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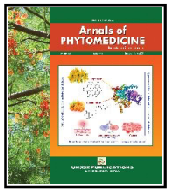

Original Article : Open Access

Ultrasonic-assisted extraction and encapsulation of *Picrorhiza kurroa* Royle ex Benth. root bioactives: A novel approach for enhancing antidiabetic potential

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Abstract

Picrorhiza kurroa Royle ex Benth. root (PKR), a highly valued medicinal herb in traditional medicine systems, is widely recognized for its potent antioxidant and antidiabetic properties. This study explores the physicochemical and bioactive characteristics of PKR extracts obtained through ultrasonic-assisted extraction (USAE) at 50% amplitude for 30 min using different solvents (ethanol, methanol, petroleum ether, and distilled water). Methanolic extracts exhibited the highest antioxidant activity, as evidenced by DPPH inhibition (57.77%) and FRAP assay (111.31 µmol TEAC/g), followed by ethanolic extracts. To optimize the retention of bioactive compounds, freeze-drying was performed at varying temperatures (– 60°C, – 70°C, – 80°C), with lower temperatures enhancing the preservation of phytochemicals. Furthermore, encapsulation of PKR extract was carried out using gum arabic (GA), carboxymethyl cellulose (CMC), and calcium alginate (CA), which significantly influenced powder properties. GA-based encapsulation showed the highest bulk (695.03 kg/m³) and tapped density (555.57 kg/m³), whereas CA-encapsulated powder demonstrated superior flowability and compressibility, with a Hausner ratio of 1.19 and Carr's index of 14.44%. Notably, CA-encapsulated PKR powder freeze-dried at – 80°C displayed the highest total phenolic content of 99.56 mg GAE/g and DPPH inhibition of 76.21%. Additionally, CA-encapsulated PKR exhibited strong antidiabetic potential, with significant inhibition of α-amylase (70.43%) and α-glucosidase (77.27%). These findings underscore the critical role of solvent selection, encapsulation material, and freeze-drying conditions in modulating the physicochemical and functional properties of PKR extracts. The study highlights the potential of PKR as a promising candidate for functional foods and nutraceutical applications. Further *in vivo* bioavailability studies are recommended to validate these findings.

1. Introduction

Picrorhiza kurroa Royle ex Benth., popularly called Kutki, is a highly valued medicinal herb indigenous to the Northwestern Himalayas. It grows in the challenging alpine and sub-alpine climates of regions like Kashmir, Himachal Pradesh, and Uttarakhand. Flourishing at altitudes of 3,000 to 5,000 meters, this hardy herb is uniquely adapted to extreme conditions, growing in rocky crevices, moist slopes, and well-drained soils. It endures freezing temperatures and survives under snow cover during winter, showcasing its resilience. However, its limited natural habitat and the growing demand in traditional Ayurvedic and Tibetan medicine have led to overexploitation, pushing *P. kurroa* toward vulnerability and raising urgent conservation concerns (Kumar *et al*., 2021). The therapeutic power of *P. kurroa* lies in its rich phytochemical composition, particularly iridoid glycosides like picroside I, picroside II, and kutkoside (Panday *et al*., 2022). These potent bioactive compounds are prized for their powerful antioxidant, anti-inflammatory, and hepatoprotective effects, playing a crucial role in promoting health and preventing disease. The herb is highly valued for its liver-protective effects, aiding in hepatitis, cirrhosis, and fatty liver disease. Its active compounds support liver cell regeneration, reduce oxidative stress, and boost bile secretion, making it essential in hepatology (Panday *et al*., 2022).

Beyond liver health, *P. kurroa* exhibits strong antioxidant activity, countering oxidative stress linked to cardiovascular and neurodegenerative diseases (Nipanikar *et al*., 2017). Its anti-inflammatory and immunomodulatory properties aid arthritis, autoimmune disorders, asthma, and infections (Tiwari *et al.,* 2018). Additionally, it supports digestion by enhancing bile flow, appetite, and alleviating dyspepsia, constipation, and ulcers (Cheema *et al.,* 2021).

Recent research highlights *P. kurroa*'s potential in metabolic disorders, improving insulin sensitivity and blood glucose regulation (Daga *et al.,* 2024). Its antimicrobial properties add to its versatility, but overharvesting threatens its survival (Ahmad *et al.,* 2023). Conservation through *in situ* cultivation and sustainable harvesting is vital. Ultrasonic-assisted extraction (UAE) enhances bioactive compound yield while preserving antioxidant and hepatoprotective properties (Sharma *et al.,* 2022). Freeze-drying encapsulation stabilizes compounds, protecting them from degradation (Rezvankhah *et al.,* 2020). Encapsulation with carriers like maltodextrin and gum

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arabic improves bioavailability, making it suitable for functional foods and pharmaceuticals (Meena *et al.,* 2022).

The study highlights the need to optimize extraction protocols for preserving polyphenols and bioactive compounds from *P. kurroa*. Using UAE, it enhances extraction efficiency, antioxidant properties, and antidiabetic potential. Advancing extraction and encapsulation strategies bridges traditional knowledge with modern science, positioning this Himalayan herb as a sustainable solution for global health challenges.

2. Materials and Methods

2.1 Raw materials procurement

Fresh *Picrorhiza kurroa* Royle ex Benth. roots were harvested in September in post flowering and seed setting stage, from their natural habitat at Sonmarg (34.32°N, 75.35°E; elevation approx. 4,500 mts above sea level). The collected specimen (Herbarium Accession No. 21545) underwent taxonomic verification by Dr. Tahir, Assistant Professor of Forestry at SKUAST Kashmir (Voucher No. FOF-57). Following collection, roots were subjected to meticulous processing: thorough surface cleaning, shade drying at ambient temperature, and mechanical reduction to fine powder using a standardized laboratory hammer mill.

2.2 Methods

2.2.1 Preparation of extract

Preliminary trials were conducted using various solvent concentrations (100:0, 75:25, 50:50, 25:75 ratios of ethanol, methanol, petroleum ether and distilled water) to determine the optimal extraction yield. It was found that the 50:50 solvent-to-water ratio yielded the highest extraction efficiency and yield % of bioactive compounds. Consequently, this solvent concentration was selected for use in the ultrasonic-assisted extraction (USAE) method. The amplitude was varied from 20% to 80% and sonication time from 10 to 30 min. Among the tested conditions, it was observed that 50% amplitude and 30 min of sonication consistently yielded the highest antioxidant activity of the extract. Based on these observations, a 13 mm ultrasonic probe operated at 50% amplitude for 30 min with 5s on/3s off pulses. Water at 25°C was circulated in the ultrasonic bath to prevent overheating during extraction.

2.2.2 Encapsulation of the extract

The bioactive extracts were microencapsulated employing three distinct wall material systems: calcium alginate (CA), gum Arabic, and carboxymethyl cellulose (CMC), maintaining an 80:20 core-to-wall material ratio. Each formulation underwent homogenization via magnetic stirring (30 min, ambient temperature) to ensure complete dissolution of particulate matter before lyophilization. The solutions were then ultrasonicated (Cole-Parmer Instruments, Model CV334) to enhance molecular dispersion. Lyophilization was performed in a programmable freeze dryer (ILShinBioBase TFD8501) with sequential temperature ramping (– 60°C, – 70°C and – 80°C) over a 20 h cycle to obtain stable encapsulates. Post-lyophilization, the resulting microencapsulated powders were aseptically packaged in amber Eppendorf tubes with desiccant packs and maintained at 4°C for storage stability.

