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Original Article

Antimicrobial Properties and Melissopalynology, Proximate and Elemental Analyses of Honey Samples from Three Different Ecozones in Nigeria

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Abstract

Honey samples from three different ecozones - coastal freshwater (Ogba), lowland rainforest (Oka-Akoko) and montane Sudan savanna (Mambilla plateau) – were subjected to melissopalynological, proximate and elemental analyses, as well as antimicrobial studies. The aim of the research was to determine the contribution of pollen, if any, in the antimicrobial activities of the studied honey samples. Standard preparation methods were adopted for these studies. The Mambilla honey recorded the highest pollen diversity, while that of Oka-Akoko and Ogba regions had similar diversity, both having lower values. The Ogba honey however contained the highest abundance of pollen. Proximate analysis showed that the Mambilla honey also recorded the highest values of moisture, ash, protein, fats and oil, as well as potassium and reducing sugars. Antimicrobial investigation revealed the highest antimicrobial activities for the Mambilla honey, followed by Oka-Akoko and Ogba against *Staphylococcus aureus* (gram positive) and *Pseudomonas aeruginosa* (gram negative). Moisture content, proteins and carbohydrates were significantly different, with positive and negative trends respectively, when related with the antimicrobial activities of the honey samples. The pollen contents were also qualitatively different. This is the first time the antimicrobial activity of honey is ever traced to pollen contents. More conclusions can be accurately made only after further research upon pollen grains directly.

Keywords: carbohydrates, diversity, Mambilla plateau, Ogba, Oka-Akoko

Introduction

Honey is a sweet yellowish or brownish viscid fluid produced by honey bees, using nectar from flowers, through a process of regurgitation and evaporation (e.g. they transform nectar into honey) (Molan, 1992). The variety of honeys produced by honey bees (*Apis melifera*) is the most commonly collected by most beekeepers and consumed by people in Nigeria (Sowunmi, 1976; Agwu and Akanbi, 1985; Adeonipekun, 1989; Adekanmbi and Ogundipe, 2009).

The study of pollen mainly, but with a focus also on spores, as well as other constituents such as fungal spores and hyphae in honey, is referred to as melissopalynology. A lot is known all over the world about botanical and ecological origins of honey samples, the honey plants and honey quality. In Nigeria, Sowunmi (1976), Agwu and Akanbi (1985), Adeonipekun (1989), Ayodele *et al.* (2006), Njokuocha and Ekweozor (2007), Adekanmbi and Ogundipe (2009), Adeonipekun (2010, 2012), Ige and Modupe (2010), Aina and Owonibi (2011), Aina *et al.* (2015) are the major works on melissopalynology. These workers have supplied the available information about the botanical and geographical origins and studied the biochemistry of honey, as well as its quality determination across the country.

Typical plants whose pollen has been found in Nigerian honey samples include *Lannea microcarpa, Senna* spp., *Daniellia oliveri, Parkia biglobosa*, *Hymenocardia acida, Lophira lanceolata, Syzygium guineensis, Parinari* spp., *Elaeis guineensis, Alchornea cordifolia* and members of Combretaceae/Melastomaceae in South-Eastern Nigeria (Njokuocha and Ekweozor, 2007); *Elaeis guineensis, Berlinia grandifolia, Tridax procumbens, Chromolaena odorata, Combretum* spp., *Nymphaea lotus*, *Syzygium guineensis*, Rutaceae (*Citrus* spp.) *Talinum triangulare, Entada abysinica*, *Polygonum* sp., *Boerhavia diffusa*, *Drepanocarpus* sp., *Mussaenda polita, Luffa echinata* and *Chlorophora excelsa* in South-Western Nigeria (Agwu and Akanbi, 1985; Adeonipekun, 1989; Adekanmbi and Ogundipe, 2009; Adeonipekun, 2010, 2012) and *Parinari kerstingii, Lannea* spp., *Syzygium* spp, Poaceae, *Elaeis guineensis, Entada abyssinica, Vitellaria paradoxa* in North-central Nigeria (Ige and Modupe, 2010).

