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Scientific Assessment of Antimicrobial Activity of Bee Glue Samples in three Selected Zones of Enugu State, Nigeria

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Abstract

This study evaluated the chemical composition and antimicrobial efficacy of bee glue from three ecological zones in Enugu State, Nigeria: Nsukka (Enugu North), Nkanu West (Enugu East), and Udi (Enugu West). Bee glue samples were collected, extracted, and analyzed for phytochemical content, followed by an assessment of their antimicrobial properties using the broth microdilution method against selected bacterial and fungal pathogens. The samples revealed significant levels of dry matter, potassium, tannins, zinc, and vitamin A, with tannins contributing to potent antioxidant activity. The ANOVA results demonstrated significant differences ($p < 0.05$) in antimicrobial activity across the three regions, with the most pronounced inhibition zones observed in Nsukka for Pseudomonas aeruginosa at 50 mg/ml (3.21 ± 0.023 mm) and in Nkanu for Aspergillus fumigatus at 150 mg/ml (6.31 ± 0.076 mm). These findings indicate that geographic origin significantly influences the chemical composition and biological activity of bee glue, suggesting its potential for pharmaceutical or food preservation applications.

Introduction: Bee glue, commonly referred to as propolis, is a resinous substance produced by honeybees, particularly *Apis mellifera adansonii* (West African Honeybee), a subspecies predominantly found in West Africa, including Nigeria. This species is the only subspecies of *Apis mellifera* present in Nigeria, where it is domesticated in a variety of traditional hives such as basket hives, skeps, drum hives, clay pot hives, and gouge hives. These hives are typically maintained by a small number of traditional beekeepers who harvest not only honey but also other valuable bee products, including royal jelly, beeswax, pollen, and propolis (Fasasi *et al.*, 2018).

Propolis is particularly valued for its antimicrobial properties, which have garnered significant attention in both traditional and modern medicine. The bees collect propolis from various plant sources and use it as a sealant within the hive, effectively preventing the decomposition of any intruders killed by the bees (Amoros *et al.*, 2013; Brumfitt *et al.*, 2015). Due to its complex composition, propolis must be purified through extraction processes to remove inert materials, thereby preserving its active polyphenolic fraction. This purified substance is known for its lipophilic nature, which causes it to be hard and brittle when cold but soft and sticky when warm. Propolis also emits a pleasant aromatic scent and varies in color depending on its source and age (Brumfitt *et al.*, 2015).

Given the diverse ecological zones in Enugu State, Nigeria, the composition and potency of propolis collected from different regions may vary, influencing its antimicrobial efficacy. Understanding these variations is crucial for optimizing the use of propolis in medicinal applications, particularly in the development of natural antimicrobial agents.

Materials and Methods: The study was conducted in Enugu State, located in the southeastern region of Nigeria. Geographically, the state is positioned between latitudes 6° 25′ 0″ N and 6° 29′ 0″ N, and longitudes 7° 28′ 0″ E and 7° 32′ 0″ E (Fig. 1). Enugu State is geologically situated within the Anambra sedimentary basin, a prominent geological feature in eastern Nigeria. The escarpment of Enugu is predominantly formed by the Ajali Sandstone, along with the sandstone units of the Mamu Formation Egesi (2017). The study area is underlain by the Enugu Shale, with outcrops of the Mamu Formation visible in certain locations. The Mamu Formation consists primarily of shale and sandstone, contributing to the region's distinct geological characteristics.