2.2.3 Yield % of the encapsulated extract (g)

The yield of microcapsules (g) derived after freeze-drying was used for the calculation of encapsulation yield using the formula (Panday and Mishra 2022).

Extraction yield (%)

$$= \frac{\text{Weight of freeze dired microcapsules (g)}}{\text{Solid content in feed suspension before drying (g)}} \times 100$$

2.2.4 Total-phenolic content (mg GAE/g)

The Folin-Ciocalteu assay (Sacchetti *et al*., 2009 modified protocol) was employed to determine total phenolic content in both *P. kurroa* root extract (PKRE) and microencapsulated formulations. Sample aliquots (0.1-0.4 ml) of microencapsulated powders were reconstituted in deionized water (final volume: 5 ml) prior to analysis. The reaction was initiated by adding 0.5 ml Folin-Ciocalteu reagent, followed by 3 min dark incubation. Alkalinization was achieved with 1.5 ml Na, CO_2 (25% w/v), with subsequent dilution to 10 ml final volume. After 60 min dark incubation at ambient temperature (25 ± 1°C), absorbance was recorded at 765 nm (UV-Vis spectrophotometer). Quantification was performed against a gallic acid standard curve (Sigma-Aldrich), with results expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g).

2.2.5 Total flavonoid content (mg QE/g)

The total flavonoid content (TFC) of both native PKRE and its microencapsulated forms was determined using an aluminum chloride colorimetric assay adapted from Dadi *et al*. (2019). In this procedure, 0.5 ml of each sample was mixed with 0.15 ml of 5% sodium nitrite ($NaNO_2$) and 2.5 ml distilled water, followed by 6 min of incubation at room temperature. Subsequently, 0.3 ml of 10% aluminum chloride ($AlCl_2$) and 1 ml of 1 M sodium hydroxide (NaOH) were added, followed by 0.55 ml distilled water. The mixture was vortexed thoroughly and allowed to stand for 15 min to develop the chromophore. A reagent blank and quercetin standard solutions (0-100 µg/ml) were processed identically to construct the calibration curve. Absorbance was measured at 510 nm using a UV-Vis spectrophotometer, and results were calculated as mg quercetin equivalents per gram dry weight (mg QE/g).

2.2.6 % DPPH radical scavenging activity (%)

The DPPH radical scavenging activity of samples was evaluated according to the method of Sahu *et al*. (2013) with some modifications. Briefly, 100 µl of samples was mixed with 3.9 ml of ethanolic solution of DPPH (0.2 mM). The obtained mixture was left at room temperature for 30 min in the dark, then centrifuged at 4000g for 5 min. The absorbance was measured at 517 nm. Finally, the DPPH radical scavenging activity was measured using the subsequent formula:

% DPPH radical scavenging activity

$$= \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.2.7 Ferric ion reducing antioxidant power (mM TE/g dry matter)

The ferric reducing antioxidant power (FRAP) of PKRE and microencapsulated powders was evaluated according to Fawole *et*

al. (2024) with slight modifications. The FRAP reagent was freshly prepared by combining 25 ml of sodium acetate buffer (300 mM, pH 3.6), 2.5 ml of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution, and 2.5 ml of 20 mM ferric chloride ($FeCl_2 \cdot 6H_2O$). For the assay, 150 µl of each sample was mixed with 2850 µl of the FRAP reagent in 10 ml volumetric flasks, and the volume was adjusted with redistilled water. After 20 min of incubation at 37°C in the dark, absorbance was measured at 593 nm using a UV-Vis spectrophotometer, with a reagent blank as reference. A Trolox standard curve (0-2.0 mM, $R^2 = 0.999$) was used for quantification, and results were presented as millimolar Trolox equivalents per gram of dry matter (mM TE/g).

2.2.8 Evaluation of encapsulated powder of phytochemical-rich fraction

The powder properties were calculated following Bhusari *et al*. (2014) with slight modifications:

2.2.8.1 Bulk density

The bulk density (ρB) of microencapsulated *P. kurroa* extract was determined gravimetrically. Precisely 2 g (mo) of each sample was poured under free-flow conditions into a 50 ml graduated cylinder. The occupied volume (vo) was recorded after settling, and bulk density was calculated using the fundamental relationship.

$$\text{Bulk density } (\rho b) = \frac{\text{Weight of the sample (mo)}}{\text{Volume occupied (vo)}}$$

2.2.8.2 True (particle) density

The true density (ρP) of microencapsulated *P. kurroa* extract was determined via liquid displacement pycnometry using toluene. A 1.000 g (mn) sample was introduced into a calibrated cylinder with toluene (v). Volume displacement was recorded after immersion, and true density was calculated using the fundamental relationship.

$$\text{True density } (\rho p) = \frac{\text{Weight of the sample (mn)}}{\text{Volume occupied in a calibrated cylinder with toulene (vl)}}$$

2.2.8.3 Tapped density

The tapped density (ρ_τ) of microencapsulated powders was determined using a manual tapping method. The pre-measured sample (mo) in a graduated cylinder was tapped from 10.0 ± 0.5 cm until volume stabilized (≈ 100 taps). The final volume (vt) was recorded after ≤1% variation, and tapped density was calculated using the mass-volume relationship.

$$\text{Tapped density } (\rho_\tau) = \frac{\text{weight of the sample (mo)}}{\text{Final volume after tapping (vt)}}$$

2.2.9 Flowability and compressibility

The flowability and compressibility properties of the encapsulated powder were calculated by following formulas (Premi and Sharma, 2017).

2.2.9.1 Hausner ratio

Hausner ratio (HR) correlates the flowability of powder material. HR for the encapsulated SBTL powder samples was calculated by using tapped and bulk density as:

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

2.2.9.2 Carr's index

The flow properties of microencapsulated *P. kurroa* extract were assessed using Carr's compressibility index (CI), a key powder rheology parameter. CI was calculated based on the relationship between bulk and tapped densities using the standard formula.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$$

2.2.10 *In vitro* method to study the antidiabetic potential of encapsulated extract

The *in vitro* antidiabetic activity was done for the samples with the highest TPC and % DPPH inhibition assay.

2.2.10.1 α-Amylase inhibition (%)

The alpha-amylase inhibitory activity of *P. kurroa* extracts was assessed using a modified protocol (Anga *et al*., 2015). In a 96-well plate, 10 µl sodium phosphate buffer (0.02 M, pH 6.9), 50 µl alpha-amylase (40 U/ml), and 50 µl test sample at varying concentrations were mixed and vortexed for 30 sec. After 15 min of incubation at 37°C, 40 µl of 1% (w/v) soluble starch was added, followed by a 20 min incubation. The reaction was stopped with 40 µl 1 M HCl and 10 µl iodine reagent (5 mM). Absorbance at 620 nm was measured using a BioTek Epoch 2 microplate reader, and inhibition was calculated.

$$A\,(\%) = (B - C/D - C) \times 100$$

where,

A represents the percentage inhibition of alpha-amylase activity.

B is the absorbance of the test sample at 620 nm.

C is the absorbance of the negative control (without the sample) at 620 nm.

D is the absorbance of the positive control (with the enzyme) at 620 nm.