In Nigerian traditional medicine honey is widely used as an antimicrobial agent and also as a base for many herbal drugs. This is due to the following factors inherent in honeys which all together contribute to its antimicrobial properties: osmotic effect, acidity (honey pH is 3.5-3.9), hydrogen peroxidase and phytochemicals present in the flower-nectar (Molan, 1992). Honey possesses powerful

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antimicrobial properties that can be utilized at low cost and at no risk. Various studies have reported the antimicrobial activities of honey (Asadi-Pooya *et al*., 2002; Al-Waili, 2004; Agbaje *et al*., 2006; Chambers, 2006; Vilma *et al*., 2007; Mekky, 2007; Israili, 2014). The intrinsic properties of honey have been reported to affect the growth and survival of microorganisms by bacteriostatic or bactericidal actions (Iurlina and Fritz, 2005).

In spite of all these efforts, the contribution of pollen assemblages which are characteristic in honey in all these previous studies has not been evaluated. Emphasis has always been on the nectar of foraged plants (Jantakee and Tragoolpua, 2015); meanwhile, pollen grains are known to contain a good number of minerals, vitamins and proteins, as well as lipids (Stanley and Linskens, 1974). These make them excellent for bees' diet. De Groot (1953) observed that addition of pollen to bees' diet greatly increased their longevity, while Kropacova *et al*. (1968) observed that the development of the ovaries of bees was favored by pollen in their diets, as cited by Stanley and Linskens (1974). Taylor (1974) reported that bees feed their young ones and the larval queen with pollen in the form of ordinary jelly, while royal jelly – a secretion from the pharyngeal and mandibular glands of worker bees is fed to the queen. The adult bees use pollen directly as food to get essential elements, proteins and lipids.

In Nigeria, the few published works on the antimicrobial properties of honey are those of Omojasola (2002), Olaitan *et al*. (2007) and Agbagwa and Frank-Peterside (2010). Interestingly, traditional medicine practitioners in Nigeria generally prefer honey from the savanna to other regions in treating ailments. Their reason is that these savanna honeys serve as better herbal bases than those from other ecological zones. Agbagwa and Frank-Peterside (2010) in their work on commercial honeys across Nigeria discovered that an excellent antibacterial activity was observed with respect to honey from the northern Nigeria. This was followed by honeys from southern, eastern and western Nigeria in descending order of zones of inhibition.

To verify this popular belief of Nigerian traditional medicine practitioners and the results of Agbawa and Frank-Peterside (2010), honey samples from three ecological zones of montane Sudan savannah, rainforest and coastal freshwater vegetation types were studied melissopalynologically along with their proximate and mineral compositions, pH and antimicrobial activities. The aim of this work was to assess the contributory roles of the floral (pollen) components and indirectly the ecological factors if any in their bactericidal activities through the comparison of the melissopalynological components, proximate and mineral compositions, and antimicrobial activities of the honey samples, using two bacteria strains.

Materials and Methods

Sample collection

Honey samples were open market-sourced from Oka-Akoko in Ondo state (lowland rainforest zone) and Mambilla plateau in Taraba state (montane Sudan savanna zone), as well as apiary-sourced from Ogba in Lagos state (coastal freshwater vegetation zone) of Nigeria (Fig. 1).

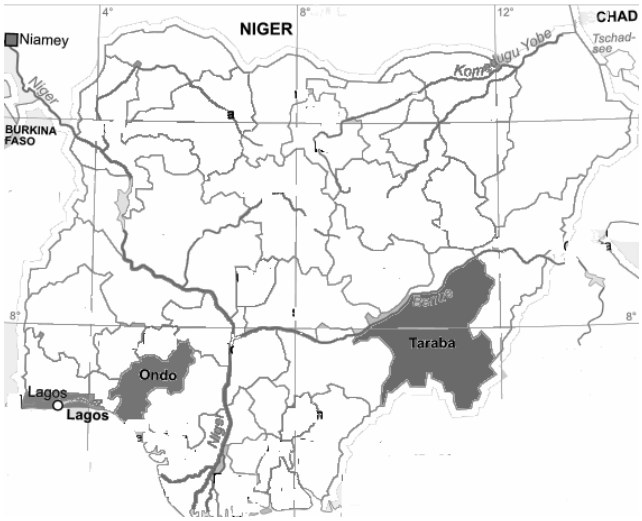

Fig. 1. Map of Nigeria showing the three sample collection locations

Sample preparation (Melissopalynology)