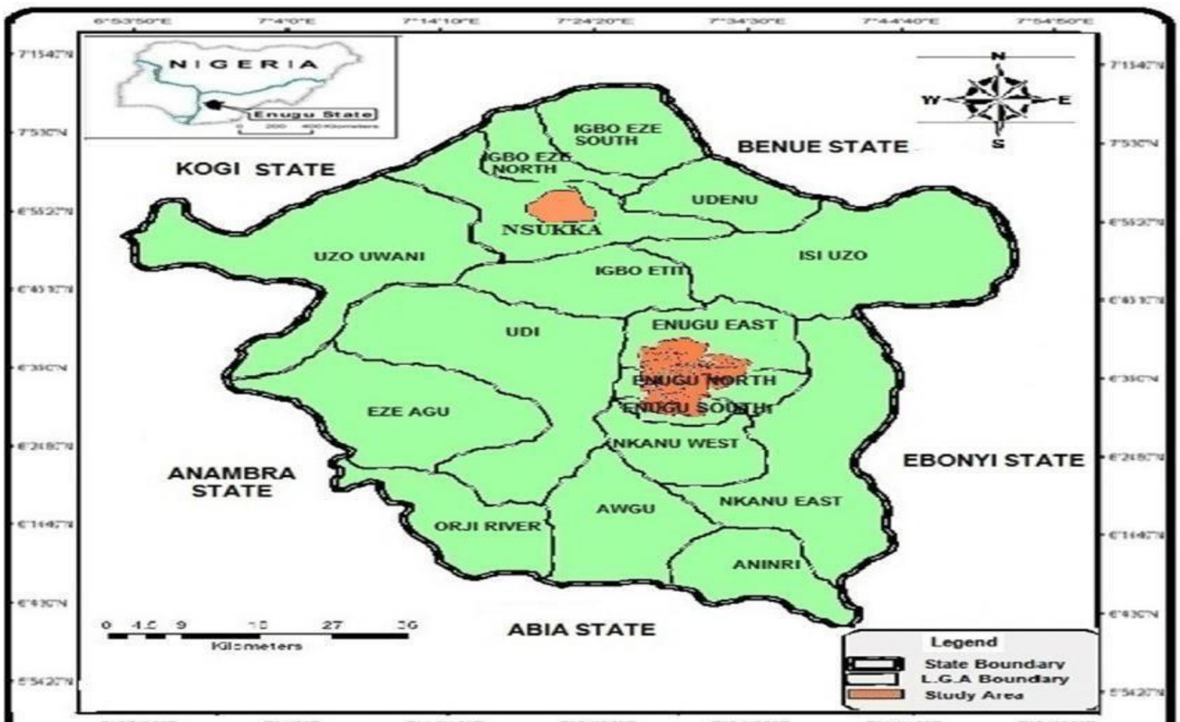

Fig.1 MAP OF ENUGU STATE SHOWING THE STUDY AREA
SOURCE: MINISTRY OF LAND SURVEYS ENUGU STATE.

Collection of bee glue and organisms: A stratified random sampling technique was employed to assess the antimicrobial properties of bee glue (propolis) across three distinct ecological zones. The study area was first divided into these zones based on environmental and geographical characteristics to capture ecological diversity. Within each zone, a comprehensive list of beekeeping sites was located and documented, from which sites were randomly selected to ensure unbiased sampling. Propolis was then collected from the chosen hives using a standardized protocol across all zones, allowing for accurate comparisons of antimicrobial efficacy while accounting for the ecological variability inherent to the different regions.

Table 1: Zones and sampled local government areas in the study area

S/N	ZONES	SAMPLED LGAs
1.	Enugu North	Nsukka
2.	Enugu East	Nkanu West
3.	Enugu West	Udi

Source of test organisms: The test organisms used in this study comprised both bacterial and fungal species. The fungal organisms included *Fusarium peritonitis*, *Candida albicans*, *Aspergillus fumigatus*, and *Rhizopus spp.*, while the bacterial organisms included *Streptococcus mutans*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Pure clinical isolates of these organisms were obtained from the Biochemistry Laboratory of the Federal University of Technology, Owerri.

Extraction of bee glue: Bee glue (propolis) was extracted using a combination of ethanol and water. Various concentrations of the extracts were prepared for antimicrobial testing. A concentration of 50 mg/ml was prepared by dissolving 5 ml of the stock extract in 10 ml of ethanol. Similarly, concentrations of 100 mg/ml and 150 mg/ml were prepared by dissolving 10 ml and 15 ml of the stock extract in 10 ml of ethanol, respectively. These ethanol extracts of bee glue were then applied at varying concentrations to assess their antimicrobial activity against the selected pathogenic organisms.

Data analysis: The data collected from the antimicrobial assays were analyzed using analysis of variance (ANOVA) to test for significant differences in means. The paleontological statistical tool, PAST, was employed for this analysis, while Microsoft Excel was used to generate graphical representations of the data.

