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Original Article

Effect of Solvent Polarity on the Nutritional and Phytochemical Composition of *Cocos nucifera* Bee Pollen

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Abstract

Background: Monofloral bee pollen is increasingly recognized as a nutritionally valuable natural product with promising applications in functional foods and herbal medicine. The present study evaluated the physicochemical, phytochemical, and nutritional characteristics of *Cocos nucifera* bee pollen extracted using solvents of varying polarity, namely acetone, ethanol, methanol, aqueous, and hydroethanolic solvents. **Methods:** *Cocos nucifera* bee pollen (CNBP) pellets were extracted by cold maceration, and the obtained extracts were subjected to physicochemical analysis, qualitative phytochemical screening, and quantitative nutritional evaluation using standard biochemical methods. **Results:** Extraction yield varied significantly with solvent polarity, with methanol extract exhibiting the highest yield (38%), indicating efficient recovery of polar bioactive constituents. Physicochemical parameters confirmed the stability and quality of the extracts. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, and proteins in different solvent extracts. Quantitative analysis demonstrated that CNBPis a rich source of carbohydrates, proteins, amino acids, dietary fibre, vitamins, lipids, cardiac glycosides, and essential minerals. Aqueous extracts exhibited higher protein (159.65 ± 0.41 mg BSA/g) and flavonoid contents (366.6 ± 0.57 mg QE/g), whereas methanolic extracts showed elevated phenols and alkaloids. Hydroethanolic extracts demonstrated higher amino acid concentrations, suggesting improved extraction efficiency for mixed-polarity biomolecules. **Conclusion:** The findings indicate that solvent polarity strongly influences the extraction efficiency and phytochemical composition of CNBP. Overall, the study highlights the nutraceutical potential and supports its prospective application in functional food and herbal formulations. Further pharmacological and clinical investigations are warranted to validate its therapeutic potential.

Keywords: *Cocos Nucifera Bee Pollen; Functional Foods; Phytochemicals; Nutritional Profiling*

Introduction

Apitherapy is a branch of complementary medicine that uses bee-derived products, such as honey, bee pollen, propolis, royal jelly, beeswax, and bee venom, for the prevention and management of various health conditions. Owing to their rich nutritional and bioactive composition, these products have gained increasing scientific attention for their medicinal and therapeutic properties. Bee pollen is a combination of plant pollen, honeybee gut secretions, and floral nectar (Guo *et al*., 2026). Honeybees collect pollen from flower buds and carry it attached to their hind legs for the nourishment of the beehive and the production of bee pollen. During pollen collection, honeybees moisten the pollen with their oral secretions, which helps organize the bee pollen and enables it to adhere to the corbiculae. Bee pollen is regarded as a valuable functional food because of its nutritional and therapeutic properties (Sanyal *et al*., 2023).

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Bee pollen exhibits several biological activities such as antibacterial, antiviral, immune-stimulating, antioxidant, anti-fungal, and hepatoprotective (Nandhini *et al*., 2026). Moreover, it has many beneficial healing traits in numerous pathological situations, including wound healing (Maurya *et al*., 2025). *Cocos Nucifera* Bee Pollen (CNBP) is one of the nutrient-rich mono-floral pollen, abundant in medicinal value with added benefits from bee enzymes (Embark, 2025). *C.nucifera*is rich in crude fats, carbohydrates, moisture, proteins, ash, dietary fiber, and trace elements of magnesium, calcium, potassium, sodium, phosphorus, and copper, along with zinc, manganese, and iron. The biological properties of *C. nucifera* include anti-helminthic, anti-inflammatory, antinociceptive, antioxidant, anti-fungal, antimicrobial, and anti-tumor activities. The main aspects include skin care, weight management, hydration, bowel health, and regulating blood pressure (Omimakinde, 2024).

Although the nutritional and therapeutic potential of bee pollen has been widely recognized, limited information is available regarding monofloral bee pollen, particularly the influence of solvent polarity on the extraction efficiency and phytochemical composition of CNBP. Therefore, this study was undertaken to evaluate the physicochemical characteristics comparatively, nutritional profile, and phytochemical constituents of CNBP extracted using solvents of different polarities to identify the most suitable extraction system for maximizing its nutraceutical value.

Materials and Method

Collection of Sample

CNBP pellets were collected from the DR Honeybee Farm, Ayakudi, Palani District, Tamil Nadu, India. The pellets were collected using the pollen trap at the entrance of the beehive. Collected pollen pellets were air-dried in the shade for 10 days at RT and subsequently processed for extraction.

Extraction of Bee pollen

Dried pollen pellets were extracted by employing cold maceration using various solvents, such as acetone, ethanol, methanol, aqueous, and hydroethanol. Hydroethanolic extraction was performed using an ethanol: distilled water solution (50:50 v/v). The pollen pellets were powdered using a laboratory blender, and 50g of bee pollen powder was soaked in the above-mentioned solvents of 250ml (1:5 w/v ratio). Extraction was performed at RT (25 ± 2°C) under dark conditions. The extraction process was repeated in three batches until the residue turned colorless. Later, the supernatant was filtered by double filtration using muslin cloth followed by Whatman filter paper (No. 1 of 148 mm diameter), and the concentrated filtrates were freeze-dried using a laboratory lyophilizer (Christ Alpha 1-2 LDplus Freeze Dryer (Germany)) at −50°C under 0.01 mbar vacuum pressure for 24 hrs (Nandhini *et al*., 2026).

Total Yield of the Extract

The total yield of the extract was calculated using the formula,

$$\% \text{ Yield of Extract} = \frac{Weig \quad of\ dried\ extract}{Weig \quad of\ initial\ biomass} X\ 100$$

Preliminary Physicochemical Screening

The CNBP extracts were screened for physicochemical parameters to screen the physical and chemical characteristics, such as nature, color, odor, pH, temperature, moisture, ash content, insoluble ash, melting point, and boiling point.

The nature and color of the extracts can be determined using ocular inspection. Organoleptics can help anticipate the odor of the extracts. The pH of the extracts was measured using a digital pH meter (Systronics Digital pH Meter Model 335). The temperature of the extracts was determined using a digital thermometer. The moisture of the sample was detected by drying the sample in an air forced draft oven, and the results were calculated using the obtained residue. The total ash content was determined by the incineration of the sample inside a porcelain crucible at 550°C for 6 hours using a muffle furnace (Thermolyne F62700 Muffle Furnace (USA)) until the formation of ash, and it was

weighed. Further, the insoluble ash was determined by ashing the sample with HCl, and the soluble components were dissolved, filtered and ignited, and the residue was weighed. The soluble ash was determined by boiling the total ash with distilled water, which was then filtered, and the dried residues were weighed. The melting and boiling points of the samples were determined using the capillary method and the Thiele tube method, respectively (Ahmed *et al*., 2023).