Five grams of honey samples from each location were weighed and poured into test-tubes and acetolyzed according to Erdtman's (1969) method. Glycerine (0.5 ml) was added to each sample and the storage bottles were labelled respectively. Micropipette was used to transfer 0.1 ml of the residue on glass slides and cover-slipped with nail

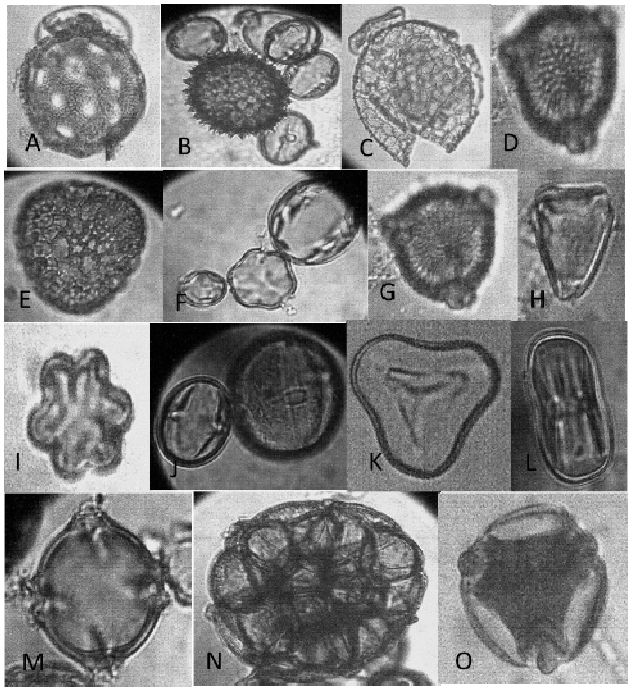

Fig. 2. A - *Amaranthus* sp., B - Asteraceae surrounded by five *Mussaenda polita* pollen; C - *Bombax* sp., D – *Hippocratea* sp., E - *Ceiba pentandra*, F - *Combretum* sp. with *Mussaenda*, G- *Hyppocratea*, H- *Syzygium guineensis,* I - *Combretum* sp., J - Large grain: *Grewia* sp with *Mussaenda* sp. K- *Elaeis guineensis*, L - *Sapotaceae*, M – *Mussaenda polita*, N- *Parkia clappertoniana*, O - *Senna* sp. Mag.x400. Bar 35 μ

polish used as sealant. Each slide was observed under Olympus 2.0 light microscope and the recovered pollen were counted and recorded. The view count method was used in the palynological analysis, of which 20 representative focal points were picked on each slide and studied. Identification was done using published floras and atlases such as Sowunmi (1976), Agwu and Akanbi (1985), Adeonipekun (1989, 2010, 2012) and Gosling *et al*. (2013), as well as the reference slide collection of the Laboratory of Palaeobotany/Palynology, Department of Botany, University of Lagos, Akoka, Lagos. Photomicrographs of some important pollen were taken with a Motic camera 2.0 and displayed in a photomicrographic album (Fig. 2).

Antibacterial assay

Pure cultures of *Staphylococcus aureus* (gram positive) and *Pseudomonas aeruginosa* (gram negative) bacteria were obtained from the Department of Microbiology, University of Lagos. Commercially produced Nutrient Agar (NA) was used to culture the bacterial species and run the antibacterial assay. To prepare 250 ml of NA, 7 g of NA were dissolved in 250 ml of distilled water in a sterile 250 ml conical flask. The mouth of the flask was plugged with cotton wool wrapped in aluminum foil and taped round with a masking tape. The conical flask was gently shaken to avoid air bubbles and then heated in a water bath to homogenize the agar at about 100 °C. The medium was then sterilized in an autoclave at 121 °C for 15 min and then allowed to cool before pouring into sterile Petri dishes.

