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Analysis of Selected Antioxidant and Anti-nutrient Content and Palynological Evaluation of Honey Samples from Southern Guinea Savanna Vegetation of Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: To investigate the presence of selected antioxidant and anti-nutrient and pollen profile of four honey samples from the southern guinea savanna vegetation of Nigeria.
Study Design: Purposive sampling method was used in selecting the study areas for the honey samples
Place and Duration of Study: The study was done in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, between June to October, 2019.
Methodology: The analyzed antioxidants (flavonoids, total phenols, vitamin C and alkaloids) and anti-nutrient (phytate and oxalate) were subjected to standard chemical treatment in four replicates. The stock samples of the honeys were thoroughly shaken and 10g collected from each sample for pollen analysis. Each sample was dissolved in acidified warm (40°C) water, centrifuged at 2000 rpm and the residues collected. Subsequently, they passed through standard acetolysis treatment. The polliniferous deposits were put in specimen bottles containing glycerol-alcohol. Routine pollen counts and identification were done at x400 magnifications. The pollen counts were presented as percentage values, while results obtained from the chemical analyses were subjected to analysis of variance and mean separation test using Duncan's New Multiple Range Test at P=.05.

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Results: All the selected antioxidants (flavonoids, total phenols, vitamin C and alkaloids) and anti-nutrients (phytate and oxalate) analyzed were detected in variable quantities in the samples. The honeys were acidic having pH range within acceptable internationally standard. Eighty-two pollen types (39 families) were recorded and composed of nectariferous (90.2%) and non-nectariferous (9.8%) plants. All the honey samples were polyfloral and the most common pollen types occurring across the samples were *Elaeis guineensis*, Asteraceae and *Phyllanthus muellerianus.*
Conclusion: The honey samples were acidic and contained the selected antioxidants and anti-nutrients parameters in variable quantities. The honey samples were polyfloral with a high number of nectariferous and few non-nectariferous honey plants.

Keywords: Anti-nutrient; antioxidant; Nigeria; pollen profile; Southern Guinea Savanna.

1. INTRODUCTION

Honey is a natural organic substance comprising many constituents such as carbohydrate, proteins, polyphenols, organic acids, amino acids, mineral elements, vitamins, flavonoids, anti-nutrients, enzymes as well as aromatic compounds [1,2,3]. It is a very good source of energy, containing about 80 – 95% carbohydrate [4] made up mainly of glucose and fructose which are readily assimilated into the blood stream. Honey has been suggested to contain more than 500 bioactive compounds in trace or small amounts which are believed to contribute to the role of honey as antioxidant, anti-ulcer, antimicrobial, anti-allergic, anti-tumor, anti-cancer, anti-inflammation and anti-browning among others [1,5,6]. Honey composition is dependent highly on the botanical composition and geographical source region [7]. It may originate from monofloral, bifloral or polyfloral sources, hence its physicochemical properties vary according to the floral species foraged [8], although environmental factors can make meaningful contribution. Equally, Kivrak and Kivrak, [9] also pointed out that multiple factors such as the floral, seasonal, geographical, soil, climate and method of processing contributes importantly in influencing the composition and antioxidant capacity as well as bioactivity of honey.

Since prehistoric times, honey has been used by humans as medicine and source of nutrition. It has been widely used in alternative medicine as antimicrobial agent, management of asthma, wound healing, cough and gastrointestinal disorder among others. Nutritionally, honey is consumed because of its numerous useful compositions such as minerals, amino acids, phenolic compounds, flavonoids, proteins, enzymes, carotenoids, reducing sugars and organic acids [10].

Studies have demonstrated that honey contains natural antioxidants which have been shown to be responsible for minimizing risks of cancer, cardiovascular diseases, immune system deficiency, cataracts and inflammation [8,10,11]. A high percentage of the bioactive compounds in honey are reported to contain compounds such as phenolic acids, flavonoids, anthocyanins, procyanidins, vitamins as well as alkaloids [12,13]. In fact, the antioxidant and antimicrobial activities of honey have been correlated with colour pigments and total phenolic composition in honeys [13,14]. Gheldof et al. [15] in their study reported significant correlation between the phenolic content of honey, its antioxidant effect and the prevention of lipoprotein oxidation of human serum. In fact, the effectiveness of antioxidant properties of honey to control the ravaging effects of free radicals was demonstrated by the works of Schramm et al. [16] and Beretta et al. [17]. One study was able to show that the consumption of honey contributed significantly to the rise in plasma antioxidant and reducing capacity, and the plasma total-phenolic composition [16]. Beretta et al. [17] were able to demonstrate the protective function of honey by subjecting a multifloral honey to oxidative stress. They achieved this by standardizing the honey for total antioxidant power and profiling it systematically in antioxidants in a cultured endothelial cell line.

The free radicals formed in the human system may be molecules, ions and or compounds that are highly unstable because of their molecular or ionic structures that make them highly reactive, pairing with other atoms, electrons, molecules or compounds to create a stable compound [10,18]. Such free radicals as reactive oxygen species (ROS) may disrupt the structure of cellular molecules or cause molecular transformations and gene mutation in many organisms resulting in cell damage, premature aging, cancer and

other health problems [12]. In fact, because of the important roles of antioxidants and the growing need to incorporate them into food products the demand for honey is becoming widespread since it is a natural source of antioxidants [11].

Apart from the antioxidants and antimicrobial potentials of honey as well as other bioactive components which are known to be beneficial to humans, honey also contain anti-nutrients such as oxalic acid/oxalate and phytic acid/phytate. These anti-nutrients are naturally incorporated into honey from nectar or extra-floral nectar and pollen grains which the honey bees harvest from different plant sources. Oxalates are organic compounds found in many plant-based foods including honey. It can also be produced by the human body or through the breakdown of vitamin C [19]. Oxalate is known to bind to minerals like iron and calcium forming compounds such as iron and calcium oxalates, an action which prevents the minerals from being absorbed into the body. Oxalate has also been linked to increased risk of kidney stone [20].

