract with retention times from 1.489 to 51.799 min (**Figure 1** and **Table 2**). The early eluting peaks revealed phenolic acids including quinic acid (Rt = 1.489 min) and gallic acid (Rt = 4.187 min, score 90.77), along with gallic acid derivatives such as 1-O-Galloylglycerol (Rt = 5.899 min) and methyl 4,6-di-O-galloyl-beta-D-glucopyranoside (Rt = 10.358 min). Several flavonoids and their glycosides were identified: Bergenin (Rt =

8.095 min, score 96.38), kaempferol 4'-glucoside (Rt = 11.603 min), epicatechin 7-O-glucuronide (Rt = 12.513 min, score 98.96), isovitexin (Rt = 13.014 min, score 90.37), (-)-amurensisin (Rt = 17.516 min), and quercetin (Rt = 18.651 min, score 99.11). Other phenolic derivatives included ethyl-p-coumarate (Rt = 22.685 min, score 97.32) and fumaroprotocetaric acid (Rt = 15.520 min). The later-eluting compounds were predominantly fatty acids, including pinolenic acid (Rt = 44.382 min, score 87.98), 9(Z),11(E)-conjugated linoleic acid (Rt = 47.289 min, score 79.41), isopalmitic acid (Rt = 50.208 min, score 76.27) and vaccenic acid (Rt = 51.799 min, score 74.26). The structures of the main bioactive compounds identified in *G. perakense* extracts are presented in **Figure 2**. The two most abundant compounds were kaempferol 4'-glucoside (26.22%) and isovitexin (26.18%), along with other bioactive compounds including bergenin, gallic acid, and (-)-amurensisin.

Table 1 Percentage yields, DPPH radical scavenging activity, and PPO inhibitory activity of crude extracts obtained by ultrasound-assisted extraction from nine plant species. All values are expressed as mean ± SD (n = 3).

Sample	Scientific name	% Yields	Antioxidant activity (DPPH)		PPO Inhibitory activity	
			% Inhibition[a]	IC_{50} (μg/mL)	% Inhibition[b]	IC_{50}(μg/mL)
Sacha inchi (seed-coated)	*Plukenetia volubilis* L.	15.05 ± 0.30	69.07 ± 0.12	44.31 ± 0.25	0	ND
Sacha inchi (mature leaves)	*Plukenetia volubilis* L.	11.12 ± 0.16	27.58 ± 0.24	ND	0	ND
Sacha inchi (seed pods)	*Plukenetia volubilis* L.	16.57 ± 0.18	21.27 ± 0.29	ND	0	ND
Man pu (young leaves)	*Glochidion perakense*	32.2 ± 1.49	88.11 ± 0.34	3.04 ± 0.17	89.93 ± 0.61	278.00 ± 2.04
Watercress (mature leaves)	*Nasturtium officinale* R.Br.	22.63 ± 0.12	12.19 ± 0.30	ND	0	ND
Cashew (young leaves)	*Anacardium occidentale* L.	25.98 ± 0.33	81.56 ± 0.22	9.45 ± 2.97	7.13 ± 0.12	ND
Kratom (mature leaves)	*Mitragyna speciosa* Korth.	20.51 ± 0.21	74.89 ± 0.14	49.41 ± 1.36	6.40 ± 0.1	ND
Betel Piper (mature leaves)	*Piper betle* L.	26.78 ± 2.08	68.38 ± 0.23	14.16 ± 0.04	17.60 ± 0.93	ND
Wild betal (mature leaves)	*Piper sarmentosum* Roxb.	24.61 ± 0.60	22.87 ± 0.09	ND	8.43 ± 1.27	ND
Garlic (bulbs)	*Allium sativum* L.	13.71 ± 0.4	22.95 ± 0.25	ND	2.15 ± 0.76	ND
Rambutan (peel)	*Nephelium lappaceum* L.	9.83 ± 0.89	86.89 ± 0.46	5.67 ± 0.47	17.96 ± 2.97	ND
Ascorbic acid	-	-	90.91 ± 0.26	2.84 ± 0.02	ND	ND
Kojic acid	-	-	ND	ND	82.121 ± 0.95	311.58 ± 1.83

[a]Percentage DPPH inhibition measured at 80 μg/mL extract concentration.

[b]Percentage PPO inhibition measured at 500 μg/mL extract concentration.

IC_{50} = half-maximal inhibitory concentration; lower values indicate stronger activity., ND = not determined.

The previously reported compounds in Glochidion genus, including quinic acid, gallic acid, bergenin, isovitexin, and quercetin, are well-documented for their antioxidant properties [33]. Among these compounds, gallic acid and quercetin demonstrate significant PPO inhibitory activity, with quercetin exhibiting competitive inhibition through copper-chelating mechanisms at the active site of PPO [34,35]. Among

the newly identified compounds, methyl 4,6-di-O-galloyl-beta-D-glucopyranoside shows potential antitumor activity [36]. Kaempferol and epicatechin derivatives have demonstrated considerable PPO inhibitory effects in previous studies [37]. Kaempferol displays strong competitive inhibition similar to quercetin. These compounds likely contribute significantly to the anti-browning activity observed in the extract. Amurensisin and isopalmitic acid primarily demonstrate antioxidant activity [38]. This diverse composition of bioactive compounds, particularly the combination of phenolic acids, flavonoids, and their derivatives, suggests significant potential for PPO inhibitory activity and antioxidant property, supporting the application of *G. perakense* as a natural PPO inhibitor in fruit and vegetable products.