Preliminary Phytochemical Screening

The CNBP extracts were subjected to preliminary phytochemical screening for alkaloids, anthraquinones, carbohydrates, anthocyanins, cardiac glycosides, carotenoids, quinones, saponins, steroids, phenols, proteins, flavonoids, glycosides, tannins, and terpenoids (Bhattacharjee *et al*., 2013).

The Alkaloids were tested with Mayer's test and Kraut's test, whereas the anthocyanin was identified with the HCl test. Molisch's and Fehling's tests were conducted to determine the presence of carbohydrates. Whereas the carotenoids and anthraquinone were tested according to the chloroform and Borntrager tests, respectively. Three tests were conducted for flavonoids, such as the alkaline reagent test, concentration of H_2SO_4, and the lead acetate test. Glycosides and proteins were tested with aqueous NaOH and the xanthoproteic test, then the phenols were tested in two ways for the presence of $FeCl_3$ and the gelatin test. Further, conc. HCl test and alcoholic KOH test were conducted to determine the existence of quinones. The presence of tannin and saponin was put to the test, such as by using brayers and saponification accordingly. Libermann tests were conducted to determine the presence of two phytochemicals, such as sterols and terpenoids (Shaikh & Patil, 2020).

Quantification of Phytochemicals

The major phytochemicals of CNBP, including carbohydrates, proteins, amino acids, vitamins, alkaloids, flavonoids, phenols, saponins, tannins, steroids, fats, sugars, fiber, cardiac glycosides, and minerals, were quantified using standard biochemical methods to determine total nutritional composition.

Carbohydrates were estimated by the anthrone method, in which samples/standards were reacted with anthrone reagent, heated, cooled, and absorbance was measured at 630 nm (Bhattacharjee *et al*., 2013). Protein content was determined using the Lowry method following reaction with reagent C and Folin-Ciocalteu reagent, and absorbance was recorded at 630 nm (Vershinina *et al*., 2025). Total amino acids were quantified by the ninhydrin method, with absorbance measured at 570 nm after heating and dilution (Vershinina *et al*., 2025).

Ascorbic acid (Vitamin C) was estimated by the DNPH method and measured at 540 nm following osazone crystal formation (Bakır*et al*., 2025). Tocopherol (Vitamin E) was determined using the Emmerie-Engel reaction, with absorbance recorded at 460 and 520 nm (Bakır*et al*., 2025). Alkaloids were quantified gravimetrically using the ammonia precipitation method (Yaneva *et al*., 2025). Flavonoids were estimated by the $AlCl_3$ colorimetric method at 425 nm (Lenny *et al*., 2025), while total phenols were determined using the Folin-Ciocalteu method (Lenny *et al*., 2025).

Saponins were estimated by the acetic acid method and measured at 527 nm (Halimatushadyah*et al*., 2025). Tannins were quantified using the Folin-Denis method at 700 nm (Aborisade *et al*., 2017). Steroids were determined by the sulfuric acid method with absorbance measured at 540 nm (Bhattacharjee *et al*., 2013). Cardiac glycosides were estimated using Baljet's reagent and measured at 495 nm (Singh *et al*., 2022).

Fat content was determined by Soxhlet extraction using petroleum ether and expressed as a percentage of sample weight (Hait *et al*., 2024). Total sugars were estimated by the Lane-Eynon volumetric method following acid hydrolysis (Bhattacharjee *et al*., 2013). Crude fibre content was determined by acid–alkali digestion, followed by drying and ashing, and calculated from the weight difference (Hait *et al*., 2024).

Mineral content was determined using AOAC methods. Samples (1 g) were acid-digested using a mixed acid solution (HNO_3, $HCLO_4$ and H_2SO_4), heated until a clear digest formed, and diluted with distilled water. Mineral concentrations were analyzed by Atomic Absorption Spectrometry (AAS) using calibration curves from standard solutions and expressed as percentages. Phosphorus was estimated separately by the molybdate method. The mineral digest was reacted with TCA, molybdate reagent, and H_2SO_4, and the absorbance was measured at 660 nm using a spectrophotometer (Achi *et al*., 2017). Mineral analysis was performed using an Atomic Absorption Spectrophotometer (PerkinElmer Analyst 400, USA).

For all spectrophotometric assays, concentrations were calculated from standard curves and expressed as percentage or mg/g of sample. Absorbance measurements were recorded using a UV-visible spectrophotometer (Shimadzu UV-1800 (Japan)). The detailed methodology will be provided in the supplementary file on request. Calibration curves were prepared using standard compounds, including bovine serum albumin (BSA), quercetin, gallic acid, ascorbic acid, and α-tocopherol, ranging from 10 to 100 µg/ml depending on the assay type. All quantitative analyses were performed in triplicate (n = 3), and the mean values were used for statistical evaluation.

Determination of Total Energy Value of CNBP

The total energy value of CNBP was determined by the total multiplication of protein content and carbohydrate content by 4, individually and fat content by 9 (Bhattacharjee *et al*., 2013).

Energy value = [Crude protein × 4] + [Total carbohydrate × 4] + [Crude fat × 9]

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0. Data are expressed as mean ± standard deviation (SD) of three independent experiments (n = 3). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare means among solvent extracts. Differences were considered statistically significant at $p< 0.05$.

Results

Yield of Extracts

The total yield of the extraction depends on the polarity of the solvent used, as it dissolves compounds with similar polarity. Polar solvents (hydroethanol, ethanol, methanol, water) extract polar bioactive compounds, while nonpolar solvents (acetone) extract lipophilic ones. Thus, the option of solvent directly influences both the yield and the type of phytochemicals obtained. The highest extraction yield was obtained with methanol (38%), followed by hydroethanol, ethanol, aqueous, and acetone extracts (Figure01).

