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# Phytochemical Profiling and Cardiometabolic Potential of *Cocos nucifera* Bee Pollen: Antioxidant Insights from FTIR, LC-MS and GC-MS Analyses

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**Abstract**

Cardiometabolic disorders driven by dyslipidemia, oxidative stress and chronic inflammation, remain one of the major global health burdens demanding a safe, natural and multifunctional therapeutic alternatives. Bee pollen is widely recognized for its nutritional richness and bioactive potential. However, the metabolomic profile and cardioprotective relevance of monofloral *Cocos nucifera* bee pollen remain largely unexplored. The present study provides an integrated phytochemical and spectroscopic characterization of the cold-macerated ethanolic extract of *C. nucifera* bee pollen, with emphasis on its antioxidant and lipid-modulating potential. Preliminary phytochemical screening revealed abundant alkaloids, phenolics, flavonoids, sterols, tannins, carbohydrates and glycosides. The extract demonstrated strong antioxidant potency with an 86.7% DPPH radical inhibition, indicating effective free-radical scavenging activity. UV-Vis absorption profiles confirmed the presence of polyphenolic constituents, while FT-IR analysis revealed characteristic functional groups corresponding to hydrox ortality rate is due to coronary affliction with conditions such as inflammation, insulin resistance, oxidative stress, hypertension and dyslipidemia Hypercholesterolemia or dyslipidemia is admitted by immoderate levels of cholesterol in the blood by limiting its flow which results in coronary diseases and stroke. It is a condition of lipid metabolic disorder with a cordial relationship associated with CMD (1).

Apitherapy was given thought to the most productive treatment with bees and their products. It has ancient origins: the first known prescription using honey, which aids various physical and emotional health issues. Honey bee products are considered one of the finest and oldest medications gifted by honey bees to the universe. Bee pollen is the most nourishing and balanced nutrient produced by the worker bee of the honey bee community, which has been historically used as an apitherapeutic (2). Bee pollen is an agglomerate honey bee derivative of floral pollen collected by worker bees mixed with the secretions of hypopharyngeal glands or nectar. Bee pollen loads are collected and stored by the worker's bees (Apis spp.). It is used as food and also for the construction of the hive, which was collected at the hive's entrance (3).

Bee pollen is used as a diet supplement due to its therapeutic action for human diseases. Some researchers also consider it mother nature's perfect food because it contains nearly all the potential sources of vital nutrients such as proteins, lipids, vitamins, minerals and carbohydrates, trace elements, and a considerable number of polyphenols, mainly flavonoids (4).

Pollen can also be distinguished as a supplementary source with a diversified complement in human health due to its enriched possessions (5). Bee pollen has highly balanced nutrients with biological activities such as anti-allergic, anti-inflammatory, immunomodulatory, appetite stimulation, anti-bacterial, anti-fungal, anti-viral, anti-arthritic, anti-mutagenic, colitis, memory, skin care, weight loss, benign prostatic hypertrophy and also alleviate premenstrual symptoms (1,5,6).

In such a way bee pollen collected from *Cocos nucifera* contains a rich source of protein, vitamins and nearly all the nutrients for optimal health. Also, *Cocos nucifera* possesses anthelmintic, antidotal, antiseptic, aperient, aphrodisiac, astringent, bactericidal, depurative, diuretic, hemostat, and pediculicide (7). The diverse uses of this species such as vermifuge properties, stomachic, styptic, purgative, suppurative, and refrigerant (8) are also made valuable. Moreover, it is used as a remedy for Alzheimer's disease, asthma, bronchitis, bruises, burns, calculus, colds, constipation, cough, dysentery, fever, flu, gonorrhea, jaundice, nausea, obesity, phthisis, pregnancy, rash, scabies, sore throat, stomachache, swelling, syphilis, toothache, tuberculosis, tumors, typhoid and wounds (9,10,11).

Although bee pollen from *Cocos nucifera* has vital pharmacological effects, its limited availability drops its nutritional and clinical application in the medical field. So, it is essential to prove and focus more on the beneficial aspects of bee pollen in the medical field and to give more importance to such products for the welfare of healthy beings. My present study is one such attempt for it.

## Materials and Methods

### *Collection of sample*

The sample of bee pollen loads from *Cocos nucifera* was collected directly from the bee hive of *Apis mellifera sp.* using the sterilized pollen trap from DR Honey bee farm, Aayakudi, Palani district, Tamil Nadu. Collected samples were authenticated and identified as *Cocos nucifera* from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

### *Preparation of extract*

Bee pollen loads were extracted by the process of cold maceration technique to analyze the phytochemical constituents present in them. Collected pellets were shade-dried inside the dark room for around 15 days and powdered for the process of cold maceration. The samples were immersed in ethanol for about 72 hrs inside the dark room. Eventually, the extract was double-filtered using the clean muslin cloth followed by the Whatman filter paper 110 mm. The filtrate was then condensed by open air for 24 hrs and the extracted sample was stored at 4°C for future use (12,13). The prepared *Cocos nucifera* Bee Pollen Cold-macerated Ethanolic (CNBPCE) extract was further analyzed for preliminary phytochemical screening.

### *Preliminary phytochemical screening*

Various preliminary tests were performed in CNBPCE extract to know the presence of the phytochemicals present in them. The compounds such as alkaloids, flavonoids, sterols, terpenoids, anthraquinone, anthocyanin, proteins, phenolic compounds, quinones, carbohydrates, tannin, saponin, cardiac glucosides, glycosides, lignins, coumarins and volatile oils were ascertained (14).

### *DPPH anti-oxidant assay*

The DPPH spectrophotometric method is one of the most widely used analyses to evaluate the antioxidant activity of drugs to measure the ability of free radical scavengers. CNBPCE extract was prepared into different concentrations and processed with DPPH solution and the absorbance was measured at 517 nm. The standard used was Ascorbic acid and these measurements were repeated and recorded.

The Percentage of Inhibition ($P_i$) was calculated using the formula,

$$Pi = \frac{(Ab\text{-}As)}{Ab} \times 100\%$$

Whereas '*Pi'* is the percentage of inhibition, '*Ab'* is the abs of control and '*As'* is the abs of the sample.

***UV- VIS spectroscopic analysis***

CNBPCE extract was subjected to UV-VIS Spectroscopic analysis to determine the compounds available. The extract was measured using a UV-VIS spectrophotometer of Perkin Elmer, USA Model: Lambda 950 using a 10mm cell, with a slit width of 2nm at room temperature. The extract was centrifuged for 10 min at 3000 rpm filtered via Whatman No. 1 filter paper and diluted to ethanol at the ratio of 1:10. The extract was examined under both visible and UV light from wavelength ranging 300-800nm for imminent examination.

***FT-IR***

CNBPCE extract was loaded to FT-IR to elucidate the functional group present. 1mg of the pollen extract was encapsulated in 10mg of KBr pellet, for devising the translucent sample discs. KBr encapsulated pollen extract was injected and measured using Shimadzu 8400S, with a resolution of $4cm^{-1}$. The spectra were recorded between the region of $4000\text{-}400cm^{-1}$ in the transmittance mode at room temperature (25 ± 2 °C).

