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ORIGINAL ARTICLE

PHYTOCHEMICAL CONTENTS OF HONEY PRODUCED IN SOUTHWESTERN NIGERIA

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ABSTRACT

Honey, a natural sweet product of bee *Apis mellifera,* is a useful nutrient source and a therapeutic agent. It relieves discomfort. However, the human body is healed majorly by bitter substances. The bio-oxidative characteristics of honey's phytochemicals are acclaimed as responsible. Although, bitter honey with a far-reaching healing effect does exist. Hence, this study considered the level of phytochemicals as potential antioxidants in honey produced in southwestern Nigeria. In a two-stage, sampling procedure, three states were selected purposively, based on their prevalent honey production. Three localities were chosen per state to collect in each, capped-comb honey between October and April from a beekeeper willing to harvest for the study. The samples were processed and analysed for phytochemicals: alkaloid; flavonoid; tannin; phenol; and saponin, using standard methods. Descriptive statistics and ANOVA at $\alpha_{0.05}$ were used to analyse collected data. Results in Oyo, Ogun, and Osun for Alkaloids were 3.58±0.54 (%), 3.74±0.40 (%) and 4.22±0.28 (%), respectively. Flavonoid was highest in Osun (3.03±1.17mgQE/g), Oyo 2.60±0.65, and Ogun (2.07±0.43mgQE/g). Phenol in Osun was 0.554±0.0278 (mgGAE/g), Oyo 0.507±0.123 (mgGAE/g) and Ogun 0.479±0.095 (mgGAE/g). Tannin was highest in Osun at 0.372±0.019 (mgGAE/g), Ogun at 0.368±0.029 (mgGAE/g) and Oyo at 0.343±0.013 (mgGAE/g). Saponin in Ogun was 0.134±0.017 (%), Osun 0.081±0.028 (%) and Oyo 0.076±0.009 (%). All the screened phytochemicals were detected in honey produced in the study areas. Meanwhile, Iwo honey had the highest antioxidant potential based on phytochemical concentration (Alkaloids at 4.55±0.16%).

Keywords: Antioxidants, Concentration, Free radicals, Human health, Southwestern Nigeria

INTRODUCTION

Honey, a natural sweet substance produced by honey bees, *Apis mellifera*, from the nectar and pollen of flowering plants, is acclaimed as being nutritious and therapeutic. The valuable role is attributed to the anti-inflammatory and antimicrobial properties of honey. This is due to honey's high acidity, and high sugar, principally of fructose and glucose components (Amril and Ladjama, 2013; Ndife *et al*., 2014). Bogdanov *et al*. (2008), reported that honey comprised water, protein, vitamins, minerals, enzymes and polyphenols which include flavonoids from pollen. Honey, though not qualified as a main source of traditional nutrients by the food ranking system, has emerged as a genuine source of sugar, vitamin B_6, vitamin B_2, manganese and iron (Alvarez-Suarez *et al*., 2010; Vanhanen *et al*., 2011; Ndife *et al*., 2014).

Phytochemicals like flavonoids and phenolic acids, present in bee honey were reported to be responsible for the characteristics of honey, including its antioxidant properties (Costa *et al*., 2019). The antioxidants found in natural honey includes organic acids, amino acids, proteins, polyphenols and carotenoids (Mohamed *et al*., 2009; Khalil *et al*., 2010). Palmer-Young *et al.* (2017) reported that the nectar and pollen consumed by bees contain phytochemicals, which positively or negatively influence pollinator's fitness in the ecosystem. Some phytochemicals can decrease human and animal parasite concentrations (Bogdanov *et al*., 2008; Faizal and Geelen, 2013). Bees tolerate the levels of phytochemicals documented in nectar, honey, and pollen, even though clove oil and thymol, at high doses, amplify bee mortality (Evans *et al*., 2006; Palmer-Young *et al*., 2017).

Phytochemicals are secondary metabolites that often play an important role in plant defence against herbivores and other interspecies defences. Humans use these secondary metabolites as medicines, flavourings, pigments, and recreational drugs (Bogdanov *et al*., 2008). The presence of such compounds in honey was speculated to protect human health by reducing the damages that could be caused by various oxidizing agents called free radicals (Gheldof *et al*., 2002; Lachman *et al*., 2010). However, some secondary metabolites are anti-nutritional, especially Phytate or Phytic acid (Reddy and Pierson, 1994; Astley and Finglas, 2016). The purpose of this study was to expatiate on phytochemical components of honey produced in southwestern Nigeria and provide more data on honey's potential as an antioxidant agent.

