Exploration of the Phytochemical Constituents in Honey from Southwestern Nigeria

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ABSTRACT

Honey is a product of bees (Apis mellifera); it is nutritional, a source of income, and medicinal. The high demand for honey has necessitated curious research into the importance of this cherished resource. Its insufficient production makes it scarce and exorbitant. However, this paper assesses honey quality in light of its possession of phytochemical compounds and its relevance to livestock and human health in Southwestern Nigeria (SN). Using a multistage sampling procedure, Oyo, Ogun, and Osun states were selected purposively based on their prevalent honey production activities. Three localities were later chosen per state. Each of the localities: Aba Oka, Oke Onitii, Iyemogun, Iwoye, Alabata, Ijagun, Oke Orogun, Laniba and Badeku were selected from a senatorial district for even sampling distribution. An emergent sampling technique was employed to obtain capped honeycomb from one hive per locality. Honey was harvested between October and April and was used for phytochemical constituents' analysis using standard procedures. Data were analysed using descriptive statistics. In Oyo, Ogun, and Osun states, phytochemicals (Saponin, Tannin, Flavonoid, Steroid, Terpenoid, Coumarin, Chalcones, Quinone, Alkaloid, Cardiac Glycosides, Phenols and Protein) were confirmed in all the honey samples except few (Anthocyanins and Emodin) that were affirmed absent. However, Di-terpenes were present in all honey samples except Ijagun and Oke Orogun honey. Conclusively, SN honey was affirmed to contain phytochemicals that could dislodge free radicals responsible for livestock and human ailments. This assures a solution to poor health observed in livestock and humans.

INTRODUCTION

Bee honey is acclaimed as food and medicine: its importance is growing as more beneficiaries testify to its services. Honey is the natural sweet substance, processed by honey bees (*Apis mellifera*) from the nectar of flowering plants, secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants. The bees collect and transform the nectar by combining it with specific substances of their own, deposit and dehydrate in honeycombs to ripen and mature, and is stored by capping (Codex Alimentarius for Honey, 2001; FAO, 2014). The colour of honey varies from nearly colourless to dark brown. Honey's consistency can be fluid, viscous, or partly or entirely crystallised. The flavour and aroma vary but usually derive from the plant origin (CAC, 2022).

Phytochemicals like flavonoids and phenolic acids present in bee honey are responsible for the characteristics of honey, including its antioxidant properties (Costa *et al*., 2019). The presence of such compounds in honey protects animal and human health by reducing the damage that could be caused by various oxidising agents (Gheldof *et al*., 2002; Ajani, 2009; Khalil *et al*., 2010; Lachman *et al*., 2010). The antioxidants found in natural honey include organic acids, amino acids, proteins, polyphenols and carotenoids (Mohamed *et al*.,

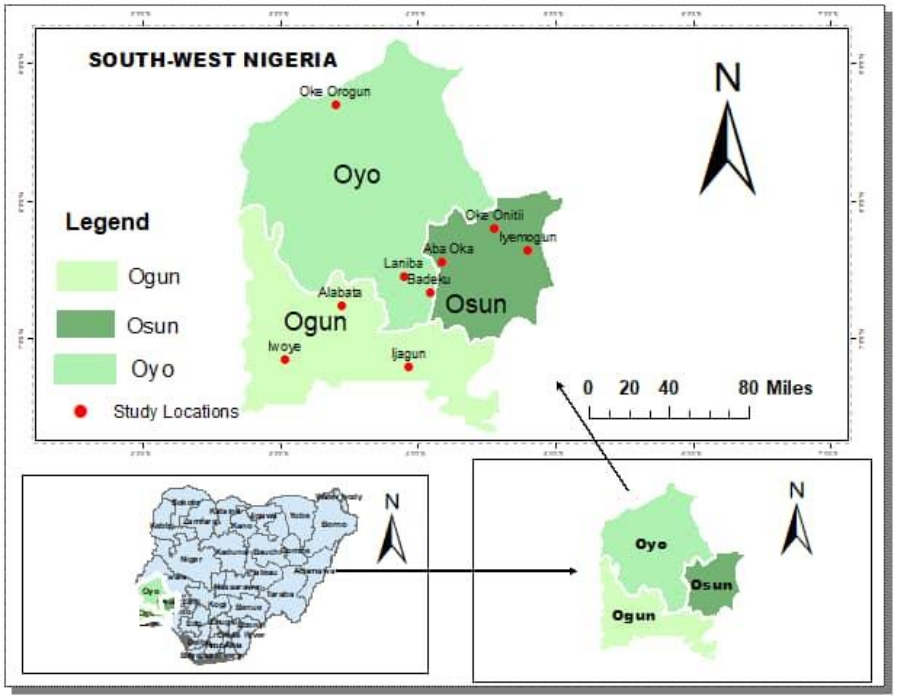
2009; Khalil *et al*., 2010). Phytochemicals or secondary metabolites are important in plant defence against herbivory and other interspecies defences. Livestock uses secondary metabolites to tackle pathogens along their gut, and humans use secondary metabolites as medicines, flavourings, pigments, and recreational drugs (Bogdanov *et al*., 2008), though some secondary metabolites are anti-nutritional.

