Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 14, Issue 2, 2026

Total Phenolic and Flavonoid Compositions and Free Radical Scavenging Activity of Nigerian Bee Pollen Samples

[*1]Ifeoma S. Iyen, [1]Nelson Meor, [1]Ayuba Stephen, [2]Kingsley O. Nwaogu

[1]Department of Chemistry, Federal University Wukari, Nigeria

[2]Youth for Apiculture Initiative, Umuahia, Nigeria

*Corresponding author: udegbunam@fuwukari.edu.ng,

Accepted: *April 23, 2026.* **Published Online**: *April 27, 2026*

ABSTRACT

Bee pollen is rich nutritionally and contains bioactive secondary metabolites. Bee pollen samples collected from Abia, Ekiti, and Ondo States in Nigeria were evaluated for total phenolic and flavonoid compositions as well as their free radical scavenging activity. Samples were first de-fatted with n-hexane and successively extracted using ethyl acetate, acetone, ethanol, and water. Spectrophotometric techniques were used to quantify total phenolic content (TPC) and total flavonoid content (TFC). There was significant variation in the TPC and TFC across extracts. Ekiti samples showed higher TPC in ethanolic and water extracts (36.34 ± 0.12 and 25.87 ± 0.48) than acetone and ethyl acetate (15.23 ± 12.64 and 5.62 ± 0.39) while Ondo samples had higher TPC in acetone and ethanolic extracts (46.32 ± 0.58 and 21.87 ± 0.05). Abia samples recorded the highest TPC in acetone extracts (77.82 ± 0.08). For TFC, whereas ethanolic and acetone extracts were highest in Ekiti (341.42 ± 36.21 and 283.92 ± 1.31), while water extracts dominated in Abia and Ondo samples (341.99 ± 0.68 and 298.92 ± 2.51). DPPH assay showed concentration-dependent activity, with water extracts exhibiting lowest IC_{50} values: Ekiti (7.99), Ondo (9.63), Abia (11.78). The results confirmed strong solvent -dependent variability and antioxidant potential of the bee pollen from these locations.

Keywords: Bee pollen, total phenolic content, total flavonoid content, antioxidant properties, solvents extract

INTRODUCTION

Bee pollen is a natural product collected by bees from various flowering plants. It serves as a nutritional source for bee colonies and exhibits a rich composition of nutrients, including proteins, carbohydrates, vitamins, and minerals [1]. Bee pollen is widely recognized for its potential health benefits and has been consumed by humans from time due to its rich dose of medicinal properties.

Additionally, bee-collected pollen is an important source of vitamins and polyphenolic compounds, particularly flavonoids, renowned for their potent antioxidant properties [2-3]. Honey bee pollen has been utilized in both traditional medicine and dietary regimens, boasting diverse applications ranging from treating conditions such as colds, flu, and ulcers to its incorporation into cosmetics for its vitamin-enriching properties [4-5].

Studies have demonstrated various therapeutic potentials of bee pollen, including its significant antiradical-scavenging activity [6]. Moreover, bee pollen has been investigated for its role in regulating insulin-like growth factor I (IGF-I) release in porcine ovarian granulosa cells, with findings suggesting a dose-dependent regulation potentially important for ovarian functions [7]. Additionally, bee pollen has shown promise in preventing postoperative intra-abdominal adhesions attributed to its anti-inflammatory and antioxidant properties [8-9]. Furthermore, studies have indicated its potential in lowering cholesterol levels and exhibiting hypolipidemic effects when consumed alongside honey [10].

Phenolic compounds, are essential components found in plants, contributing significantly to their nutritional and sensory characteristics, as well as those of their derived products. These compounds are categorized as secondary metabolites The most prominent classes of polyphenols are flavonoids and phenolic acids, encompassing over 5,000 compounds [11]. They display a diverse array of biological activities, including antibacterial, anti-inflammatory, anticarcinogenic, anti-atherogenic, anti-thrombotic, anti-allergic, immune-modulating, analgesic, and antioxidant properties [12-15].

The limited research on the composition and antioxidant activity of Nigerian bee pollen presents a significant knowledge gap. Understanding the phenolic and medicinal properties of Nigerian bee pollen is essential for harnessing its potential benefits for human health and economic development. This research seeks to fill the existing gap in knowledge and provide valuable information for promoting the use of Nigerian bee pollen in healthcare and nutrition.