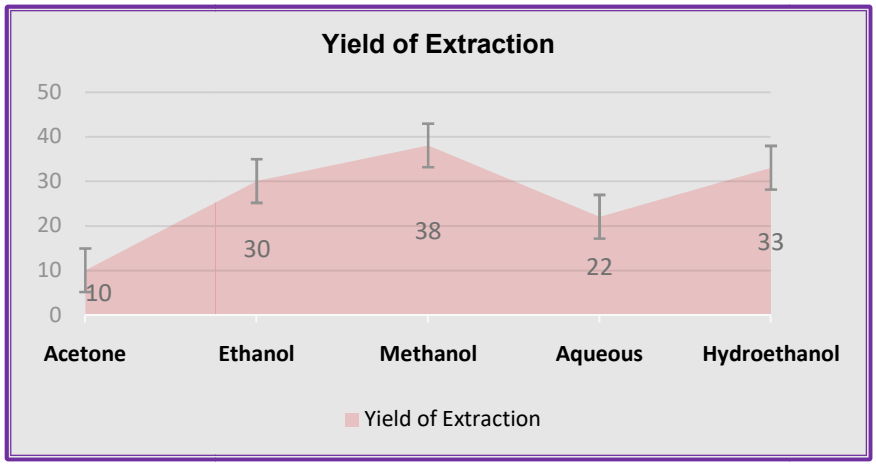

Figure 1: *Total Yield of the CNBP Extracts from Disparate Solvents*

Preliminary Physicochemical Screening

The physicochemical screening, such as nature, color, odor, pH, temperature, moisture, total ash, insoluble ash, soluble ash, melting point, and boiling point, is accessed and tabulated in Table 01. As it ensures the compound's identity, purity, stability, and safety, which are essential for it to act reliably as a drug, it also helps to confirm quality and to detect impurities and predict biological compatibility.

Table 1: *The Preliminary Physicochemical Screening of All Cnbp Extracts*

Sl. No	Physiochemical parameters	Acetone	Ethanol	Methanol	Aqueous	Hydro ethanol
1	Nature	Viscous & Sticky	Viscous & Sticky	Viscous & Sticky	Liquid	Viscous & Sticky
2	Color	Orange	Reddish Orange	Reddish Orange	Orange	Reddish Orange
3	Odor	Honey	Honey	Honey	Honey	Honey
4	pH	7.1	7.3	7.1	7	7.2
5	Temperature	15°C	15°C	15°C	23°C	15°C
6	Moisture	6.46%				
7	Total Ash	8.80%				
8	Insoluble ash	2.26%				
9	Soluble ash	0.45%				
10	Melting point	50°C - 80°C				
11	Boiling point	85°C - 100°C				

Qualitative Phytochemical Screening

The qualitative screening, like alkaloids, flavonoids, sterols, terpenoids, anthraquinones, anthocyanins, proteins, phenols, quinones, carbohydrates, tannins, saponins, cardiac glycosides, and carotenoids, was carried out to identify the beneficial bioactive compounds for the development of new drugs by changing color or texture. The phytochemical screening of the disparate extracts was processed, and further, the results were presented as shown in table 02 using the signs of positive and negative.

Table 2: *The Qualitative Phytochemical Screening of all CNBP Extracts*

Sl. No	Phyto chemicals	Name of Test	Acetone	Ethanol	Methanol	Aqueous	Hydro Ethanol
1	Alkaloid	Mayers	+	+	+	++	+
		Krauts	-	+	-	+	+
2	Flavonoids	Alkaline	+	-	-	+	+
		Conc. H_2SO_4	+	++	+	++	++
		Lead acetate	+	+	+	+	+
3	Sterols	Libermann	+	+	+	+	+
4	Terpenoids	Libermann	-	-	-	-	-
5	Anthroquinone	Borntrager	-	-	-	-	-
6	Anthocyanin	HCl test	-	-	-	-	-
7	Proteins	Ninhydrin	+	-	-	++	+
		Xanthoproteic	+	++	+	-	+
8	Phenols	$FeCl_3$	-	+	+	-	-
		Gelatin	+	+	+	-	+
9	Quinone	Conc. HCl	-	-	-	-	-
		Alcoholic KOH	-	-	-	-	-
10	Carbohydrates	Molish	+	+	+	+	+
		Fehling	+	+	+	+	++
11	Tannin	Braymers	-	+	+	-	-
		Gelatin	+	++	+	-	+
		NaOH	+	+	+	-	+
12	Saponin	Saponification	+	+	+	+	+
13	C.glycosides	Baljet reagent	-	-	-	+	+
		Br water	+	+	+	+	++
		Kellar Killani	+	++	+	++	+
14	Glycosides	NaOH	-	-	-	-	-
		Xanthoproteic	+	+	+	+	+
15	Carotenoids	Chloroform	-	+	-	+	+

(++ strongly present; + present; - absent)

Quantitative Phytochemical Screening

Quantitative phytochemical screening is very crucial as it predicts the precise value of specific classes of compounds like flavonoids, carbohydrates, proteins, amino acids, ascorbic acid, tocopherol, alkaloids, phenols, saponin, tannin, steroids, fat, sugar, fiber, cardiac glycosides, and minerals like calcium, iron, copper, manganese, zinc, sodium, potassium, cobalt, chromium, and magnesium, as shown in figures 02 and 03 and table 03. The table depicts that the concentration of certain phytochemicals, such as carbohydrates, ascorbic acid, and saponin, tends to be high in ethanolic extracts. In contrast, the proteins, flavonoids, and tannins are at higher rates in aqueous extracts. Tocopherol, alkaloids, phenols, and steroids tend to be more in methanolic extracts, and amino acids are in hydroethanolic extracts. % of all the functional phytochemicals of each extract were shown in figures 04, 05, 06 and 07.