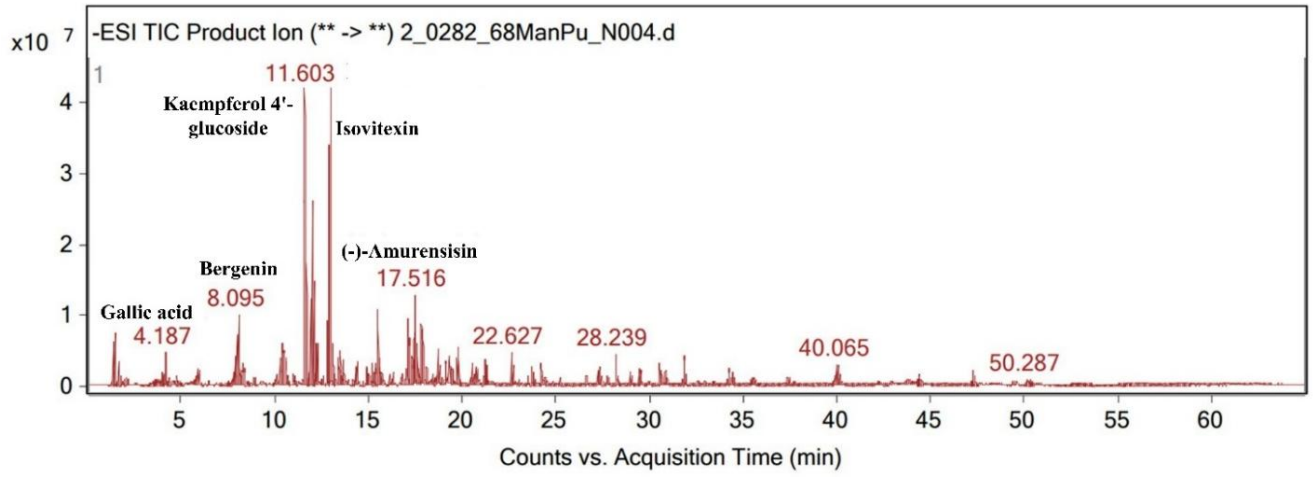

Figure 1 LC-MS/MS chromatogram in negative ion mode showing bioactive compounds identified in *G. perakense* young leaf extract.

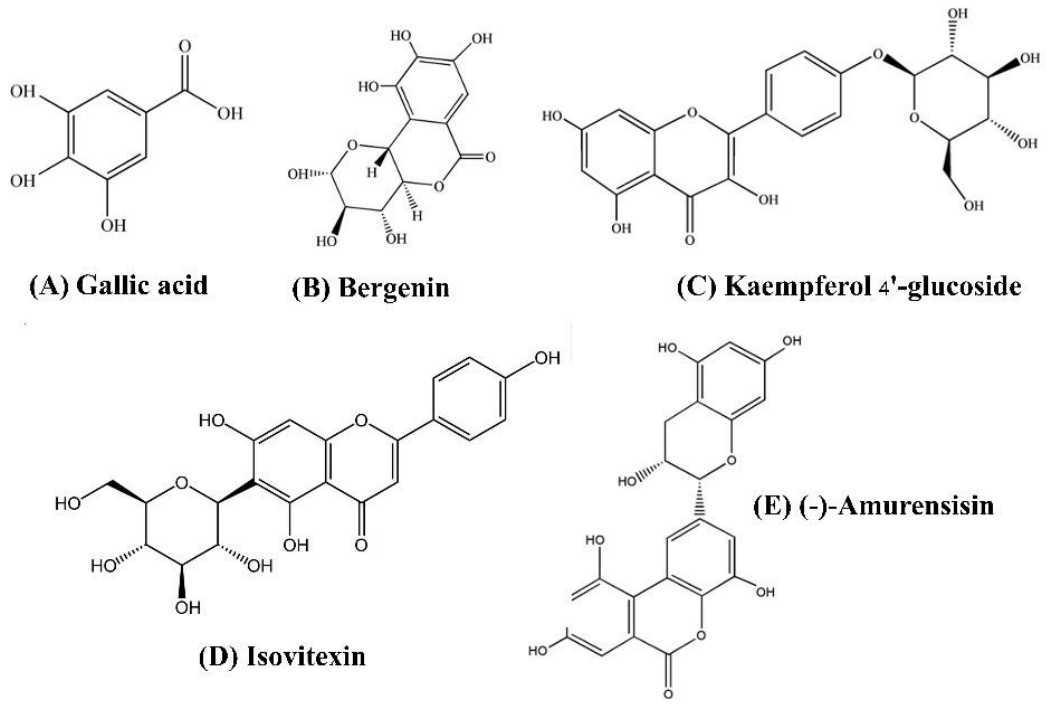

Figure 2 Structures of the main compounds found in the *G. perakense* extracts.

Table 2 Compounds identified in *G. perakense* leaf extract using LC-MS/MS analysis.

Peak no.	Compound name	Retention time (min)	Observed mass (m/z)	Mass (g/mol)	Score (Lib)	Relative abundance (%)
1	Quinic acid	1.489	191.0564	192.0637	0	4.69
2	Gallic acid	4.187	169.0142	170.0215	90.77	2.99
3	1-O-Galloylglycerol	5.899	243.0511	244.0584	0	1.57
4	Bergenin	8.095	327.0723	328.0796	96.38	6.22
5	Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside	10.358	497.094	498.1013	0	3.84
6	Kaempferol 4'-glucoside	11.603	447.0943	448.1015	0	26.22
7	Epicatechin 7-O-glucuronide	12.513	511.1095	466.1113	98.96	0.65
8	Isovitexin	13.014	431.0994	432.1065	90.37	26.18
9	Fumarprotocetraric Acid	15.520	471.057	472.0643	0	6.76
10	(-)-Amurensisin	17.516	485.0758	440.0746	0	5.48
11	Quercetin	18.651	301.0357	302.0431	99.11	0.46
12	3,5,7-Tris(acetyloxy)-2-[4-(acetyloxy)-3- hydroxyphenyl]-4H-1-benzopyran-4-one	19.829	469.0778	470.0851	0	3.49
13	Ethyl-p-coumarate	22.685	191.0713	192.0786	97.32	0.13
14	Soyasapogenol B 3-O-[α-L-rhamnosyl-(1→4)-β-D-galactosyl-(1→4)-β-D-glucuronide]	28.297	1001.5326	942.5188	0	2.85
15	Avermectin B1a	31.905	917.4904	872.4922	0	0.29
16	Mupirocin	34.260	559.3124	500.2986	0	1.62
17	PI(18:2(9Z,12Z)/0:0)	35.488	595.289	596.2961	0	0.80
18	Dolichyl diphosphate	37.793	579.2843	520.27	0	0.43
19	Fexaramine	39.973	555.2846	496.2704	0	1.89
20	Pinolenic Acid	44.382	277.2174	278.2246	87.98	1.10
21	9(Z),11(E)-Conjugated Linoleic Acid	47.289	279.233	280.2403	79.41	1.42
22	Isopalmitic acid	50.208	255.2331	256.2404	76.27	0.70
23	Vaccenic acid	51.799	281.2482	282.2555	74.26	0.22

