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Nutritional Compositions of the Nectar of Melliferous Plants and their Impact on Honey Production in Selected Vegetation Zones in Nigeria

Ifiok D. Uffia, C. O. Akachuku & O. E. Udofia

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Abstract *Nutritional compositions of the nectar of melliferous plants and their impact on honey production were studied for 48 months in the mangrove vegetation (in Akwa Ibom and Rivers States in Nigeria) and rainforest vegetation (in Abia and Imo states in Nigeria). Nectar of melliferous plants was collected randomly for three years (2015-2017) and from two states in each of the vegetation zones during dry and raining seasons. Samples were also taken in the early and later stages of flowering and the plants selected for study were Helicteres ixora, Musa paradisiaca, Thevetia peruviana, Costus afer, Allamanda cathartica, Canna generalis and Setcreasea pallida. In each of the zones, nectar was extracted from the flowers directly, using a 10 µl capillary tube from the floral cup of 200-3000 flowers of melliferious plants. Measured concentrations of sugar and vitamins indicated that vitamin B2 contents had the least mean value of 0.066 mg/100g while vitamin C contents had highest mean value of 72.971 mg/100g. Mean concentrations of glucose, fructose and sucrose were 172.164 mg/100g, 172.893 mg/100g and 173.296 mg/100g respectively. Mean seasonal variability revealed that higher concentrations of vitamins and sugar in nectar of melliferous plants was in the dry season indicating that the rate of biochemical processes within plants that produces nectar increases with increase in temperature.*

Key words: *Nectar, Melliferous Plants, Mangrove, Rainforest, Vegetation Zone*

***I. D. Uffia**
[1]Department of Genetics and Biotechnology,
Akwa Ibom State University, P. M. B. 1167,
Uyo, Akwa Ibom State, Nigeria
Email: ifiok.uffia@yahoo.com

C. O. Akachuku
[2]Department of Forestry and environmental Management,
Michael Okpara of Agriculture, Umudike, Abia State

O. E. Udofia
Department of Genetics and Biotechnology,
Akwa Ibom State University, P. M. B. 1167,
Uyo, Akwa Ibom State, Nigeria.

1.0 Introduction

Melliferous plant species are plants that are visited by the bees and produce substances that can be harvested by honeybees for the production of honey (Akachuku, 2002). Osborne *et al.* (2000) stated that a plant is a melliferous plant if it is visited by honeybees for nectar or pollen collection. According to Burtchnell (2000), honeybees can collect nectar and/ or pollen from only a few flowering plants because of their body size and shape and length of proboscis. Adekanmbi and Ogundipe (2009) explained that melliferous plants contribution to honey production depends mostly on its nectar quality and quantity. Adekanmbi and Ogundipe (2009), further explained that not all nectar produced by flowers is accessible to honeybees. Even if accessible, the amount and quality of nectar varies from plant to plant and over time. Ben (2004) stated that bees extract nectar from different flowers and combine it with enzymes to produce honey which is then stored in their honeycombs. Kaal (1991) stated that the type of vitamins, minerals and their quantities found in any honey sample depends on the type of flowers visited by the honeybees. This implies that the chemical composition of honey, its quality and quantity depend on the floral source. The need for an alternative source of revenue in Nigeria apart from crude oil has become imperative because there is a serious demand for economic diversification. Projects that can promote self-reliance with the aim of reducing unemployment is grossly inadequate in Nigeria especially those that are geared towards understanding the value of melliferous plants for honey production as well as for the conservation of such multipurpose honey plants. According to National Honey Board of Nigeria (2008), the availability of good quality honey in the country has declined tremendously in the last two decades. This could be as a result of factors that affect honey production which include deforestation, uncontrolled bush burning, pesticide usage, hydrocarbon pollution, poor waste management system and unsustainable agricultural practices (Akachuku. 2002). Akachuku (1996) and Ben (2004) reported that studies on effects of environmental degradation on distribution and nectar quality of melliferous plants and subsequent effect on honey production have received very limited consideration. Available data on melliferous plant species richness, nectar characterization and their influence on all aspects of honey production remain largely unlimited in Nigeria. Despite the importance of such studies, very little research has been considered especially on the richness of such species and nectar quality in different vegetation zones of Nigeria. The aim of the study is to assess the nutritional composition of the nectar of melliferous plant species in the selected vegetation zones in Nigeria.