Results and Discussions

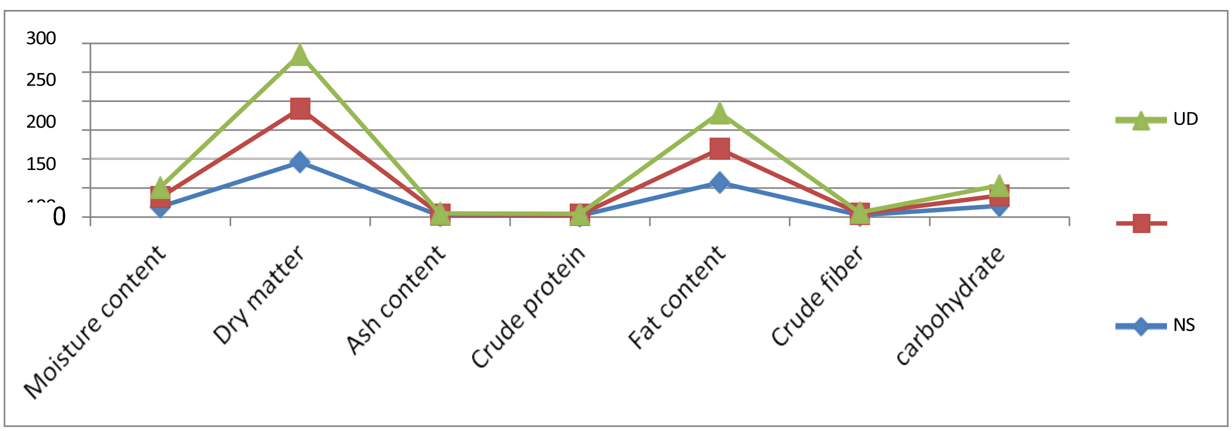

Figure 2: Mean Proximate Composition of Bee glue Collected from Three Different local areas in Enugu state.

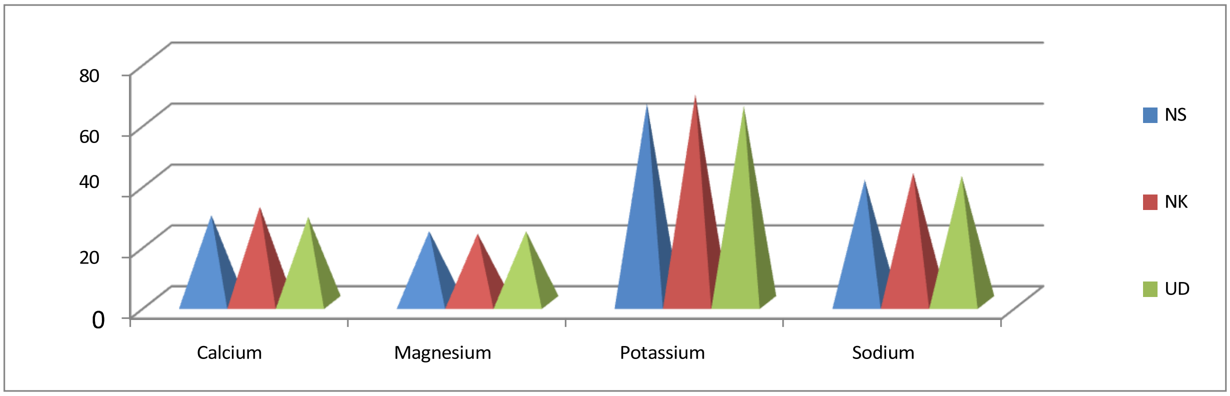

Figure 3: Mineral Composition of Bee glue Collected from Three Different local government areas in Enugu state.