2.2.10.2 α-glucosidase inhibition (%)

The alpha-glucosidase inhibitory activity of *P. kurroa* extracts was evaluated using a modified method (Ahmad *et al*., 2019). In a 96-well plate, 50 µl extract at various concentrations was mixed with 75 µl 0.1 M phosphate buffer (pH 7.0) and 25 µl alpha-glucosidase (5 µg/ml). After 5 min of pre-incubation at 37°C, the reaction was initiated by adding 25 µl 15 mM pNPG substrate and incubated for 30 min. The reaction was stopped with 100 µl 0.2 M sodium carbonate. Absorbance at 405 nm was measured using a BioTek Epoch 2 microplate reader, and inhibition was calculated relative to the control.

$$A\,(\%) = (B - C/B) \times 100$$

where,

A denotes the inhibition rate (%) of alpha-glucosidase.

B denotes the negative control's absorbance at 405 nm.

C denotes the test sample's absorbance at 405 nm.

3. Results

3.1 Physicochemical analysis of *P. kurroa* root (PKRE) extracts

The physicochemical analysis of *P. kurroa* root extracts (PKRE) obtained through ultrasonic-assisted extraction (USAE) revealed significant variations based on the solvent used. Among the tested solvents, methanol exhibited the highest extraction yield (34.73%) and the highest total phenolic content (TPC) of 84.85 mg GAE/g, followed by ethanol (32.65%, 81.13 mg GAE/g) and distilled water (26.99%, 76.21 mg GAE/g). Methanolic extract also showed the strongest antioxidant activity, with a DPPH inhibition of 57.77% and a ferric reducing antioxidant power (FRAP) of 111.31 µmol TEAC/g, whereas ethanol and distilled water showed slightly lower but comparable values (Table 1). The highest TFC was observed in the methanol extract (79.71 mg QE/100 g), followed by distilled water (70.42 mg QE/100 g) and ethanol (69.55 mg QE/100 g). In contrast, petroleum ether demonstrated the lowest extraction efficiency, with a significantly lower yield (13.69%), TPC (16.15 mg GAE/g), DPPH inhibition (18.32%), FRAP (17.85 µmol TEAC/g), and TFC(16.55 mg QE/100 g), indicating its ineffectiveness in extracting bioactive compounds. These findings highlight methanol as the most efficient solvent for extracting antioxidant-rich compounds from *P. kurroa* roots using USAE.

3.2 Powder properties

3.2.1 Bulk density

The bulk density of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) exhibited the highest bulk density (695.03 kg/m³), followed by carboxymethyl cellulose (CMC) (634.52 kg/m³), while calcium alginate (CA) had the lowest (497.08 kg/m³). Lower freeze-drying temperatures (– 60°C to – 80°C) slightly reduced bulk density (616.98-604.72 kg/m³). Statistical analysis showed significant variations (CD = 2.568, $p \leq 0.05$) and interaction effects (CD = 5.136). These findings highlight the crucial role of encapsulating material and freeze-drying conditions in PKRE powder formulation.

Table 1: Physicochemical properties of *P. kurroa* root extract (PKRE) USAE

Parameter solvent	Yield %	TPC (mgGAE/g)	% DPPH inhibition	FRAP (µmol TEAC/g)	TFC (QE/100 g)
Methanol	34.73	84.85	57.77	111.31	79.71
Ethanol	32.65	81.13	56.46	99.85	69.55
Distilled water	26.99	76.21	48.71	86.83	70.42
Petroleum ether	13.69	16.15	18.32	17.85	16.55
SE(d)	0.50	0.32	0.62	0.77	0.47
C.D.	1.01	0.65	1.25	1.54	0.95

*Mean values in the columns with different superscripts are significantly different at $p \leq 0.05$. CD = Critical difference.

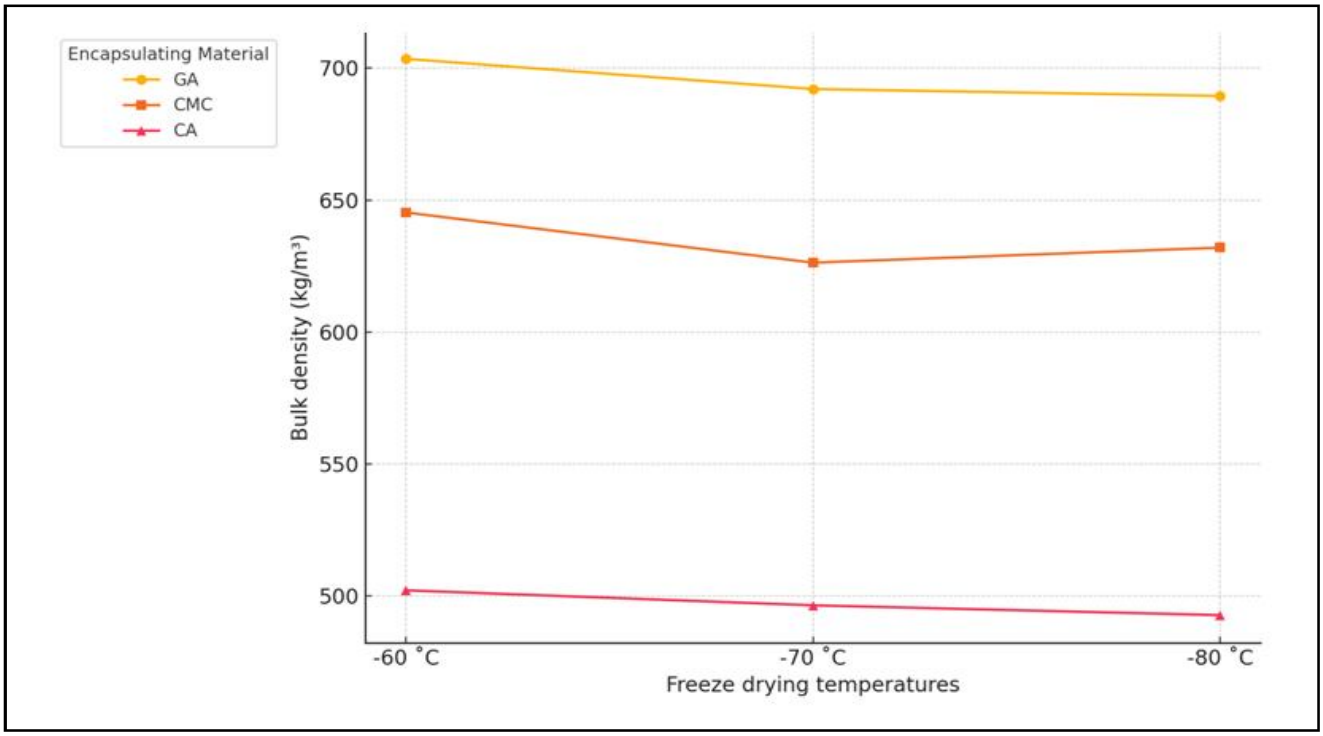

Figure 1: Effect of encapsulation materials and freeze drying temperatures on bulk density.

3.2.2 Tapped density

The tapped density of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) had the highest tapped density (555.57 kg/m³), followed by carboxymethyl cellulose (CMC) (518.22 kg/m³), while calcium alginate (CA) had the lowest (415.41 kg/m³). Lower freeze-drying temperatures (– 60°C to – 80°C) gradually reduced tapped density (506.49-489.90 kg/m³). Statistical analysis confirmed significant variations (CD = 1.685, $p \leq 0.05$) and interaction effects (CD = 3.37). These findings highlight the role of encapsulating material and freeze-drying conditions in PKRE powder formulation.