Phytate is one of the anti-nutrients that decreases the absorption of minerals like copper, iron, zinc, magnesium and calcium from ingested food in the gut because of its six reactive phosphate groups [21,22]. Phytate is formed when phytic acid is bound to minerals. Phytate serves as the key storage structure of phosphorus in several plants. It forms indigestible complexes with compatible mineral elements or proteins negatively affecting their bioavailability in the gastrointestinal tract. The fact remains as to whether the quantity of these anti-nutrients present in honey is enough to raise concerns for those honey consumers that have mineral deficiency.

This study was therefore, designed mainly to determine the presence and possible differences in the quantity of selected antioxidants and anti-nutrient content of honey samples from the southern guinea savanna vegetation of Nigeria, and ascertain the plants that contributed in the honey production palynological.

2. MATERIALS AND METHODS

2.1 Source Areas of Honey Samples

The honey samples were collected from genuine honey dealers in Ogige Market, Nsukka, Enugu State, Ankpa in Kogi State and Ukum and Kwande in Benue State, Nigeria. The study was conducted in the four sampling areas located in the major honey producing areas of Nigeria and have noticeable phytogeographical differences. The complex and diverse vegetation flora of these areas favour large scale production of honey. Presently, the vegetation is rapidly depleted, fragmented and deforested due to expansion in agriculture, wood harvest for fuel wood, indiscriminate bush burning, urbanization and other infrastructural development. It becomes imperative that the existing honey bee plants which are now threatened be identified and properly documented so that they can be harnessed for future apicultural development.

Nsukka lies with the Derived savanna mosaic adorned with a mixture of lowland rainforest and savanna species. It is a narrow east-west band of vegetation derived largely from the lowland rainforest due to persistent anthropogenic activities spanning thousands of years. It transited into the southern boundaries of the southern guinea savanna. Ankpa, Ukum and Kwande are situated within the southern guinea savanna with abundant woodland and grass vegetation. Enugu, Benue and Kogi States share common boundaries and have similar vegetation composition particularly at boundary areas. About 60 percent of honey produced in Nigeria annually comes from this vegetation zone. The ecosystem is adorned with diverse and abundant plant species which are important nectar sources, extra-floral nectar and pollen grains to honey bees and other nectar loving insects. The honeys which were collected for the study were marked and stored at room temperature pending when they will be analyzed.

2.2 Determination of Flavonoids

The flavonoid content of the honey samples was determined with colorimetric assay [23]. Four mils of water was mixed with 1ml of the honey sample to make up 5ml of the solution. At the baseline, about 0.3 ml of Na_2NO_2 (5% w/v) was added to the solution of honey followed by 3ml $AlCl_3$ 5 minutes later. After 6 mins, 2 ml of 1 mol $litre^{-1}$ NaOH was added and made up to 10 ml with distilled water. The mixture was thoroughly agitated to ensure even mixture and the absorbance read at 510 nm. The total flavonoid concentration was assessed from the routine standard curve.

2.3 Determination of Vitamin C

One gram of the honey sample was weighed and mixed evenly with 20 ml of 0.4% oxalic acid and

then filtered. One ml of the filtrate was added into 9 ml of indolphenol reagent. The absorbance was read at 520 nm and the concentration of vitamin C calculated based on ascorbic acid standard curve [24].

2.4 Determination of Total Phenolic Content

The total phenolic content was assessed by means of a modified method of spectrophotometric Folin-Cioucalteu. One ml of Folin and Cioucalteu's phenol reagent was mixed with 1ml of honey. One ml of Na_2CO_3 (10%) solution was added to the mixture after 5 minutes and then made up to 10 ml with distilled water. The reaction was kept in the dark for 90 minutes and the absorbance at 725 nm was read using a UV/Vis spectrophotometer. The total phenolic concentration was determined as gallic acid equivalent [25,26].

2.5 Determination of Alkaloids

Two grams of honey sample were put in a beaker and 100 ml of 10% acetic acid in alcohol solution was added to each of the samples. The mixture was thoroughly agitated and allowed to stand at room temperature for 3 hours and was shaken at every interval of 30 minutes. The mixture was heated in order to concentrate and reduce it to the quarter of its original volume. Ten mil of concentrated ammonia solution was added to the mixture drop-wise in order to precipitate the alkaloids. Ammonia was added continuously until it was in excess. Whatman No 1 filter paper was used in filtering off the precipitated alkaloid and then, washed with 1% NH_4OH solution, and dried. The filter paper was dried and the weight obtained [27]. Percentage alkaloids content was calculated as follows:

% alkaloid = weight of alkaloid / weight of sample x 1000

2.6 Determination of Phytate

About 0.5 g of the honey sample was put in a 50 ml flat bottom flask and the flask was placed in a shaker and extraction was done with 100 ml of 24% HCl for 1 hour at room temperature and the solution was filtered. Five ml of the filtrate was pipetted into a beaker and diluted to 25 ml with distilled water. Fifteen millilitre of sodium chloride was added into 10 ml of the diluted sample and the solution passed through an amplet raisen grade 260-400 mesh to cool in organic phosphorus before adding 15 ml of 0.7M sodium chloride. The absorbance was taken at 520 nm wavelength [28].

2.7 Determination of Oxalate

To determine oxalate content, 2 g of each honey sample were put into 300 ml flasks and 20 ml 30% HCl was introduced into the sample and allowed to stand for 20 minutes. The solution was filtered into 250 ml volumetric flask and was made up to 250ml with 30% HCl. 10 ml of the filtrate was put into 100 ml centrifuge tube and 30ml diethyl ether was added and adjusted to pH7.0 with ammonium hydroxide. It was centrifuged (10,000 rpm) for 15 minutes and the filtrate was put into 250 ml conical flask. The filtrate was titrated with 0.1m potassium tetraoxomanganate ($KMnO_4$) and the volume recorded [28].

$$\% \text{ oxalate} = \frac{\text{titre} \times \text{mol KMnO4} \times \text{DF}(12.5) \times 100}{\text{Weight of sample}}$$

2.8 Determination of pH

This was done with the help of a pH meter. Ten percent of the honey solution was prepared by diluting 10 g of the sample with 90 ml of distilled water. The pH meter electrode was inserted into the honey solutions and the reading taken in triplicates.