Anti-nutritional factors are found in bee honey. Anti-nutrients are either natural or synthetic compounds that interfere with the absorption of nutrients. Examples include Phytate, Protease inhibitors and Lipase inhibitors Also, Amylase inhibitors, Phytic acid, Oxalic acid and Oxalates (Astley and Finglas, 2016). Some traditional preparation methods, like fermentation, reduce the anti-nutrients, such as phytic acid. Fermentation increases the nutritional quality of plant foods and is widely used in societies where cereals and legumes are a significant part of their diet (Reddy and Pierson, 1994). For example, cassava is fermented to reduce levels of hydrocyanide, which is both toxic and anti-nutrient. Hence, this study was designed to evaluate the phytochemical constituents of honey to reveal more data about their presence in honey from different localities in SN states.

METHODOLOGY

The Study Area: The Southwestern zone of Nigeria comprises Oyo, Ekiti, Osun, Ondo, Lagos and Ogun. Osun, Ogun and Oyo were chosen purposively, based on their prevalent honey production activities, to conduct the study (Figure 1). The weather conditions vary between the rainy season (March–October) and the dry season (November–February), accompanied by Harmattan dust and cold, dry wind from the northern desert. The southwestern Nigeria is sighted between Longitude 30^0 and 7^0E and Latitude 4^0 and 9^0N (Oni and Odekunle, 2016). Its rainfall is 2000 to 3000mm, and its temperature is over 17^0C (Uzoh, 2021).

Sampling Technique: Using a multistage sampling procedure (Figure 1), three (3) states were selected purposively based on their prevalence of apicultural activities. Three localities were chosen per state. In each of the nine localities (Aba Oka, Oke Onitii, Iyemogun, Iwoye, Alabata, Ijagun, Oke Orogun, Laniba and Badeku), a capped honeycomb was harvested from one hive, using an emergent sampling technique.



Source. The study area map was digitised (ArcGIS software, 2023).

Figure 1. The study area map shows Nigeria, the chosen South-west states and the nine study localities.

Data Collection: The honey collected (Plates 1 and 2 in the Appendices) during the honey flow period (October to April) were analysed (Plate 3 in the Appendices) for their constituents to ascertain the presence of phytochemicals using standard procedures (Harborne, 1973; Boham and Kocipai-Abyazan, 1974; AMPR, 1992; Obadoni and Ochuko, 2001; Mythili *et al.*, 2014; Yadav *et al*., 2014).

Data Analysis: Data were analysed using descriptive statistics to show the presence or absence of various phytochemicals in honey samples from the chosen localities in SN states.

RESULTS

Qualitative phytochemical screening (Plate 3) of honey samples from localities in Oyo, Osun and Ogun States are revealed in Table 1. The results show that Saponin was confirmed in all nine honey samples using Froth's test. Likewise, using Braymer's test, it was confirmed that Tannin was present in all nine honey samples. A Lead acetate test was used to determine the presence of Flavonoid in all nine honey samples. Salkowaski's test method confirmed that both Steroids and Terpenoids were present in all nine honey samples. Coumarin was confirmed present using reactions with 10% sodium hydroxide (NaOH). Chalcones were present in all nine honey samples in the reaction with ammonium hydroxide. In the presence of hydrochloric acid (HCL), Quinone was confirmed to be present in all nine honey samples. However, the reaction with acid and ammonia used to establish the presence of Anthocyanins showed that Anthocyanin was absent in all the nine honey samples.

Table 1. Qualitative phytochemical screening of honey samples from localities in Oyo, Osun and Ogun States.