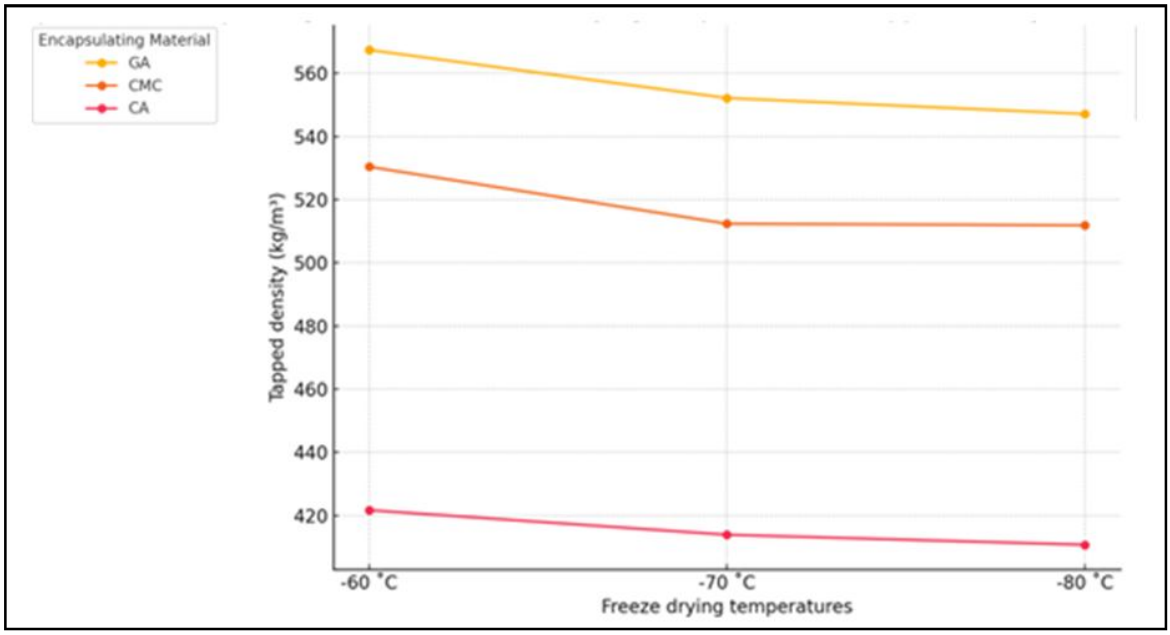

Figure 2: Effect of encapsulation materials and freeze drying temperatures on tapped density.

3.2.3 True density

The true density of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) had the highest true density (1364 kg/m³), followed by carboxymethyl cellulose (CMC) (1222 kg/m³), while calcium alginate (CA) had the lowest (1060 kg/m³). True density slightly increased from 1205 kg/m³ at -60°C to 1222 kg/m³ at -80°C. Statistical analysis confirmed significant variations (CD = 10.85, $p \leq 0.05$) and interaction effects (CD = 21.60). These findings highlight the importance of encapsulating material in determining PKRE powder's true density.

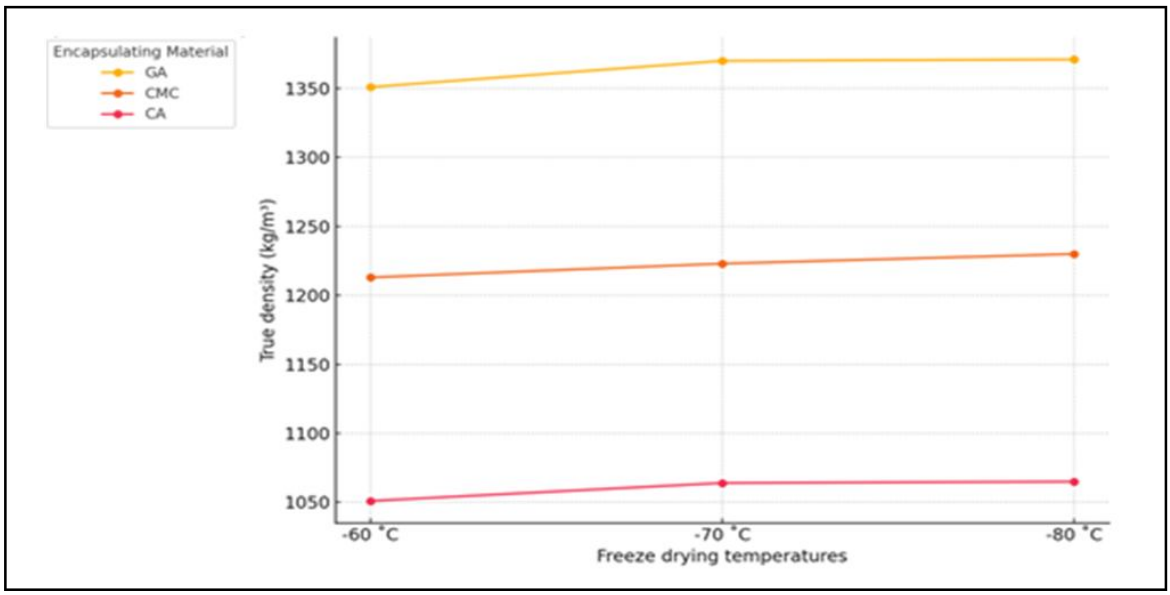

Figure 3: Effect of encapsulation material and freeze drying temperatures on true density.

3.2.4 Hausner ratio

The Hausner ratio (HR) of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) had the highest HR (1.25), followed by carboxymethyl cellulose (CMC) (1.22), while calcium alginate (CA) had the lowest (1.19). HR slightly increased from 1.21 at -60°C to 1.23 at -80°C. Statistical analysis confirmed significant variations (CD = 0.03, $p \leq 0.05$) and interaction effects (CD = 0.06). These findings highlight the role of encapsulating material and freeze-drying conditions in PKRE powder's flow properties, with GA showing lower flowability than CA.

Table 2: Effect of encapsulating material and freeze drying temperatures on Hausner ratio (HR) of encapsulated of PKRE powder

Encapsulating materials (EM)	Temperatures			
	-60°C	-70°C	-80°C	Mean (E.M)
GA	1.24	1.25	1.26	1.25
CMC	1.21	1.22	1.23	1.22
CA	1.18	1.19	1.20	1.19
Mean (Temp.)	1.21	1.22	1.23	
Factors				C.D.
Encapsulating material				0.03
Freeze drying temperature				0.03
Encapsulating material × Freeze drying temperature				0.06

*Encap. mat. =encapsulation material, temp. = temperature CD= Critical difference ($p \leq 0.05$).

3.2.5 Carr's index

The Carr's index (CI) of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) had the highest CI (18.53), followed by carboxymethyl cellulose (CMC) (16.80), while calcium alginate (CA) had the lowest (14.44). CI increased slightly with lower freeze-drying temperatures (– 60°C to -80°C). Statistical analysis confirmed significant variations (CD = 0.865, $p \leq 0.05$) and interaction effects (CD = 1.73). These findings highlight the impact of encapsulating material and freeze-drying conditions on PKRE powder compressibility, with GA exhibiting poorer flow properties than CA.