2.0 Materials and Methods

The study was carried out in mangrove and rainforest vegetation zones in Nigeria. Akwa Ibom and Rivers State were selected in this vegetation zone for the study. Mangrove vegetation zone is located along the Atlantic Coast with Latitude 4˚15 'N and 4˚55' N and longitude 6˚ 10 and 8˚ 16'E (UNEP. 2007. According to Ukpong (2007), the mangrove forest is known to be rich in both aquatic and terrestrial biodiversity, as such, a major source of rural life sustenance not only in Akwa Ibom and Rivers States but also in many other regions in Niger Delta and elsewhere (Meteorological services, 1980). Abia and Imo States were representative states for rainforest forest vegetation zone which is located on latitude 05°02′ N and 05°04′N and on longitude 8°07′ and 8°22′E. The proximity of the Atlantic Ocean has a moderating effect on temperature with highest average daily maximum of 35 °C and recorded mean actual temperature of 26 °C. The area has an average relative humidity of 80–90% at 10 am during the raining season (source: Metrological Station, National Root Crop Research Institute (NRCRI) Umudike, Nigeria).

The determination of honeybee plants was done through direct observation of foraging honeybee workers on its flowers. These observations were made during field trips, which lasted from dawn to dusk, organized periodically in all seasons. Only plants on which many foragers sustained foraging for nectar and/or pollen were recorded.

Nectar of melliferous plants were collected randomly from two vegetation zones in the two states of Nigeria, during dry and wet seasons for three years (2015-2017), in the early and later stages of flowering which were *Helicteres ixora*, *Musa paradisiaca*, *Thevetia peruviana, Costus afer*, *Allamanda cathartica*, *Canna generalis* and *Setcreasea pallida.* In each of the zones, nectar was extracted from the flowers directly, using a 10 µl capillary tube: a sterile technique according to Harrigan (1976) and Kearns and Inouye (1993) from the floral cup of 200-3000 flowers of melliferious plants, depending on nectar yield of each plant. The tubes of the micropipettes and micro-syringes were used for extracting nectar of many plant species where floral nectar was highly viscous, or was produced in low volumes (<1 µl) (Corbet, 2003). Thus, the number of flowers in a tree species from which nectar was collected indicates the nectar quantity. Samples collected were transferred to a refrigerator at -4°C.

For analysis of various sugars (glucose, fructose, sucrose) from extracted nectar of melliferious plants, Sample cleanup to remove less polar impurities was done through solid-phase extraction on C18 columns then dried in a vacuum centrifuge, diluted with 200 µl distilled water, and filtered using a WATERS™ high performance carbohydrate column to avoid contamination. Samples were injected directly after filtration. The injection volume was 10 µl, and elution took place with an acetonitrile–water mixture (71: 29) at a flow rate of 1·4 ml min^{-1} and a temperature of 40 °C. Glucose, fructose, and sucrose were detected with a refraction index detector and quantified with the WATERS Millenium Software™. Concentrations were converted from µg $µl^{-1}$ to sucrose-equivalent, percentage weight per total weight (Weast, 1969: AOAC, 2003: Čižmárik, 2004).

Vitamins (A-C) contents was determined using Utra-performance liquid chromatography (UPLC) which is an easy and accurate way to qualify and quantify specific sugars and other chemicals within a nectar sample. After centrifugation and filtration (with a 0.20 µm syringe filter), the sample was analyzed using a UPLC system (Waters, USA), an Acquity UPLC® HSS T3 (2.1 x 100 mm, 1.8 µm) column, and a PDA detector (Waters, USA) set at a wavelength of 254 nm. The mobile phase used was 99% methanol and 1% distilled water with 0.1% formic acid solution at a flow rate of 0.3 mL min^{-1}. Authentic vitamins standard was used for identification and quantification of the peak. (Kearns and Inouye, 1993: Spinola *et al*. (2012).

3.0 Results and Discussion

Results obtained for overall sugar and vitamin contents in nectars extracted from different flowers of melliferious plants are recorded in Table 1. The results revealed that vitamin B2 contents had the least mean value of 0.066 mg/100g while vitamin C contents had overall highest mean value of 72.971 mg/100g. Glucose, fructose and sucrose contents recorded overall mean values of 172.164, 172.893 and 173.296 mg/100g respectively. Glucose content had the least overall mean value amongst the measured sugar in the study area.