Score: Spectral match percentage with METLIN/PCDL database references. Zero indicates identification based on accurate mass and fragmentation patterns without available library spectra.

Relative abundance: Percentage of total peak height among the 23 compounds; values represent semi-quantitative indicators due to varying ionization efficiencies in ESI mode.

In silico docking study of *G. perakense* compounds with PPO

The structural properties of PPO present a challenge due to the high polarity of its active sites. The substrate binding site contains highly polar amino acid residues, including histidine, asparagine and serine, which play crucial roles in substrate recognition and binding [39]. To understand potential inhibitor interactions with these residues, ten major compounds

with different polarities identified from LC-MS analysis were selected for the *in silico* docking study.

Molecular docking analysis revealed distinctive binding interactions between *G. perakense* leave compounds and PPO (**Table 3**). (-)-Amurensisin demonstrated the highest binding affinity (–7.13 kcal/mol), surpassing the reference inhibitor tropolone (–5.20 kcal/mol), with a conformational cluster of 35%. The compound formed key interactions with GLU256, ASN260, HIS263, MET280 and SER282, suggesting stable binding within the PPO active site. Catechin exhibited the second strongest binding (–6.36 kcal/mol), interacting with HIS61, MET280 and GLY281 with 37% cluster formation. Bergenin displayed substantial binding affinity (–5.84 kcal/mol) with notably high conformational stability (73% cluster), forming interactions with HIS61, ASN260, MET280, GLY281 and SER282. Kaempferol 4'-glucoside showed moderate binding energy (–5.66 kcal/mol) with 48% cluster formation, interacting with HIS85, THR261, SER282 and VAL283.

The recurring involvement of specific polar residues, particularly ASN260, HIS263 and non-polar MET280, across multiple compounds indicates their crucial role in PPO inhibition mechanisms. The molecular visualization revealed that these interactions were predominantly stabilized through hydrogen bonding with polar residues, as illustrated in the 2D and 3D binding models (**Figure 3**). The superior binding affinity of (-)-amurensisin can be attributed to its ability to form multiple interactions with both polar (GLU256, ASN260, HIS263) and non-polar (MET280) residues, suggesting its structural compatibility with the active site. Similarly, bergenin's high conformational stability (73% cluster) and interactions with polar residues (HIS61, ASN260) indicate its effectiveness in overcoming the polarity challenges of the binding site.

Given its reported antioxidant activity surpassing vitamin E, amurensisin likely prevents lipid peroxidation through free radical scavenging [38]. The strong binding affinities and stable interactions with polar residues demonstrated by (-)-amurensisin, catechin, bergenin and kaempferol 4'-glucoside support their potential as PPO inhibitors, leading to further investigation of suitable delivery systems to enhance their stability and effectiveness.

Table 3 Binding energies, conformational clusters (%) and interacting residues of bioactive compounds from *G. perakense* leaf extract with PPO.

Compounds	Binding energy (kcal/mol)	% Cluster	Interacted residues
(-)-Amurensisin	–7.13	35	GLU256, ASN260, HIS263, MET280, SER282
Catechin	–6.36	37	HIS61, MET280, GLY281
Bergenin	–5.84	73	HIS61, ASN260, MET280, GLY281, SER282
Kaempferol 4'-glucoside	–5.66	48	HIS85, THR261, SER282, VAL283
Quercetin	–5.47	62	HIS61, HIS224, ASN260
Isovitexin	–4.84	51	HIS61, ASN260, GLY281
Fumarprotocetraric Acid	–4.66	58	ASN260, HIS263, ARG268, GLY281
Gallic acid	–3.62	62	ASN260, MET280, SER282
Isopalmitic acid	–3.47	38	GLY281
Quinic acid	–2.77	82	ASN260, GLY281, VAL283
Tropolone	–5.20	100	HIS263