Table 3: Effect of encapsulating material and freeze-drying temperatures on Carr's ratio (CI) of encapsulated of PKRE powder

Encapsulating materials (EM)	Temperatures			
	-60°C	-70°C	-80°C	Mean (EM)
GA	17.21	18.93	19.47	18.53
CMC	15.62	16.99	17.97	16.8
CA	13.97	15.01	14.36	14.44
Mean (Temp.)	**15.6**	**16.97**	**17.26**	
Factors				C.D.
Encapsulating material				0.865
Freeze drying temperature				0.865
Encapsulating material × Freeze drying temperature				1.73

*Encap. mat. =encapsulation material, temp. = temperature CD= Critical difference ($p \leq 0.05$).

3.3 Total phenolic content (mg GAE/g)

The total phenolic content (TPC) of encapsulated *P. kurroa* root extract (PKRE) powder was influenced by the extraction method, encapsulating material, and freeze-drying temperature. Calcium alginate (CA) showed the highest TPC (98.06 mg GAE/g), followed by gum arabic (GA) (95.80 mg GAE/g) and carboxymethyl cellulose (CMC) (94.97 mg GAE/g). The highest TPC was recorded at – 60°C (98.06 mg GAE/g), followed by – 80°C (96.99 mg GAE/g), with the lowest at -70°C (96.10 mg GAE/g). CA at – 80°C exhibited the highest TPC (99.56 mg GAE/g). These findings suggest that CA combined with optimized freeze-drying conditions and ultrasonic assisted extraction enhances phenolic retention in PKRE powder.

3.4 %DPPH inhibition

The %DPPH inhibition of encapsulated *P. kurroa* root extract (PKRE) powder was influenced by extraction method, encapsulating material, and freeze-drying temperature. Calcium alginate (CA) showed the highest antioxidant activity (75.92%), followed by gum arabic (GA) (74.62%) and carboxymethyl cellulose (CMC) (72.98%). Freeze-drying at – 80°C retained the highest % DPPH inhibition (75.04%), followed by – 70°C (74.30%) and -60°C (74.18%). CA at – 80°C

exhibited the highest inhibition (76.21%), indicating better bioactive compound retention. These findings highlight CA as the optimal encapsulating material and -80°C as the preferred freeze-drying temperature for preserving PKRE powder's antioxidant properties.

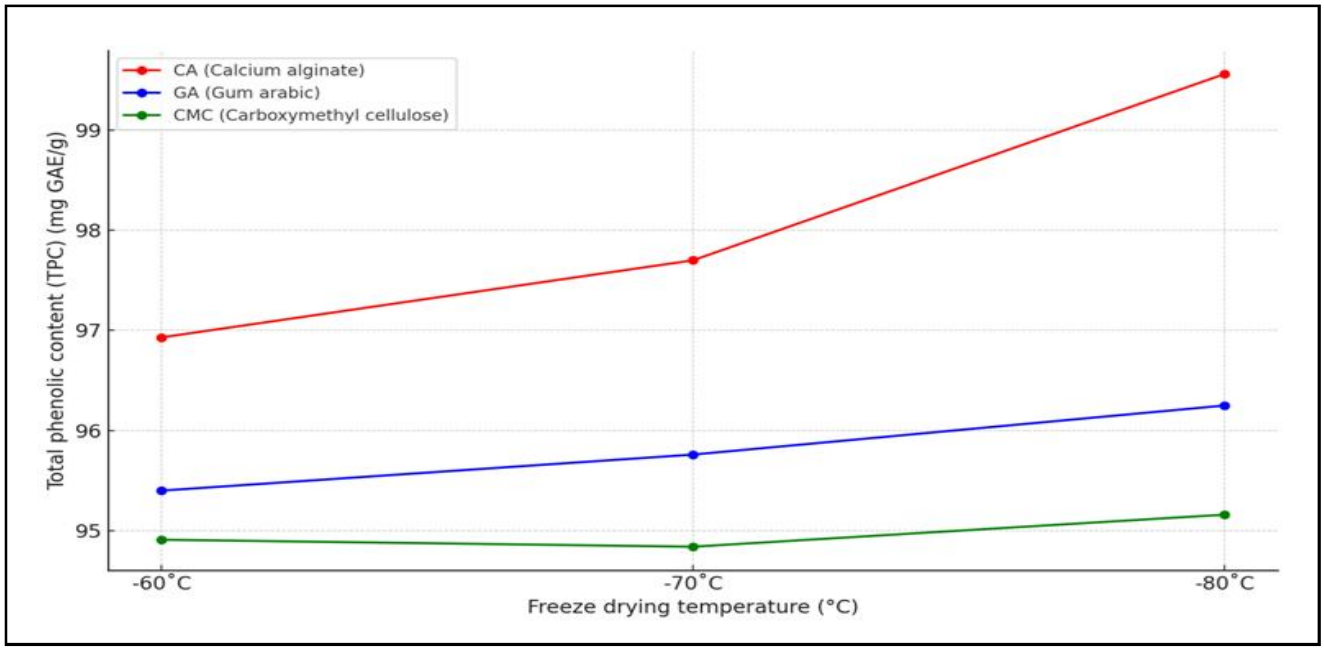

Figure 4: Effect of extraction method, encapsulation materials and freeze drying temperatures on total phenolic content (TPC) of encapsulated PKRE.

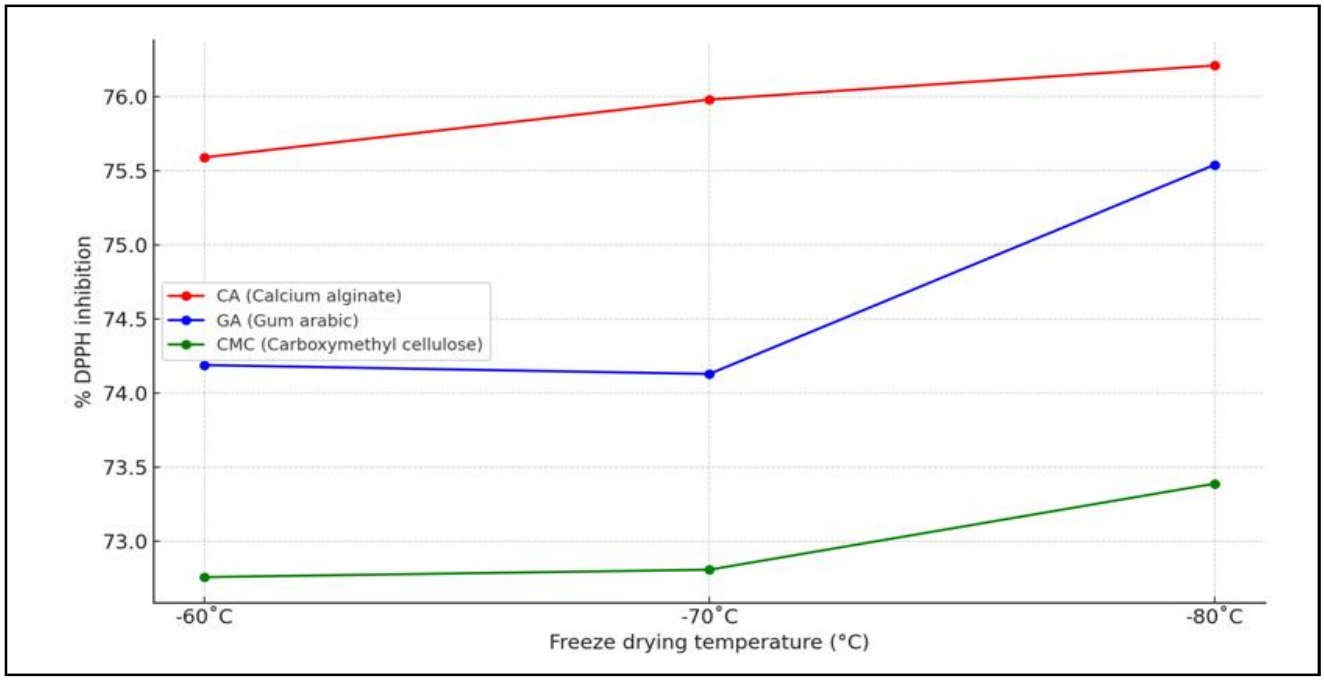

Figure 5: Effect of extraction method, encapsulation materials, and freeze drying temperatures on %DPPH inhibition.