***LC-MS***

The classification of non-volatile compounds in the CNBPCE extracts can be identified using LC-MS analysis. The sample was performed using Shimadzu CBM-20A using a PDA detector with a total run time of 30 minutes. The pump used here was binary mode with a flow rate of 0.3 ml/min and a B concentration of 10% with 0.1% formic acid and acetonitrile. The sample was injected in 51L 30 AC of autosampler system with a total injection volume of 5µl and wavelength ranges between 190 to 800 nm. The HPLC eluted was detected by MS with ESI along the maintained oven temperature at 40°C and the interface temperature of 350°C. The scanning range was between 100 to 1000 m/z with a speed of 3750/ sec. Absorved chromatograms were matched with the library and the results were interpretated.

***GC-MS***

The characterization of phytochemicals and the identification of the compounds in the CNBPCE extract were identified by GC-MS using GC-2010 and MS-QP 2010 plus, respectively. The sample injecting process was done with an autosampler system- 7693. The carrier gas used here was helium. The flow rate of linear velocity of 2 mins at 14.4 mL/min, with the column flow of 1.03 mL/min. The split ratio was 10.1 with the capillary column of Rxi 1MS with a length of 30 m and an internal diameter of 0.25mm and 0.25 micron of thickness was used. The Injector temperature was set at 280°C, the column was initially set at 70°C for 10 mins and held at 5°C/rate to 280°C for 10 mins. 1µl of CNBPCE extract was injected with a total run time of 60 mins. The temperature of the MS Ion source and interferace were 250°C and 200°C, respectively. The total event was held at the interval of 0.5 sec at the scan speed of 2000 with the start m/z of 35.00 and end m/z of 850.00. The resulting spectra of the chromatogram were compared to the mass spectral library NIST (National Institute of Science and Technology) for the identification of the eluted compounds in CNBPCE extract.

## Results and Discussion

***Preliminary phytochemical screening***

The Preliminary phytochemical screening was performed in CNBPCE extract to confirm the presence of metabolites such as alkaloids, flavonoids, sterols, terpenoids, anthraquinone, anthocyanin, proteins, phenolic compounds, quinones, carbohydrates, tannin, saponins, cardiac glycosides, glycosides, lignin, coumarins and volatile oils based on the technique by Harborne method of plant analysis. The results show the presence of certain compounds as tabulated in Table 1.

Table 1. Preliminary phytochemical screening of the cold macerated ethanolic extract of *Cocos nucifera* bee pollen.

| S.No | Metabolite | Test performed | Observation | Results |
|---|---|---|---|---|
| 1 | Alkaloids | Mayers test | Cream precipitate | ++ |
| 2 | Flavonoids | Lead acetate test | White precipitate | + |
| 3 | Sterols | Libermann test | Reddish brown ring | ++ |
| 4 | Terpenoids | Libermann test | Absence of green color | _ |
| 5 | Anthraquinone | Borntragers test | Absence of reddish orange | _ |
| 6 | Anthocyanin | HCl Test | No colour change | _ |
| 7 | Proteins | Xanthoproteic | Yellow coloured | + |
| 8 | Phenolic compounds | Gelatin test | White precipitate | ++ |
| 9 | Quinones | Conc HCl test | No yellow precipitate | _ |
| 10 | Carbohydrates | Fehling's test | Red precipitate | ++ |
| 11 | Tannin | Braymer's test | Bluish-green color | ++ |
| 12 | Saponins | Saponification | Foam formation | + |
| 13 | Cardiac glycosides | Keller-killani test | Brown ring formed | + |
| 14 | Glycosides test | Aq. NaOH test | Yellow coloured | + |
| 15 | Lignin | Labat test | No olive green colored | _ |
| 16 | Coumarins | Fluorescence test | No yellow fluorescence | _ |
| 17 | Volatile oils | Fluorescence test | No pink fluorescence | _ |

**(++)** Strongly present; (**+**) Present; (-) Absent

***DPPH anti-oxidant assay:***

The DPPH assay was performed with certain concentrations like 100, 200, 400, 800 and 1000 µg/ml for both the sample and the standard drug. The standard used was ascorbic acid, and the abs of the UV Spectrophotometer were noted at the wavelength of 517 nm. The Inhibitory percentage of the CNBPCE was compared with the standard as tabulated in Table 2. It shows that the % Inhibition of CNBPCE extract was 86.7±0.15% at 1000 µg/ml concentration which was slightly lower when compared to that of the standard with 95.7±0.15% at particular concentration.

Table 2. 2,2-Diphenyl-1-picrylhydrazyl antioxidant assay for the cold-macerated ethanoic extract of *Cocos nucifera* bee pollen

| S. No | Concentration (µg/ml) | Standard (% Inhibition) | CNBPCE (% Inhibition) |
|---|---|---|---|
| 1 | 100 | 62.1 ± 0.17 | 50.8 ± 0.09 |
| 2 | 200 | 70.1 ± 0.32 | 58.8 ± 0.09 |
| 3 | 400 | 83.6 ± 0.13 | 78.5 ± 1.06 |
| 4 | 800 | 90.1 ± 0.03 | 81.2 ± 0.12 |
| 5 | 1000 | 95.7 ± 0.15 | 86.7 ± 0.15 |

Standard: Ascorbic acid

Values are expressed in mean ± SD (n=3) statistically significant test for comparison by ANOVA followed by Dunnett's t-test which was compared between the standard ascorbic acid and the extract CNBPCE.

***UV- VIS spectroscopic analysis:***

The UV-Vis spectroscopic analysis detects the maximum number of peaks between the wavelength of 440 to 240 nm as shown in Figure 1 and Table 3.

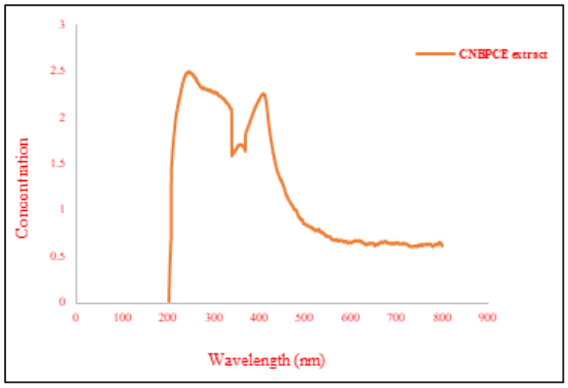



Figure 1. UV-Vis spectra of the cold-macerated ethanolic extract of the *Cocos nucifera* Bee pollen.