2.9 Pollen Extraction

The honey samples were shaken thoroughly and each diluted with 35 ml warm (40^oC) acidified water (997 ml of distilled water and 3 ml Conc. H_2SO_4) to enhance the dissolution of colloidal matters and sugars in the honeys. The honey solutions were centrifuged (2000 rpm) for ten minutes and the precipitates acetolysed [29,30]. The resulting palyniferous residues were stored in 2 ml glycerol-alcohol in specimen bottles. From there samples were taken for microscopic examination - identification and pollen counts under the light microscope at X400 magnification. Identifications and pollen counts were carried out on the entire area (484 cm^2) of the cover slip aided by photomicrographs in Bonnefille and Riolett [31], Y'bert [32], APLF [33] and pollen slides in the Environment and Palynology Research Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.10 Statistical Analysis

The analysis of variance was used in determining the significant difference at P=.05, while Duncan's New Multiple Range Test was used for the mean separation test in IBM SPSS Statistics 20 software package. The values of the phytonutrients were in mean ± SD and the pollen values were converted to percentage.

3. RESULTS

3.1 Phytonutrients

The results of the study on selected antioxidant and anti-nutrient composition of honey samples from the southern guinea savannah vegetation of Nigeria is presented in Table 1. All the honey samples contained flavonoids in varied quantity. The values of the flavonoids content of honey samples between Ankpa and Ukum and between Kwande, Ukum and Nsukka did not have any significant difference (P=.05), but that of Ankpa varied significantly (P=.05) from those of Kwande and Nsukka honey samples. The highest concentration of total phenolic content was recorded in the honey sample from kwande and it varied significantly (P=.05) from that of Ukum, Ankpa and Nsukka honey samples. Similarly, that of Nsukka sample varied significantly (P=.05) from that of Ankpa and Ukum and there was no statistical difference (P=.05) between the total phenolic content in Ankpa and Ukum honey samples. The vitamin C content of the honey sample from Kwande, Ukum and Nsukka did not vary significantly (P=.05), but the values recorded for Kwande and Nsukka varied statistically (P=.05) from that of Ankpa. The value of vitamin C content recorded in honey samples from Ukum and Ankpa did not have any significant difference (P=.05). The highest amount of oxalate was recorded in honey sample from Ankpa and it varied significantly (P=.05) from that of Ukum, Kwande and Nsukka. The value recorded for Kwande sample was statistically different (P=.05) from that of Ukum and Nsukka honey samples. The amount of oxalate recorded in Ukum and Nsukka honey samples did not vary significantly (P=.05). The honey sample from Ankpa had the highest content of phytate and this varied statistically (P=.05) from that of Ukum, Kwande and Nsukka samples. No statistical difference (P=.05) was observed in the values of phytate recorded in the honey samples from Kwande and Nsukka and between Ukum and Nsukka, however significant difference (P=.05) was observed in the amount of phytate recorded in the honey samples from Kwande and Ukum. Regarding the alkaloids content, the amount recorded in honey sample from Ankpa varied statistically (P=.05) from that of Kwande, Ukum and Nsukka. Statistically, there was statistical difference (P=.05) in the quantity of alkaloids in honey samples from Ukum and Nsukka, however, they varied statistically (P=.05) from that of Kwande. The pH values did not show any significant difference (P=.05) in the pH values of honey samples from Ankpa, Kwande and Ukum, but they varied statistically (P=.05) from that of Nsukka (Table 1).

3.2 Results of Pollen Analysis

From the palynological results, 82 (41 – 47) pollen types classified into 39 (25 – 27) plant families were identified in the four honey samples (Table 2). These comprised nectariferous and non-nectariferous plants with the nectariferous pollen types comprising 90.2 % of the total identified pollen types and the non-nectariferous pollen types comprising 9.8%. An average of 55,549 pollen grains was counted in the honey samples. Of these pollen types Combretaceae-Melastomataceae, *Irvingia gabonensis*, *Elaeis guineensis*, Asteraceae, *Bombax buonopozense*, *Parinari* sp., *Psorospermum* sp., *Euphorbia hirta, Senna* sp., Moraceae, Poaceae, *Phyllenthus muellerianus*, *Nauclea latifolia, Crossopteryx febrifuga* and *Ziziphus* sp. were identified in all the honey samples. These are among the common plants found in the study locations. Fabaceae family contributed the highest percentage of identified pollen types in the entire honey samples generally and in the individual samples (Figs. 1 – 5). In terms of botanical origin, all the honey samples were polyfloral. This is because none of the pollen types had pollen frequency above 44%. The range in class of their pollen frequency varied from secondary pollen (16-44%) to minor pollen (≤ 3%) abundance.

In the honey sample from Kwande, a total of 47 pollen types classified to 26 families were recorded. Asteraceae, *Phyllanthus muellerianus*, *Senna* sp., *Alchornea cordifolia*, *Prosopis africana*, *Elaeis guineensis*, *Trichilia* sp. and Moraceae were among the predominant pollen types recorded (Table 1). They constitute the major nectar and pollen sources for the honey production. Fabaceae family was the major source of pollen types contributing 23% of the recorded pollen types in the sample followed by Euphorbiaceae (6.4%) (Fig. 2). In the

palynological results from Ukum honey sample 45 pollen types identified were classified to 25 families. The most dominant pollen types were Asteraceae, *Phyllanthus muellerianus, Alchornea cordifolia, Elaeis guineensis*, Moraceae, *Euphorbia hirta* and *Trichilia* sp. Notable nectariferous plant species recorded in this sample included *Phyllanthus muellerianus, Crossopteryx febrifuga, Trichilia* sp, Combretaceae-Melastomataceae, Asteraceae, *Senna* sp., *Tephrosia* sp., *Parkia biglobosa, Syzygium guineense* and *Nauclea latifolia* despite low pollen abundance (Table 1) and they constituted 88.9% of the identified pollen types in this sample. Also, the non-nectarifrous pollen types such as *Elaeis guineensis, Alchornea cordifolia*, Moraceae and Poaeae were also recorded in considerable quantity and constituted 11.1% of the identified pollen types (Fig. 4). Fabaceae (26.7%) and Euphorbiaceae (8.9%) contributed the highest pollen types in the pollen spectrum.