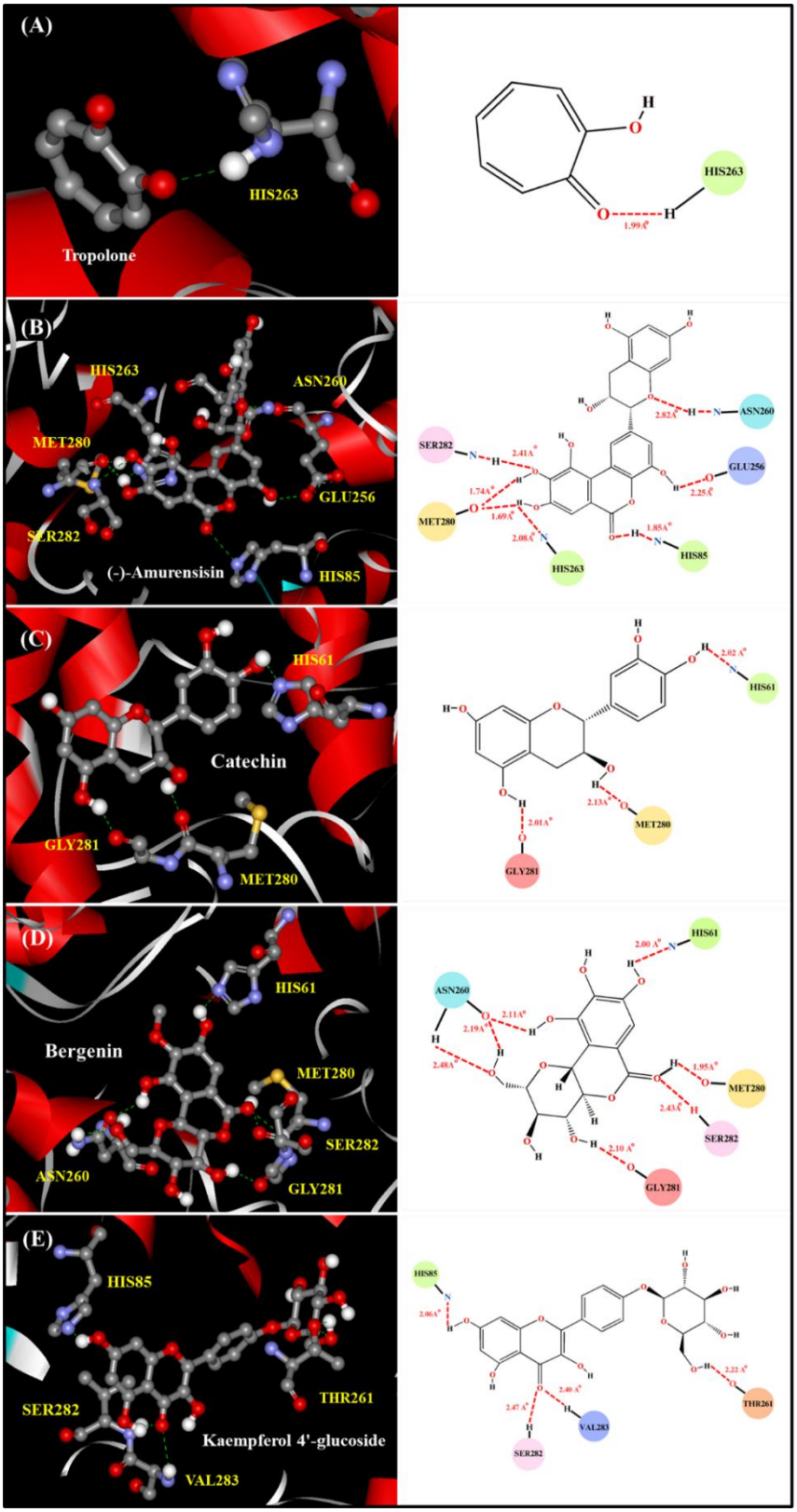

Figure 3 Three-dimensional (3D) and two-dimensional (2D) binding interactions of (A) tropolone, (B) (-)-amurensisin, (C) catechin, (D) bergenin, and (E) kaempferol 4'-glucoside with polyphenol oxidase (PPO).

Physicochemical and morphological characterization of chitosan-*G. perakense* microcapsules

The bioactive compounds from *G. perakense* extract, including (-)-amurensisin, catechin, bergenin and kaempferol 4'-glucoside possess both hydrophilic and hydrophobic regions that facilitate their encapsulation within the microcapsule core. Chitosan has also demonstrated PPO inhibitory activity in different food matrices [20,40]. Chitosan-loaded *G. perakense* microcapsules were developed using 2 different chitosan-to-extract ratios (w/w): 1:1 (C1MP1) and 1:0.5 (C1MP0.5), along with their corresponding unencapsulated controls (MP1 and MP0.5). The microcapsules were formed through self-assembly between chitosan and Tween 80, where Tween 80 created micellar structures encapsulating the polyphenolic compounds in its hydrophobic core, while chitosan provided an outer protective layer through electrostatic forces and hydrogen bonds [41].

As shown in **Table 4**, physicochemical characterization revealed that extract incorporation significantly affected microcapsule properties. Particle size increased with extract content, from 530.3 ± 2.93 nm in chitosan-only microcapsules to 661.4 ± 8.79 nm in C1MP1 and 744 ± 5.73 nm in C1MP0.5. Notably, extract-loaded formulations demonstrated improved homogeneity with lower PDI values (0.33 ± 0.01 for C1MP1 and 0.41± 0.01 for C1MP0.5) compared to chitosan microcapsules (0.63 ± 0.08). Both extract formulations exhibited significantly enhanced zeta potential (approximately 46 mV) compared to chitosan-only microcapsules (23.2 ± 4.39 mV), indicating excellent colloidal stability [42]. Among the extract-loaded formulations, C1MP1 showed the most favorable properties with smaller particle size and lower PDI, suggesting optimal extract-to-chitosan ratio for microcapsule formation.

Morphological analysis (**Figure 4**) confirmed the formation of distinct spherical microcapsules in chitosan-coated formulations, with C1MP0.5 exhibiting well-dispersed particles and C1MP1 showing higher particle density with some clustering. In contrast, uncoated formulations displayed significantly fewer visible particles with irregular distribution, suggesting poor stability without chitosan coating. These observations demonstrated that chitosan coating plays a crucial role in maintaining structural integrity, making these stable micro-sized carriers promising candidates for applications requiring PPO inhibitory activity. These findings were consistent with Zhu *et al*. [43], who reported that in chitosan-coated *Toddalia asiatica* (L.) Lam. extracts, the bright spots inside the microcapsules represented the core material, while the black circles outside indicated the chitosan shell material [43].