Table 3. Characterization using Ultraviolet-Visible spectroscopic analysis of the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen

| S.No | Wavelength (nm) | Abs |
|---|---|---|
| 1 | 446.50 | 1.34 |
| 2 | 412.00 | 2.25 |
| 3 | 410.50 | 2.26 |
| 4 | 408.00 | 2.26 |
| 5 | 391.00 | 2.12 |
| 6 | 355.50 | 1.71 |
| 7 | 349.50 | 1.67 |
| 8 | 324.00 | 2.20 |
| 9 | 308.00 | 2.27 |
| 10 | 303.50 | 2.28 |
| 11 | 301.50 | 2.28 |
| 12 | 300.00 | 2.28 |
| 13 | 290.00 | 2.30 |
| 14 | 286.00 | 2.31 |
| 15 | 277.50 | 2.32 |
| 16 | 276.50 | 2.32 |
| 17 | 261.50 | 2.43 |
| 18 | 255.00 | 2.47 |
| 19 | 246.50 | 2.50 |
| 20 | 242.5 | 2.49 |

***FT-IR:***

CNBPCE extract was characterized with FT-IR for the identification of compounds with the help of the resulting peaks as shown in Figure 2. The peaks were detected along with the area and intensity which can be used for the conformation of functional groups and compounds present in the sample as tabulated in Table 4. The Peaks were ascertained at 3327.32, 2974.33, 2879.82, 1423.51, 1379.15, 1087.89, 1045.45, 879.57 $cm^{-1}$ with their respective area and intensity.

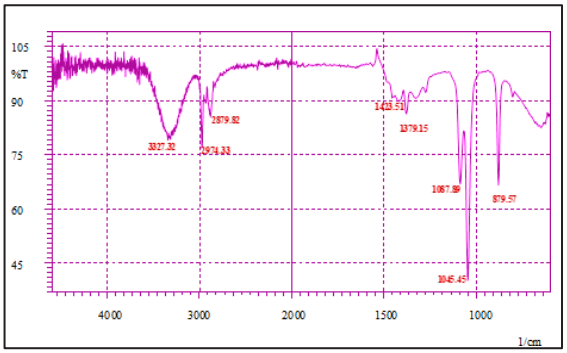



Figure 2. FT-IR peaks of the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen

Table 4. Characterization using Fourier Transform- InfraRed spectroscopic analysis of the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen

| S.No | Peak ($cm^{-1}$) | Area (%) | Intensity | Functional group | Compounds |
|---|---|---|---|---|---|
| 1 | 3327.32 | 1.11 | 79.83 | -C=C-H:C-H stretch | Alkynes |
| 2 | 2974.33 | 3.02 | 77.02 | C-H stretch | Alkanes |
| 3 | 2879.82 | 1.04 | 85.55 | C-H stretch | Alkanes |
| 4 | 1423.51 | 0.83 | 90.02 | C-C stretch | Aromatic |
| 5 | 1379.15 | 1.64 | 86.43 | C-H rock | Alkanes |
| 6 | 1087.89 | 5.39 | 66.74 | C-O stretch | Alcohol, Acid, Ester |
| 7 | 1045.45 | 9.78 | 40.11 | C-O stretch | Alcohol, Acid, Ester |
| 8 | 879.57 | 3.41 | 66.67 | C-H oop | Aromatic |

***LC-MS:***

LC-MS eluted the major non-volatile compounds present in CNBPCE extracts using Shimadzu CBM-20A using a PDA detector. With the total run time of 30 mins 8 major positive peaks were detected with their specific retention time, base peaks and mass peaks. The compounds detected were dihexosylquercetin, 5-O-caffeoyl shikimic acid, 6b-naltrexol 3-O-b-D-glucuronide, leukotriene D4, ornithine, xanthoangelol and quercitrin with the base peaks of 625, 659, 518, 496, 413, 391 and 756, respectively as shown in table 5 & 7 and figure 3.

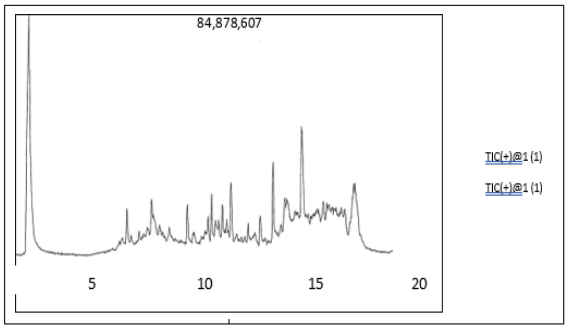
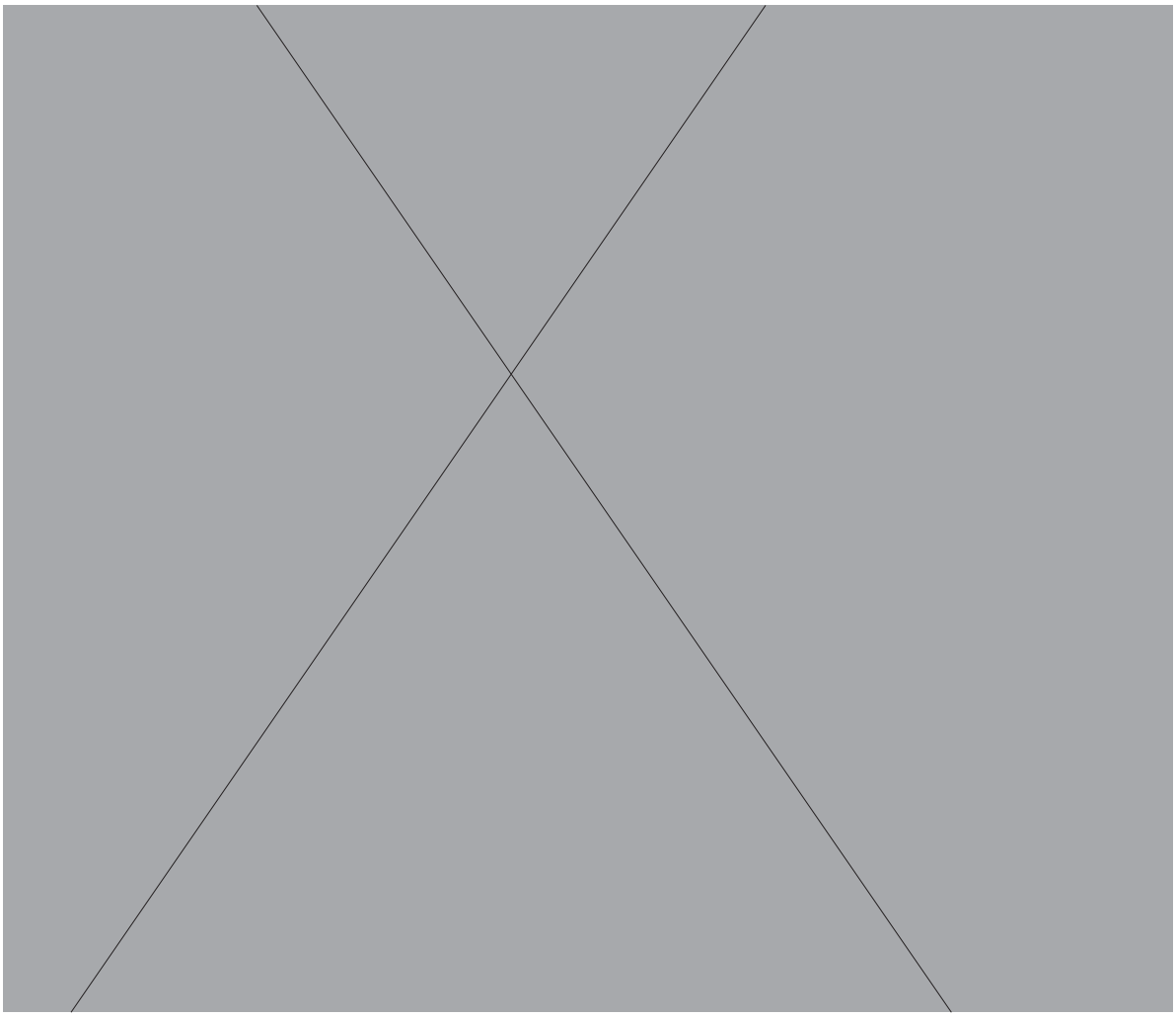