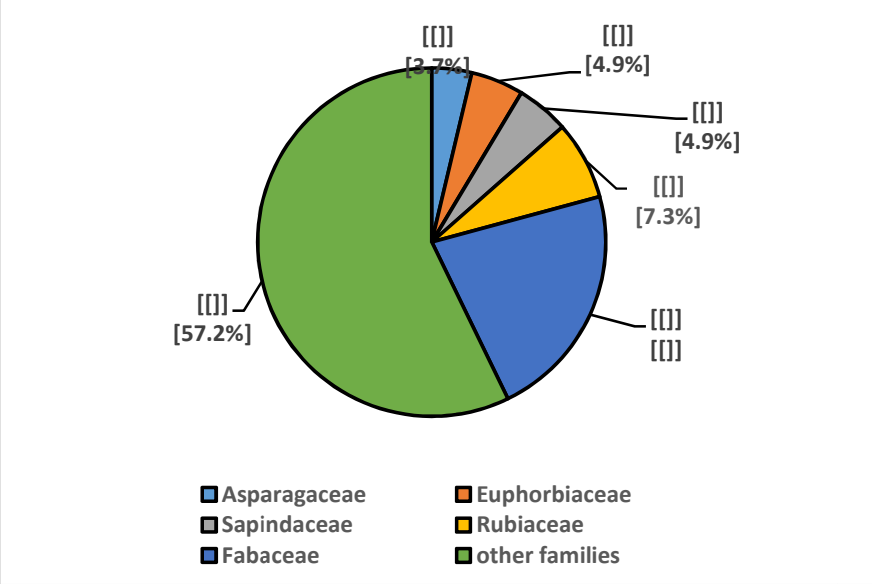

Fig. 1. Percentage of dominant families in the honey samples

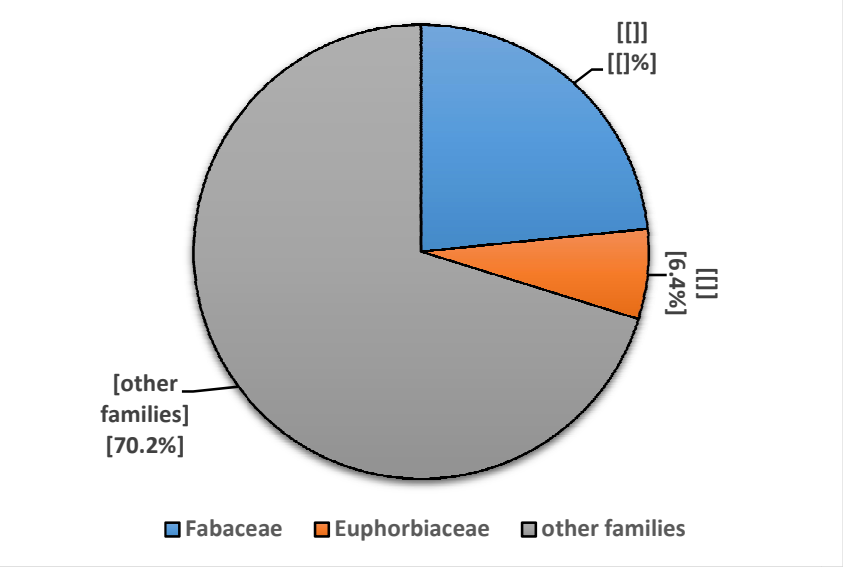

Fig. 2. Percentage of dominant families in the honey sample from Kwande

Table 1. The mean ± SD values of the of the selected phytonutrients

Source	Flavonoids (%)	Phenols (mg/100 g)	Vitamin C (mg/100 g)	Oxalate (%)	Phytate (%)	Alkaloids (%)	pH
Ankpa	13.30 ± 0.10^{a}	183.42 ± 0.01^{c}	2.80 ± 0.00^{bc}	9.25 ± 0.05^{a}	10.50 ± 0.00^{a}	5.49 ± 0.00^{a}	4.20 ± 0.00^{a}
Kwande	12.15 ± 0.05^{b}	233.36 ± 0.00^{a}	3.55 ± 0.05^{a}	8.10 ± 0.10^{b}	9.65 ± 0.05^{b}	3.97 ± 0.01^{c}	4.30 ± 0.00^{a}
Ukum	12.47 ± 0.01^{ab}	183.54 ± 0.01^{c}	3.05 ± 0.05^{ab}	7.65 ± 0.05^{c}	9.00 ± 0.00^{c}	4.83 ± 0.01^{b}	4.10 ± 0.00^{a}
Nsukka	12.34 ± 0.00^{b}	207.99 0.01^{b}	3.25 ± 0.05^{a}	7.05 ± 0.05^{c}	9.35 ± 0.05^{bc}	4.13 ± 0.01^{b}	3.55 ± 0.01^{b}