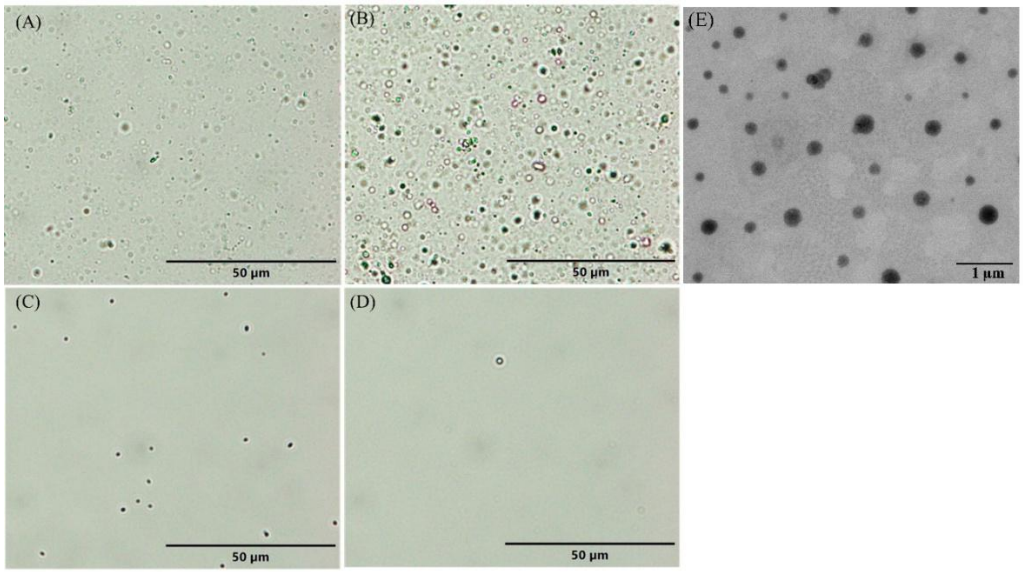

Figure 4 Morphological characterization of chitosan–*G. perakense* microcapsules. (A–D) Optical microscope images (400×) of chitosan-coated microcapsules containing *G. perakense* extract at (A) 1:0.5 and (B) 1:1 (w/w), and non-encapsulated extract at (C) 0:0.5 and (D) 0:1 (w/w). (E) STEM image of the C1MP1 formulation.

Table 4 Physicochemical properties of chitosan-coated microcapsules containing *G. perakense* extract at different ratios (C1MP1 and C1MP0.5) and chitosan microcapsules without extract. Values are expressed as mean ± SD (n = 3).

Microcapsules	Z-average size diameter (nm)	Polydispersity index (PDI)	Zeta-potential (mV)
C1MP0.5	744±5.73^{c}	0.41±0.01^{a}	45.9±0.76^{b}
C1MP1	661.4±8.79^{b}	0.33± 0.01^{a}	45.7±0.83^{b}
Chitosan	530.3±2.93^{a}	0.63±0.08^{b}	23.2±4.39^{a}

C1MP0.5 = chitosan:*G. perakense* extract at 1:0.5 (w/w), C1MP1 = chitosan:*G. perakense* extract at 1:1 (w/w) and Chitosan = chitosan microcapsules without *G. perakense* extract. Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Phenolic content and antioxidant activity during microcapsule storage

The total phenolic contents of chitosan-coated microcapsules (C1MP1 and C1MP0.5) and their uncoated counterparts (MP1 and MP0.5) after 30 days of storage at various temperatures (4 °C, room temperature (28 °C) and 45 °C) are shown in **Figure 5(A)**. Chitosan-coated microcapsules demonstrated significantly higher retention of phenolic compounds compared to uncoated ones. C1MP0.5 maintained 2.7 - 3.3 µg GAE/mL, while MP0.5 only retained 0.3 - 0.7 µg GAE/mL. Similarly, C1MP1 preserved 3.8 - 4.5 µg GAE/mL compared to 0.9 - 1.1 µg GAE/mL in MP1. Chitosan microencapsulation resulted in approximately 4-fold higher phenolic content compared to unencapsulated forms. The enhanced stability could be attributed to the protective barrier formed by chitosan through electrostatic interactions between its protonated amino groups (NH_3^+) and negatively charged phenolic compounds. Additionally, the surfactant properties of Tween 80 helped maintain structural integrity by preventing microcapsule aggregation and coalescence during storage. The higher phenolic retention in C1MP1 compared to C1MP0.5 suggested that increased extract concentration promoted more efficient complex formation between phenolic compounds and chitosan.

The DPPH radical scavenging assay (**Figure 5(B)**) revealed superior antioxidant activity in chitosan-coated samples, with C1MP1 showing the highest inhibition (85% - 95%) across all storage temperatures, followed by MP1 (85% - 90%). At lower concentration, C1MP0.5 maintained moderate activity (55% - 65%), while MP0.5 showed lower activity (15% - 35%). This synergistic interaction between chitosan, Tween 80 and the extract components created a robust encapsulation system that effectively preserved both phenolic content and antioxidant activity during storage, regardless of temperature conditions.