Figure3. LC-MS Chromatogram of the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen

Table 5. Phytochemical constituents detected in the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen using Liquid Chromatography- Mass Spectroscopy analysis



***GC-MS***

To specify the compounds, present in CNBPCE extract the sample was subjected to GC-MS analysis. The sample was performed using GC-2010 and MS-QP 2010+. From the total run time of 60 mins, 28 eluted compounds were identified along with peak number, peak area and retention time as shown in Figure 4 and Table 6. The dominant compounds such as linolenic acid, linolenyl alcohol, methyl and ethyl linolenate, 9,12- Octadecadienoic acid, ethyl ester, palmitic acid, l (+)-Ascorbic acid, 2,6-dihexadecanoate, pentadecyclic acid, 5-hydroxymethylfurfural, ascabiol, n-tetrapeatacontane, bicetyl, n-tetrapentacontane, hexatriacontane, palmitic acid; beta monoglyceride, monostearin, stearic acid, 1,2cyclopentanedione, sitosterol, linolein-1-mono, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6 methyl, 1-1-dimethyl tetradecyl hydrosulfide, octatriacontyl pentafluoro propionate, canophyllal, stigmasta-5-24 (28)dien-3-ol-3btea, tocopherol, hexatriacontane and furanmethanol were identified. Table 7 interprets the nature of the dominating phytoconstituents and their desired pharmacological activities. Figure 5 demonstrstes the total percent of functional groups depicted from both GCMS and LCMS.

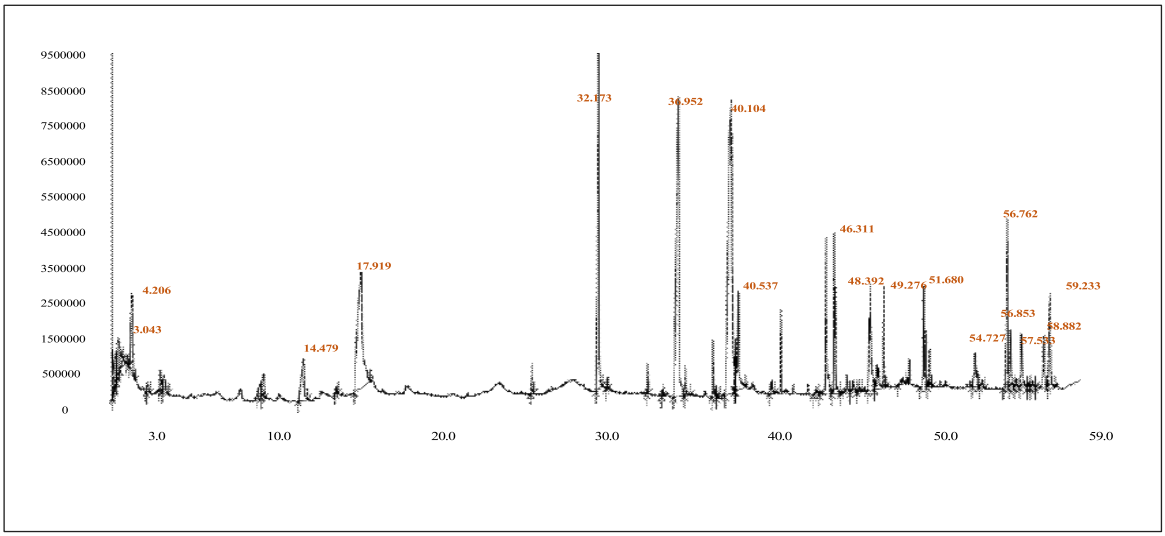
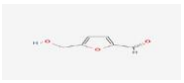
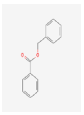
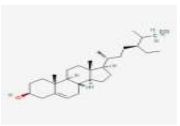
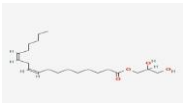
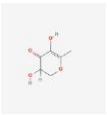
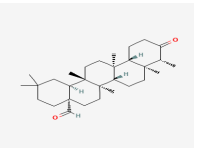
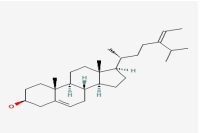
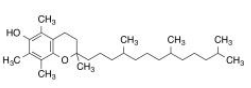



Figure 4. GC-MS: Chromatogram of the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen

Table 6. Phytochemical constituents detected in the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen using Gas Chromatography-Mass Spectroscopy analysis