METHODOLOGY

The Study Area: The southwestern zone of Nigeria comprised 6 states: Oyo, Ekiti, Osun, Ondo, Lagos and Ogun; but Osun, Ogun and Oyo states were purposively chosen based on prevalent honey production. The weather conditions vary between the rainy season (March–October) and the dry season (November–February) which is accompanied by Harmattan dust, cold dry wind from the northern desert, and blown into the southern regions. The location of the study area lies between Longitude 30^0 and 7^0E and Latitude 4^0 and 9^0N, respectively (Oni and Odekunle, 2016). Its rainfall is 2000–3000mm, and its temperature is over 17^0C (Uzoh, 2021).

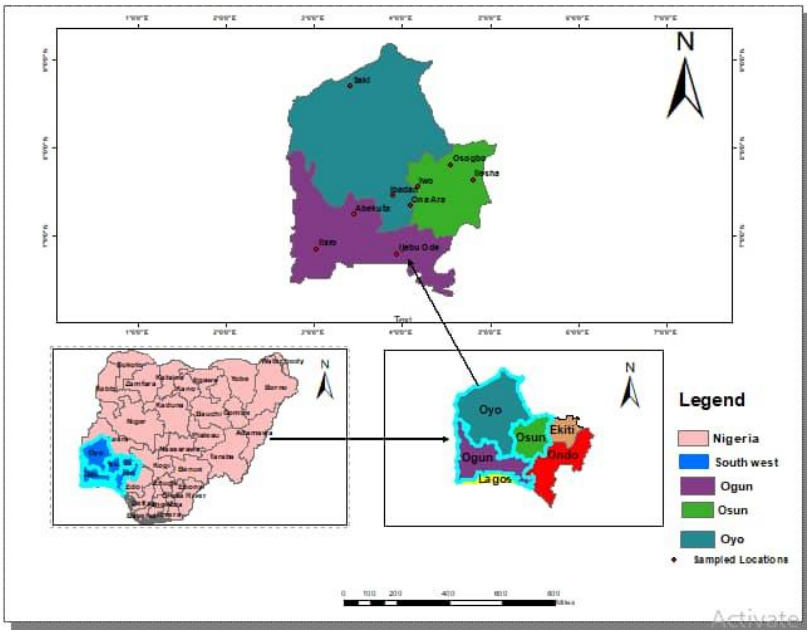

Figure 1. Map of the study area showing Nigeria, Southwest region and 9 study locations.

Source: This map was digitised using ArcGIS software in 2023.

Sampling Technique:

In a two-stage, sampling procedure (Figure 1), three states were selected purposively based on their prevalent honey production activities. Three Local Government Areas (LGAs) were chosen per state based on their senatorial district. In each of the 9 LGAs, a capped-comb honey sample from Osun comprising Iwo, Osogbo and Ilesa; Ogun comprising Ilaro, Abeokuta and Ijebu-Ode; Oyo comprising Saki, Ibadan and Ona-Ara were collected in the month of October-April, using emergent sampling techniques, from an associated beekeeper willing to harvest for the study.

Data collection and Analysis:

Using standard methods, the honey collected from various localities were screened for phytochemicals like alkaloids (Harborne, 1973), flavonoids (Mythili *et al.*, 2014), tannins (Mythili *et al.*, 2014), phenols (Mythili *et al.*, 2014) and saponins (Obadoni and Ochuko, 2001), using a spectrophotometer. The data collected were analysed using descriptive statistics according to Hayes (2024) and ANOVA at $\alpha_{0.05}$. Also, using 2-2-Diphenyl-1-picrylhydrazyl (DPPH) reagent, a preliminary test was conducted to observe the reactions of ascorbic acid, an antioxidant agent often used as a standard reference, and subsequently, the DPPH reagent was used with honey produced in Oyo, Osun and Ogun states to ascertain honey's antioxidant potentials.

RESULTS

Table 1 shows the antioxidant absorbance capacity of ascorbic acid that was first tested and its values used as reference standard to compare the scavenging capacity of the honey samples studied. The scavenging capacity for ascorbic acid was highest (96.40±0.00 %) at the highest concentration of 400.00 µg/g and lowest (94.46±0.02 %) at the lowest concentration of 25.00 µg/g.