The agar diffusion test method of Aneja and Joshi (2009) was applied. About 10 ml of NA was poured into Petri dishes and allowed to solidify. The medium was inoculated with bacteria by streak plate method. Wells were prepared in the plates with a cup-borer (0.85 cm) and 100 µl of the test compound was pipetted directly into the well. Two controls were used: sterilized distilled water and tetracycline. Tetracycline control was prepared by dissolving 12.5 g of tetracycline powder in 10 ml of sterile distilled water. Prior to incubation at 37 °C for 24 h, the Petri dishes were kept at room temperature for 15 min in order to promote diffusion of the extracts into the agar.

Measurements were taken using a meter rule from the position of the extract to the point of no inhibition. This was done for all four wells in each Petri dish. The mean was then calculated. It is important to state that none of the tested honeys was diluted.

Proximate and mineral analysis

Aliquots were made from 0.5 g of fresh weight from each sample analyzed. Moisture, ash, crude protein, fat contents and dietary fiber were evaluated by the methods described in AOAC (1999). Moisture was determined using the drying oven method, by drying a representative 5 g sample in an oven at 105 °C for 3 hrs. The ash content was determined by the incineration of a 4 g sample in a muffle furnace at 600 °C for 6 h until the ash turned white. Crude protein was estimated by the Kjeldahl method (1883). Total protein was calculated by multiplying the evaluated nitrogen by 6.25. The fat content was determined by petroleum ether extraction in a Soxhlet apparatus. A representative 3 g of sample was extracted for 6 h. Dietary fibre was evaluated by an enzymatic gravimetric method using the Tecator Fibertec E System (Foss Tecator, Sweden). Carbohydrates (g/100 g) were estimated by using a different method, as described by Dara (2006), by subtracting the sum of the percent of protein, moisture, fat and ash from 100.

The ash of each honey sample obtained was digested by the addition of 5 ml of 2 M HCL in a crucible and heated to dryness on a heating mantle. Five microliters of 2 M HCL were added afterwards, heated to boil and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up with distilled water stopper and made ready for reading. These diluents were aspirated into the Buck 211 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at its respective wavelength with their respective cathode lamps using appropriate fuel and oxidant combination (AOAC, 1999).

Determination of pH

The pH of honey samples were measured using a pH meter (Hanna Instruments, HI8424, Denver, USA).

Statistical analyses

For the antibacterial analysis, the tests were made in duplicates and the mean diameters of the inhibition zones were analyzed, as well as their standard errors. Using One-way ANOVA, their levels of significance were analyzed at $p < 0.05$. For the proximate analyses, samples were tested once and One-way ANOVA was used to analyze their level of significance at $p < 0.05$. For the pH, tests were in triplicates, the mean and standard errors were calculated, while One-way ANOVA was used to calculate their level of significance at $p < 0.05$. SPSS software 16 was used for all statistical analyses.

Results

Melissopalynology

In total, 1,001 pollen grains were counted, belonging to 23 families with 34 species. Mambilla Plateau honey had the highest palynomorph diversity (24 different species), while Ogba and Oka-Akoka honey samples had lower values (16 species each). The Ogba honey however recorded the highest abundance (577), while the Oka-Akoko had the least (Table 1). Dominant pollen types recovered from the Mambilla Plateau honey included *Alchornea cordifolia* and *Senna* spp.; in Oka-Akoko

Table 2. Antibacterial results of honey samples

Extracts	Zone of Inhibition (mm)	
	S. aureus	*P. aeruginosa*
Mambilla honey sample	20.9 ± 0.7^{b}	13.5 ± 1.3^{b}
Oka Akoko honey sample	7.3 ± 0.26^{a}	3.3 ± 0.6^{a}
Ogba honey sample	6.0 ± 0.4^{a}	1.7 ± 0.2^{a}
Distilled water	0	0
Tetracycline	31.3 ± 1.1^{c}	20.7 ± 0.8^{c}

Means in the same column with different letters were significantly different at p=0.05