3.5 *In vitro* antidiabetic activity (Alpha amylase and Alpha glucosidase inhibition assay)

Calcium alginate (CA) encapsulated *P. kurroa* root extract (PKRE) exhibited strong α-amylase and α-glucosidase inhibitory activity, demonstrating antidiabetic potential. The α-amylase inhibition assay showed 70.43% ± 0.0163 inhibition, reducing starch breakdown and postprandial glucose spikes. Encapsulation preserved bioactive compounds, enhancing enzyme interference and carbohydrate digestion modulation. α-Glucosidase inhibition was higher at 77.27% ± 0.228, delaying glucose absorption by suppressing disaccharide conversion. These findings suggest encapsulated PKRE's role in diabetes management through enzyme inhibition.

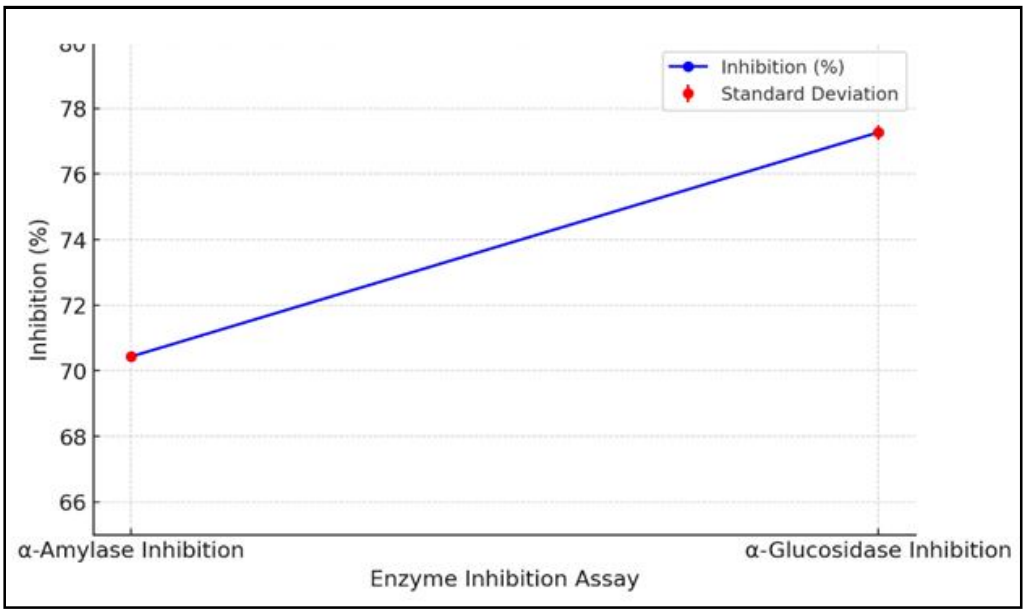

Figure 6: *In vitro* antidiabetic activity of calcium alginate encapsulated *P. kurroa* root extract.

4. Discussion

The choice of solvent significantly influences the extraction efficiency and antioxidant potential of *P. kurroa* root (PKRE) extracts. Solvent polarity plays a crucial role in the solubility of bioactive compounds, particularly phenolics and flavonoids, which contribute to antioxidant activity (Rajhard *et al*., 2021). Polar solvents tend to extract higher amounts of these compounds due to better solubility and cell wall penetration. Methanol, being highly polar, is known for its superior ability to extract phenolic compounds, while ethanol, though slightly less polar, also demonstrates effective extraction (Quitério *et al*., 2022). Water, despite being a universal solvent, may be less efficient due to the limited solubility of certain bioactive compounds (Gullón *et al*., 2020)

In contrast, non-polar solvents like petroleum ether are ineffective in extracting phenolics and flavonoids, leading to lower antioxidant activity. The strong correlation between total phenolic content and antioxidant assays suggests that phenolic compounds are key contributors to the extract's bioactivity (Shi *et al*., 2022). These findings highlight the importance of selecting an appropriate solvent to optimize bioactive compound recovery. Further research focusing on refining extraction conditions, such as temperature, sonication time, and solvent concentration, can enhance the functional properties of PKRE extracts, making them more suitable for nutraceutical and pharmaceutical applications.

The bulk density of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by the choice of encapsulating material and freeze-drying temperature. Encapsulating agents affect powder structure and particle arrangement, impacting density. Gum arabic (GA) exhibited the highest bulk density, likely due to its superior film-forming ability and compact structure (Wang *et al*., 2024). In contrast, calcium alginate (CA) resulted in the lowest bulk density, suggesting a more porous structure (Cuadros *et al*., 2015).

Freeze-drying temperature also influenced bulk density, with a slight reduction as the temperature decreased from -60°C to -80°C. This may be attributed to increased porosity at lower temperatures, leading to reduced powder compaction (Shanmugam *et al*., 2015). The significant interaction between encapsulating material and freeze-drying temperature highlights the need for optimization to achieve the desired powder properties. These findings emphasize the importance of selecting appropriate encapsulating agents and processing conditions to enhance the physical characteristics of PKRE powder for potential applications in functional foods and pharmaceuticals.

The tapped density of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by encapsulating material and freeze-drying temperature. Gum arabic (GA) exhibited the highest tapped density, followed by carboxymethyl cellulose (CMC), while calcium alginate (CA) had the lowest. Lower freeze-drying temperatures (– 80°C) resulted in reduced tapped density, likely due to increased porosity from larger ice crystals (Balaxi *et al*., 2010). The significant interaction effect suggests that both factors must be optimized to enhance powder flowability and compaction. These findings are crucial for improving the formulation and processing of PKRE powder in food and pharmaceutical applications.

The true density of encapsulated *P kurroa* root extract (PKRE) powder was significantly influenced by both encapsulating material and freeze-drying temperature. Gum arabic (GA) exhibited the highest true density, followed by carboxymethyl cellulose (CMC), while calcium alginate (CA) had the lowest. Freeze-drying temperature had a minor effect, with density slightly increasing at lower temperatures, likely due to reduced structural collapse (Nowak *et al*., 2020). The significant interaction effect suggests that optimizing both parameters is essential for maintaining powder integrity. These findings are important for ensuring the stability and application potential of PKRE powder in functional food and pharmaceutical formulations.

The Hausner ratio (HR) of encapsulated *P. kurroa* root extract (PKRE) powder was significantly affected by both encapsulating material and freeze-drying temperature. Gum arabic (GA) exhibited

the highest HR, followed by carboxymethyl cellulose (CMC), while calcium alginate (CA) had the lowest. Higher HR values indicate poorer flowability, suggesting that gum arabic-encapsulated powders are more cohesive than calcium alginate encapsulated powders due to their complex branched polysaccharide structure, which promotes stronger interparticle adhesion and moisture retention (Jeyakumari *et al*., 2018).