In the honey sample from Ankpa, 41 pollen types were identified and classified to 25 families. The most frequently occurring pollen types were *Elaeis guineensis, Phyllanthus muellerianus, Senna* sp., *Irvingia gabonensis, Ziziphus* sp. and *Alchornea cordifolia*. The pollen profile showed that both nectariferous and non-nectariferous plants were foraged by the honey bees. The nectariferous plants such as *Senna* sp., *Irvingia gabonensis, Phyllanthus muellerianus, Ziziphus* sp., Asteraceae, Combretaceae-Melastomataceae, *Trichilia* sp., *Crossopteryx febrifuga, Citrus* sp. and *Kohautia* sp. constituted 90.2% of the pollen types recorded, while the non-nectariferous plants such as *Elaeis guineensis, Alchornea cordifolia, Mimosa pudica* and Poaceae constituted 9.8%. The families of Fabaceae, Euphorbiaceae and Rubiaceae contributed more pollen types to the pollen spectrum compared to other families (Fig, 4). The pollen profile results of Nsukka honey sample indicated that both nectariferous and non-nectariferous plants were foraged by the bees for nectar and or pollen during the honey production. The necariferous plants dominated by pollen grains of *Phyllanthus muellerianus, Irvingia gabonensis, Nauclea latifolia, Senna* sp., *Eugenia uniflora* and *Citrus* sp. constituted 90.5% of the recorded pollen types, while the non-nectariferous pollen types (*Elaeis guineensis,* Cyperaceae, Moraceae and Poaceae) constituted 9.5% of the plants identified. Fouty-two pollen types classified to 27 families were identified in the sample. *Phyllanthus muellerianus, Irvingia gabonensis, Nauclea latifolia, Senna* sp., *Elaeis guineensis, Eugenia unflora, Citrus* sp., Moraceae, *Crossopteryx febrifuga, Detarium microcarpum, Euphorbia hirta, Hymenocardia acida* and Combretaceae-Melastomataceae were among the dominant pollen types recorded (Table 1). The dominant families that contributed more pollen types in the honey sample were Fabaceae and Rubiaceae (Fig. 5).

Table 2. Percentage pollen profile of honey samples from the southern guinea savanna vegetation of Nigeria

			Percentage frequency occurrence of pollen counts in the honey samples			
S/N	**Family**	**Taxa**	**Kwande**	**Ukum**	**Ankpa**	**Nsukka**
1	Acanthaceae	*Hypoestes* sp.			0.04	
2		Justicia sp.				0.06
3	Anacardiaceae	*Lannea* sp.	1	0.1	0.2	
4		*Spondias mombin*				0.4
5	Apocynaceae	*Rauwolfia vomitoria*		0.03		
6		*Motandra* sp.				0.7
7	Arecaceae	*Elaeis guineensis*	6.9	9.3	19.8	14.2
8	Asparagaceae	*Urgenia* sp.			0.06	
9		*Asparagus* sp.			0.05	
10		*Dracaena* sp				0.2
11	Asteraceae		9.5	11.1	4.9	0.8
12	Aristolochiaceae	*Aristolochia* sp.	23(0.03			
13	Bombacaceae	*Bombax buonopozense*	0.05	0.05	0.1	1.5
14		*Ceiba pentandra*	0.05	0.2		0.04
15	Boraginaceae	*Cordia africana*	0.04			
16	Combretaceae-Melastomataceae		0.6	0.04	2.7	2.6
17	Chrysobalanaceae	*Parinari* sp.	0.09	0.1	0.06	1.2
18	Clusiaceae	*Psorospermum* sp.	0.05	0.1	0.07	0.15
19		*Garcinia kola*	0.09			
20	Commelinaceae	*Aneilema* sp.	0.03			
21		*Cyanotis rubescens*		0.03	0.05	0.1
22	Cyperaceae					0.06
23	Dioscoreaceae	*Dioscorea* sp	0.3			

			Percentage frequency occurrence of pollen counts in the honey samples			
S/N	**Family**	**Taxa**	**Kwande**	**Ukum**	**Ankpa**	**Nsukka**
24	Ebenaceae	*Diospyros* sp.	1.5			
25	Euphorbiaceae	*Alchornea cordifolia*	7.8	9.8	5.3	
26		*Manihot esculentus*	1.1	0.3	0.04	
27		*Jatropha* sp		0.04		
28		*Euphorbia hirta*	0.5	5.9	1.3	3.1
29	Fabaceae	*Piliostigma thonningii*	0.4	0.07		
30		*Prosopis africana*	7.3	3.3		
31		*Senna* sp.	8.3	4.2	10.9	7.2
32		*Tephrosia* sp.	3.8	3.3		0.4
33		*Parkia biglobosa*		0.03		0.2
34		*Sesbania sesban*	4.7	0.1		
35		*Crudia* sp.		0.1		
36		*Dalbergia* sp.		0.05	0.2	1.7
37		*Afzelia africana*	0.1	0.2		0.1
38		*Acacia* sp.	0.2	0.2	0.05	
39		*Pentaclethra macrophylla*	0.09			0.8
40		*Brachystegia eurycoma*	0.4	0.06		
41		*Mimosa pudica*	0.06		0.04	
42		*Detarium microcarpum*	0.01	0.1	1.3	3
43		*Indigofera* sp.			1.6	
44		*Danielia oliveri*			0.1	
45		*Albizia* sp.				0.07
46		*Erythroxylum* sp.				0.7
47	Hymenocardiaceae	*Hymenocardia acida*		0.4	0.7	2.6
48	Icacinaceae	*Leptaulus* sp.		0.1		
49	Irvingiaceae	*Irvingia gabonensis*	0.8	0.2	7.7	7.5
50	Liliaceae	*Lilium* sp.	0.07			
51		*Gloriosa superba*	0.07			
52	Malvaceae	*Hibiscus* sp.		0.02	0.05	
53		*Corchorus olitorius*			0.06	0.05
54	Meliaceae	*Trichilia* sp.	5.6	5.6	2.7	
55		*Azadirachta indica*			1.7	
56		*Khaya senegalensis*	3.7	0.5		0.7
57	Moraceae		5.05	8.3	3.3	3.7
58	Myrtaceae	*Syzygium guineense*		0.04	0.08	0.5
59		*Eugenia uniflora*			0.05	6.8
60	Ochnaceae	*Lophira lanceolata*	4.6	1.8		
61	Olacaceae	*Olax* sp.	0.1			
62	Pandanaceae	*Microdesmis* sp.				2.5
63	Poaceae		1	0.6	0.08	1.2
64	Phyllanthaceae	*Bridelia* sp.	0.04			
65		*Phyllanthus muellerianus*	8.9	10	13.7	10.1
66	Proteaceae	*Protea madiensis*				0.09