Table 3: *The Quantitative Phytochemical Screening of the Various Solvents from CNBP Extracts*

Sl No	Phyto Chemicals	Aqueous	Ethanol	Methanol	Hydro ethanol	Units
1	**Carbohydrates**	115.68±0.2^{a}	122.88±0.2^{b}	98.08±0.2^{c}	119.68±0.7^{ab}	mg G equivalents/g
2	**Proteins**	159.65±0.41^{a}	103.65±0.41^{b}	63.65±0.3^{c}	140.87±.36^{d}	mg BSA equivalents/g
3	**Amino acids**	33.9±0.09^{a}	22.09±0.09^{b}	16.27±0.09^{c}	35.07±0.07^{d}	mg L equivalents/g
4	**Ascorbic acid**	123.7±0.53^{a}	140.48±0.17^{b}	101.92±0.09^{c}	130.65±0.2^{d}	mg AA equivalents/g
5	**Tocopherol**	151.5±0.05^{a}	149.07±0.15^{b}	157.08±0.46^{c}	152.6±0.23^{d}	mg T equivalents/g
6	**Alkaloids**	28.5±0.26^{a}	36.5±0.26^{b}	47.5±0.26^{c}	30.07±0.87^{d}	mg /g
7	**Flavonoids**	366.6±0.57^{a}	350.0±0.51^{b}	186.1±0.76^{c}	340.1±1.07^{d}	mg QE equivalents/g
8	**Phenols**	120.5±1.32^{a}	145.3±0.28^{b}	158.8±1.21^{c}	135.96±0.8^{d}	mg GAE equivalents/g
9	**Saponin**	125.66±0.28^{a}	226.6±0.76^{b}	96.5±0.5^{c}	206.9±0.57^{d}	mg OA equivalents/g
10	**Tannin**	86.66±0.88^{a}	70.25±0.88^{b}	37.43±0.88^{c}	83.67±0.05^{d}	mg TA equivalents/g
11	**Steroids**	30.96±0.07^{a}	40.15±0.15^{b}	43.98±0.15^{c}	35.92±0.84^{d}	mg SG equivalents/g
12	**Fat**	35.2±0.5				mg/g
13	**Sugars**	380.5±0.52				mg/g
14	**Fibers**	216.06±0.31				mg/g
15	**Cardiac glycosides**	100.48±0.1^{a}	82.06±0.2^{b}	115.64±0.2^{c}	95.06±0.75^{d}	mg S equivalents/g
16	**Minerals**					
a	**Calcium**	0.97				mg/g
b	**Iron**	0.04				mg/g
c	**Copper**	0.005				mg/g
d	**Manganese**	0.07				mg/g
e	**Zinc**	0.04				mg/g
f	**Sodium**	0.02				mg/g
g	**Potassium**	0.04				mg/g
h	**Cobalt**	0.0004				mg/g
i	**Chromium**	0.01				mg/g
j	**Magnesium**	0.07				mg/g

Values are expressed as Mean ± SD of three independent experiments (n = 3). Different superscript letters within the same row indicate statistically significant differences at $p< 0.05$.

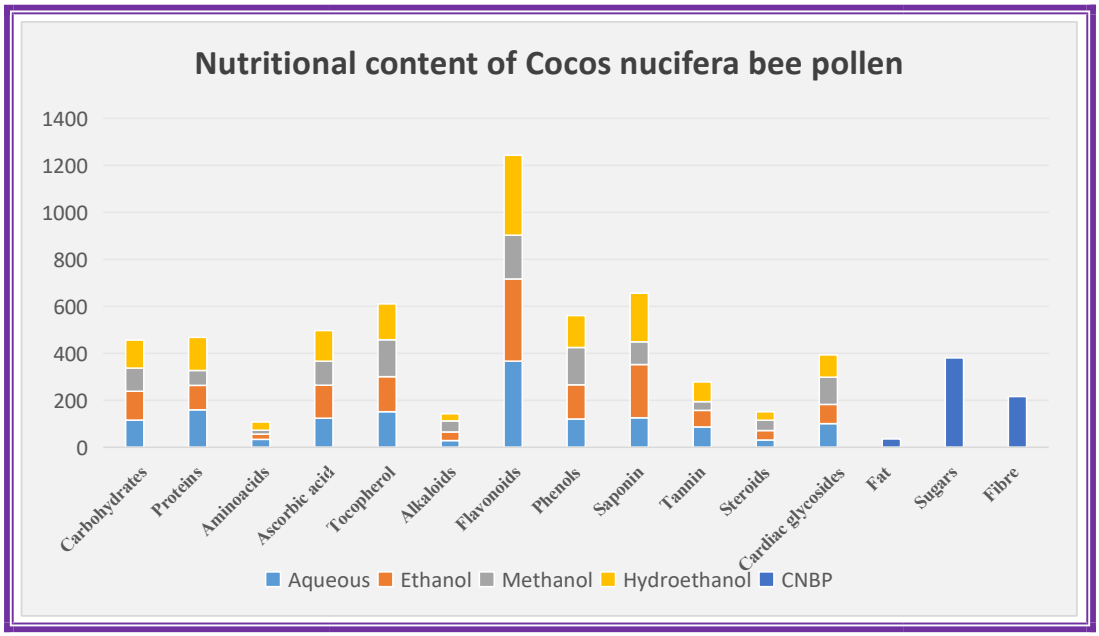

Figure 2: *Quantitative Phytochemical Composition of CNBP Extracts Obtained Using Solvents of Varying Polarity*

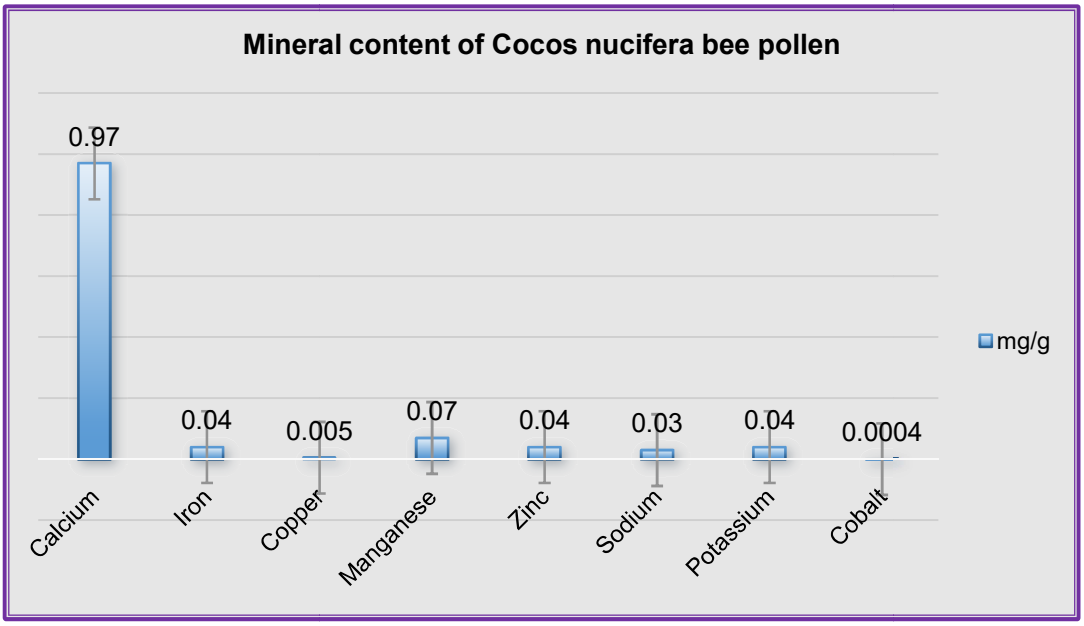

Figure 3: *The Quantitative Phytochemical Screening of Mineral Content in CNBP Extracts*