Therefore, this study aims to investigate the total phenolic and flavonoids composition, as well as the free radical scavenging activity of selected Nigerian bee pollen samples.

MATERIALS AND METHODS

Sample Collection, Preparation, and Extraction

Bee pollen samples from Abia, Ondo, and Ekiti States in Nigeria, were collected using hive entrance traps, labeled, sieved, and stored in airtight containers. Approximately 7 g of dried pollen per sample was weighed and soaked in 120 mL n-hexane for 24 h to remove fatty components, followed by filtration. The solvent was evaporated using a rotary evaporator (Bibby Scientific, RE300/MS) to obtain viscous extracts and the residues underwent sequential extraction with ethyl acetate, acetone, ethanol, and water in increasing polarity. The resulting extracts were stored in clean, dry glass containers for further analyses.

Determination of Phenolic Content

The total phenolic content of the extracts was determined according to the method outlined by Molole *et al* [16]. Aliquots (1 mL) and standard gallic acid (10 -100 mg/mL) were placed into the test tubes and 5 mL of distilled water with 0.5 mL of Folin Ciocalteu's reagent were mixed and shaken. After 5 minutes, 1.5 mL of 20 % sodium carbonate was added and the volume made up to 10 mL with distilled water. It was incubated for 2 hours at room temperature. Intense blue colour developed. After incubation, absorbance was measured at 517 nm using UV-visible spectrophotometer (Jasco *V- 630* instrument). The extractions were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of *C. maxima* leaves were expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass.

Determination of Total Flavonoid Content

To determine the total flavonoid content, the aluminum chloride colorimetric method described by Pallab *et al* [17] was employed. Aliquots (1 mL) and 1 mL standard quercetin solution (10 -100 mg/mL) were placed into test tubes and 4 mL of distilled water with 0.3 mL of 5 % sodium nitrite solution w added into each. After 5 minutes, 0.3 mL of 10 % aluminium chloride was added. At the 6^{th} minute, 2 mL of 1 M sodium hydroxide was added. Finally, the volume was madeup to 10 mL with distilled water and properly mixed. An orange yellowish colour developed. The absorbance was measured at 517 nm spectrophotometer using UV-visible (Jasco V-630) instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin.

The data of total flavonoid of the leaves powder were expressed as mg of quercetin equivalents/ 100 g of dry mass

Free Radical Scavenging Activity

The antioxidant activity of fractions of methanol extract of the plant was assayed by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method described by Karadag, *et al* [18]. The assay mixture contained 2 mL of 1.0 mM DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (1.25 – 20 mg/mL). The solution was rapidly mixed and incubated in dark at 37 ^{o}C for 20 minutes. The decrease in absorbance of each solution was measured at 517 nm using UV-visbile spectrophotometer (Jasco V-630) instrument. Ascorbic acid was used as positive control while 2 mL of 1.0 mM DPPH radical solution with 1 mL ethanol was taken negative control.

The percentage of radical scavenging (%) was calculated by:

% Free Radical Scavenging Activity = $A_c – A_s/A_c$ x 100

Where, A_c = Absorbance of control at 517 nm

A_s = Absorbance of sample at 517 nm

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percentage inhibitions plotted against the respective concentrate

Effect of Solvent on the Extraction Yield of Abia, Ekiti and Ondo Pollen Extracts

The extraction yield of bee pollen from Abia (ABPL), Ekiti (EKPL), and Ondo (ONPL) States (Table 1) varied with solvent, reflecting the role of solvent polarity. Yields ranged from 0.20–2.29% (ABPL), 0.20–1.64% (EKPL), and 0.38–22.78% (ONPL). In ABPL, aqueous extract showed the highest yield (2.29%), followed by acetone (1.99%) and ethanol (1.88%), while ethyl acetate was lowest (0.20%). EKPL followed a similar pattern, with ethanol highest (1.64%), then acetone (0.85%) and water (0.72%), and ethyl acetate lowest (0.20%).

ONPL extracts showed markedly higher yields, especially ethanol (22.78%) and water (20.45%), while acetone (2.89%) and ethyl acetate (0.38%) were lower. The higher yields with ethanol and water are attributed to their polarity, enhancing extraction of polar constituents [19-20]. Water extracts may also reflect co-extraction of proteins and carbohydrates [21], improving recovery of

soluble bioactive compounds [22]. The elevated ONPL yields may reflect regional botanical diversity [23-24].