| S. No | Compound Name/ IUPAC | Peak No. | Peak Area (%) | Rate time (min) | Mol. formula | Mol. weight (g/mol) | Chemical structure |
|---|---|---|---|---|---|---|---|
| 1 | **Linolenic acid /** (9Z,12Z,15Z)-octadeca-9,12,-15-trienoic acid | 24 | 19.73 | 40.104 | $C_{18}H_{30}O_2$ | 278.40 | |
| 2 | **Linolenyl alcohol /** (9E,12E,15E)-octadeca-9,12,-15-trien-1-ol | 24 | 19.73 | 40.104 | $C_{18}H_{32}O$ | 264.40 | |
| 3 | **Methyl linolenate /** methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate | 24 | 19.73 | 40.104 | $C_{19}H_{32}O_2$ | 292.50 | |
| 4 | **Ethyl linolenate /** ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate | 24 | 19.73 | 40.104 | $C_{20}H_{34}0_2$ | 306.50 | |
| 5 | **9,12- Octadecadienoic acid, ethyl ester /** ethyl (9E,12E)-octadeca-9,12-dienoate | 24 | 19.73 | 40.104 | $C_{20}H_{36}0_2$ | 308.50 | |
| 6 | **Palmitic acid /** hexadecanoic acid | 20 | 12.72 | 36.952 | $C_{16}H_{32}0_2$ | 256.40 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7 | **L-(+)-Ascorbic acid, 2,6-di-hexadecanoate /**<br>((2S)-2-((2R)-4-hexadeca-noyloxy -3-hydroxy-5-oxo-2H-furan-2-yl) -2-hydroxyethyl) hexadecanoate | 20 | 12.72 | 36.952 | $C_{38}H_{68}0_8$ | 652.90 |  |
| 8 | **Pentadecylic acid /**<br>pentadecanoic acid | 20<br>26 | 12.72<br>3.14 | 36.952<br>40.537 | $C_{15}H_{30}0_2$ | 242.40 |  |
| 9 | **5-Hydroxymethylfurfural** /<br>5-(hydroxymethyl)furan-2-car-baldehyde | 14 | 10.43 | 17.919 | $C_6H_60_3$ | 126.11 |  |
| 10 | **Ascabiol** /<br>benzyl benzoate | 16 | 8.53 | 32.173 | $C_{14}H_{12}0_2$ | 212.42 |  |
| 11 | **n-Tetrapentaconate** /<br>tetrapentaconate | 41<br>38 | 3.99<br>1.22 | 56.762<br>54.727 | $C_{54}H_{110}$ | 759.40 |  |
| 12 | **Bicetyl** /<br>dotriacontane | 41<br>34<br>39 | 3.99<br>3.54<br>1.22 | 56.762<br>48.392<br>54.727 | $C_{32}H_{66}$ | 450.90 |  |
| 13 | **n-Tetraconatane** /<br>tetracontane | 41<br>34 | 3.99<br>3.54 | 56.762<br>48.392 | $C_{40}H_{82}$ | 506.10 |  |
| 14 | **n-Hexatriacontane** /<br>hexatriacontane | 34 | 3.54 | 48.392 | $C_{36}H_{74}$ | 507.01 |  |
| 15 | **Palmitic acid-β- monoglycer-ide/**<br>1,3-dihydroxypropan-2-yl hexa-decanoate | 30 | 3.46 | 46.311 | $C_{19}H_{38}O_4$ | 330.50 |  |
| 16 | 2- **Monostearin** /<br>2,3-dihydroxy propyl octade-canoate | 30 | 3.46 | 46.311 | $C_{21}H_{42}O_4$ | 358.60 |  |
| 17 | **Stearic acid** /<br>octadecanoic acid | 26 | 3.14 | 40.537 | $C_{18}H_{36}O_2$ | 284.50 |  |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 18 | **1,2-Cyclopentanedione** / cyclopentane-1,2-dione | 6 | 2.66 | 4.206 | $C_5H_6O_2$ | 98.10 |  |
| 19 | **Gamma-sitosterol** / (3S,8S,9S,10R,13R,14S,17R)-17-((2R,5S)-5-ethyl-6-methyl heptan -2-yl)-10,13-di-methyl,2,3,4,7,8, 9,11,12,14,15,16,17-dodecahy-dro 1H-cyclopenta(a)phenan-thren-3-ol | 48 | 2.59 | 59.233 | $C_{29}H_{50}O$ | 415.70 |  |
| 20 | **Linolein,1-mono** / 2,3-dihydroxypropyl (9Z,12Z)--octadeca-9,12-dienoate | 35 | 2.30 | 49.276 | $C_{21}H_{38}O_4$ | 354.50 |  |
| 21 | **4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6 methyl** / 3,5-dihydroxy-6-methyl-2,3-di-hydropyran-4-one | 12 | 2.20 | 14.479 | $C_6H_8O_4$ | 144.12 |  |
| 22 | **1,1-Dimethyltetradecyl hy-drosulfide** / 2-methylpentadecane-2-thiol | 37 | 2.12 | 51.680 | $C_{16}H_{34}S$ | 258.5 |  |
| 23 | **Octatriacontyl pentafluoro propionate** / octatriacon- | 37 | 2.12 | 51.680 | $C_{41}H_{77}F_5O_2$ | 697.01 |  |
| 24 | **Canophyllal** / (4aS,6aS,6aR,6bS,8aS ,9R,12aS,14aS,14bS)--2,2,6a,6a,8a,9, 14a-hepta-methyl-10-oxo-3,4,5,6,6b, 7,8,9,11,12,12a,13,14,14b,te-trad cahydr0-1H-picene-4a-car-baldehyde | 47 | 1.58 | 58.882 | $C_{30}H_{48}O_2$ | 440.70 |  |
| 25 | **Stigmasta-5,24(28)dien-3-ol-3β** / (3S,10R,13R)-10,13-di-methyl-17-((E,2R)-5-pro-pan-2-ylhept-5-en-2-yl)-2,3,4,7,8,9,11,12,14,15, 16,17-dodecahydro-1H-cyclo-penta, (a)phenanthren-3-ol | 42 | 1.53 | 56.853 | $C_{29}H_{48}O$ | 412.71 |  |
| 26 | **α-Tocopherol** / (2R)-2,5,7,8-tetramethyl-2-((4R,8R)-4,8,12-trimethyltride-cyl)-3,4,dihydrochromen-6-ol | 44 | 1.37 | 57.533 | $C_{29}H_{50}O_2$ | 430.70 |  |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 27 | **Hexatriacontane** / hexatriacontane | 39 | 1.22 | 54.727 | $C_{36}H_{74}$ | 507.00 | |
| 28 | 3- **Furanmethanol** / furan-3-ylmethanol | 1 | 1.08 | 3.043 | $C_5H_6O_2$ | 98.10 |  |

Table 7. Pharmacological activity of the detected phytochemical constituents from the cold-macerated ethanolic extract of *Cocos nucifera* bee pollen using LCMS & GCMS

| S.No | Compound Name | Compound Nature | Pharmacological activity |
|---|---|---|---|
| 1 | Linolenic acid | Fatty acid | Anti-inflammatory (15,16), Anticarcinogenic (17,18), Immune modulation (19), Antimicrobial (20), Anti-oxidant (18,21), Decreases cardiovascular disease, decreases arrhythmias, decreases thrombosis, decreases serum triglycerides level, Increases vascular endothelial level, decreases inflammation (16,22), Anti-arthritic, Antiandrogenic, Hepatoprotective, Antieczemic, 5α-reductase Inhibitor, Hypocholesterolemic, Anti-acne, antihistaminic (23,24,25). |
| 2 | Linolenyl alcohol | Alcohol | Anti-oxidant, Hypocholesterolemic (26,27,28), Antibacterial against B.subtilis, S.aureus (27,28), Antiradial, Antitumour, Antidepressant, Anti-inflammatory (29), Antianxiety (30), Anti-carcinogenic, (29,31), Hepatoprotective, Anti-arthritic, surpass blood brain barrier (29), Antimicrobial (32), Inhibits protein thyrosine phosphatase (33), Antibacterial, Antifungal (34). |
| 3 | Methyl linolenate | Fatty esters | Antimicrobial (35), Anti-carcinogenic, Hepatoprotective, Anti-arthritic (35,36), Anti-asthma, Dieuretic (35), Anti-inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Anti-histaminic, Anti-eczemic, Anti-acne, 5α-reductase Inhibitor, Antiandrogenic, Anticoronary (36). |
| 4 | Ethyl linolenate | Fatty esters | Antibacterial against E.coli, P.aeruginosa, B.subtilis, S.aureus (37), Hypocholesterolemic, Nematicide, Anti-arthritic, Hepatoprotective, 5α-reductase Inhibitor, Anti-histaminic, Insectifuge, Anti-coronary, Anti-eczemic, Antiacne, Anti-androgenic (35,38,39). |
| 5 | 9,12- Octadecadienoic acid, ethyl ester | Fatty esters | Hypocholesterolemic, Nematicide, Anti-arthritic, Hepatoprotective, 5α-reductase Inhibitor, Anti-androgenic, Anti-microbial, Anti-acne, Anti-histaminic, Insectifuge, Anti-coronary, Anti-eczemic (24,35,40,41). |
| 6 | Palmitic acid | Fatty acids | Anti-quorum sensing against A.baumannii (42), Anti-inflammatory (43,45), Anti-oxidant, Anti-androgenic (43,44,35), Hypocholesterolemic, Nematicide, 5α-reductase Inhibitor (35,43,44,45), Hemolytic (43), Treats Rheumatic symptoms, Mosquito larvicide (43), Pesticide (43,35), Anti-fungal (44), Anti-malaria (44,45), Lubricant, Antipsychotic (35), Haemolithic, Anti-viral, Anticancer (45). |