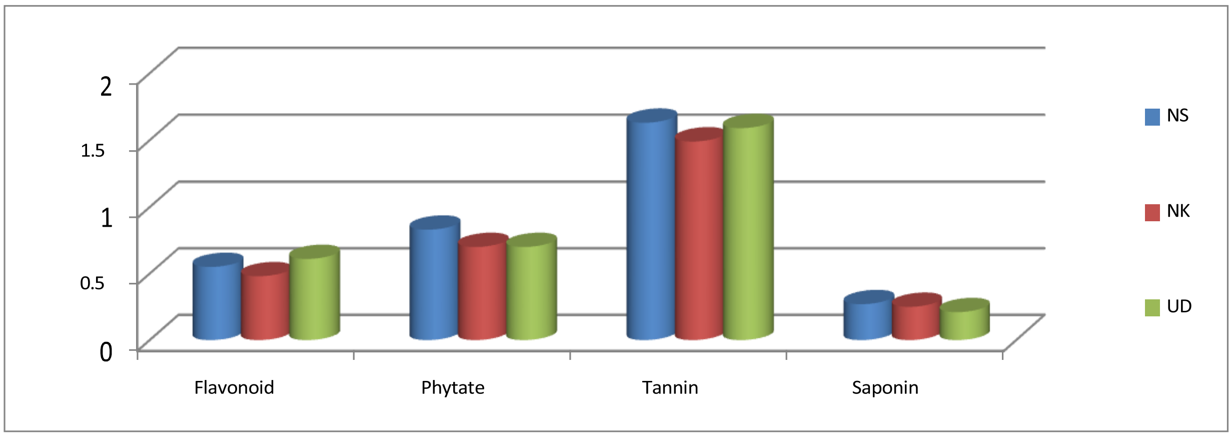

Figure 4: Phytochemical Composition of Bee glue Collected from 3 different local government areas in Enugu state.

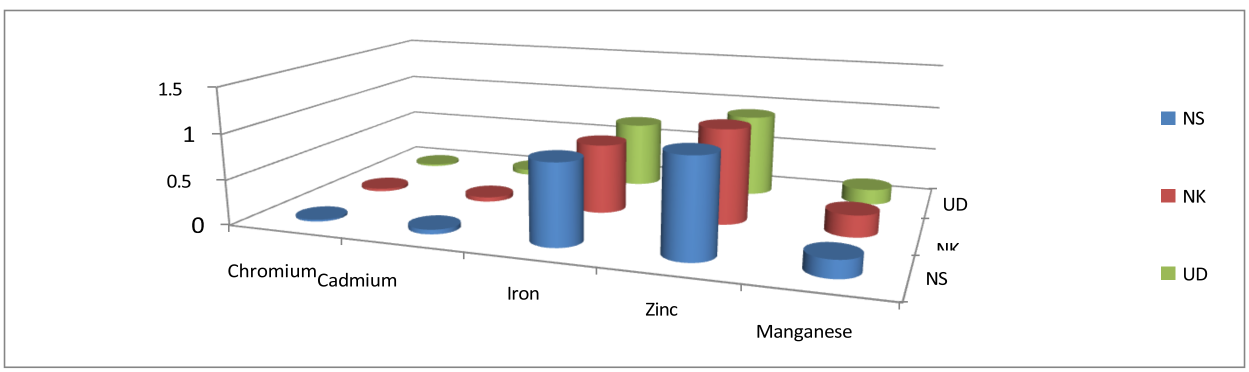

Figure 5: Heavy Metal Composition of Bee glue Collected from three local government areas in Enugu state.

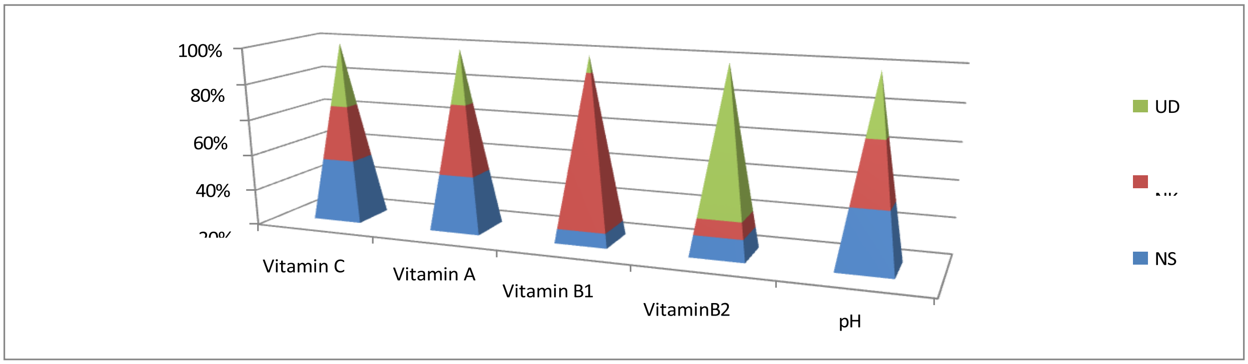

Figure 6: Vitamin Compositions of Bee glue Collected from three Different Vegetation Zones of Nigeria.Note: Where NS = bee glue collected Nsukka local government area, Enugu state. NK=bee glue collected from Nkanu local government area, Enugu state. UD = bee glue collected from Udi local government area, Enugu state.