Table 1. Palynomorphs recovered from Mambilla plateau, Oka-Akoko and Ogba honey samples

Palynomorphs	Families	Mambilla	Oka-Akoko	Ogba
Acacia senegal	Mimosaceae	-	-	4
Acacia sieberiana	Mimosaceae	3	-	-
Alchornea cordifolia	Euphorbiaceae	38	6	-
Amaranthaceae	Amaranthaceae	1	1	-
Arecaceae	Arecaceae	-	1	-
Asteraceae	Asteraceae	2	-	-
Berlinia glandifolia	Fabaceae	-	-	9
Bombax sp.	Bombacaceae	1	8	10
Borreiria sp.	Rubiaceae	-	-	2
Ceiba sp.	Malvaceae	-	4	2
Celtis sp.	Cannabaceae	8	-	-
Chromolaena odorata	Asteraceae	-	15	-
Combretum spp.	Combretaceae	13	9	108
Cyperus sp.	Cyperaceae	1	-	-
Elaeis guineensis	Arecaceae	19	80	-
Euphorbia reticulata	Euphorbiaceae	1	-	-
Grewia cf *bicolor*	Tiliaceae	17	7	-
Corchorus olitorus sp.	Tiliaceae	3	-	76
cf. Hippocratea sp. (small)	Hippocrateaceae	-	-	51
Hippocratea sp.	Hippocrateaceae	8	8	4
Hyphaene thebaica	Arecaceae	-	6	2
Hyptis sp.	Lamiaceae	-	-	2
Indeterminate pollen	-	-	2	-
Ipomoea sp.	Convolvulaceae	1	-	1
Milicia excelsa	Moraceae	-	7	-
Mussaenda polita	Rubiaceae	7	9	266
Nymphaea lotus	Nymphaeaceae	-	-	2
Parinari kerstigii	Chrysobalanaceae	4	-	-
Parkia clappertoniana	Mimosaceae	2	-	-
Paullinia pinnata	Sapindaceae	1	-	-
Poaceae	Poaceae	5	1	28
Polygonum sp.	Polygonaceae	5	-	-
Psilatricolporate pollen 1	-	5	-	-
Psilatricolporites pollen 2 (small)	-	27	-	-
Sapotaceae	Sapotaceae	-	-	5
Senna sp.	Caesalpinaceae	170	3	-
Syzygium guineensis	Myrtaceae	52	6	3
Uapaca togoensis	Euphorbiaceae	22	-	-
Vernonia sp.	Asteraceae	5	-	-
Verrumonocolpate "crassus" pollen	-	-	-	2
Total Abundance		247	177	577
Species Diversity		24	16	16

honey, *Elaeis guineensis* dominated, while *Combretum* sp., *Mussaenda polita* and *Corchorus olitorus* pollen dominated in the Ogba honey sample.

Antibacterial analysis

Antibacterial activity test revealed that Mambilla Plateau honey was the most active against both tested bacteria, though this was significantly lower than the control (Tetracycline), it was also significantly higher than Ogba and Oka-Akoko honey samples.

None of the samples was inactive against the tested bacteria (Table 2).

Proximate and minerals analysis

The Mambilla honey recorded the highest moisture content, ash, protein, fats and oil, calcium, potassium and reducing sugars. Oka-Akoko honey recorded the highest fibre, magnesium, iron and sodium content, while Ogba honey recorded the highest carbohydrates. The moisture content, protein, carbohydrates, magnesium and sodium were significantly different in the three honeys, while the reducing sugar, ash, fibre, fats, calcium, iron and potassium contents were not significantly different (Table 3).

pH measurements

The Mambilla honey recorded the highest acidity followed by the Ogba honey, while the Oka-Akoko honey had the least.