| | | | |
|---|---|---|---|
| 7 | L-(+)-Ascorbic acid, 2,6-dihexa-decanoate | Fatty esters | Wound healing (46,51,52), Vitamin C Immunomodulator (46), Anti-tumour (46,47,49,52), Anti-bacterial (46,47,52), Anti-oxidant (46,48,49,50,51), Anti-microbial (49), Reduces triglycerides, protects LDL against peroxidation and inhibits the progression of artherosclerosis (50), Anti-inflammatory, Anti-scorbutic, Anti-nociceptive, Antimutagenic (51), Antiallergic, Anti-anemic, Anti-axiety, Antibronchitis, Anti-cataract, Anti-coagulant, Anticonvulsant, Anti-diabetic, Anti-diarraheic, Antifatigue, Anti-fertility, Anti-gastric, Antimalarial, Antistress, Anti-ulcer, Anti-artheroscelerotic, Anti-cold, Anti-glaucomic, Anti-hepatic, Antihypertensive, Antiplague, Antiproliferant, Antiprotozoal, Antiseptic, Antistroke, Antituberculic, CNSS stimulant, Chelator, Chemopreventive, Cytochrome P450 Inducer, Deodrant, Hypolipidimic, Neuroprotective, Neurotransmiter, Termiticide, Anti-viral (53). |
| 8 | Pentadecylic acid | Fatty acid | Anti-asthmatics, Anti-abortive (54), Anti-bacterial, Anti-allergic (55), Lubricants, Adhesive agents (56), Anti-oxidant (57), Anti-cancer (22,54,58). |
| 9 | 6-Hydroxymethyl-furfural | Aldehydes | Anti-oxidant (59,62,64,65), Antiproliferative (59,64), Antibiofilm (64), Preservative (60), Antimicrobial (60,64), Antifungal (63,67), Antibacterial (61,63,65,67), Pesticide, Cosmetics (67), Anti-allergenic, Anti-diabetic (66). |
| 10 | Ascabiol | Phenols | Anti-parasitic, Antifungal, Antibacterial, Fragrance ingredients, Pesticides, Solvents (68), Spasmolytic, Calmodulin inhibitor, tyrosinase inhibitor, treatment of angina pectoris and scabies (69). |
| 11 | n-Tetrapentacon-ate | Fatty esters | Antibacterial (70), Anti-inflammatory (71,74), Anti-oxidant (71,72), Antimicrobial (72,75), Antimutagenic (72), Hydroxylation of the liver enzyme during phase 1 metabolism (73), Hair growth, uric acid production, arachidonic acid inhibitor in the human body, Anticancer (73). |
| 12 | Bicetyl | Ketones | Antimicrobial (76,77), Anti-oxidant (77,79), Antispasmodic (77,81), Antibacterial (77,79), Antiviral (77,80), Antifungal (78,81), Anti-inflammatory (78), Cytotoxic activity against hepatocarcinoma cell line (78,80). |
| 13 | n-Tetraconatane | Hydrocarbons | Antimicrobial (76,86), Anti-inflammatory (82,83), Analgesic (82,83), Antibacterial (84,85), Antioxidant (84), Anti-tumour, Antidiabetic (73). |
| 14 | n-Hexatriacontane | Hydrocarbons | Anti-inflammatory, Analgesic (87), Radical scavenger (87,82), Anti-oxidant (87,88,89), Antimicrobial (88,90), Antidepressant, Hypocholesterolemic (90). |
| 15 | Palmitic acid-β-monoglyceride | Fatty esters | Antibacterial against E.coli (91,93), Biomarker of type 2 diabetes, Visco elastic property, Non-ionic surfactant for vaccines, Liquid crystal delivery system (92), Sugar-phosphate inhibitor, Lipid metabolism regulator, Anti-infective, Anti-inflammatory, Intestine histamine release inhibitor, Antiprotozoal (93). |