			Percentage frequency occurrence of pollen counts in the honey samples			
S/N	**Family**	**Taxa**	**Kwande**	**Ukum**	**Ankpa**	**Nsukka**
67	Rhamnaceae	*Lasiodiscus* sp.	1.2			
68		*Ziziphus* sp.	1	8.9	7.1	1.3
69	Rubiaceae	*Nauclea latifolia*	5	1.8	0.5	7.4
70		*Crossopteryx febrifuga*	4.6	9.9	4.7	3.6
71		*Mitragyna* sp.		2.3	1.6	0.6
72		*Tarenna* sp.	0.8			1.2
73		*Kohautia* sp.			3	3.1
74		*Borreria* sp.				0.04
75	Rutaceae	*Citrus* sp.			3.6	6.7
76	Sapindaceae	*Allophyllus* sp	0.01		0.06	
77		*Blighia* sp.		0.04		
78		*Pancovia turbinata*	0.06	0.6		
79		*Paullinia pinnata*			0.1	
80	Scrophulariaceae	*Scoparia dulcis*		0.06		
81	Tiliaceae	*Grewia* sp		0.03	0.06	
82	Ulmaceae	*Celtis* sp.	2.3			
	Indeterminate		0.03	0.01	0.02	0.02
	Absolute total count		84,194	63,509	35,750	38,742

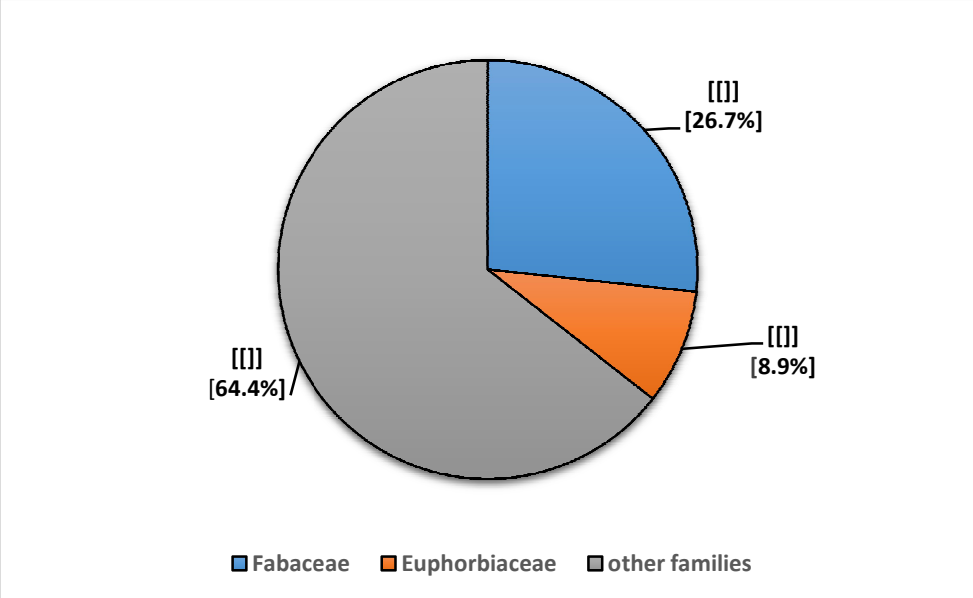

Fig. 3. Percentage of dominant families in the honey sample from Ukum

4. DISCUSSION

Honey is an invaluable natural syrup containing multiple phytochemical constituents. A small proportion of these complex physicochemical constituents are biologically potent compounds that have endeared the use of honey as antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral as well as wound healing agents. The present investigation has provided an insight into the content of the selected antioxidants and anti-nutrients as well as pollen profile of the honeys from the southern guinea savanna vegetation of Nigeria. The pH of the honey samples showed that they are acidic and therefore capable of inhibiting microbial growth and expression of other qualities of an acidic honey. The range of pH of the samples showed that they fell within the range (3.2 – 5.0) set by Codex Alimentarius Commission [34]. An acidic pH plays vital role in ensuring honey freshness and long shelf life because it is capable of

preventing the growth of microorganisms and fermentation (6). This finding is comparable to the results of Saxena et al. [35], El Sohaimy et al. [36], Nweze et al. [2] and Bako et al. [37]. The presence of considerable quantity of flavonoids in the honey samples is in line with previous reports that flavonoids are part one of the phyto-constituents found in honey. The quantity of flavonoids detected in this study is comparable to the findings of Can et al. [7] in honey samples from Turkey. However, the concentration is comparably lower than that recorded by Nweze et al. [2] in honey samples from Nsukka, Nigeria.