Table 1. The antioxidant absorbance capacity of ascorbic acid was used as a standard reference.

S/N	ANTIOXIDANT ABSORBANCE CAPACITY OF ASCORBIC ACID	
	*DPPH Conc. (µg/g)	ASCORBIC ACID (%)
1	400.00	96.40±0.00
2	200.00	96.27±0.00
3	100.00	96.12±0.02
4	50.00	96.00±0.01
5	25.00	94.46±0.02

***DPPH: 2-2-Diphenyl-1-picrylhydrazyl.**

Source: Laboratory assay to establish standard using ascorbic acid as reference (Brand-Williams *et al*., 1995; Sanchez-Mareno, 2002).

Table 2. Honey samples from Oyo, Osun and Ogun States' phytochemical components' absorbance capacity as an antioxidant agent in 2-2-Diphenyl-1-picrylhydrazyl (DPPH).

S/N			1	2	3	4	5
DPPH	Conc.(µg/g)		400	200	100	50	25
SAMPLES' ANTIOXIDANT ABSORBANCE MEAN±SD (%)	1**(OSIW)	*Mean±SD	60.59±0.07	35.03±0.07	10.41±0.07	6.08±0.15	2.30±0.33
	2 (OSOS)	Mean±SD	74.59±000	68.18±0.04	40.42±0.08	20.40±0.22	9.40±0.13
	3 (OSIL)	Mean±SD	58.10±0.11	37.09±0.04	13.33±0.04	6.15±0.11	2.37±0.25
	4 (OGIL)	Mean±SD	60.21±0.04	30.70±0.05	8.83±0.07	6.54±0.25	1.13±0.04
	5 (OGAB)	Mean±SD	62.31±0.00	33.81±0.07	15.43±0.00	9.19±0.22	4.27±0.17
	6 (OGIO)	Mean±SD	83.49±0.12	43.84±0.11	14.86±0.07	8.54±0.15	3.44±0.13
	7 (OYSA)	Mean±SD	63.22±0.04	37.83±0.15	17.28±0.04	9.53±0.04	5.96±0.00
	8 (OYIB)	Mean±SD	63.37±0.04	40.58±0.08	20.05±0.15	10.17±0.11	6.80±0.15
	9 (OYOA)	Mean±SD	79.63±0.08	66.50±0.21	25.79±0.12	12.95±0.10	7.34±0.06

***Data are mean values of triplicate determinations per sample ± standard deviation (SD). **{Osun [OSIW=Iwo], [OSOS=Osogbo], [OSIL=Ilesa]}; {Ogun [OGIL=Ilaro], [OGAB=Abeokuta], [OGIO=Ijebu-ode]}; {Oyo [OYSA=Saki], [OYIB=Ibadan], [OYOA=Ona-ara]}.**

In Table 2, the values of the absorbance capacity of honey from Oyo, Osun and Ogun States as an antioxidant agent in 2-2-Diphenyl-1-picrylhydrazyl (DPPH) are displayed; whereas Figure 2 shows the concentration of the screened phytochemicals. There was a highly significant difference in the phytochemical concentration. The scavenging capacity for honey was highest (83.49±0.12 %) at the highest concentration of 400.00 µg/g and lowest (1.13±0.04 %) at the lowest concentration of 25.00 µg/g.