		SAMPLES' INFERENCE								
S/N	**PHYTO-CHEMICAL PARAMETERS**	**1 (Aba Oka)**	**2 (Oke Onitii)**	**3 (Iyemogun)**	**4 (Iwoye)**	**5 (Alabata)**	**6 (Ijagun)**	**7 (Oke Orogun)**	**8 (Laniba)**	9 (Badeku)
1	Saponin (Froth's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
2	Tannin (Braymer's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
3	Flavonoid (Lead acetate Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
4	Steroid (Salkowaski's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
5	Terpenoid (Salkowaski's test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
6	Coumarin (Reaction with 10 % NaOH)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
7	Chalcones (Ammonium Hydroxide)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
8	Quinone (HCL)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
9	Anthocyanins (Reaction with Acid and Ammonia)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	**-ve**	**-ve**
10	Alkaloid (Hager's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
11	Cardiac Glycosides (Legal's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
12	Phenols (Ferric Chloride's Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
13	Di-terpenes (Cupric Acetate's Test)	+ve	+ve	+ve	+ve	+ve	-ve	-ve	**+ve**	**+ve**
14	Protein (Conc. HNO_3)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	**+ve**	**+ve**
15	Emodin (With Benzene)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	**-ve**	**-ve**

+ve = Parameters present -ve = Parameters absent

Results further revealed that Alkaloid was confirmed in all nine honey samples using Hager's test. Likewise, Cardiac glycosides were confirmed in all the nine honey samples using Legal's test. A Ferric Chloride test was used to ascertain the presence of Phenols in all nine honey samples. A Cupric acetate test was used to determine the presence of Di-terpenes in all nine honey samples, and it was discovered that all the honey samples had Di-terpenes except the honey from Ijagun and Oke Orogun. Using concentrated tri-oxo-nitrate-v-acid ($HNO_{3)}$, Protein was confirmed in all nine honey samples. However, using the benzene test for confirmation, Emodin was confirmed absent in all nine honey samples.



Plate 1. The nine Capped honeycomb samples.

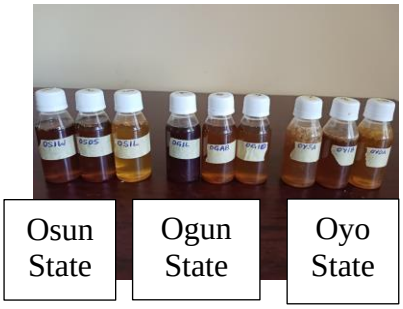


Plate 2. The nine sealed honey samples in sterilised plastic bottles ready for phytochemical constituent analysis in the laboratory.



Plate 3. Cuvette that hold honey sample in the Ultraviolet-Visible Spectroscopy (Spectrophotometer) to detect presence of phytochemicals in qualitative screening.

S/N		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PHYTOCHEMICAL PARAMETERS		Saponin (Froth's Test)	Tannin (Braymer's Test)	Flavonoid (Lead acetate Test)	Steroid (Salkowaski's Test)	Terpenoid (Salkowaski's test)	Coumarin (Reaction with 10 % NaOH)	Chalcones (Ammonium Hydroxide)	Quinone (HCL)	Anthocyanins (Reaction with Acid and Ammonia)	Alkaloid (Hager's Test)	Cardiac Glycosides (Legal's Test)	Phenols (Ferric Chloride's Test)	Di-terpenes (Cupric Acetate's Test)	Protein (Conc. HNO_3)	Emodin (With Benzene)
SAMPLES' INFERENCE	1 (Aba Oka)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	2 (Oke Onitii)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	3 (Iyemogun)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	4 (Iwoye)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	5 (Alabata)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	6 (Ijagun)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
	7 (Oke Orogun)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
	8 (Laniba)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve
	9 (Badeku)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve

DISCUSSION

Qualitative phytochemical screening of bee honey from localities in Osun, Ogun and Oyo states confirmed that saponin, tannin, flavonoid, steroid and terpenoid were present. This is consistent with the affirmations of Ilodibia *et al*. (2015) and Otmani *et al*. (2019) that bee honey contains these components. Also, coumarin, chalcones, quinone, alkaloid and cardiac glycosides were present. Likewise, Phenols and proteins were present. This is in line with the findings of Otmani *et al.* (2019), who reported that phenolic substances were the main factors responsible for the biological activities of honey and that unifloral bitter honey is richer in total polyphenols with better antioxidant potential. Also, Floris *et al.* (2021) stated that honey, as a functional food, contributes to the reduction of oxidative stress due to the presence of antioxidant molecules in its composition, including phenolic compounds. However, anthocyanins and emodin were absent in virtually all the bee honey samples, while Diterpenes were present in all the bee honey samples except those from Ijagun and Oke Orogun. This confirms the definition of the components of honey as containing antioxidants by the CODEX standard for honey (1987). This is also in line with the reports of Vazquez *et al.* (2021) and Fernandes *et al*. (2022) that honey contains phytochemicals.

CONCLUSION

This study confirmed that honey truly comprised phytochemicals: the free availability of these chemicals in nature, particularly in honey, should be further explored to discover more potential benefits for the sustenance of the livestock industry and the betterment of humanity. Likewise, conservation efforts should be geared towards southwestern Nigeria's natural ecosystem, thereby making the environment conducive to sustaining the continual honey processes and availability of the phytochemicals.

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