| | | | |
|---|---|---|---|
| 16 | 4- Monostearin | Fatty esters | Emulsification (94), Anti-static, Anti-fogging (95), Increased drug permeability, Self-emulsifying drug delivery system (96). |
| 17 | Stearic acid | Fatty acids | Anti-oxidant (97,98), Antidiabetic (97), Neuroprotective (97), Anti-tumour (99,101), Cytotoxicity (99), Antimicrobial (98, 100), Anti-inflammatory (98,103), Diuretic activity (98), Antibacterial (44,101), Antifungal (56,101), Antiviral against Measles and Parainfluenzavirus (102), Anti-arthritic (103), Hypocholesterolemic (56,103), Anticonvulsant, Antianalgesic, Anti-asthmatic, Anti-amoebic, Anti-gastric, Anti-malaria, treat obesity (104), Cosmetic, 5α-reductase Inhibitor, Flavor (85). |
| 18 | 1,2-Cyclopentanedione | Ketones | Prevention of gastrointestinal tumor (105), Anti-bacterial (106), Anti-diabetic (107), Anti-oxidant (108). |
| 19 | Gamma-sitosterol | Sterols | Anti-diabetic (109,110), Hypocholesterolemic activity (109,110), Cytotoxic against colon and liver cancer cell line (109), Extrinsic apoptotic pathway in human lung and breast adenocarcinoma cell (110), Anti-cancerous (49,111), Hepatoprotective (49,111), Biomarker for cancer prevention (49), Anti-microbial, Anti-arthritic, Anti-asthma (111). |
| 20 | Linolein,1-mono | Fatty esters | Treatment of breast cancer (112), Hypocholesterolemic activity, Anti-eczemic, Nematicide, Hepatoprotective, Anti-oxidant, Anti-acne, Haemolytic, Pesticide, Flavour (113). |
| 21 | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6 methyl | Ketones | Antifungal (114,116), Inhibition of colon cancer cell growth (115), Anti-oxidant (115,116,117), Anti-microbial, Automative nerve activity, Anti-inflammatory, Anti-proliferative, Anti-cancer, Anti-diabetic, Proapoptic effects, Cytotoxicity, Antipyretic, Anthelmentic, Anti-tumour, Antibronchitis, Anti-tuberclosis, Dysepsia, Constipation, Anemia, Throat disease, Elephantiasis, Anti-diabetic, Anti-diarrhoeal (117), Anti-arthritic (117,118), Anti-asthma (117,118). |
| 22 | 1,1-Dimethyltetradecyl hydrosulfide | Organosulfur | Anti-tumour, Anti-fungal, Insecticidal (43), Enzyme activators (119), Anti-microbial (120), Anti-oxidant (43,79,80,121), Antibacterial (79,80,121). |
| 23 | Octatriacontyl pentafluoro propionate | Fatty esters | Anti-viral drugs in the treatment of cancer (122,124), Antibacterial (123), Anti-MDR (123), and Antiviral against Covid-19 (124). |
| 24 | Canophyllal | Terpenoids | Anti-inflammatory, Antioxidant, Hypocholesterolemic (125,126) |
| 25 | Stigmasta-5,24(28)-dien-3-ol-3β | Sterols | Antidiabetic (127), Synthetic progesterone (56,83), Anti-hepatotoxic, Cancer prevention (56,129,130), Hypocholesterolemic, Anti-viral (56,129), Anti-oxidant (56,128,129,130), Antiosteoarthritic, Anti-inflammatory, Anti-photoaging, Immunomodulatory, Antineurological, Algicidal, Anti-obesity, Antimicrobial (130). |
| 26 | α-Tocopherol | Phenols | Antioxidant (131,132), Anti-inflammatory, Antibacterial (133), Anti-bronchitic, Anti-coronary, Anti-arthritic, Hepatoprotective, Antimicrobial, Antiasthma (134), Anti-cancer (134,135). |

| | | | |
|---|---|---|---|
| 27 | Hexatriacontane | Hydrocar-bons | Anti-inflammatory, Analgesic activity, Radical Scavenger (87), Anti-oxidant (87,136,137). |
| 28 | 5- Furanmethanol | Alcohols | Anti-bacterial (106), Anti-viral (106,138,139), Malignancy preventive (118), Anti-fungal (138), Moderate toxic, Flavouring agents, the aroma of tea, coffee, etc., Adhesive agents, Anti-oxidants (140), Eye mucous irritation, abdominal pain, Diarrhoea, Headache, Vomiting (141). |
| 29 | Dihexosylquer-cetin | Flavonoids | Antioxidant, Anti-inflammatory, Antimicrobial and Antiprolifera-tive (142) |
| 30 | 5-O-Caffeoylshi-kimic acid | Phenols | XOD Inhibitor (143), Strong activity related to DPPH, ABTS (144), uricacid lowering effects in hyperuricemic (145) |
| 32 | 6b-Naltrexol 3-O-b-D-Glucuro-nide | Alkaloids | No activity reported |
| 34 | Ornithine | Amino acid | Urea cycle/ ammonia detoxification (146), Precursor for polyamines and amino acids (147), Metabolic regulation and homeostasis (148) |
| 35 | Xanthoangelol | Flavonoids | Anticancer / Antitumor / Antimetastatic Activity (149) |
| 36 | Quercitrin | Flavonoids | Antioxidant activity,Antiinflammatory and immunomodulatory effects,Antiparasitic, Antimetastatic and Anticancerrelated activity (150) |

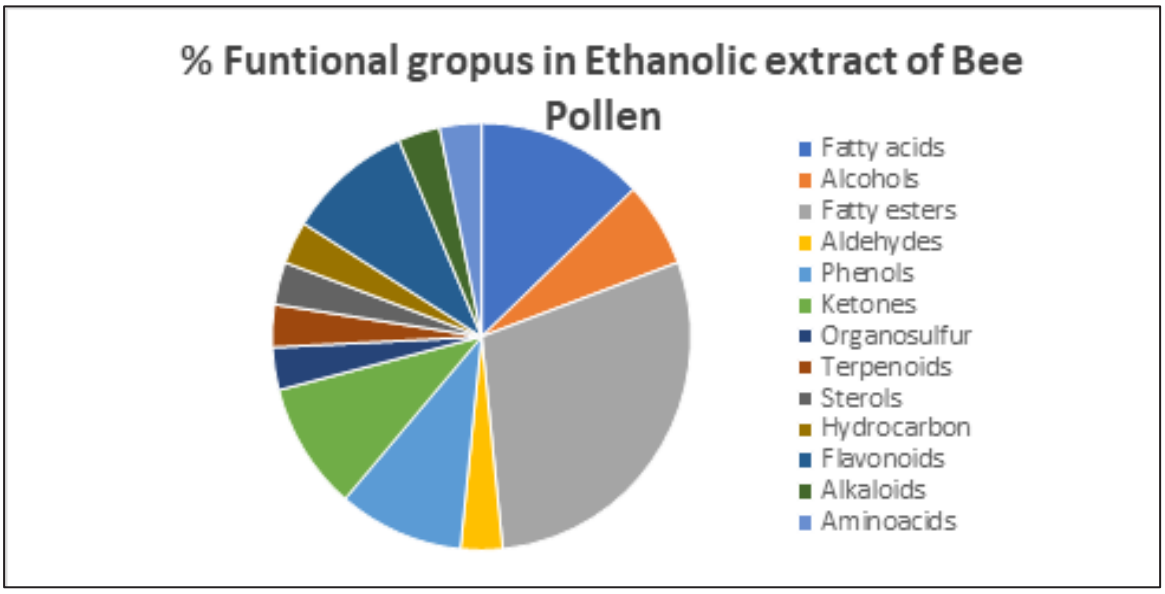



Figure 5. % of the total Functional group detected from both GCMS and LCMS

**Discussion**

Traditional medicine from natural sources is adopted in the medical field to prevent and treat abundant life-threatening diseases owing to their systematic therapeutic potential. Noticeably, Bee pollen is one of the natural sources aided by honey bees with an enormous quantity of chemical compounds and eluted possessions of pharmacological activities. Therefore, the bee pollen from *Cocos nucifera* was collected manually using the pollen trap. The purpose is to identify and isolate the pharmacologically important phytochemicals and to elucidate the medicinal values present in them. For the process of extraction, the cold maceration technique was employed to acquire the pharmacologically important phytochemicals without the loss of thermolabile compounds.

The present investigation provides a comprehensive biochemical profile of *Cocos nucifera* bee pollen, revealing a rich array of metabolites with strong antioxidant, anti-inflammatory, and lipid-regulating potential. The preliminary phytochemical screening confirmed the presence of key secondary metabolites, including alkaloids, sterols, phenolics, flavonoids, tannins and glycosides compounds that are well recognized for their physiological relevance in maintaining metabolic balance and protecting against oxidative and inflammatory disorders. The abundance of these compounds supports the longstanding use of bee pollen as a natural therapeutic supplement and underscores the unique biochemical richness of pollen derived specifically from *C. nucifera*.