Discussion

Melissopalynology and ecology

From the melissopalynology, the Mambilla honey recorded the highest diversity and relatively high abundance though not as much as the abundance in the Ogba honey (Table 1). This high diversity is a reflection of the diverse

Table 3. Proximate composition, mineral contents and pH of honey samples

Parameters	Mambilla	Oka Akoko	Ogba
Moisture content (%)	23.64[a]	19.00[b]	16.39[b]
Ash (%)	0.81[a]	0.72[a]	0.64[a]
Protein (%)	2.38[a]	0.44[b]	0.38[b]
Fibre (%)	0.002[a]	0.004[a]	0.003[a]
Fats & oil (%)	0.005[a]	0.003[a]	0.002[a]
Carbohydrate (%)	73.16[a]	79.83[b]	82.58[b]
Reducing sugar (%)	85.00[a]	81.20[a]	80.70[a]
Calcium (mg/kg)	0.1[a]	0.06[a]	0.09[a]
Magnesium (mg/kg)	0.18[a]	0.34[a]	0.06[b]
Iron (mg/kg)	0.22[a]	0.43[a]	0.23[a]
Potassium (mg/kg)	3.26[a]	2.93[a]	1.55[a]
Sodium (mg/kg)	11.44[a]	13.01[a]	4.63[b]
pH	3.78±0.01[a]	3.89±0.04[a]	3.69±0.04[a]

Means in the same row with different letters are significantly different at p=0.05

vegetation on the Mambilla plateau which consists of montane wooded and grassland savanna, rainforest-like and riparian vegetation types, as reported by Keay (1959). The low diversity of the Ogba honey could probably have resulted from the secondary nature of the vegetation of the Lagos coastal area due to urbanization and industrialization or due to age and nature of bees that produced the honey. It is important to note that the Ogba honey was sourced from an apiary within a farm land where *Corchorus olitorus* - a popular vegetable in Southwest Nigeria - was part of the readily available potential forage. Nonetheless, the diversity was the same with Oka-Akoko (lowland rainforest), indicating the presence of vegetation regrowth/regeneration around the apiary of the Ogba honey.

The age of bees has been reported by Adeonipekun (1989, 2012) as an important factor determining their activity. In the palynological study of an apiary in Ibadan Southwest Nigeria, it was observed that an old and defensive colony of bees recorded higher abundance of pollen grains, while a young and gentle colony recorded lesser pollen grains but had higher diversity – a reflection of difference in their experience and nature. Thus, as consideration of the present work, bees in each zone investigated cannot be said to be of same age hence their activities and experience were different. Vegetation in the Ogba area is not as rich as the Oka-Akoko area, but most likely the bees of Oka-Akoko were older and more experienced than the Ogba and then more specific in foraging. This is apart from the fact that the *Elaeis guineensis* plants, whose pollen dominates in the Oka-Akoko honey, is abundantly available, hence there was no need for the bees in the area to go after other plants as such. This is unlike the Ogba area, whose vegetation has been greatly disturbed, leaving only *Mussaenda polita* and *Combretum* spp. as main bee pollen in the coastal freshwater area where the bees might have foraged many different plants and thus less specific. The high pollen diversity and population of Mambilla honey is a reflection of the luxuriant montane Sudan savanna vegetation which is less disturbed. This may also be due to age related factor which cannot be verified in the current study.

All three honey samples have different pollen dominating in them, which clearly reflects the vegetation types in their locations. Pollen of *Senna* spp. and *Alchornea cordifolia* dominate in Mambilla honey. The uniqueness of this area is once again highlighted as no previous published report on honey in Northern Nigeria has shown the dominance of pollen of *Senna* spp. Works from regions close to the Mambilla plateau, such as report on Kogi (Aina *et al*., 2015) and North central area (Ige and Modupe, 2009) indicated the dominance of pollen of species such as *Lannea,* Poaceae and *Elaeis guineensis* among others, but not the *Senna* taxus; Njokuocha and Ekweozor (2007) however recorded an abundance of *Senna* spp. among other pollen from honey of South-Eastern Nigeria