The slight increase in HR at lower freeze-drying temperatures may be due to reduced particle agglomeration. These findings highlight the importance of selecting appropriate encapsulating materials and processing conditions to optimise PKRE powder flow characteristics.

The carr's index (CI) of encapsulated *P. kurroa* root extract (PKRE) powder was significantly influenced by both encapsulating material and freeze-drying temperature. Gum arabic (GA) exhibited the highest CI, indicating greater compressibility and lower flowability, while calcium alginate (CA) had the lowest CI, suggesting better flow properties. A decrease in freeze-drying temperature resulted in a slight increase in CI, possibly due to changes in particle structure affecting powder cohesion (Ergin *et al*., 2022). These findings highlight the impact of encapsulating material and processing conditions on PKRE powder's flow characteristics, with CA being the most favourable for improved flow properties.

The total phenolic content (TPC) of encapsulated *P. kurroa* root extract (PKRE) powder was significantly affected by the extraction method, encapsulating material, and freeze-drying temperature. Calcium alginate (CA) demonstrated the highest TPC retention, suggesting its superior protective ability against phenolic degradation (Wongverawattanakul *et* al., 2022). The freeze-drying temperature also played a critical role, with -60°C yielding the highest TPC, followed by -80°C and -70°C. The highest TPC was recorded in CA-encapsulated PKRE at -80°C, highlighting its effectiveness in phenolic preservation (Buljeta *et al*., 2022). These findings suggest that calcium alginate, and optimized freeze-drying conditions, enhances phenolic compound stability in PKRE powder.

The antioxidant activity of encapsulated *P. kurroa* root extract (PKRE) powder is influenced by the extraction method, encapsulating material, and freeze-drying temperature. Encapsulation protects bioactive compounds from oxidation, with different materials varying in their ability to retain antioxidants. The choice of encapsulant affects the stability and efficiency of antioxidant preservation.

Freeze-drying is crucial for maintaining bioactivity, with lower temperatures minimizing thermal degradation and enhancing antioxidant retention (Kandasamy *et al.,* 2022). The interaction between encapsulating material and freeze-drying conditions further influences antioxidant stability (Ballesteros *et al*., 2017). Optimizing both encapsulation and drying parameters ensures better preservation of PKRE's functional properties. Identifying the most effective encapsulating material and freeze-drying conditions can enhance antioxidant stability and bioavailability, making PKRE powder suitable for functional food and nutraceutical applications. Further studies should explore its long-term stability and health benefits for commercial viability. The USAE-extracted PKRE encapsulation is highly scalable and feasible for industrial use. Ultrasound-assisted extraction reduces time, solvent use, and boosts yields, while encapsulation with materials like gum arabic ensures stability and bioavailability. The process integrates seamlessly into existing production lines, though energy optimization remains key. Ideal for nutraceuticals and functional foods, offering a cost-effective, high-efficiency solution for bioactive delivery (Esclapez *et al.,* 2011)

The study indicates that calcium alginate (CA) -encapsulated *P. kurroa* root extract (PKRE) exhibits significant *in vitro* antidiabetic potential by inhibiting key carbohydrate-digesting enzymes (Daub *et al*., 2025). By targeting both α-amylase and α-glucosidase, the extract interferes with the breakdown of complex carbohydrates into simple sugars, suggesting that it can help modulate postprandial blood glucose levels. The inhibition of α-amylase and α-glucosidase enzymes helps regulate postprandial glucose by delaying carbohydrate digestion. Competitive inhibitors (*e.g.,* acarbose) bind to the enzyme's active site, blocking substrate breakdown, while non-competitive inhibitors alter enzyme structure by binding elsewhere, reducing its efficiency. Mixed-type inhibitors exhibit both mechanisms. By slowing starch conversion into absorbable sugars, these inhibitors prevent rapid glucose spikes after meals. Since α-amylase breaks starch into oligosaccharides and α-glucosidase further digests them into glucose, their inhibition particularly at the intestinal brush border leads to gradual glucose absorption. This mechanism is key in diabetes management, though excessive inhibition may cause gastrointestinal side effects due to undigested carbs fermenting in the colon. Natural inhibitors (*e.g.,* polyphenols) often provide broader, milder effects compared to synthetic drugs (Li *et al.* 2022).

5. Conclusion

This study highlights the importance of ultrasonic-assisted extraction (USAE) in enhancing the extraction of bioactive compounds from the *P. kurroa* root (PKR). Among the solvents evaluated, methanol demonstrated the highest efficiency, resulting in the greatest total phenolic content (TPC) and antioxidant activity. Additionally, encapsulation and freeze-drying significantly influenced the physicochemical properties, stability, and functionality of PKR extracts. Calcium alginate (CA) emerged as the most effective encapsulating material for preserving bioactive compounds, particularly at lower freeze-drying temperatures, demonstrating superior antioxidant and antidiabetic properties. The main challenge and future research should be focused on pharmacokinetics, *in vivo* validation, and delivery strategies to improve the bioavailability of PKRE-based formulations.

The findings of this research hold considerable implications for functional food and nutraceutical applications, particularly in the development of antioxidant-rich and antidiabetic formulations. Investigating the bioavailability and metabolic pathways of PKRE extracts in human models would further strengthen their therapeutic potential. Additionally, expanding research into alternative green extraction methods, such as supercritical fluid extraction, enzyme assisted and sub critical water extraction could improve yield and sustainability. These advancements will contribute to the broader application of *P. kurroa* in food, pharmaceutical, and cosmeceutical industries.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

References

Aakash, P.; Siddharth, A. and Kirtesh R. (2014). A review on applications of maltodextrin in pharmaceutical industry. International Journal of Pharmacy and Biological Sciences. **4**(4):67-74

Ahmad, A.; Ullah, F., Sadiq, A. ; Ayaz, M.; Saeed Jan, M.; Shahid, M. and Mahmood, H. M. (2020). Comparative cholinesterase, α-glucosidase inhibitory, antioxidant, molecular docking, and kinetic studies on potent succinimide derivatives. Drug Design, Development and Therapy, pp:2165-2178.

Ahmad, S.; Faraz, M.; Farid, A. and Ghazanfar, S. (2023). Threatened and endangered medicinal and aromatic plants. Ethnobotany and ethnopharmacology of medicinal and aromatic plants: Steps Towards Drug Discovery, 1st edn. CRC Press, Boca Raton.

Anderson C, Griffin (1969). Gelatinization of corn grits by roll and extrusion cooking. Cereal Science Today. **14**:14-12

Ando, Y. and Nei, D. (2023). Comparison of potato void structures dried by air-drying, freeze-drying, and microwave-vacuum-drying, and the physical properties of powders after grinding. Food and Bioprocess Technology, **16**(2):447-458.

Balaxi, M.; Nikolakakis, I. and Malamataris, S. (2010). Preparation of porous microcrystalline cellulose pellets by freeze drying: Effects of wetting liquid and initial freezing conditions. Journal of pharmaceutical sciences, **99**(4):2104-2113.

Ballesteros, L. F.; Ramirez, M. J.; Orrego, C. E.; Teixeira, J. A. and Mussatto, S. I. (2017). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chemistry, **237**:623-631.

Bhusari, S. N.; Muzaffar, K. and Kumar, P. (2014). Effect of carrier agents on physical and microstructural properties of spray dried tamarind pulp powder. Powder Technology, **266**:354-364.