Effect of Solvent on the Total Phenolic Content and Total Flavonoid Content of Abia, Ekiti and Ondo Pollen Extracts

The total phenolic content and total flavonoid content of bee pollen extracts varied across locations and solvents (Table 1). Abia pollen (A-ABPL) showed the highest TPC in acetone (77.82 ± 0.08 mg GAE/g), followed by water (49.79 ± 0.29 mg GAE/g), ethanol (13.84 ± 0.17 mg GAE/g), and ethyl acetate (2.12 ± 0.17 mg GAE/g). For Ekiti, ethanol recorded the highest TPC (36.34 ± 0.12 mg GAE/g), followed by water (25.87 ± 0.48 mg GAE/g), acetone (15.23 ± 12.64 mg GAE/g), and ethyl acetate (5.62 ± 0.39 mg GAE/g). Ondo extracts showed highest TPC in acetone (46.32 ± 0.58 mg GAE/g), followed by water (33.98 ± 0.22 mg GAE/g), ethanol (21.87 ± 0.05 mg GAE/g), and ethyl acetate (10.09 ± 0.21 mg GAE/g). These results indicate higher phenolic extraction with acetone, ethanol, and water due to their polarity [24-26].

TFC also varied significantly. In Abia, water extract showed highest TFC (341.99 mg QE/g), followed by ethanol (323.64 mg QE/g), acetone (316.67 mg QE/g), and ethyl acetate (255.65 mg QE/g). Ekiti showed highest TFC in ethanol (341.42 ± 36.21 mg QE/g), followed by water (283.92 ± 1.31 mg QE/g), acetone (242.06 ± 2.91 mg QE/g), and ethyl acetate (231.38 ± 15.66 mg QE/g). Ondo followed a similar trend with water highest (298.92 ± 2.51 mg QE/g), then acetone (292.44 ± 0.19 mg QE/g), ethanol (289.37 ± 0.51 mg QE/g), and ethyl acetate (235.83 ± 2.33 mg QE/g). Lower TFC in ethyl acetate reflects limited extraction of polar flavonoids [26].

Abia pollen showed the highest phenolic content, suggesting richer polyphenolic composition, possibly due to botanical and environmental factors [27-28]. Overall, TFC exceeded TPC across samples. Lower TPC may reflect limitations of the Folin–Ciocalteu assay and interference by compounds such as ascorbic acid and sugars [29]. Solvent–pollen interactions also influence TFC [30], consistent with findings of Oroian *et al* [31].

Table 1: Extraction Yield, Total Phenolic Content and Total Flavonoid Content of Abia, Ekiti and Ondo Pollen extracts

Solvent Extracts	Extraction yield (% w/w)	TPC (mg GAE/g)	TFC (mg QCE/g)
EA- ABPL	0.20	2.12 ± 0.17	255.65±0.91
A-ABPL	1.99	77.82 ± 0.08	316.67±0.19
E-ABPL	1.88	13.84 ± 0.17	323.64 ± 10.27

D-ABPL	2.29	49.79 ± 0.29	341.99±0.68
EA-ONPL	0.38	10.09±0.21	235.83 ± 2.33
A-ONPL	2.89	46.32±0.58	292.44 ± 0.19
E-ONPL	22.78	21.87±0.05	289.37 ± 0.51
D-ONPL	20.45	33.98±0.22	298.92 ± 2.51
EA-EKPL	0.20	5.62 ±0.39	231.38 ± 15.66
A-EKPL	0.85	15.23±12.64	341.42 ± 36.21
E-EKPL	1.64	36.34 ± 0.12	242.06 ± 2.91
D-EKPL	0.72	25.87 ± 0.48	283.92 ± 1.31

Key: EA-EKPL = ethyl acetate Ekiti pollen extract, A-EKPL = Acetone Ekiti pollen extract, E-EKPL = Ethanol Ekiti pollen extract, D- EKPL= Water Ekiti pollen extract, A-ONPL = Acetone Ondo pollen extract, E-ONPL = Ethanol Ondo pollen extract, D- ONPL = Water Ondo pollen extract, EA-ABPL = ethyl acetate Abia pollen extract, A-ABPL = Acetone Abia pollen extract E-ABPL = Ethanol Abia pollen extract, D- ABPL= Water Abia pollen extract

Table 2 and Figure 1 show that as concentration increases, the percentage of DPPH scavenged increased for all samples, indicating a dose-dependent response. At higher concentrations, Aqueous extract (D-ABPL) demonstrated the highest activity, followed by ethanol (E-ABPL), acetone (A-ABPL), and ethyl acetate (EA-ABPL).