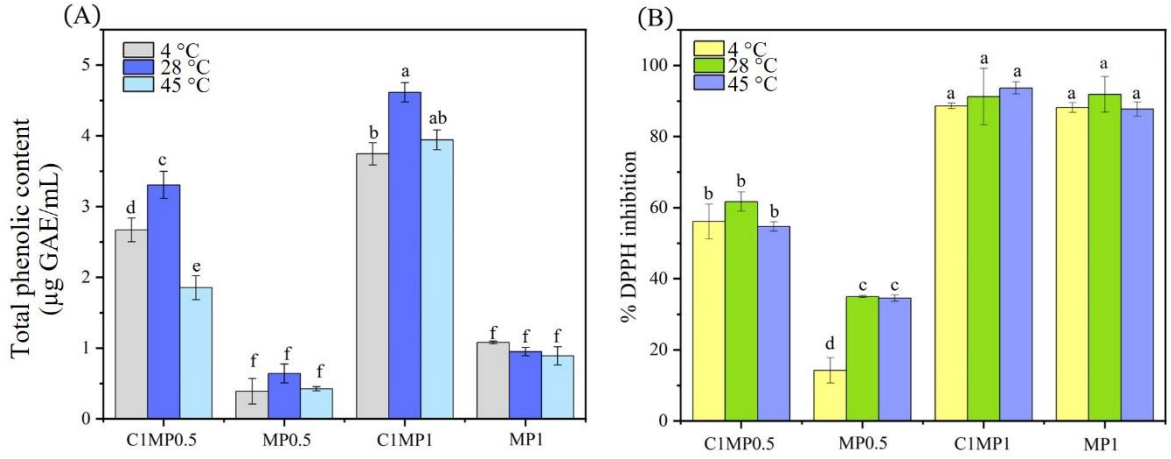

Figure 5 Total phenolic content (A) and DPPH radical scavenging activity (B) of samples stored at 4 °C, room temperature, and 45 °C for 30 days. Samples: C1MP0.5 (chitosan:*G. perakense* extract at 1:0.5 w/w), C1MP1 (chitosan:*G. perakense* extract at 1:1 w/w), MP0.5 (non-encapsulated extract at 0:0.5 w/w), and MP1 (non-encapsulated extract at 0:1 w/w). Different letters indicate significant differences ($p < 0.05$). Values: Mean ± SD (n = 3).

Effects of chitosan- *G. perakense* microcapsules on PPO activity of fresh-cut potatoes

The inhibitory effects of chitosan-*G. perakense* microcapsules on PPO activity in fresh-cut potatoes were investigated during room temperature storage for 30 min, 2 h and 18 h (**Figure 6**). The results demonstrated that chitosan-coated microcapsules (C1MP0.5 and C1MP1) exhibited significantly lower PPO activity (8-10 mUnits/mL) compared to uncoated formulations (MP0.5 and MP1; 19 - 29 mUnits/mL). Throughout the storage period, the chitosan-coated formulations maintained consistent enzyme inhibition levels (12 - 14 mUnits/mL), whereas uncoated samples, particularly MP0.5, showed considerable activity fluctuations (29 - 32 mUnits/mL) during the first 2 h before declining at 18 h. The encapsulated extract exhibited approximately 2-fold higher PPO inhibition compared to unencapsulated controls. The superior enzyme inhibition observed in chitosan-coated formulations could be attributed to the combined effects of active compounds from *G. perakense* extract and chitosan coating. As previously identified through molecular docking studies, *G. perakense* extract contained several compounds, including (-)-amurensisin, catechin, bergenin and kaempferol 4'-glucoside, with strong PPO binding affinities. The chitosan coating likely provided controlled release of these active compounds while forming a protective barrier against oxidation, resulting in sustained PPO inhibition. Additionally, chitosan's inherent ability to bind with enzymes and chelate metal ions might have contributed to the enhanced inhibitory effect observed in the coated formulations [40]. These results suggested that chitosan-based microencapsulation effectively improved the stability and functionality of *G. perakense* leaves extract as a PPO inhibitor in fresh-cut potato applications.

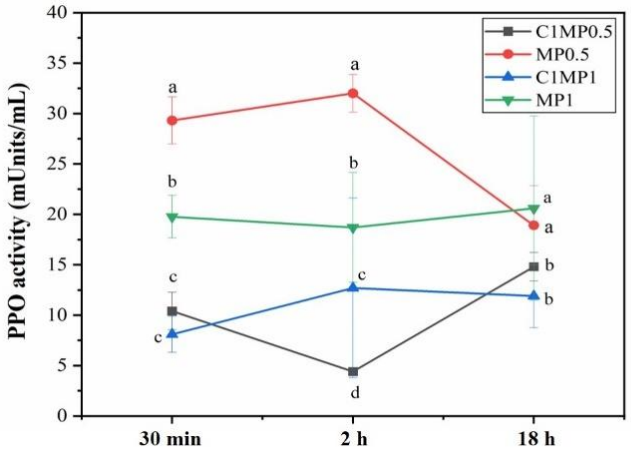

Figure 6 Polyphenol oxidase (PPO) activity in fresh-cut potatoes treated with: Chitosan-coated microcapsules with *G. perakense* extract at 1:1 w/w (C1MP1) and 1:0.5 w/w (C1MP0.5) ratios; and non-encapsulated *G. perakense* extract at 0:1 w/w (MP1) and 0:0.5 w/w (MP0.5) ratios during storage at room temperature (30 min, 2 h, and 18 h). Different letters at each time point indicate significant differences ($p < 0.05$, Duncan's multiple range test). Results are presented as mean ± standard deviation ($n = 3$).

Conclusions

This study demonstrated the exceptional potential of *G. perakense* leave extract as a natural preservative, exhibiting superior antioxidant activity and PPO inhibition. Molecular docking identified multiple potent PPO inhibitors including (-)-amurensisin (–7.13 kcal/mol), catechin (–6.36 kcal/mol) and bergenin (–5.84 kcal/mol), all demonstrating stronger binding affinity than reference compound tropolone (–5.20 kcal/mol) with favorable interaction profiles within the enzyme active site. Chitosan-based microencapsulation enhanced extract stability, maintaining high phenolic content and antioxidant activity during storage. Chitosan-*G. perakense* microcapsules demonstrated significant efficacy as anti-PPO agents, exhibiting 2.5-fold higher PPO inhibition in fresh-cut potato slices compared to unencapsulated controls. These findings provide a promising foundation for sustainable food

preservation solutions, particularly addressing post-harvest losses in fresh-cut produce through natural enzyme inhibition strategies.

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Declaration of Generative AI in Scientific Writing

Claude (Anthropic) was employed to enhance the linguistic quality and readability of this manuscript under continuous human oversight. The AI tool was not involved in research methodology, data generation, or scientific interpretation. All intellectual content and conclusions remain solely the responsibility of the authors.