Results in Table 2 summarize levels of vitamin and sugar contents in nectar extracted from flowers of melliferous plants in mangrove vegetation zone of Nigeria. The results indicated no significant difference ($p>0.05$) in vitamin A content which varied from plant to plant; *Helicteres ixora* (0.19±0.00), *Musa paradisiaca* (0.14±0.00), *Canna generalis* (0.17±0.00), *Thevetia peruviana (*0.15±0.00*)*, *Costus afer* (1.21±1.05), *Allamanda cathartica* (0.15±0.01), *Setcreasea pallida* (0.22±0.01) but significant difference ($p<0.05$) was recorded in vitamin B1, vitamin B2, vitamin B3, vitamin C, glucose, fructose and sucrose contents. The vitamin B1 contents in nectar of *Helicteres ixora*, *Musa paradisiaca*, *Canna generalis*, *Thevetia peruviana*, *Costus afer*, *Allamanda cathartica* were significantly higher ($p>0.05$) than the vitamin B1 content of *Setcreasea pallida. Allamanda cathartica* had the least content compared to vitamin B2 contents of the other six plant species. *Helicteres ixora, Canna generalis* and *Costus afer* had higher ($p>0.05$) vitamin B3 contents than *Setcreasea pallida*. However, vitamin B3 content of *Helicteres ixora*, *Musa paradisiaca*, *Canna* generalis, *Thevelia peruviana*, *Costus afer* and *Allamanda cathartica* were not from each other. Vitamin B3 contents of *Setcreasea pallida*, *Musa parasidiaca*, *Thevelia peruviana* and *Allamanda cathertica* were statistically similar. In term of vitamin C contents, *Canna generalis* and *Musa paradisiaca* had significant highest and least concentrations respectively. The statistically similar values of *Helicteres ixora* and *Canna generalis* had the highest ($p>0.05$) glucose values while *Musa paradisiaca* had the least content. The fructose contents of *Canna generalis*, *Helicteres ixora* and *Costus afer* had did not displayed any significant difference but was significantly higher than the content in *Musa paradisiaca* which had the least value. There was not statistically difference in sucrose contents of *Helicteres ixora* and *Canna generalis*. However, their sucrose contents were significantly higher than the sucrose content of *Musa paradisiaca* which had the least sucrose content.

Table 3 presents the results of various vitamin and sugar contents of nectar from melliferous plant species in the studied rainforest vegetation. Significant difference ($p<0.05$) was recorded in all the vitamin and sugar contents in nectar extracted from flowers of melliferous plants in the rainforest vegetation zone. In terms of vitamin A, the statistically similar vitamin A contents of *Canna generalis* and *Setcreasea pallida* were significantly higher ($p<0.05$) than vitamin A contents of

Helicteres ixora, *Musa paradisiaca*, *Thevetia peruviana*, *Costus afer*, *Allamanda cathartica*. The statistically similar vitamin B1 con tents *Helicteres ixora*, *Costus afer* and *Allamanda cathartica* were also significantly higher ($p<0.05$) while *Setcreasea pallida* had the least content. In the same trend, the statistically similar vitamin B2 contents of *Helicteres ixora*, *Canna generalis* and *Setcreasea pallida* were significantly higher ($p<0.05$) when compare to statistically similar vitamin B2 contents of *Musa paradisiaca*, *Thevelia peruviana* and *Allamanda cathartica*. In terms of vitamin B3, *Setcreasea pallida* had significantly the least contents when compare to the vitamin B3 contents of the other six plant species. *Canna generalis* had significantly the highest vitamin C content when compare to the rest of the six plant species. Vitamin C contents of *Thevelia peruviana* and *Allamanda cathartica* were statistically similar. Glucose contents of statistically similar *Helicteres ixora* and *Canna generalis* had the highest significant ($p<0.05$) values while *Musa paradisiaca* of glucose had the least significant values. In the same trend, *Canna generalis* had the highest significant values of fructose contents while *Musa paradisiaca* of had the least significant values. In terms of sucrose contents, the statistically similar *Helicteres ixora* and *Canna generalis* had the highest significant values while *Musa paradisiaca* of had the least significant values. Sucrose contents of *Thevelia peruviana* and *Allamanda cathartica* were statistically similar.