Table 2: Free Radical Scavenging Activity (DPPH) for ABPL Samples

Con (mg/mL)	EA-ABPL	A-ABPL	E-ABPL	D-ABPL	Ascorbic Acid
20	0.27	13.82	23.63	83.07	98.45
10	0.14	6.91	11.63	41.54	89.77
5	0.07	3.46	5.91	27.77	78.57
2.5	0.03	1.73	2.95	10.38	62.33
1.25	0.02	0.86	1.48	5.19	52.74

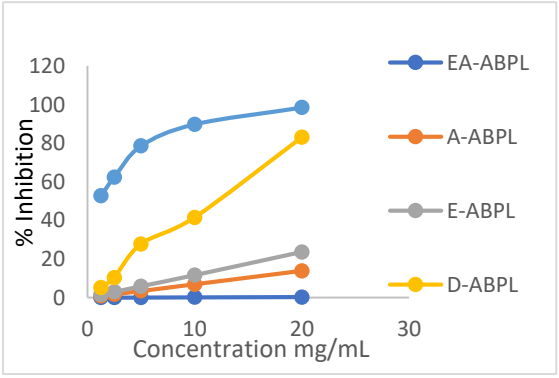

Figure 1. The Free Radical Scavenging Activity of Abia State Bee Pollen (ABPL)

Table 3 and Figure 2 present the IC_{50} values for ABPL samples. Aqueous extract (D-ABPL) exhibited the lowest IC_{50} (11.78), indicating the highest antioxidant activity. Ethanol (E-ABPL) showed a higher IC_{50} (42.36), followed by acetone (A-ABPL) (72.36), while ethyl acetate (EA-ABPL) had the highest IC_{50} (3703.61), indicating the least activity.

Table 3. Inhibition Concentration IC_{50} for ABPL Samples

Solvent	IC_{50}
EA-ABPL	3703.61
A-ABPL	72.36
E-ABPL	42.36
D-ABPL	11.78
Ascorbic Acid	-3.99

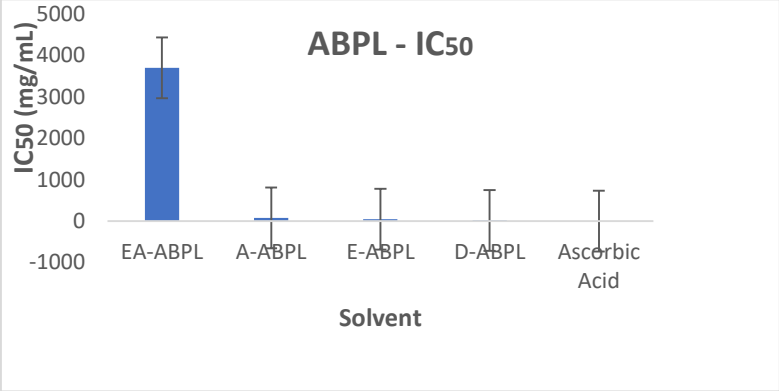

Figure 2. The Inhibition Concentration IC_{50} for Abia State Bee Pollen (ABPL)

Table 4 and Figure 3 present the Free Radical Scavenging Activity (DPPH) of EKPL samples at different concentrations using ethyl acetate (EA-EKPL), acetone (A-EKPL), ethanol (E-EKPL), and water (D-EKPL) extracts. The percentage of DPPH scavenged increased with concentration for all samples, indicating a dose-dependent response. Ascorbic Acid consistently showed higher scavenging activity across all concentrations compared to EKPL samples.