CRediT Author Statement

Kittisak Sangthongchin: Investigation; Formal analysis; Data curation; Writing - original draft; Visualization. **Panita Kongsune:** Methodology; Formal analysis; Writing - review & editing. **Parichat Thepthong:** Investigation; Data curation; Writing - review & editing. **Pimpun Chanakeat:** Resources; Formal analysis; Writing - review & editing. **Netnapa Chana:** Conceptualization; Methodology; Supervision; Project administration; Funding acquisition; Writing - review & editing.

References

[1] M Ali, S Batool, N Khalid, S Ali, MA Raza, X Li, F Li and Z Xinhua. Recent trends in hydrogen-associated treatments for maintaining the postharvest quality of fresh and fresh-cut fruits and vegetables: A review. *Food Control* 2024; **156**, 110114.

[2] H Zou, Q Xiao, G Li, X Wei, X Tian, L Zhu, F Ma and M Li. Revisiting the advancements in plant polyphenol oxidases research. *Scientia Horticulturae* 2025; **341**, 113960.

[3] G Bobo, C Arroqui and P Virseda. Natural plant extracts as inhibitors of potato polyphenol oxidase: The green tea case study. *LWT* 2022; **153**, 112467.

[4] AN Pepper, P Sriaroon and MC Glaum. Additives and preservatives: Role in food allergy. *Journal of Food Allergy* 2020; **2(1)**, 119-123.

[5] E Teshome, SF Forsido, HPV Rupasinghe and EO Keyata. Potentials of Natural Preservatives to Enhance Food Safety and Shelf Life: A Review. *The Scientific World Journal* 2022; **2022**, 9901018.

[6] W Panpipat, W Suttirak and M Chaijan. Free radical scavenging activity and reducing capacity of five southern Thai indigenous vegetable extracts. *Walailak Journal of Science and Technology* 2011; **7(1)**, 51-60.

[7] DTSL Mendes, RAD Jesus, VRDS Moraes, CF Silva, WRD Silva, A Wisniewski, MCSS Macedo and CX Resende. Extraction of *Anacardium occidentale*: A kinetic, thermodynamic, phytochemical identification, and antibacterial study. *Food Bioscience* 2024; **62**, 105336.

[8] A Thuanthong, S Klomklao, J Panyo and Y Zhang. *In-vitro* screenings for biological and antioxidant activities of aqueous extract from Sacha Inchi (*Plukenetia volubilis* L.) Husks. *Trends in Sciences* 2023; **20(11)**, 6815.

[9] P Thepthong, S Srirat, W Hiranrat, P Kongsune and N Chana. Development of Chitosan-Based microencapsulation system for *Mitragyna speciosa* alkaloids: A novel approach for alzheimer's disease treatment. *Journal of Pharmaceutical* 2025; **20**, 58.

[10] A Manzoor, S Ahmad and B Yousuf. Development and characterization of edible films based on flaxseed gum incorporated with *Piper betle* extract. *International Journal of Biological Macromolecules* 2023; **245**, 125562.

[11] N Chana, A Muengpoon, S Pethkaew, N Kulsin, A Mahasuk and S Srirat. Screening for phenolic compounds and oxidative capacity of fruit peels, agricultural waste, and traditional herbal medicine for use as biodiesel fuel additive. *Songklanakarin Journal of Science and Technology* 2022; **44(4)**, 1067-1074.

[12] R Kongkachuichai, R Charoensiri, K Yakoh, A Kringkasemsee and P Insung. Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. *Food Chemistry* 2015; **173**, 838-846.

[13] I Bakhouche, T Aliat, T Boubellouta, L Gali, A Şen and Y Bellik. Phenolic contents and *in vitro* antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte *Limonium delicatulum*. *South African Journal of Botany* 2021; **139**, 42-49.

[14] S Zhang. Recent advances of polyphenol oxidases in plants. *Molecules* 2023; **28(5)**, 2158.

[15] M Landi, E Degl'Innocenti, L Guglielminetti and L Guidi. Role of ascorbic acid in the inhibition of polyphenol oxidase and the prevention of browning in different browning-sensitive *Lactuca sativa* var. *capitata* (L.) and *Eruca sativa* (Mill.) stored as fresh-cut produce. *Journal of the Science of Food and Agriculture* 2013; **93(8)**, 1814-1819.

[16] M Hassan, S Shahzadi and A Kloczkowski. Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: A review. *Molecules* 2023; **28(1)**, 378.

[17] QY Han, F Liu, X Wen and YY Ni. Kinetic, spectroscopic, and molecular docking studies on the inhibition of membrane-bound polyphenol oxidase from Granny Smith apples (*Malus domestica* Borkh.). *Food Chemistry* 2021; **338**, 127928.

[18] A Farasat, M Ghorbani, N Gheib and H Shariatifar. *In silico* assessment of the inhibitory effect of four flavonoids (Chrysin, Naringin, Quercetin, Kaempferol) on tyrosinase activity using the MD simulation approach. *BioTechnologia* 2020; **101(3)**, 193-204.

[19] P Nokthai, VS Lee and L Shank. Molecular modeling of peroxidase and polyphenol oxidase: Substrate specificity and active site comparison. *International Journal of Molecular Sciences* 2010; **11(9)**, 3266-3276.

[20] DHU Eranda, M Chaijan, W Panpipat, S Karnjanapratum, MA Cerqueira and R Castro-Muñoz. Gelatin-chitosan interactions in edible films and coatings doped with plant extracts for biopreservation of fresh tuna fish products: A review. *International Journal of Biological Macromolecules* 2024; **280**, 135661.

[21] Y Jang and E Koh. Characterisation and storage stability of aronia anthocyanins encapsulated with combinations of maltodextrin with carboxymethyl cellulose, gum Arabic, and xanthan gum. *Food Chemistry* 2023; **405**, 135002.

[22] Q Ji, Z Jin, W Ding, Y Wu, C Liu, K Yu, N Zhang, G Jin, P Lu, D Bao and L Mei. Chitosan composite films based on tea seed oil nano-microcapsules: Antibacterial, antioxidant and physicochemical properties. *Food Packaging and Shelf Life* 2023; **40**, 101212.