The overall sugar and vitamin contents in nectars extracted from various melliferious plants in this study showed that vitamin B2 contents had the least mean value of 0.066 mg/100g while vitamin C contents had overall highest mean value of 72.971 mg/100g. Glucose, fructose and sucrose contents recorded overall mean values of 172.164 mg/100g, 172.893 mg/100g and 173.296 mg/100g respectively. Glucose content had the overall least mean value (172.164 mg/100g). Like other animals, honey bees need carbohydrates as an energy source

Table 1: Vitamins and sugar contents in nectar of melliferous plants in the Mangrove and Rainforest vegetation zones during the study

Nectar parameters (mg.100 g)	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
Vitamin A	1.120	0.020	1.140	0.203	0.008	0.120	0.014
Vitamin B1	1.710	0.090	1.800	1.074	0.013	0.201	0.040
Vitamin B2	0.550	0.020	0.570	0.066	0.003	0.054	0.003
Vitamin B3	1.290	0.510	1.800	1.451	0.011	0.174	0.030
Vitamin C	67.320	31.080	98.400	72.971	1.048	16.635	276.729
Glucose	184.670	18.610	203.280	172.164	3.835	60.881	3706.474
Fructose	183.460	19.720	203.180	172.893	3.748	59.495	3539.608
Sucrose	183.130	20.000	203.130	173.296	3.785	60.088	3610.598

All carbohydrates are first converted to glucose, which enters the Krebs cycle and produces ATP, the fuel in nearly all cells, and carbon dioxide and water as by-products. A worker bee needs 11 mg of dry sugar each day (Brodschneider, 2010). This affirmed observations of Nettles (1986) and Somerville and Nicol (2002) that Glucose is important for larval growth, development, pupation and survival at low amino acid levels. Since different plant species has a specific nectar characteristic and by understanding nutritional need of honeybees and providing solutions to them, honeybees will be as healthy as possible.

Table 2: Vitamin and sugar contents in nectar extracted from flowers in the Mangrove vegetation zone of Nigeria

	Plants							
Nectar parameters (mg/100 g)	*Helicteres ixora*	*Musa paradisiaca*	Canna generalis	*Thevetia peruviana*	*Costus afer*	*Allamanda cathertica*	*Setcreasea pallida*	Significant
Vitamin A	0.19±0.00	0.14±0.00	0.17±0.00	0.15±0.00	1.21±1.05	0.15±0.01	0.22±0.01	No
Vitamin B1	1.14±0.00$_{a}$	1.10±0.01$_{a}$	1.13±0.00$_{a}$	1.11±0.00$_{a}$	1.15±0.03$_{a}$	1.12±0.01$_{a}$	0.91±0.10$_{b}$	Yes
Vitamin B2	0.07±0.00$_{b}$	0.06±0.00$_{b}$	0.07±0.00$_{b}$	0.06±0.00$_{b}$	0.06±0.00$_{b}$	0.14±0.04$_{a}$	0.06±0.00$_{b}$	Yes
Vitamin B3	1.47±0.00$_{a}$	1.40±0.01$_{ab}$	1.46±0.01$_{a}$	1.38±0.01$_{ab}$	1.45±0.01$_{a}$	1.41±0.01$_{ab}$	1.32±0.09$_{b}$	Yes
Vitamin C	83.51±0.73$_{c}$	39.57±1.05$_{f}$	94.07±0.80$_{a}$	64.45±0.82$_{e}$	89.50±0.83$_{b}$	65.22±0.58$_{e}$	78.52±0.67$_{d}$	Yes
Glucose	202.11±0.12$_{a}$	25.78±1.24$_{e}$	201.73±0.20$_{a}$	192.12±0.56$_{d}$	198.90±0.43$_{b}$	192.00±0.63$_{d}$	196.59±0.52$_{c}$	Yes
Fructose	199.42±0.31$_{ab}$	26.28±1.07$_{e}$	200.11±0.28$_{a}$	195.56±1.04$_{cd}$	197.94±0.88$_{abc}$	193.24±1.30$_{d}$	197.11±0.51$_{bc}$	Yes
Sucrose	202.31±0.06$_{a}$	27.07±1.16$_{e}$	202.08±0.13$_{a}$	191.91±1.22$_{c}$	198.05±0.55$_{b}$	186.87±1.66$_{d}$	197.87±0.28$_{b}$	Yes

Means with different subscripts along the same row are significantly different (Duncan's test) $p<0.05$

Table 3: Vitamins and sugar contents in nectar extracted from flowers in Rainforest vegetation zone of Nigeria