Table 4: Free Radical Scavenging Activity (DPPH) for EKPL Samples

Conc. (mg/mL)	EA-EKPL	A-EKPL	E-EKPL	D-EKPL	Ascorbic Acid
20	41.2	62.4	83.6	104.8	98.45
10	30.5	51	71.5	92	89.77
5	10.38	15.76	21.14	26.52	78.57
2.5	5.19	7.88	10.57	13.26	62.33
1.25	2.59	3.93	5.27	6.61	52.74

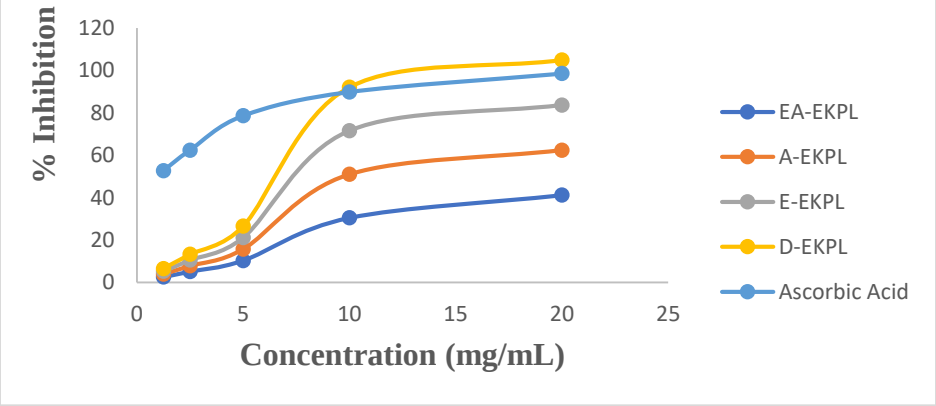

Figure 3: Free Radical Scavenging Activity of Ekiti State Bee Pollen (EKPL)

Table 5 and Figure 4 present the IC_{50} values, where lower values indicate higher antioxidant activity.

Table 5: Inhibition Concentration IC_{50} for EKPL Samples

Samples	IC_{50}
EA-EKPL	22.62266
A-EKPL	14.34362
E-EKPL	10.34697
D-EKPL	7.992537
Ascorbic Acid	-3.99

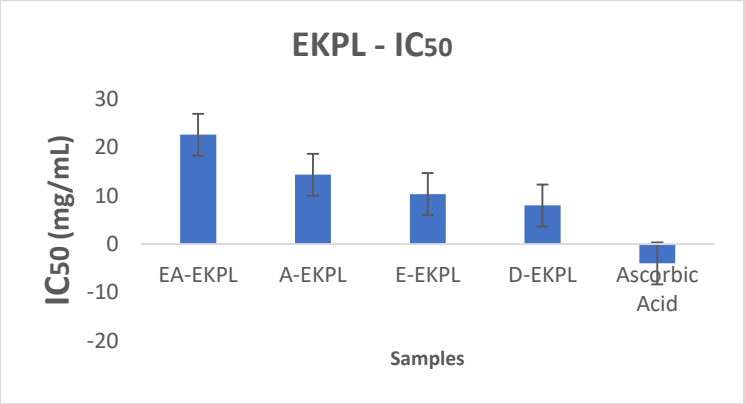

Figure 4: Chart Showing the Inhibition Concentration IC_{50} for Ekiti State Bee Pollen (EKPL)

The aqueous extract (D-EKPL) showed the lowest IC_{50} (7.99), while ethyl acetate (EA-EKPL) recorded the highest (22.62), indicating lowest activity. These findings suggest solvent extraction influences antioxidant activity, with aqueous extracts showing the highest potential, consistent with literature [32-34].

Table 6 and Figure 5 show that increasing concentration led to higher DPPH scavenging, indicating a dose-dependent response.

Table 6: Free Radical Scavenging Activity (DPPH) for ONPL Samples

Con (mg/mL)	EA-ONPL	A-ONPL	E-ONPL	D-ONPL	Ascorbic Acid
20	1.03	21.77	71.93	80.12	98.45
10	0.52	10.89	37.47	50.06	89.77
5	0.26	5.44	18.73	20.03	78.57
2.5	0.13	2.72	9.37	14.15	62.33
1.25	0.06	1.36	4.68	5.01	52.74