Of melissopalynological importance is the dominance of pollen of *Elaeis guineensis* in the Oka-Akoko honey sample. *Elaeis guineensis* is a species of palm commonly called African oil palm, which is the source of palm oil. Crane *et al*. (1983) reported that *Elaeis guineensis* pollen is abundant in honey due to the juice of the fermenting fruit which is collected by the bees, thereby making *Elaeis guineensis* their favorite in any area the plant inhabits. Its pollen is used as food by the bees since the plant does not produce nectar (Aina and Owonibi, 2011). Also, the plant is characteristic of open/secondary forest vegetation, which probably reflects the openness or secondary nature of the lowland rainforest in Oka-Akoko in Ondo state of Nigeria. *Mussaenda polita* pollen dominates the Ogba honey along with *Combretum* spp. to the extent of being 80% of pollen assemblages. Adeonipekun (2012) had earlier reported the abundance of *Mussaenda*-like pollen in honey samples from Lagos, which he erroneously referred to as cf. Olacaceae and Meliaceae due to its dimorphism. *Mussaenda polita* is a hydrophilic plant with dimorphic character (presence of two different pollen types) and hence may be difficult to be recognized correctly in honey. Its predominance in the Lagos honey may therefore be characteristic of the coastal vegetation of Lagos state.

Antimicrobial, proximate and mineral analyses

Malika *et al*. (2005) in a study of the microbiological and physio-chemical properties of Moroccan honey reported that the moisture content determines the quality of honey, since it affects storage life and processing characteristic. Even more, Oyeleke *et al*. (2010) and Buba *et al*. (2013) have found out that the moisture content of Nigerian honeys fall within 12.5-25.2%. Incidentally, all the honey samples studied in the present work also fall within that range, with the Ogba honey having the least and hence should have the best quality in terms of shelf-life. This is not surprising as it was the only one sourced from a modern apiary and professionally processed area, while the other two samples were sourced from the open market which could have been poorly processed due to over dilution with water or exposure.

From the antibacterial tests using the two bacteria strains, *Staphylococcus aureus* and *Pseudomonas aeruginosa,* the Mambilla honey was the most effective in inhibiting their growth (Table 2). Factors characteristic of the Mambilla honey as revealed from the proximate and mineral analyses are: highest values of moisture content, ash, protein, fats and oil, potassium, calcium, and reducing sugars (Table 3). Out of these indices, the high reducing sugars content is an important factor involved in the

highest antimicrobial activity, though not significantly different from the other honeys' reducing sugar values. However, notable significantly different factors were moisture content, protein, carbohydrates, magnesium and sodium. The moisture content though reported to be good when a low values is obtained, thus for a good honey shelf-life and indirectly the nutritional value; when high, it has been implicated in the antimicrobial activity of honey, for it is directly proportional to water activity caused by dilution (Chirife *et al*., 2006). This is because dilution of honey to lower concentrations has been reported to lead to the production of hydrogen peroxide through the action of glucose oxidase enzyme (Molan, 1992; Jantakee and Tragoolpua, 2015). Lower carbohydrate, fat and oil values, along with high protein and potassium values seem to favour the antimicrobial activities of the studied honey samples. Though not significantly different among the honey samples, high potassium, calcium, ash contents favor the recorded antibacterial activities. Magnesium, iron and crude fibre seem not to have any effect either positive or negative on the activity of honey as revealed from the hereby study.

The determinant of the presence and quantity of these mineral and nutritional components of each honey are the floral contents sourced through the nectar and the pollen grains the bees fed on. Thus, attention has been given to the nectar as understandable since the monosaccharide sugars are known antimicrobials. This was however done along with a neglect of the pollen contents of honey which is the food the bees fed on. Several works have shown that pollen were purposely collected as food (Adeonipekun, 1989; Aina and Owonibi, 2011) and even in honey combs, cells are packed with pollen alone and some packed together with honey (Adeonipekun, 2012). If the popular saying that "you are what you eat" is correct, then the activities of bees and their products will reflect what the bees fed on. In the present work, different pollen served as food for the bees that produced the three honey samples since they come from distant and different ecozones of Nigeria, with different climatic parameters. Since the proximate and mineral analyses have revealed that the reducing sugars of the three honeys were not significantly different, then moisture content, characteristic mineral contents and the pollen content might have served as or introduced the none hydrogen peroxide antimicrobial agents in the honeys. The more or less similar reducing sugars content was definitely not the cause of the difference in their antimicrobial activity.