	Plants							
Nectar parameters (mg/100g)	Helicteres Ixora	*Musa paradisiaca*	Canna generalis	*Thevetia peruviana*	*Costus afer*	*Allamanda cathertica*	*Setcreasea pallida*	Significant
Vitamin A	0.20±0.01$_{bc}$	0.14±0.01$_{c}$	0.39±0.06$_{a}$	0.24±0.03$_{b}$	0.17±0.01$_{bc}$	0.19±0.01$_{bc}$	0.34±0.05$_{a}$	Yes
Vitamin B1	1.12±0.01$_{a}$	1.03±0.02$_{ab}$	1.10±0.01$_{a}$	0.97±0.08$_{ab}$	1.13±0.02$_{a}$	1.10±0.05$_{a}$	0.94±0.09$_{b}$	Yes
Vitamin B2	0.07±0.00$_{a}$	0.05±0.00$_{b}$	0.07±0.00$_{a}$	0.06±0.00$_{b}$	0.06±0.00$_{b}$	0.05±0.00$_{b}$	0.07±0.00$_{a}$	Yes
Vitamin B3	1.64±0.01$_{a}$	1.42±0.02$_{bc}$	1.64±0.02$_{a}$	1.42±0.02$_{bc}$	1.43±0.03$_{bc}$	1.50±0.02$_{b}$	1.38±0.09$_{c}$	Yes
Vitamin C	80.76±1.42$_{c}$	43.79±1.08$_{f}$	91.16±0.57$_{a}$	64.49±0.46$_{e}$	86.30±0.86$_{b}$	63.66±0.33$_{e}$	76.61±0.51$_{d}$	Yes
Glucose	201.97±0.21$_{a}$	22.10±0.23$_{e}$	200.56±0.40$_{a}$	192.13±1.02$_{c}$	196.64±1.76$_{b}$	188.81±1.72$_{d}$	198.89±0.70$_{ab}$	Yes
Fructose	200.39±0.48$_{ab}$	29.69±0.84$_{e}$	201.74±0.19$_{a}$	192.07±0.38$_{d}$	195.37±2.93$_{cd}$	194.49±0.27$_{cd}$	197.09±0.34$_{bc}$	Yes
Sucrose	201.95±0.15$_{a}$	26.93±0.46$_{d}$	202.10±0.12$_{a}$	193.23±0.85$_{c}$	200.20±0.42$_{b}$	194.46±0.66$_{c}$	201.09±0.34$_{ab}$	Yes

Means with different subscripts along the same row are significantly different (Duncan's test) $p<0.05$

This affirmed observations of Akachuku and Onyenso (2009) that bee plant's contribution to honey production depends not only on its abundance but also on its nectar quality and quantity. Variations occurred spatially in concentrations level in vitamin and sugar contents in nectar of various plants in the different zones. This may be a reflection different in the genotype /makeup of individual plant in the zones or the ability to absorb optimal nutrient from the soil. Concentrations of vitamin C was significantly higher ($p<0.05$) when compare to vitamins A, B1, B2 and B3. In plants, vitamin A occurs in form of provitamin-A carotenoids which amount determine their bioavailability in animal diet (Rodriguez-Amaya, 2001). Vitamins obtained from studied melliferous flowers will be useful for honeybees since insect need vitamins for growth and synthesis of certain ones. This affirmed observations of Somerville (2005) and Brodschneider and Crailsheim (2010) that vitamins C and A as antioxidants and may play an important role in the detoxification processes, protection against microbial infection, molting process and also seems essential for honey bees brood rearing. The B vitamins obtained from studied melliferous flowers will function as cofactors for enzymes. This also affirmed observations of Strand *et al.*, (1985) that vitamin B complex is absolute or essential requirement without which the insects such as honey bees fails to grow or develop; reproduce and for brood rearing.

Amount of vitamins obtained in this study was in the range of vitamins content in food ingredients commonly used in diet formulations for farmed insect. This is in line with the work of USDA (2015) and Okwu (2004) that nectars from melliferous plants are rich in ascorbic acids which are useful for metabolic activities and general body performance.

4.0 Conclusion

Lack of floral quality and diversity is among the multiple factors causing health problems to honey bee plants, honeybees and honey production. The nectar from melliferous plants in the study area was rich in glucose, fructose, sucrose and ascorbic acids as revealed by the high values recorded in the analyzed nectar. This indicates that honey made from the studied nectar will be useful for maintaining humans' proper nervous system function, energy production and general body performance. Plant species has shown specific nectar characteristic. By understanding nutritional need of honey bees and providing solutions to them, bees will be healthy. The knowledge of plants visited by bees in the both the mangrove and rainforest vegetation zones are essential in guiding prospective beekeepers in the choice of suitable sites for locating apiaries. Establishment of a properly manage forage plant species around apiary could improve the quality and quantity of honey production because when honeybees have close access to adequate and sustained food, more energy could be conserved for honey production all year round.

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