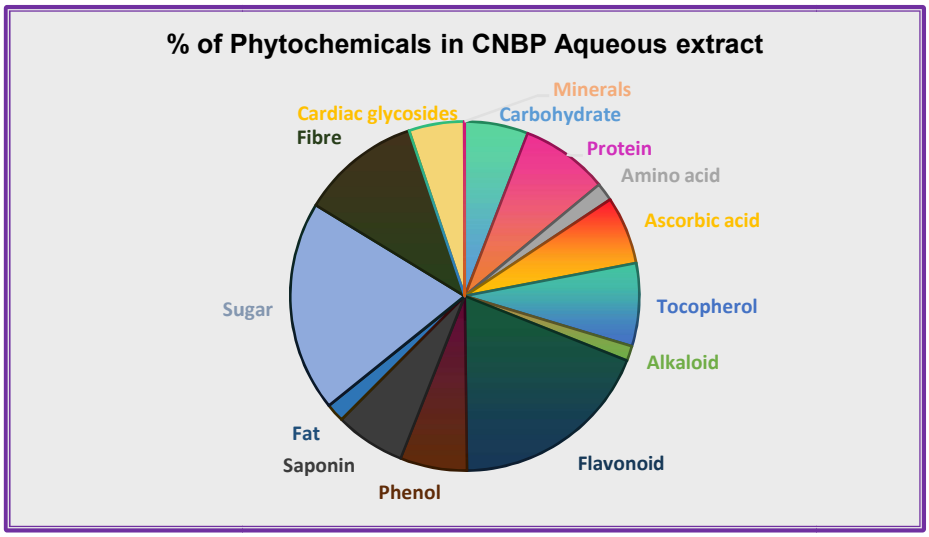

Figure 4: *Percentage (%) of All Functional Groups in the Aqueous Extract of CNBP*

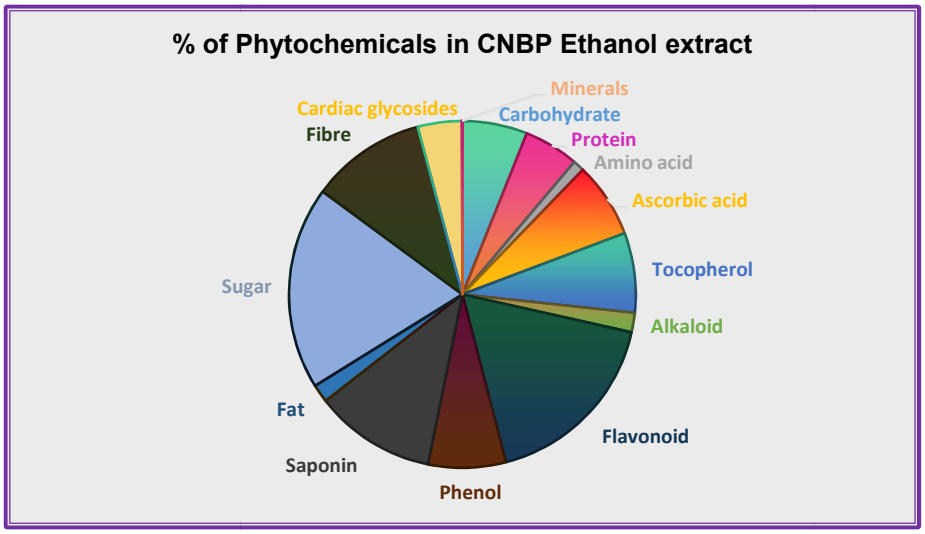

Figure 5: *Percentage (%) of All Functional Groups in the Ethanol Extract of CNBP*

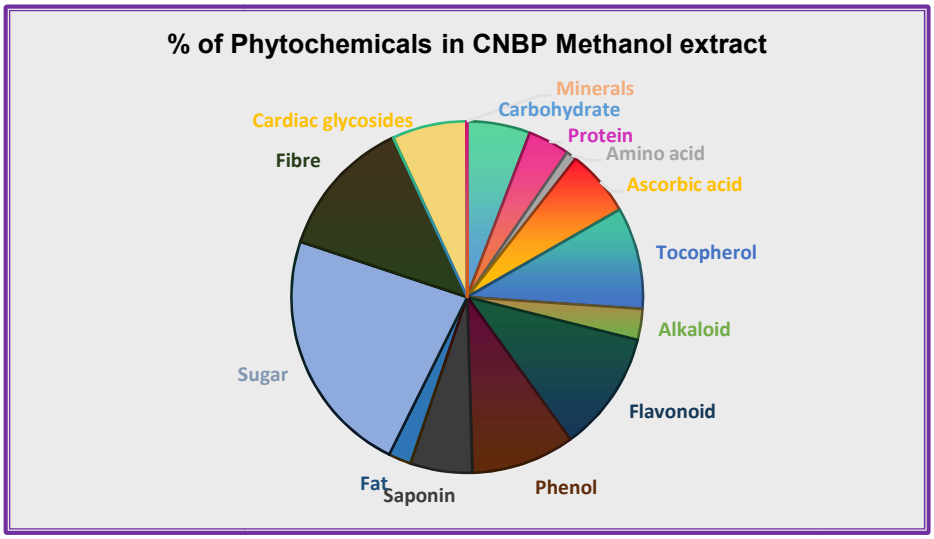

Figure 6: *Percentage (%) of All Functional Groups in the Methanol Extract of CNBP*