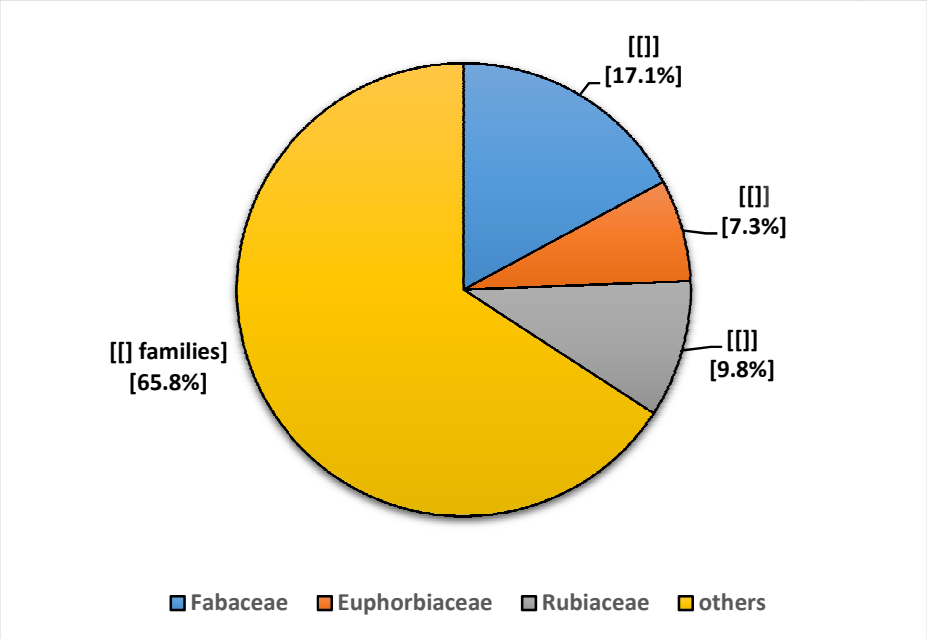

Fig. 4. Percentage of dominant families in the honey sample from Ankpa

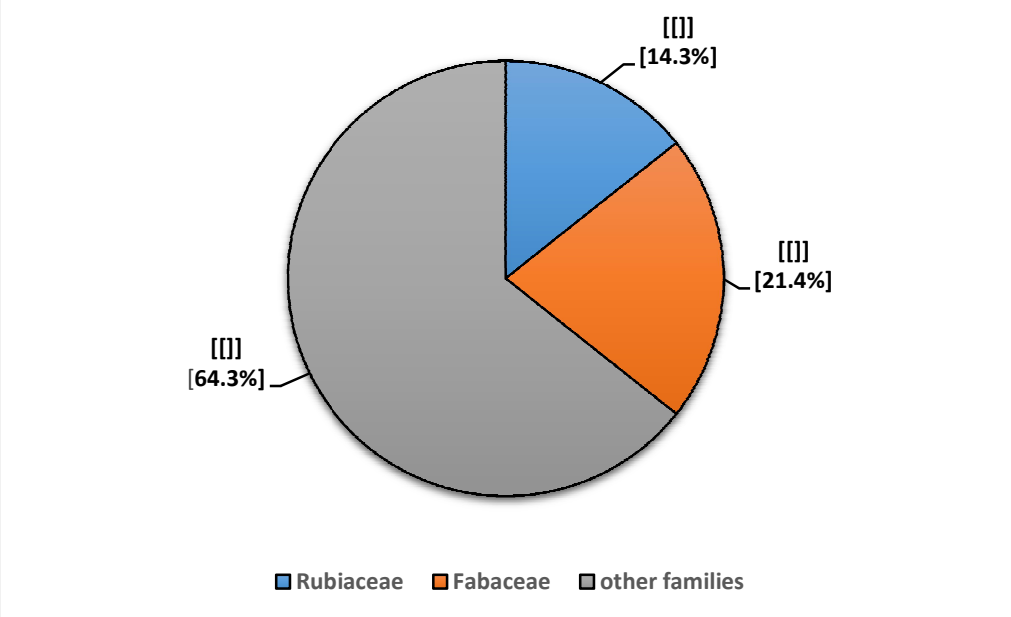

Fig. 5. Percentage of dominant families in the honey sample from Nsukka

Flavonoids are diverse group of phytonutrients that are powerful antioxidant with immune system and anti-inflammatory benefits. Pinocembrin, a flavonoid found in high concentration in honey propolis has been found to possess potential health benefits. It has been reported in a study that pinocembrin extracted from honey bee propolis was able to bring about positive improvement in the perceptive ability of rat caused by lingering cerebral hyper perfusion, and also played a protective role on brain mitochondria structure and function [38]. In another study, pinocembrin was found to decrease cerebral ischemia/reperfusion injury probably through anti-oxidative and anti-apoptic activity [39]. Equally, dietary flavonoid quercetin, was found to lower/control blood pressure [40] and restored endothelial dysfunsion in hypertensive animal models [11]. Quercetin has equally been found to be beneficial in reducing the chances of having stroke and coronary heart disease [41].

The presence of phenols in the honey samples agrees with earlier reports that honey contains phenol and polyphenolic compounds believed to originate from nectar, honeydew, pollen and or propolis [42]. The total phenolic content recorded in this study was greater than that reported by Buba et al. [26] in North-East Nigeria and in Turkey by Can et al. [7] and Boussaid et al. [43] and lower than that reported by Nweze et al. [2] in Nsukka, Nigeria. Phenolic compounds have been recognised as the ingredient responsible for the antioxidant activity of honey mainly associated with free radical scavenging activity leading to the formation of relatively stable and less toxic molecules. They play vital role in the treatment and control of cardio vascular diseases [44].

Vitamin C which is recorded in the study is regarded as the most vital vitamin for human nutrition [24]. It is widely distributed in plant cells for growth and metabolism. It occurs in plant secretions such as nectar and extra-flora nectarines and pollen grains as ascorbic acid and consequently enters into honey through the foraging activities of honey bees. Its occurrence in the present honey samples is quite natural. The honey samples have been shown to contain variable amount of the vitamin C. The relatively high concentration of vitamin C in Kwande and Nsukka samples may be accredited to the nectar and pollen sources in the study areas or other variables. This compares favourably with the results of monofloral honeys analysed by Khalil et al. [45] in Northern Region of Banbladesh. However, it has lower concentration compared to the works of Buba et al. [26] and Nweze et al. [2] in honey samples studied in North-East and Nsukka, Nigeria respectively. Vitamin C has been found to have significant impact on honey antioxidant properties. It plays a vital role in the removal of different reactive oxygen species, keeping the membrane bound antioxidant α-tocopherol in reduced state, maintaining the activity of a number of enzymes as a cofactor and plays a role in stress resistance [46,47].