The antioxidant capacity of the extract was strongly supported by the DPPH assay, where the inhibition reached nearly the activity of the standard antioxidant. This indicates substantial free radical scavenging ability, which is critical given that oxidative stress is a major driver of hypercholesterolemia, endothelial dysfunction and atherosclerotic plaque development. The high degree of scavenging suggests that the phenolic and flavonoid constituents detected in both the UV-Vis and FTIR analyses actively contribute to the neutralization of reactive oxygen species. The UV-Vis spectral pattern, characterized by broad absorptive bands typical of conjugated aromatic systems, further validates the presence of polyphenolic structures commonly associated with strong antioxidant behavior. FT-IR profiles showing the prevalence of hydroxyl, carbonyl and aromatic groups reinforce this interpretation by indicating the presence of phenolic acids, flavonoids, alcohols, esters and alkanes in the extract.

LCMS analysis revealed eight major non-volatile phytoconstituents, several of which such as flavonoid glycosides, caffeoyl derivatives and prenylated chalcones are well known for their roles in reducing oxidative burden, suppressing lipid peroxidation and modulating inflammatory pathways. Flavonoid glycosides identified in the extract are particularly noteworthy due to their established roles in preventing LDL oxidation, enhancing endothelial function and attenuating metabolic disturbances. Compounds such as caffeoylshikimic acid and xanthoangelol contribute additional anti-inflammatory and lipid-lowering effects through inhibition of oxidative signaling and suppression of inflammatory mediators. The detection of amino acid derivatives such as ornithine suggests auxiliary metabolic benefits, particularly in nitrogen metabolism and endothelial regulation.

GCMS analysis unveiled a dominant presence of polyunsaturated fatty acids, fatty acid esters, plant sterols, hydrocarbons and lipid-soluble antioxidants. The high abundance of linolenic acid, methyl and ethyl linolenate and linolenyl alcohol is significant, as these compounds are central to the regulation of lipid metabolism, reduction of serum triglycerides, improvement of endothelial function and attenuation of inflammatory responses. These fatty acids are widely recognized for lowering the risk of cardiovascular complications by modulating cholesterol transport and enhancing membrane fluidity. The detection of plant sterols, including γ-sitosterol and stigmasta-based sterols, pro-

vides further mechanistic insight into the hypocholesterolemic potential of the extract, as sterols inhibit intestinal cholesterol absorption through competitive displacement. The presence of tocopherol, a potent lipid-soluble antioxidant, enhances the extract's protective capability against lipid peroxidation and oxidative damage within vascular tissues.

Several additional compounds identified including pentadecylic acid, palmitic acid derivatives, benzyl benzoate, long-chain hydrocarbons and hydroxymethylfurfural which contribute complementary antimicrobial, anti-inflammatory and antioxidant functions. Collectively, the metabolomic profile indicates a synergistic network of bioactive molecules acting across multiple biochemical pathways associated with cardiometabolic regulation.

Taken together, the convergence of findings from phytochemical screening, antioxidant assays, UV-Vis and FT-IR spectroscopy along with LCMS and GCMS analyses demonstrates that *Cocos nucifera* bee pollen is a potent reservoir of pharmacologically significant compounds. The extract exhibits strong free radical scavenging potential, contains diverse lipid-modulating constituents and includes multiple anti-inflammatory and antioxidant molecules known to support cardiovascular health. The integration of these bioactivities suggests that *C. nucifera* bee pollen may serve as a highly effective natural agent for managing dyslipidemia, mitigating oxidative stress and improving overall cardiometabolic function.

The results presented in this study therefore not only validate the therapeutic relevance of *C. nucifera* bee pollen but also contribute novel insights into its chemical composition and biological potential. These findings justify further in vivo exploration and clinical validation to delineate dosage, bioavailability, synergistic interactions among compounds and long-term safety. With its broad spectrum of beneficial phytochemicals and strong antioxidant and lipid-regulating properties, *C. nucifera* bee pollen holds considerable promise as a natural adjunct in the prevention and management of cardiometabolic disorders.

## Conclusion

The present study demonstrates that *Cocos nucifera* bee pollen is a rich source of pharmacologically significant phytochemicals with strong antioxidant and lipid-regulating potential. The extract exhibited substantial free radical scavenging activity, confirming its ability to mitigate oxidative stress, a key factor in cardiometabolic disorders. Spectroscopic analyses revealed abundant phenolic, flavonoid and lipid-derived functional groups, supporting its biochemical complexity. LCMS profiling identified several bioactive non-volatile metabolites contributing to anti-inflammatory and hypolipidemic effects. GCMS analysis further highlighted the dominance of polyunsaturated fatty acids, sterols and lipid antioxidants crucial for cholesterol regulation. The synergistic action of these compounds suggests meaningful therapeutic potential in managing dyslipidemia and cardiometabolic dysfunctions. The findings validate the traditional relevance of bee pollen and emphasize the uniqueness of the *C. nucifera* floral source. This study also adds new metabolomic insights for future natural-product-based interventions. Further in vivo and clinical studies are warranted to evaluate its efficacy, safety and bioavailability. Overall, *C. nucifera* bee pollen emerges as a promising natural candidate for cardiometabolic health management.

## Abbrevation

1. % : Percentage
2. °C : Degree Celsius
3. µg : microgram
4. µl : microlitre
5. Ab : Absorbance of Control
6. Abs : Absorbance
7. ANOVA : Analysis Of Variance
8. As : Absorbance of the Extract
9. cm : Centimeter

10. CNBPCE extract : Cocos nucifera Bee Pollen Cold-macerated Ethanolic extract
11. CNSS System : Caudal Neuro Secretory
12. DPPH : 2,2-diphenyl-1-picrylhydrazyl
13. FT-IR : Fourier Transform- InfraRed
14. g : gram
15. GC-MS : Gas Chromatography-Mass Spectrometry
16. hrs : Hours
17. KBr : Potassium Bromide
18. kPa : Kilopascal
19. m : metre
20. m/z : Mass to Charge ratio
21. min : Minute
22. mL : Millilitre
23. mm : Millimeter
24. NIST : National Institute of Science and Technology
25. nm : Nanometer
26. Pi : Percentage of Inhibition
27. rpm : Revolution per minute
28. SD : Standard Deviation
29. TBA : Thiobar Bituric Acid
30. UV-VIS : UltraViolet-Visible Spectrophotometer

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**Author contributuion**

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**Thangapandiyan S:** Formal analysis,

**Thirumalai V:** Methodology analysis, framing concepts

**Conflict of interest**

The authors declare that they have no conflicts of interest.

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**Data availability statement**

The datasets generated and/or analyzed during the current study are availabe from the corresponding author on reasonable request.

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