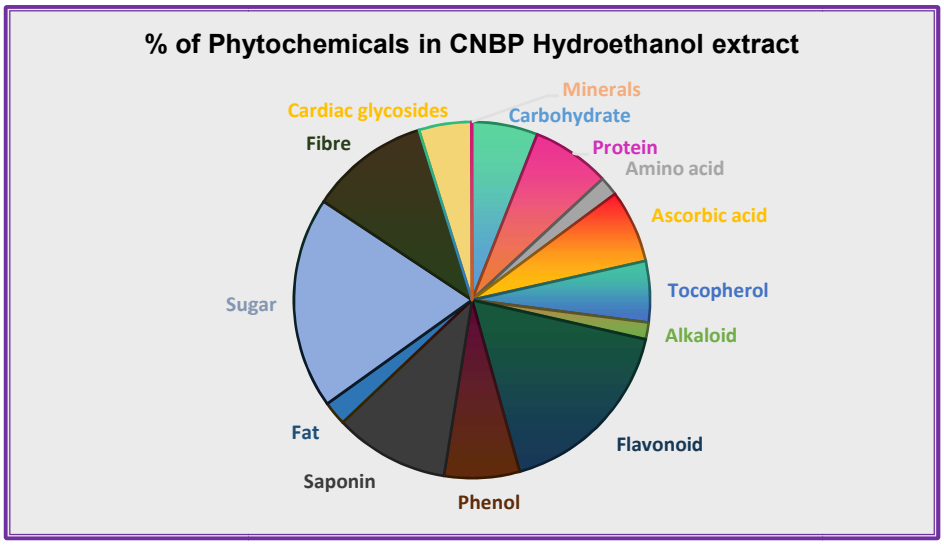

Figure 7: *Percentage (%) of All Functional Groups in the Hydroethanol Extract of CNBP*

Total Energy Value of CNBP

The aqueous extracts of CNBP showed the highest total energy content, followed by the methanol extract, which had the lowest value. This suggests that aqueous and hydroethanol solvents are more effective in extracting energy-rich components, as shown in figure 08.

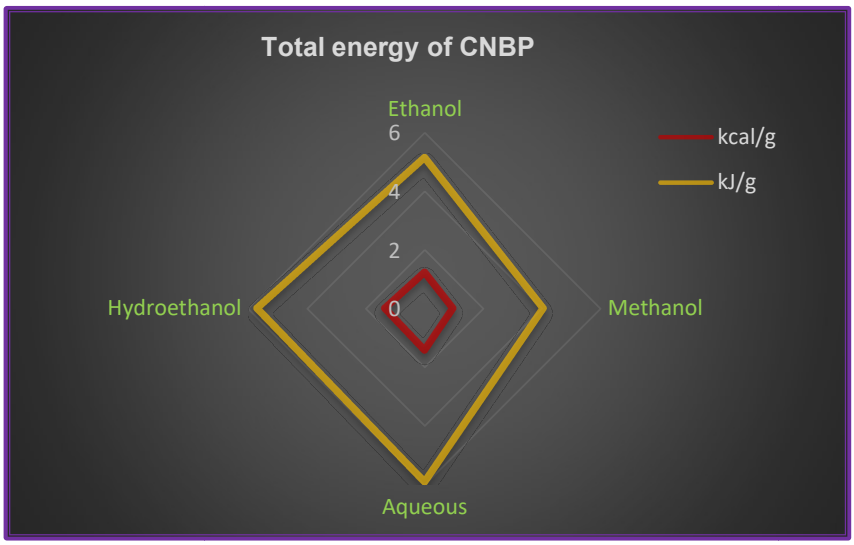

Figure 8: *The Total Energy Content of All CNBP Extracts*

Discussion

Bee pollen is a nutrient-rich apicultural product recognized for its nutritional and bioactive composition with enormous activities like antioxidant, antifungal, antibacterial, anti-inflammatory, anti-hypercholesterolemic, anti-diabetic, and anti-obesity mainly due to the presence of compounds like an alkane, alkene, esters, alcohols, and amine groups and was confirmed by testing the pollen by extraction followed by the phytochemical and physicochemical screening (El-Seedi *et al*., 2022). The bee pollen contains a broad spectrum of macro and micronutrients like proteins, carbohydrates, lipids, vitamins, minerals, along with bioactive phenolics or flavonoids and other secondary metabolites, which collectively underlie the reported antioxidant, anti-inflammatory, and antimicrobial properties as early as reported by Bobis *et al*. (2025).

The pellets of bee pollen from *C. nucifera* were collected from the hive of Italian bees by placing the pollen trap at the entrance of the beehive (Boni *et al*., 2025). Collected pellets were initially shade-

dried, ground, and extracted by the process of cold maceration (Nandhini *et al*., 2026) for the acquisition of thermolabile compounds essential for the therapeutic properties (Kalaskar *et al*., 2025). When compared to other traditional methods of extraction, the yield from cold maceration is comparatively low. According to Nawaz *et al*. (2020), the total yield of the extract depends on the polarity of the solvents, while the highly polar solvent yields a huge extract compared to that of the low-polarity solvents.

The physicochemical nature of the extracts was studied. The extracts obtained with ethanol, methanol, and acetone remained viscous and sticky after condensation and semi-viscous for hydroethanol and aqueous extracts. The color of the extracts was orangish-red for the maximum number of extracts due to the presence of flavonoids and carotenoids in the bee pollen. The odor of all the extracts smelled the same as that of the honey, as it is naturally coated with the floral nectars and enzymes, and the pH was generally neutral (Verellen *et al*., 2025). The temperature of the aqueous extract was the same as that of RT, with a minimum range of 23℃, and all other extracts exhibit a low of about 15℃ due to cold extraction (Dagher *et al*., 2025). The moisture content, total ash, soluble ash, and insoluble ash of the pollen were 6.46%, 8.80%, 0.45% and 2.26%, respectively. The melting and boiling points of the pollen were between 50℃ and 80℃ and 85℃ to 100℃, respectively.

Coconut bee pollen likely contains high levels of alkaloids, flavonoids, proteins, tannins, and cardiac glycosides because these compounds are naturally concentrated in pollen grains and serve protective, metabolic, and antioxidant functions in plants. The minimal or absent presence of compounds such as terpenoids, anthraquinones, quinones, sterols, and certain glycosides may be due to the specific botanical origin of coconut pollen, environmental factors, and the selective accumulation of phytochemicals during pollen formation.