Cheema, H. S. and Singh, M. P. (2021). The use of medicinal plants in digestive system related disorders: A systematic review. J. Ayurvedic Herb. Med., **7**(3):182-187.

Cuadros, T. R.; Erices, A. A. and Aguilera, J. M. (2015). Porous matrix of calcium alginate/gelatin with enhanced properties as scaffold for cell culture. Journal of the Mechanical Behavior of Biomedical Materials, **46**:331-342.

Dadi, D. W., Emire, S. A.; Hagos, A. D. and Eun, J. B. (2019). Effect of ultrasound-assisted extraction of *Moringa stenopetala* leaves on bioactive compounds and their antioxidant activity. Food Technology and Biotechnology, **57**(1):77.

Daga, S.; Shrivastava, P. and Sharma, D. (2024). Krishna's diabic care: An evidence-based review of ingredients for diabetes control. International Journal of Ayurveda and Pharma Research, pp:106-117.

Daub, C. D.; Michaels, A. L.; Mabate, B.; Mkabayi, L.; Edkins, A. L. and Pletschke, B. I. (2025). Exploring the inhibitory potential of sodium alginate against digestive enzymes linked to obesity and type 2 diabetes. Molecules, **30**(5):1155.

Ergin, F. (2022). Effect of freeze drying, spray drying and electrospraying on the morphological, thermal, and structural properties of powders containing phage Felix O1 and activity of phage Felix O1 during storage. Powder Technology, **404**:117516.

Esclapez, M. D.; García-Pérez, J. V.; Mulet, A. and Cárcel, J. A. (2011). Ultrasound-assisted extraction of natural products. Food Engineering Reviews, **3**: 108-120.

Gullón, P.; Gullón, B.; Romaní, A.; Rocchetti, G. and Lorenzo, J. M. (2020). Smart advanced solvents for bioactive compounds recovery from agri-food by products: A review. Trends in Food Science and Technology, **101**: 182-197.

Jambamma, U.; Udaykumar, N.; Sharanagouda, H.; Mathad, P. F.; Srinivasakumar, K.; Swamy, M. and Saroja, R. (2024). Phytochemical screening and pharmacological benefits of *Parthenium hysterophorus* L.: *In vitro* anticancer cell line evaluation. Ann. Phytomed., **13**(2): 491-503. https://doi.org/10.54085/ap.2024.13.2.49

Jeyakumari, A.; Parvathy, U.; Zynudheen, A.; Murthy, L. N.; Visnuvinayagam, S. and Ravishankar, C. (2018). Microencapsulation of bioactive food ingredients: Methods, applications, and controlled release mechanism: A reviews, pp:711-731.

Kandasamy, S. and Naveen, R. (2022). A review on the encapsulation of bioactive components using spray drying and freeze drying techniques. Journal of Food Process Engineering, **45**(8):e14059.

Kumar, A.; Sathyakumar, S.; Goraya, G. S.; Gupta, A. K.; Adhikari, B. S. and Rawat, G. S. (2021). Sustainable harvesting and cultivation protocols of threatened medicinal and aromatic plants of the Western Himalaya. Wildlife Institute of India.

Meena, S.; Prasad, W.; Khamrui, K.; Mandal, S. and Bhat, S. (2021). Preparation of spray-dried curcumin microcapsules using a blend of whey protein with maltodextrin and gum arabica and its *in vitro* digestibility evaluation. Food Bioscience, **41**: 100990.

Misra, S.; Pandey, P.; Dalbhagat, C. G. and Mishra, H. N. (2022). Emerging technologies and coating materials for improved probiotication in food products: A review. Food and Bioprocess Technology, **15**(5):998-1039.

Mukwevho, P. L.; Kaseke, T. and Fawole, O. A. (2024). Journal of Agriculture and Food Research. Journal of Agriculture and Food Research, **18**:101301.

Nipanikar, S. U., Chitlange, S. S. and Nagore, D. (2017). Pharmacological evaluation of hepatoprotective activity of AHPL/AYTAB/0613 tablet in carbon tetrachloride, ethanol, and paracetamol-induced hepatotoxicity models in Wistar albino rats. Pharmacognosy Research, **9**(Suppl 1):S41.

Noreen, H.; Semmar, N.; Farman, M. and McCullagh, J. S. (2017). Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. Asian Pacific Journal of Tropical Medicine, **10**(8):792-801.

Nowak, D. and Jakubczyk, E. (2020). The freeze-drying of foods: The characteristic of the process course and the effect of its parameters on the physical properties of food materials. Foods, **9**(10):1488.

Oluwafunmilayo, O. O. (2019). Inhibitory effects of sorghum-cowpea composite biscuit on starch-hydrolysing enzymes (Master's Thesis, Kwara State University (Nigeria)).

Pandey, R. (2022). Molecular characterisation of picrosides biosynthetic machinery components in populations of a medicinal herb, *Picrorhiza kurroa* Royle ex Benth. (Doctoral Dissertation, Bennett University).

Parveen, S.; Amin, Q. A.; Jabeen, A.; Khan, I. M.; Wani, T. A. and Shah, R. (2025). Proximate analysis and storage stability of spice tikki (Vaer) influenced by oil using different packaging materials. Biological Forum, **14**(1):1-10.

Premi, M. and Sharma, H. K. (2017). Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (*Moringa oleifera*) oil. International Journal of Biological Macromolecules, **105**:1232-1240.

Quitério, E.; Grosso, C.; Ferraz, R.; Delerue-Matos, C. and Soares, C. (2022). A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds. Marine Drugs, **20**(11):677.

Rajhard, S.; Hladnik, L.; Vicente, F. A.; Srèiè, S.; Grilc, M. and Likozar, B. (2021). Solubility of luteolin and other polyphenolic compounds in water, nonpolar, polar aprotic and protic solvents by applying FTIR/ HPLC. Processes, **9**(11):1952.

Rezvankhah, A.; Emam-Djomeh, Z. and Askari, G. (2020). Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: A review. Drying Technology, **38**(1):235-258.

Rosland Abel, S. E.; Yusof, Y. A.; Chin, N. L.; Chang, L. S.; Mohd Ghazali, H. and Manaf, Y. N. (2020). Characterisation of physicochemical properties of gum arabic powder at various particle sizes. Food Res, **4**(1): 107-115.

Sahu, R. K.; Kar, M. and Routray, R. (2013). DPPH free radical scavenging activity of some leafy vegetables used by tribals of Odisha, India. Journal of Medicinal Plants Studies, **1**(4):21-27.

Shanmugam, S. (2015). Granulation techniques and technologies: recent progresses. BioImpacts: BI, **5**(1):55.

Sharma, P. and Raju, L. (2022). Ethnomedicinal insight among local tribes in Seraj valley, Himachal Pradesh, India.

Shi, L.; Zhao, W.; Yang, Z.; Subbiah, V. and Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research, **29**(54):81112-81129.

Tiwari, R.; Latheef, S. K.; Ahmed, I.; Iqbal, H. M.; Bule, M. H.; Dhama, K. and Farag, M. R. (2018). Herbal immunomodulators remedial panacea for designing and developing effective drugs and medicines: Current scenario and future prospects. Current Drug Metabolism, **19**(3):264-301.

Wang, X.;Xue, J.; Wang, Y.; Zhu, H.; Chen, S.; Xiao, Z. and Luo, Y. (2024). Development and characterization of zein/gum Arabic nanocomposites incorporated edible films for improving strawberry preservation. Advanced Composites and Hybrid Materials, **7**(6):1-19.

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