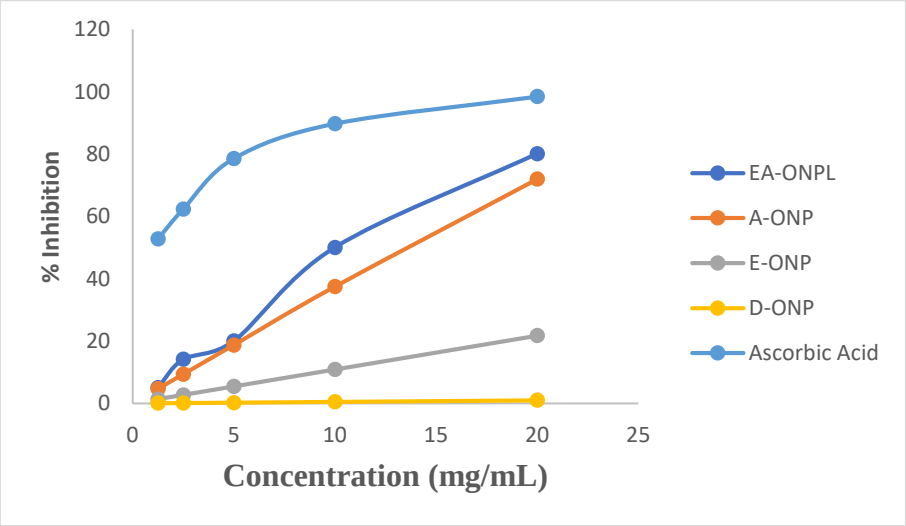

Figure 5: The Free Radical Scavenging Activity of Ondo State Bee Pollen (ONPL)

At higher concentrations, aqueous extract (D-ONPL) showed the highest activity, followed by ethanolic extract (E-ONPL), while ethyl acetate recorded the least inhibition.

Table 7 and Figure 6 show that aqueous extract (D-ONPL) had the lowest IC50 (9.63), indicating the highest antioxidant activity. Ethanol (E-ONPL) followed with a higher IC50 (13.76), showing lower activity. These results indicate that solvent extracts influence the antioxidant activity of ONPL samples.

Table 7: Inhibition Concentration IC_{50} for ONPL Samples

Samples	IC_{50}
EA-ONPL	9.63
A-ONPL	13.76
E-ONPL	45.93
D-ONPL	49.95
Ascorbic Acid	-3.99

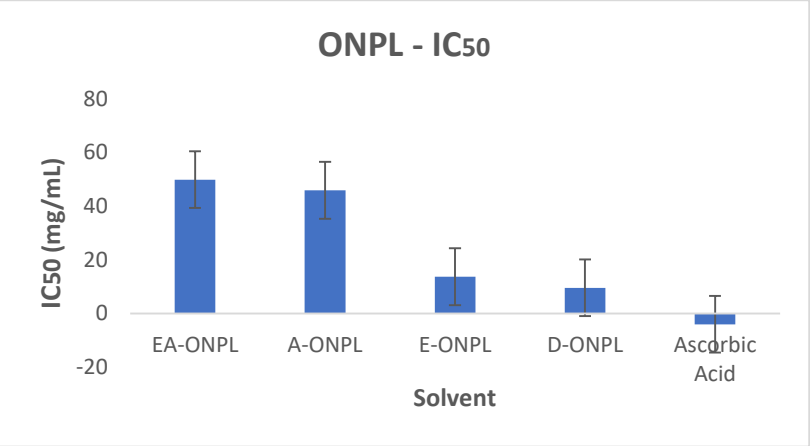

Figure 6: The Inhibition Concentration IC_{50} for Ondo State Bee Pollen (ONPL)

CONCLUSION

This study investigated the total phenolic and flavonoid compositions, as well as the free radical scavenging activity of selected bee pollen samples from Abia, Ekiti and Ondo States, Nigeria. The results demonstrated significant variations in phenolic and flavonoid contents among the samples, influenced by the type of extraction solvent used. Water and ethanol emerged as effective solvents for extracting phenolic compounds, whereas acetone and water demonstrated superior efficiency for flavonoid. Comparatively, ethyl acetate extraction consistently yielded lower phenolic and flavonoid contents. The free radical scavenging activity varied across the samples, with aqueous extract exhibiting the highest antioxidant activity. These findings emphasize the importance of solvent selection in optimizing the extraction of bioactive compounds from bee pollen.

This study highlighted the pharmacological and medicinal properties of Nigerian bee pollen, emphasizing its potential as a natural source of antioxidants with potential health-promoting benefits. Further research is necessary to explore its applications in healthcare and nutrition.