[23] S Baliyan, R Mukherjee, A Priyadarshini, A Vibhuti, A Gupta, RP Pandey and CM Chang. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules* 2022; **27(4)**, 1326.

[24] M Sierocka and M Świeca. Natural inhibitors of the polyphenol oxidase activity isolated from shredded stored iceberg lettuce (*Lactuca sativa* L.). *Applied Sciences* 2024; **14(21)**, 9980.

[25] W Innok, A Hiranrat, N Chana, T Rungrotmongkol and P Kongsune. *In silico* and *in vitro* anti-AChE activity investigations of constituents from *Mitragyna speciosa* for Alzheimer's disease treatment. *Journal of Computer-aided Molecular Design* 2021; **35(3)**, 325-336.

[26] LRG Kumar, NS Chatterjee, CS Tejpal, KV Vishnu, KK Anas, KK Asha, R Anandan and S Mathew. Evaluation of chitosan as a wall material for microencapsulation of squalene by spray drying: Characterization and oxidative stability studies. *International Journal of Biological Macromolecules* 2017; **104**, 1986-1995.

[27] A Kyriakoudi, IR Redovniković, S Vidović, K Radošević, T Andreou, I Mourtzinos and MC Bubalo. Coupling deep eutectic solvents with

innovative extraction techniques towards plant derived bioactive compositions. *RSC Sustainability* 2024; **2(6)**, 1675-1691.

[28] S Núñez, MS Valero, AM Mustafa, G Caprioli, F Maggi, C Gómez-Rincón and V López. Ultrasound-assisted extraction versus traditional Soxhlet apparatus for the obtention of polyphenols, carotenoids and tocopherols from *Tagetes erecta* L. flowers. *Journal of the Science of Food and Agriculture* 2024; **105(6)**, 3453-3462.

[29] YJ Chan, CS Chiu, PH Li and WC Lu. Evaluation of different roasting condition on yield, physico-chemical characteristics, and antioxidant activity of cold-pressed sacha inchi (*Plukenetia volubilis*) oil. *LWT* 2024; **203**, 116343.

[30] A Smeriglio, V D'Angelo, M Denaro, D Trombetta, FM Raimondo and MP Germanò. Polyphenol characterization, antioxidant and skin whitening properties of *Alnus cordata* stem bark. *Chemistry & Biodiversity* 2019; **16(9)**, e1900314.

[31] S Zolghadri, A Bahrami, MTH Khan, J Munoz-Munoz, F Garcia-Molina, F Garcia-Canovas and AA Saboury. A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 2019; **34(1)**, 279-309.

[32] T Sae-leaw and S Benjakul. Prevention of quality loss and melanosis of Pacific white shrimp by cashew leaf extracts. *Food Control* 2019; **95**, 257-266.

[33] NN Linh, NQ Hop, PTT Nhung, PTB Dao, VQ Manh, TV Pham and NT Son. *Glochidion* species: A review on phytochemistry and pharmacology. *Natural Product Communications* 2024. https://doi:10.1177/1934578X241276962

[34] AM Mendoza-Wilson, RR Balandrán-Quintana, MÁ Valdés-Covarrubias and JL Cabellos. Potential of quercetin in combination with antioxidants of different polarity incorporated in oil-in-water nanoemulsions to control enzymatic browning of apples. *Journal of Molecular Structure* 2022; **1254**, 132372.

[35] Y Han, Y Liu, Z Li, J Sun, F Song and J Chen. Effects of chitosan grafted with gallic acid treatment on the postharvest physiology and biochemistry of Saimaiti apricots. *Journal of Stored Products Research* 2024; **108**, 102390.

[36] CW Li, HJ Dong and CB Cui. The synthesis and antitumor activity of twelve galloyl glucosides. *Molecules* 2015; **20**, 2034-2060.

[37] X Tian, Y Lv, Z Wang, L Rao and Y Wang. Identification of natural inhibitors targeting polyphenol oxidase through machine learning-assisted virtual screening and investigation of molecular mechanisms using multispectral experiments. *LWT* 2024; **208**, 116699.

[38] JN Wang, YJ Chen, Y Hano, T Nomura and RX Tan. Antioxidant activity of polyphenols from seeds of vitis amurensis *in vitro*. *Acta Pharmacologica Sinica* 2000; **21(7)**, 633-636.

[39] J Li, ZY Deng, H Dong, R Tsao and X Liu. Substrate specificity of polyphenol oxidase and its selectivity towards polyphenols: Unlocking the browning mechanism of fresh lotus root (*Nelumbo nucifera* Gaertn.). *Food Chemistry* 2023; **424**, 136392.

[40] M Ghasemnezhad, S Zareh, M Rassa and RH Sajedi. Effect of chitosan coating on maintenance of aril quality, microbial population and PPO activity of pomegranate (*Punica granatum* L. cv. Tarom) at cold storage temperature. *Journal of the Science of Food and Agriculture* 2012; **93(2)**, 368-374.

[41] JC Antunes, JM Domingues, CS Miranda, AFG Silva, NC Homem, MTP Amorim and HP Felgueiras. Bioactivity of Chitosan-based particles loaded with plant-derived extracts for biomedical applications: Emphasis on antimicrobial fiber-based systems. *Marine Drugs* 2021; **19(7)**, 359.

[42] S Soleymanfallah, Z Khoshkhoo, SE Hosseini and MH Azizi. Preparation, physical properties, and evaluation of antioxidant capacity of aqueous grape extract loaded in chitosan-TPP nanoparticles. *Food Science & Nutrition* 2022; **10(10)**, 3272-3281.

[43] Y Zhu, Y Wang and X Yan. Effect of microcapsules of Chitosan-Coated *Toddalia asiatica* (L.) Lam extracts on the surface coating properties of poplar wood. *Coatings* 2024; **14(8)**, 1013.