That the acidity of honey is an important factor in its antibacterial activity has been stressed by Molan (1992), Mandal and Mandal (2011), Jantakee and Tragoolpua (2015). However, from the results of the acidity test of all the hereby tested honeys, the Ogba honey recorded the highest acidity, even though there was no positive correlation between acidity and the antibacterial activities of the honeys and ironically, it was the one with the least microbial inhibition. This was because, unexpectedly, the Oka-Akoko honey with the lowest acidity had a higher antibacterial activity than that of Ogba (Table 3). These results then point to the clearly different pollen assemblages through which some of the minerals and proximate contents might have been introduced, which could be the main source of the significantly different antibacterial activities.

To assess indirectly the contribution of pollen to the proximate components of honey, proximate analyses of pollen grains of *Cynodon dactylon, Panicum maximum, Cyperus rotundus, Mariscus alternifolius, Alchornea cordifolia, Amaranthus hybridus* and *Tridax procumbens* were carried out. The result of this effort showed that their reducing sugar contents varied between 40-60%, while the protein varied between 17 and 22% (Table 4). This confirms that the total reducing sugar of honey is also augmented by the reducing sugars of the pollen of plants foraged, since through the digestive system of the bees, some of these sugars would have been introduced. The overall consequence of this fact is the addition or the increase of already implicated reducing sugars within the honey. However, as noted, different pollen had different percentages of reducing sugars (Table 4), which means that a particular plant with a pollen high in reducing sugars will contribute with more sugar to the honey than those with lesser percentages. Hence, the need to give more attention to the pollen contents of honey. Similarly and more importantly is the overall protein contribution from pollen grains. Different pollen contribute in different proportions of proteins through the pollen to honey, since these proteins have been implicated in the inhibitory activities of honeys; as noted in the present work, a plant with high protein pollen in honey will confer a high antimicrobial activity on such honey. The pollen of *Senna* sp., *Syzygium guineensis*, *Corchorus olitorius* and *Alchornea cordifolia*, that are dominant in the Mambilla honey need to be critically evaluated to assess their protein contents and antimicrobial activities.

Agbagwa and Frank-Peterside (2010) were the first to investigate and compare the antimicrobial activities of honey from different geographical zones in Nigeria. The researchers examined different honey samples and found out that honey from the northern part, with characteristic different savannah vegetation type, had higher antimicrobial activities compared with the Southern part with wet rainforest vegetation. This observation was also recorded in the current work with the Mambilla honey having the highest antimicrobial activities. The suposistion

Table 4. Proximate analysis of some pollen grains

Plant	Families	Protein (%)	Reducing sugar (%)
Mariscus alternifolius	Cyperaceae	18.0	60.0
Alchornea cordifolia	Euphorbiaceae	20.0	40.0
Tridax procumbens	Asteraceae	21.0	48.0
Panicum maximum	Poaceae	19.0	54.0
Cynodon dactylon	Poaceae	17.0	46.0
Cyperus rotundus	Cyperaceae	22.0	47.0
Amaranthus hybridus	Amaranthaceae	18.0	42.0

here is that the pollen of *Senna* spp., *Syzygium guineensis* and *Alchornea cordifolia* alone or in conjunction with others might have been responsible for the significant elemental and nutritional components. Conclusion on this cannot be accurately made until the pollen grains of these suspected plants are biochemically and antimicrobial investigated directly. Climatic factors of these three areas might also have been indirectly responsible. Savanna pollen is commonly thicker in their exine structure and hence may contain higher concentration of elements and substances. These hypotheses of differential thickness and mineral components however must be investigated scientifically for a concrete inference to be made.

Conclusions

The pollen contents of studied honeys revealed to some extent the characteristic floristic composition of the ecological regions of the source areas, in that they are totally different from one another. Thus, the different antibacterial activities is more attributable to the significantly different pollen components, moisture content, proteins and carbohydrates, and less to the potassium, fat and oil, magnesium, sodium, ash contents and the reducing sugar contents. The pH does not seem to have any contributory role in the antibacterial activity of the honeys.

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