Alkaloids are classes of nitrogenous organic compounds of plant origin which have diverse and distinct physiological effects on humans. Like other compounds of plant origin, they occur in the nectar and pollen of some honey plants (such as members of Fabaceae, Boraginaceae, Asteraceae and Solanaceae) from where they become part of the honey chemical components. The concentration of honey alkaloids is reliant on the plant species foraged by honey bees during the process of honey production. Alkaloid was detected in the honey samples studied especially that from Ankpa, Ukum and Nsukka. Alkaloids are known to have unique and diverse health benefits on humans. They can be used in the treatment of certain ailments such as immune disorders, inflammation and pains, although it can be toxic to the human system. In a study conducted by Dübecke et al. [48] on honey samples and bee pollen, pyrrolizidine alkaloids were detected in 66% of raw honey not yet packaged for sale, 94% of honey displayed in supermarkets and 60% of honey bee samples analyzed. Pyrrolizidines alkaloids are potentially toxic to the liver (hepatotoxic) and are mutagenic and carcinogenic [49]. In another study, some alkaloids extracted from *Catharanthus roseus* were found to induce considerable increase in glucose uptake (hypoglycemic activity) in β-TC6 and C2C12 of mouse pancreatic cells. They were also found to show antioxidant potential by reducing H_2O_2-induced oxidative damage in β-TC6 cells [50]. This makes it imperative to analyze honey samples for the presence and concentration of some of these toxic alkaloids before they are made available for public consumption.

Oxalate occurs naturally in many foods including honey in variable concentrations. The small percentage of oxalate detected in the honey samples may be associated with the floral sources of the nectar and other plant secretions used for the honey production. Comparatively,

the amount recorded in this work was higher in relation to findings of Moosbeckhofer et al. [51]. However, oxalate was not detected in honey samples studied in Cross River State, Nigeria [52]. Oxalate is regarded as anti-nutrient because it binds to certain minerals such as calcium (forming calcium oxalate) and magnesium limiting their absorption in the gut and bioavailability to the system. Oxalates will also crystalize in tissues if consumed regularly, creating arthritis-like symptoms and increased risk of kidney stone [20].

Another anti-nutrient detected in all the honey samples was phytate which was recorded in relatively higher percentage in honey sample from Ankpa. Phytate as anti-nutrient binds to essential minerals in the gut such as iron, zinc, calcium, phosphorus and magnesium forming insoluble and indigestible complexes, thereby making them biologically unavailable for absorption in the human system [22]. However, on the contrary phytate has been reported to have some beneficial properties as an antioxidant, shows anticancer properties and is believed to have positive effect on cholesterol and blood sugar level [22,53,54]. Based on *in vitro* and *in vivo* studies, it has been demonstrated that increase in phytate plays significant part in averting the formation of oxalate crystals of calcium and phosphate responsible for kidney stone formation [55,56].

The pollen composition of the honey samples was found to originate from two main sources: wild plants and cultivated crops. The pollen types of wild plants are those derived from southern guinea savanna vegetation typical of the source locations such as *Elaeis guineensis*, Combretaceae-Melastomataceae, *Prosopis africana, Parkia biglobosa, Acacia* sp., *Afzelia africana, Detarium microcarpum, Hymenocardia acida, Parinari* sp., *Khaya senegalensis, Trichilia* sp., *Syzygium guineense, Lophira lanceolata, Phyllanthus muellerianus, Nauclea latifolia, Ziziphus* sp. and *Crossopteryx febrifuga*. These are some of the characteristic or markers plants of the vegetation of the southern guinea savanna flora. Similar characteristic plants have been reported in studies conducted in Enugu and Kogi States [30,57,58]. The cultivated economic crops recorded in this study are those whose pollen were foraged by honey bees such as *Citrus* sp., *Elaeis guineensis, Irvingia gabonensis, Manihot esculenta, Pentaclethra macrophylla* and *Hibiscus* sp. These are plants that are either planted or conserved specially for their economic benefits as cash, food and subsistence crops in the areas where the honeys were produced.

Based on the frequency of family occurrences and relative abundance of pollen grains of some species, the most important honey bee plant groups or taxa in study areas include members of the Fabaceae (e.g. *Senna* spp., *Prosopis africana, Tephrosia* sp., *Acacia* sp., *Parkia biglobosa, Afzelia africana, Dalbergia* sp., *Detarium microcarpum, Brachystegia euricoma* and *Pentaclethra macrophylla*), *Elaeis guineensis, Phyllanthus muellerianus, Alchornea cordifolia*, Moraceae, *Ziziphus* sp., *Crossopteryx febrifuga, Euphorbia hirta, Citrus* sp. and Combretaceae-Melastomataceae. Similar characteristic honey plants have also been recorded in southern guinea savannah vegetation of Nigeria [59,60,61]. These plants characterize the vegetation areas of Enugu, Kogi and Benue States which are located in the southern guinea savannah vegetation of Nigeria. Surprisingly relatively high frequency of the members of Asteraceae was recorded in all the honey samples. This may be an indication of increase in deforestation and expansion of agricultural landscapes which promoted increase and extension in agricultural weeds especially members of the Asteraceae family. This explanation is strengthened by the relative number and quantity of farmland indicator weed pollen types such as Asteraceae, *Hypoetes* sp., *Justicia* sp., *Aneilema* sp., *Cyanotis* sp., *Euphorbia hirta*, *Senna* spp., *Borreria* sp. and *Mimosa pudica* recorded in this study.

5. CONCLUSION

The study showed that the analysed honey samples were acidic and contains the selected antioxidants and anti-nutrient parameters in variable quantities. The study indicated that the honey samples are polyfloral with a high number of nectariferous and non-nectariferous honey plants available for the foraging activities of the honey bees. Majority of the identified pollen types are reflections of the characteristic flora of the southern guinea savannah vegetation of the study locations.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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