REFERENCES

1. El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C. & Lyoussi, B. (2023). Bee Pollen as Functional Food: Insights into Its Composition and Therapeutic Properties. *Antioxidants*, *12*(3), 557.
2. , C., Candal-Uslu, C., Özkök, A., Koç, A., Özhanlı, H., Akdeniz, G. & Erbaş, M. (2025). Comprehensive analysis of botanical origin and amino acid composition of bee pollen samples from various regions of Turkey. *Journal of Apicultural Research*, *64*(4), 1142-1150.
3. Mărgăoan, R., Stranț, M., Varadi, A., Topal, E., Yücel, B., Cornea-Cipcigan, M., ... & Vodnar, D. C. (2019). Bee collected pollen and bee bread: Bioactive constituents and health benefits. *Antioxidants*, *8*(12), 568
4. Kowalczuk, I., Gębski, J., Stangierska, D. & Szymańska, A., (2023). Determinants of Honey and other bee products use for culinary, cosmetic, and medical purposes. *Nutrients*, *15*(3), p.737.
5. Zambrano, T.Y.M., Joza, J.M.A., Cobeña, G.V.S., Anchundia, S.E.P. & Zambrano, Y.D.M., 2022. Honey, pollen and propolis in therapeutic treatments for COVID-19. *International journal of health sciences*, *6*(1), pp.196-213.
6. Alcalá-Orozco, M., Lobo-Farfan, I., Tirado, D. F. & Mantilla-Escalante, D. C. (2024). Enhancing the nutritional and bioactive properties of bee pollen: A comprehensive review of processing techniques. *Foods*, *13*(21), 3437.
7. Sirotkin, A. V., Tarko, A., Alexa, R., Fakova, A., Alwasel, S. & Harrath, A. H. (2020). Bee pollens originating from different species have unique effects on ovarian cell functions. *Pharmaceutical Biology*, *58*(1), 1101-1106.
8. Elsegood, L. (2020). *The LDN Book, Volume Two: The Latest Research on How Low Dose Naltrexone Could Revolutionize Treatment for PTSD, Pain, IBD, Lyme Disease, Dermatologic Conditions, and More*. Chelsea Green Publishing.
9. Rashidi, Z., Khosravizadeh, Z., Talebi, A., Khodamoradi, K., Ebrahimi, R. & Amidi, F. (2021). Overview of biological effects of Quercetin on ovary. Phytotherapy research, 35(1), 33-49.

10. Münstedt, K., Hoffmann, S., Hauenschild, A., Bülte, M., von Georgi, R. & Hackethal, A. (2009). Effect of honey on serum cholesterol and lipid values. *Journal of medicinal food*, *12*(3), 624-628.
11. Du, J., Zhong, B., Subbiah, V., Barrow, C. J., Dunshea, F. R. & Suleria, H. A. (2021). Lc-esi-qtof-ms/ms profiling and antioxidant activity of phenolics from custard apple fruit and by-products. *Separations*, *8*(5), 62.
12. Ahmad, R. S., Hussain, M. B., Saeed, F., Waheed, M. & Tufail, T. (2017). Phytochemistry, metabolism, and ethnomedical scenario of honey: A concurrent review. *International journal of food properties*, *20*(sup1), S254-S269.
13. Patel, D. K. (2023). Ethnopharmacological Uses, Pharmacological Activities, and Therapeutic Applications of Tectochrysin in Medicine: An Important Class of Dietary Flavonoid. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders)*, *23*(1), 11-20.
14. Ikewuchi, C. C., Ikewuchi, J. C. & Ifeanacho, M. O. (2015). Phytochemical composition of Tridax procumbens Linn leaves: potential as a functional food. *Food and Nutrition Sciences*, *6*(11), 992.
15. Guiné, R. P., Florença, S. G., Barroca, M. J. & Costa, C. A. (2025). Bee Brood as a Food for Human Consumption: An Integrative Review of Phytochemical and Nutritional Composition. *Insects*, *16*(8), 796.
16. Molole, G. J., Gure, A. & Abdissa, N. (2022). Determination of total phenolic content and antioxidant activity of *Commiphora mollis (Oliv.)* Engl. resin. *BMC chemistry*, *16*(1), 48.
17. Pallab, K., Tapan, B., Tapas, P. & Ramen, K. (2013). Estimation of total flavonoids content (TPC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. *Journal of Drug Delivery and Therapeutics,* 3(4): 33-37.
18. Karadag, A., Ozeelik, B. and Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food Analytical Methods*, 2: 41-60.
19. Çelik, S., Kutlu, N., Gerçek, Y. C., Bayram, S., Pandiselvam, R. & Bayram, N. E. (2022). Optimization of ultrasonic extraction of nutraceutical and pharmaceutical compounds from bee pollen with deep eutectic solvents using response surface methodology. *Foods*, *11*(22), 3652.