Table 2: Antimicrobial zone of inhibition at 50mg/ml of Bee glue collected from Three Different local government area in Enugu state.

Bacteria organisms (Mg/ml)	*Salmonella Typhii*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Streptococcus mutan*
NS	$1.9^{C}\pm0.023$	$1.87^{C}\pm0.030$	$3.21^{A}\pm0.023$	$2.42^{B}\pm0.025$
NK	$2.14^{C}\pm0.020$	$1.92^{D}\pm0.025$	$2.86^{A}\pm0.034$	$2.53^{B}\pm0.061$
UD	$1.87^{D}\pm0.026$	$2.15^{C}\pm0.035$	$3.15^{A}\pm0.020$	$2.42^{B}\pm0.138$
Control	$8.44^{C}\pm0.005$	$9.61^{A}\pm0.020$	$8.70^{B}\pm0.005$	$8.20^{D}\pm0.005$

Table 3: Antimicrobial zone of inhibition at 100mg/ml of Bee glue Collected from Three Different local government area in Enugu state.

Bacteria organisms (Mg/ml)	*Salmonella typhii*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Streptococcus mutans*
NS	$3.24^{C}\pm0.040$	$3.31^{C}\pm0.079$	$4.84^{A}\pm0.017$	$4.18^{B}\pm0.023$
NK	$4.21^{B}\pm0.036$	$3.55^{C}\pm0.086$	$4.54^{A}\pm0.121$	$4.28^{B}\pm0.028$
UD	$3.61^{C}\pm0.028$	$2.98^{C}\pm0.144$	$4.71^{A}\pm0.036$	$4.57^{A}\pm0.064$
Control	$11.35^{C}\pm0.086$	$12.47^{A}\pm0.110$	$12.38^{A}\pm0.076$	$11.78^{B}\pm0.076$

Table 4: Antimicrobial zone of inhibition at 150mg/ml of Bee glue Collected from Three Different local government areas in Enugu state.

Bacteria organisms (Mg/ml)	*Salmonella typhii*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Streptococcus mutans*
NS	$5.65^{C}\pm0.081$	$5.75^{B}\pm0.040$	$6.28^{A}\pm0.034$	$6.23^{A}\pm0.028$
NK	$5.85^{A}\pm0.041$	$5.47^{B}\pm0.110$	$5.88^{A}\pm0.034$	$5.40^{B}\pm0.011$
UD	$5.24^{C}\pm0.050$	$4.78^{D}\pm0.160$	$5.96^{A}\pm0.057$	$5.72^{B}\pm0.105$
Control	$14.51^{A}\pm0.076$	$14.66^{A}\pm0.076$	$13.64^{B}\pm0.144$	$14.21^{B}\pm0.529$

Where NS = bee glue collected Nsukka local government area, Enugu state.

NK = bee glue collected from Nkanu local government area, Enugu state.

UD = bee glue collected from Udi local government area, Enugu state.

Values within the column with different superscript are significantly ($p<0.05$) different. Values are presented as Mean ± SD.

Control (Ciprofloxacin 100mg/ml against each of the samples).

Table 5: Fungi zone of inhibition 50mg/ml of Bee glue Collected from Three local governments area in Enugu state.

Fungiorganisms (Mg/ml)	*Fusarium peritonitis*	*Aspergillus fumigatus*	*Candida Albicans*	*Rhizopus*
NS	$2.74^{B}\pm0.017$	$3.17^{A}\pm0.011$	$2.44^{C}\pm0.011$	$2.21^{D}\pm0.028$
NK	$2.67^{B}\pm0.025$	$2.93^{A}\pm0.036$	$1.93^{D}\pm0.017$	$2.58^{C}\pm0.034$
UD	$2.80^{A}\pm0.011$	$2.63^{B}\pm0.015$	$2.27^{C}\pm0.025$	$2.79^{A}\pm0.036$
Control	$6.77^{C}\pm0.005$	$5.86^{D}\pm0.034$	$6.86^{B}\pm0.028$	$7.09^{A}\pm0.079$

Table 6: Fungizoneofinhibition100mg/mlOfBeeglueCollectedfromthreel ocal governmentareas in Enugu state.