The extracts were subjected to quantitative phytochemical analysis, and the concentrations of major constituents were determined. Carbohydrates are the primary source of energy, especially for the brain, nervous system, and muscles (Fioramonti & Pénicaud, 2019). Carbohydrates produce glucose, which acts as the primary source of fuel and helps in digestion (Sandhu *et al*., 2025) and regulates blood cholesterol levels. Carbohydrates help in producing some kinds of vitamins and minerals, which are rich in antioxidants and other beneficial nutrients (Ibrahim *et al*., 2025). The total carbohydrates present were higher in the ethanolic extract, with 122.88±0.2 mg G/g, followed by hydroethanol and aqueous extracts, with 119.68±0.7 mg G/g and 115.68±0.2 mg G/g, respectively. This suggests that mixed solvent systems may more efficiently solubilize carbohydrate components, enhancing extract yield.

Proteins are the most important biomolecule of amino acids that the body requires to function (Deutz *et al*., 2025). Proteins are needed daily to produce hormones, enzymes, and energy (Wang *et al*., 2025). It is also needed for the growth of cells and tissue and provides support for bone and muscle restoration (Smith *et al*., 2025). The high level of proteins was found to be 159.65±0.41 mg BSA/g, which was followed by hydroethanol, ethanol, and methanol at 140.87±0.36 mg BSA/g, 103.65±0.41 mg BSA/g, and 63.65±0.3 mg BSA/g, respectively. These results indicate that water is more effective for extracting proteinaceous compounds, as hydrophilic proteins are likely to remain insoluble in organic solvents. Amino acids are the organic compounds acting as the building blocks of proteins (Blachier, 2025) and other products like hormones and neurotransmitters (Mensah *et al*., 2025). The number of amino acids varies from 33.9±0.09 mg/L/g in aqueous extracts, followed by ethanol, methanol, and hydroethanol extracts. The hydroethanolic extract shows the highest amino acid content (35.1 mg L/g), significantly surpassing the others.

Essential vitamins such as ascorbic acid (vitamin C) and tocopherol (vitamin E) are the most power-packed antioxidants, which play a vital role in maintaining the overall system and aid in the protection against oxidative damage (Singh *et al*., 2025). Vitamin C is present in the range of 100-140 mg AA/g, and Vitamin E is present in the range of 140-160 mg T/g of pollen extracts. This points to the improved retrieval property of vitamin C using solvent combinations, and the lipid-soluble nature of tocopherols may explain the stability of yields across solvents. Beyond nutritional value, bee pollen

extracts have demonstrated significant antioxidant, antimicrobial, and anti-inflammatory effects as represented by Cavallero *et al*. (2025). Recent pharmacoinformatic studies suggest potential lipid-modulating and enzyme-inhibitory effects (Sun *et al*., 2025), which support the rationale for evaluating *C. nucifera,* which demonstrates potential relevance in the management of metabolic syndrome associated with hyperlipidemia, paralleling the bioactive and metabolic regulatory effects reported for its sister apiary by-product, propolis, as described by Colzani *et al*. (2025).

Secondary metabolites like alkaloids and flavonoids have a wide range of therapeutic potential and biological aspects (Javed *et al*., 2025). Alkaloids are nitrogen compounds with physiological effects, while flavonoids are polyphenols that improve health and have antioxidant effects. The total alkaloids present were 28.5±0.26, 36.5±0.26, 47.5±0.26, and 30.07±0.87 mg/g for aqueous, ethanol, methanol, and hydroethanol extracts, respectively. These rise from aqueous to ethanol extracts, reaching 47.5 mg/g in ethanol. This trend is consistent with alkaloids having appreciable solubility in less polar media. The total flavonoid content was approximately 350 mg QE/g for aqueous, ethanol, and hydroethanol extracts, while methanol extracts showed a moderate level of 186.1±0.76 mg QE/g. Aqueous extracts have the highest amount of flavonoids, with 366.6 mg QE/g. This suggests that most of the flavonoids in CNBPare hydrophilic. In bee pollen from disparate floral sources, typical flavonoid content ranges between 200 and 300 mg QE/g. Here, the aqueous extract of CNBP recorded 366.6 mg QE/g, surpassing those averages and reflecting high antioxidant potential. Leja *et al*. (2007) values correlate with the flavonoid values (350–366 mg QE/g in aqueous/hydroethanol extracts), which appear higher than many previously reported ranges, indicating a particularly high antioxidant potential of our *C. nucifera* pollen sample.

The other secondary compounds required for the body's needs were phenols, saponins, tannin, and steroids, with an average of 140.14 mg GAE/g, 163.91 mg OA/g, 69.5 mg TA/g, and 37.75 mg SG/g, respectively. The phytocompounds like phenols, saponins, and tannins are meant for their anti-inflammatory properties, as implied by Yatoo *et al*. (2018). As per Patra's statement, the antioxidants were found to be in phenols and tannins; further, the steroids are required for the anti-microbial activities, like those of saponins (Patra, 2012). Phenolic compounds are most abundant in ethanol extracts, indicating better solubility in moderately polar solvents (Galanakis *et al*. 2013). Published ranges for total phenolics in bee pollen are often within 100-400 mg GAE/g. The hydroethanolic extract here (158.8 mg GAE/g) falls within the lower half of that spectrum, suggesting moderate phenolic extraction efficiency. In the case of saponins, the highest concentration occurs in aqueous extracts, which aligns with expectations for these amphiphilic glycosides. Aqueous extracts yield a high tannin content, while ethanol yields the lowest, reinforcing tannin’s affinity for water-based solvents, but this contrasts with the outcome of total tannins in Oak bark (Choi & Lee, 2025). In case of steroids, the highest levels are noted in ethanol, consistent with their hydrophobic character and preference for less polar solvents.

The estimation of the most major properties, such as fat, sugars and fiber, was tested for a direct raw sample of CNBP; thus, the extracted values depict that it contains 35.2±0.5 mg/g, 380.5±0.52 mg/g, and 216.06±0.31 mg/g, respectively. The value of sugar indicates that CNBP is rich in both digestible carbohydrates and dietary fiber, contributing to its energy and functional benefits (Aylanc*et al*., 2023; Bertoncelj *et al*., 2024). Also, the fat content in bee pollen can exceed 50 mg/g. Still, the reported 35.2 mg/g suggests relatively lower lipid content, which could affect energy density and lipid-soluble nutrient supply (Lau *et al*., 2022). The most important phytochemical for various heart-related conditions is cardiac glycosides (Omole *et al*., 2025). They are among the various branches of drugs that can be used for the treatment of heart ailments and common causes of poisoning (Yurdakok-Dikmen & Filazi, 2025). Cardiac glycoside content ranged from 100.48±0.1 mg S/g in aqueous, 82.06±0.2 mg S/g in ethanol, and 115.64±0.2 mg S/g in methanol to 95.06±0.75 mg S/g in hydroethanol, which might appeal to bioactivity profiling.