20. Mekky, A. E., El-Barkey, N. M., Abd El Halim, H. M., Nasser, S. A., Mahmoud, N. N., Zahra, A. A. & Nasr-Eldin, M. A. (2025). Exploring the potential of hydro alcoholic crude extract of beeswax as antibacterial antifungal antiviral antiinflammatory and antioxidant agent. *Scientific reports*, *15*(1), 32512
21. Jin, T. Y., Saravanakumar, K. & Wang, M. H. (2018). In vitro and in vivo antioxidant properties of water and methanol extracts of linden bee pollen. *Biocatalysis and agricultural biotechnology*, *13*, 186-189.
22. Lawag, I. L., Yoo, O., Lim, L. Y., Hammer, K. & Locher, C. (2021). Optimisation of bee pollen extraction to maximise extractable antioxidant constituents. *Antioxidants*, *10*(7), 1113.
23. Malagnini, V., Cappellari, A., Marini, L., Zanotelli, L., Zorer, R., Angeli, G., ... & Fontana, P. (2022). Seasonality and landscape composition drive the diversity of pollen collected by managed honey bees. *Frontiers in Sustainable Food Systems*, *6*, 865368.
24. Tesfaye, O. (2025). Effect of floral variation on the proximate and mineral composition of bee pollen in Dale Sedi District, Kellem Wollega Zone, Western Ethiopia. *BMC Plant Biology*, *25*(1), 1068.
25. Adekanmbi, O. H. & Alebiosu, O. S. (2016). Dominant pollen in honey samples from four beehives in Abeokuta, southwest, Nigeria. *UNILAG Journal of Medicine, Science and Technology*, *4*(1), 140-147.
26. Farooq, S., Abdullah, Zhang, H. & Weiss, J. (2021). A comprehensive review on polarity, partitioning, and interactions of phenolic antioxidants at oil–water interface of food emulsions. *Comprehensive Reviews in Food Science and Food Safety*, *20*(5), 4250-4277.
27. Palmer-Young, E. C., Farrell, I. W., Adler, L. S., Milano, N. J., Egan, P. A., Junker, R. R., ... & Stevenson, P. C. (2019). Chemistry of floral rewards: intra-and interspecific variability of nectar and pollen secondary metabolites across taxa. *Ecological Monographs*, *89*(1), e01335.
28. Homrani, M., Escuredo, O., Rodríguez-Flores, M. S., Fatiha, D., Mohammed, B., Homrani, A. & Seijo, M. C. (2020). Botanical origin, pollen profile, and physicochemical properties of Algerian honey from different bioclimatic areas. *Foods*, *9*(7), 938.

29. Lawag, I. L., Nolden, E. S., Schaper, A. A., Lim, L. Y. & Locher, C. (2023). A modified folin-ciocalteu assay for the determination of total phenolics content in honey. *Applied Sciences*, *13*(4), 2135.
30. Harif Fadzilah, N., Jaapar, M. F., Jajuli, R. & Wan Omar, W. A. (2017). Total phenolic content, total flavonoid and antioxidant activity of ethanolic bee pollen extracts from three species of Malaysian stingless bee. *Journal of Apicultural Research*, *56*(2), 130-135.
31. Oroian, M., Ursachi, F. & Dranca, F. (2020). Ultrasound-assisted extraction of polyphenols from crude pollen. *Antioxidants*, *9*(4), 322.
32. Kainama H., Fatmawati S., Santoso M., Papilaya P.M., Ersam T. (2020). The Relationship of Free Radical Scavenging and Total Phenolic and Flavonoid Contents of Garcinia lasoar . PAM. Pharm. Chem. Journal, 53,1151–1157.
33. Borycka, K., Grabek-Lejko, D. & Kasprzyk, I., (2015). Antioxidant and antibacterial properties of commercial bee pollen products. *Journal of Apicultural Research*, *54*(5), .491-502.
34. Sun, C., Wu, Z., Wang, Z. *Zhang, H., (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. *Evidence-Based Complementary and Alternative Medicine*, (1), p.595393.