Fungiorganisms (Mg/ml)	*Fusarium peritonitis*	*Aspergillus fumigatus*	*Candida albicans*	*Rhizopus*
NS	3.40^{B}±0.011	4.33^{A}±0.026	2.95^{B}±0.516	3.01^{B}±0.028
NK	3.76^{B}±0.040	4.82^{A}±0.025	3.10^{C}±0.020	3.23^{B}±0.598
UD	3.65^{B}±0.005	4.68^{A}±0.052	3.76^{B}±0.595	3.49^{B}±0.096
Control	9.54^{B}±0.121	8.48^{D}±0.028	9.35^{C}±0.092	10.56^{A}±0.060

Table 7: Fungi zone of inhibition 150mg/ml Of Bee glue Collected from three local government areas in Enugu state.

Fungi organisms(Mg/ml)	*Fusarium Peritonitis*	*Aspergillus fumigatus*	*Candida albicans*	*Rhizopus*

NS	5.71^{B}±0.035	6.31^{A}±0.076	5.26^{C}±0.041	4.60^{D}±0.011
NK	5.23^{C}±0.026	5.79^{A}±0.036	5.56^{B}±0.069	4.83^{D}±0.015
UD	4.83^{C}±0.015	5.70^{A}±0.089	5.23^{B}±0.028	4.34^{D}±0.046
Control	12.60^{A}±0.180	11.71^{C}±0.076	12.79^{A}±0.026	12.44^{B}±0.052

Where NS = bee glue collected Nsukka local government area, Enugu state.

NK = bee glue collected from Nkanu local government area, Enugu state.

UD= bee glue collected from Udi local government area, Enugu state.

Values within the column with different superscript are significantly ($p<0.05$) different. Values are presented as Mean ± SD.

Control (Ciprofloxacin, 100mg/ml against each of the samples).

Discussion: The proximate composition of bee glue collected from three distinct local government areas in Enugu State—Nsukka (NS), Nkanu (NK), and Udi (UD)—revealed significant variations in chemical constituents. NS exhibited the highest levels of dry matter (93.62±0.138), carbohydrate (18.75±0.078), and crude fiber (2.61±0.015), while NK displayed the highest moisture content (17.23±0.030), ash content (1.95±0.010), and crude protein (1.73±0.032). Conversely, UD had the highest fat content (61.68±1.201) but the lowest crude protein (1.15±0.020), ash (1.71±0.015), and crude fiber (2.29±0.011). These variations in chemical composition are consistent with the findings of Fabio et al. (2018), who reported that the chemical and biological properties of bee glue are influenced by its geographic origin and type. Jonilson *et al.* (2014) similarly observed that the nutritional composition of bee products could be affected by the local environment and diet of the bees.

The mineral composition analysis of bee glue from the three regions showed that potassium was the most abundant mineral, with levels significantly higher ($p<0.05$) in NK than in NS and UD. Magnesium, on the other hand, had the lowest concentration. These results align with the study by Aksoy *et al.* (2017), who found that the mineral content of bee glue varies depending on the local flora, season, and collection time. Higher potassium levels in the bee glue samples from Enugu State are comparable to those found in various regions of Turkey, highlighting the influence of geographic factors on mineral composition. Phytochemical analysis revealed that tannins were the most prevalent compounds in the bee glue samples, with values ranging from 1.49±0.011 to 1.63±0.015, while saponins were present in the lowest concentrations. The findings correspond with Marco *et al.* (2014), who noted that tannin content in bee glue is positively correlated with total phenolic content, and the proportion of these compounds can vary significantly among different samples. This variability in phytochemical composition underscores the complex nature of bee glue and its dependence on local vegetation.

The study also examined the trace element composition, with zinc being the most prominent element, nearly twice as abundant as iron and manganese, and three times greater than chromium and cadmium. The influence of soil type on the mineral and heavy metal content of bee glue has been well documented, as observed by Grzgorz *et al.* (2012). Their research showed that the mineral content of bee products is highly dependent on the location of the apiary, leading to significant differences in concentrations of elements like zinc and iron across different regions. Vitamin analysis indicated that Vitamin C was the most abundant vitamin in the bee glue samples, while Vitamins B1 and B2 were the most limited. These findings differ from those reported by Nazife (2017), who found higher concentrations of Vitamins B1 and B2 in other bee glue samples. This discrepancy may be attributed to differences in the environmental conditions and botanical sources in Enugu State compared to other regions. The pH values of the bee glue samples varied significantly, with NK exhibiting a higher pH (5.60±0.000) compared to UD (5.46±0.011) and NS (5.45±0.005). The variation in pH levels is consistent with Marco *et al.* (2014), who noted that the pH and chemical composition of bee glue are influenced by its geographic origin, which in turn affects its antimicrobial, antioxidant, and antiviral properties.