Minerals are the most vital nutrients that help with bodily activities such as building and maintaining bones and teeth, regulating fluid balance, and assisting in enzymatic processes (Alghamdi *et al*., 2022). They also help in regulating the function of nerves, muscles, and energy metabolism. Minerals

also play a minor role in guiding the immune response of our body (Stefanache *et al*., 2023). Calcium is required for the formation of bone and teeth, as stated by Dos Santos *et al*. (2025), and it was the highest one to be present in CNBP, with 0.97 mg/g. Iron is the most important mineral required for the facilitation of oxygen transport, with 0.04 mg/g (Abner, 2025). Copper is beneficial for energy production, iron metabolism, and connective tissue formation, with a concentration of 0.005 mg/g in pollen extracts (Fu *et al*., 2025). Compounds like manganese and magnesium help in the regulation of various physiological properties, such as enzyme function, bone health, metabolism of fat and carbohydrates, regulation of blood pressure, and nerve function, to the extent of 0.07 mg/g and 0.07 mg/g, respectively (Razzaque & Wimalawansa, 2025; Wu *et al*., 2025).

Zinc is one of the vital micronutrients that help in numerous functions, including the immune system, growth, and development, especially during pregnancy, infancy, and childhood, with 0.04mg/g of bee pollen extracts (Borpatra& Saikia, 2025; Majumdar *et al*., 2025). The electrolyte minerals, such as sodium and potassium, are required for fluid balance, normal blood pressure, and heart problems (Mohamed *et al*., 2025). The estimates of sodium and potassium levels were 0.02mg/g and 0.04mg/g, respectively, in the pellets of CNBP. Finally, trace minerals like cobalt and chromium are required for the process of vitamin B12 synthesis, RBC production, DNA synthesis, glucose metabolism, and insulin production (Balabanova *et al*., 2021), respectively, with the range of 0.0004mg/g and 0.01mg/g. Standard bee pollen calcium content is often higher (around 1 to 2 mg/g) compared to the 0.97 mg/g noted here, while iron levels (0.04 mg/g) lie within the lower expected range. Overall, mineral composition trends appear broadly consistent but slightly underrepresented in this sample.

Therefore, aqueous extracts yield the highest protein, flavonoid, saponin, and tannin levels, signaling strong solubility of hydrophilic compounds in water. Ethanol extracts excel at recovering alkaloids, phenols, and steroids, reflecting the affinity of these molecules for moderately polar solvents. Hydroethanolic extraction achieves top yields for amino acid and ascorbic acid, highlighting its effectiveness at pulling both hydrophilic and slightly less polar biomolecules into solution. Flavonoid content notably exceeds typical ranges seen in other bee pollen studies, suggesting superior antioxidant qualities for CNBP. Phenolic, protein, and lipid values are on the lower-to-mid end of published ranges; these results may indicate floral-source-specific composition or extraction limitations. Mineral concentrations, particularly calcium and iron, are on par with or slightly below known benchmarks, reinforcing the importance of multi-source pollen intake for comprehensive micronutrient coverage (Sun *et al*., 2025).

The relatively higher total energy values in aqueous and hydroethanol fractions suggest the presence of carbohydrate-rich metabolites, consistent with the nutritional role of bee pollen as an energy-dense supplement. Similar reports have highlighted the caloric contribution of bee pollen as a functional food due to its high sugar and protein content. The observed values further align with earlier findings from the work of Denisow & Denisow-Pietrzyk (2016), where solvent choice significantly influences bioactive and nutritional yield from pollen extracts, indicating that *C. nucifera* pollen could serve as a potential natural energy source. Such variety and solvent-dependent variability have been widely reported, as suggested by Feás *et al*. (2020), which explains the differences observed when compared with other bee-pollen studies.

CNBP demonstrates a pronounced abundance of bioactive constituents, particularly flavonoids, amino acids, and vitamin C, with hydroethanolic and aqueous extraction methods yielding especially favorable outcomes. The compositional profile underscores its considerable antioxidant potential, although comparatively reduced levels of protein, lipids, and minerals were observed relative to certain other pollen types. These variations may be attributable to species-specific phytochemical characteristics or differential extraction efficiencies. However, as noted in comprehensive reviews by Feás *et al*. (2020), pollen composition and bioactivity are strongly influenced by floral origin, harvesting conditions, and extraction protocols, underlining the need for standardization in future studies.

Limitations

The present study has certain limitations. The investigation was primarily limited to the physicochemical, nutritional, and phytochemical profiling of CNBP extracts obtained with different solvents and did not include detailed characterization of individual bioactive compounds or biological activity assays.

Future Scope

Future studies should focus on advanced metabolomic characterization using chromatographic and spectrometric techniques, evaluation of antioxidant and pharmacological activities through in vitro and in vivo models, and assessment of seasonal and geographical variations. Such investigations would provide a more comprehensive understanding of the nutraceutical and therapeutic potential of CNBP and support its development as a functional food ingredient and natural health product.

Conclusion

This study was undertaken to evaluate the influence of solvent polarity on the extraction efficiency, nutritional composition, and phytochemical profile of CNBP, a promising yet underexplored natural nutraceutical. The findings demonstrated that solvent polarity significantly influences extraction efficiency and the recovery of nutritional and phytochemical constituents from CNBP, with methanol yielding the highest extraction efficiency, aqueous extracts exhibiting superior protein and flavonoid contents, and hydroethanolic extracts showing enhanced amino acid recovery. These results highlight the rich nutritional and phytochemical potential of CNBP and reinforce its value as a functional food ingredient and a potential source of health-promoting bioactive compounds.

The solvent-dependent extraction approach employed in this study may be broadly applied for the standardization and optimization of bioactive compound recovery from bee pollen and other natural products intended for nutraceutical, pharmaceutical, and food applications. Furthermore, the present work provides a foundational dataset for future investigations aimed at advanced chemical characterization, bioactivity validation, quality standardization, and clinical evaluation of CNBP. Such studies will contribute to the development of evidence-based functional foods and natural therapeutic formulations derived from this valuable apicultural product.

Conflict of Interest

The authors declare no conflicts of interest in this work.

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