The antimicrobial activity of bee glue against selected bacterial strains *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus mutans* was evaluated at concentrations of 50 mg/ml, 100 mg/ml, and 150 mg/ml. At 50 mg/ml, *Pseudomonas aeruginosa* showed the highest inhibition, followed by *Streptococcus mutans*, while *Staphylococcus aureus* and *Salmonella typhi* had the least inhibition. As the concentration increased to 100 mg/ml and 150 mg/ml, the inhibition zones expanded, with *Pseudomonas aeruginosa* and *Streptococcus mutans* exhibiting the most significant inhibition. These results corroborate the observations by Amna (2014), who found that the antimicrobial properties of bee glue are highly dependent on its chemical composition, which is influenced by the geographical location and local flora. Fungal inhibition by the bee glue samples was also assessed, with *Aspergillus fumigatus* showing the highest inhibition, particularly at higher concentrations. This result is consistent with the findings of Fabio *et al.* (2018), who reported that the biological activity of bee glue varies with its botanical origin, affecting its efficacy against different pathogens.

Conclusion: This study provides a comprehensive analysis of bee glue samples collected from three distinct local governments: Nsukka, Nkanu, and Udi in Enugu State. The results demonstrate that these samples contain significant concentrations of dry matter, potassium, tannins, zinc, and vitamin A, highlighting the nutritional and pharmacological potential of bee glue. Notably, the high tannin content across all samples underscores their substantial antioxidant properties, which are essential for mitigating oxidative stress and supporting overall health. The antimicrobial activity of the bee glue samples exhibited minimal variation across the different localities, suggesting that while the biological efficacy of bee glue is influenced by the geographical origin, the antimicrobial potency remains relatively consistent. This finding supports the notion that the biological activities of bee glue are closely linked to the specific ecological conditions of the collection site. Significantly, bee glue from Nsukka Local Government Area exhibited the highest concentrations of zinc (1.07±0.011) and iron (0.89±0.005), while the Udi samples contained lower levels of iron (0.73±0.023), zinc (0.92±0.011), and manganese (0.17±0.011). These differences in metal concentrations reflect the varying environmental and botanical factors influencing bee glue composition in different regions. Given its rich biochemical profile and longstanding use in traditional medicine, bee glue represents a promising natural remedy with considerable potential for therapeutic applications. Nonetheless, further research is warranted to elucidate its clinical efficacy and safety, as well as to explore its potential in addressing environmental contamination and promoting public health.

Recommendation: Based on the comprehensive analysis presented in this study, it is imperative to adopt strategies that leverage on the potential health benefits of bee glue. To this end, fostering sustainable beekeeping practices is crucial. Supporting the conservation of honeybee populations and the protection of melliferous plant species are essential measures to ensure a continuous and high-quality supply of bee glue. Such practices should include promoting biodiversity within beekeeping environments and encouraging the use of best management techniques to maintain robust bee colonies. The findings highlight the substantial mineral content and notable antioxidant properties of bee glue, suggesting significant potential for its use in the medical field. It is recommended that further research be directed towards exploring the therapeutic applications of bee glue, particularly in developing natural health products. Investigating its efficacy in clinical settings could validate its role in medicinal formulations, potentially integrating it into treatments and preventive measures due to its high concentrations of beneficial compounds such as zinc and tannins.

Moreover, it is vital to conduct in-depth studies on the impact of environmental factors on the chemical composition and biological activity of bee glue. Understanding how soil quality, plant diversity, and environmental contaminants influence the properties of bee glue will provide valuable insights for optimizing its quality and efficacy. Enhanced quality control measures in the production and processing phases will also be necessary to ensure the consistency and reliability of bee glue used in therapeutic applications. Educating beekeepers about the ecological and economic advantages of bee glue, alongside best practices for its collection and use, will further support the sustainable management of this resource. Training programs aimed at improving the quality of bee glue through better beekeeping techniques can benefit both producers and consumers.

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