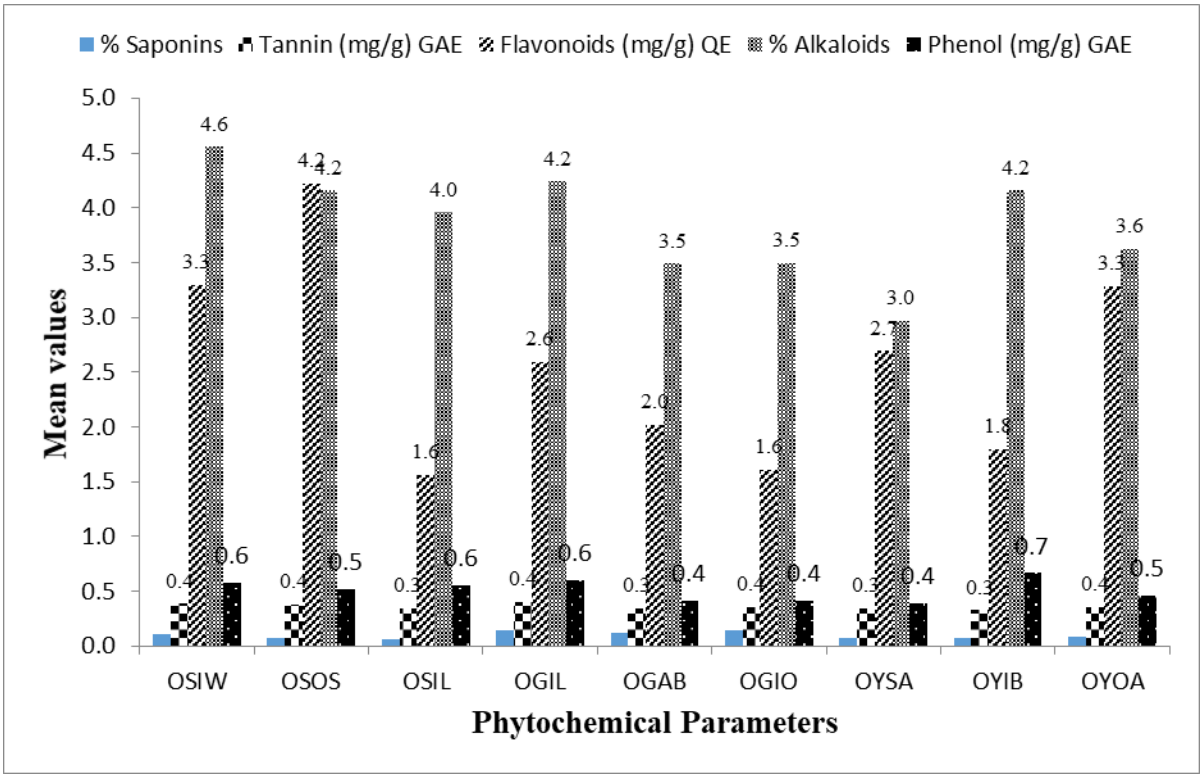

Figure 2. Phytochemical parameter's content in honey samples from 9 study locations in southwestern Nigeria.

The analysis of variance result in Table 3 shows the concentration and statistical difference of phytochemicals in honey samples 1-9 and Ascorbic acid. It revealed that there was no statistical difference ($p>0.05$) in the phytochemicals in honey samples across the sample locations except for honey sample 8. The follow-up test (Table 4) revealed that the Duncan Multiple Range Test separated the means for the DPPH antioxidants capacity of the honey samples. It further shows that the higher the concentration, the higher the DPPH radicals scavenged. However, DPPH radicals scavenged was highest in some locations, for instance, at 400 µg/g concentration, it was highest at honey sample 6 (Ilaro), followed by honey sample 9 (Ona Ara), whereas honey sample 3 (Ilesa) was lowest at 83.49 %; 79.63 % and 58.10 %, respectively.

Table 3. ANOVA for concentration of phytochemicals in Honey Samples (HS) 1-9 and Ascorbic acid.

Source	HS	Type III Sum of Squares	df	Mean Square	F	Sig.	R
%IH	1	0.064	2	0.032	1.117	0.373 ^{ns}	0.998
	2	0.013	2	0.007	0.415	0.674 ^{ns}	0.999
	3	0.029	2	0.014	0.758	0.500 ^{ns}	0.987
	4	0.027	2	0.013	0.908	0.441 ^{ns}	0.989
	5	0.013	2	0.006	0.356	0.711 ^{ns}	0.998
	6	0.000	2	0.000	0.010	0.990 ^{ns}	0.996
	7	0.014	2	0.007	1.522	0.275 ^{ns}	0.979
	8	0.091	2	0.046	10.580	0.006^{s}	0.998
	9	0.004	2	0.002	0.105	0.901 ^{ns}	0.997
	A.ACID	0.000	2	0.000	1.161	0.361 ^{ns}	0.988
Error	1	0.230	8	0.029			
	2	0.126	8	0.016			
	3	0.151	8	0.019			
	4	0.117	8	0.015			
	5	0.146	8	0.018			
	6	0.136	8	0.017			
	7	0.038	8	0.005			
	8	0.035	8	0.004			
	9	0.153	8	0.019			
	*A.ACID	0.002	8	0.000			
Total	1	7293.125	14				
	2	9795.370	14				
	3	6697.889	14				
	4	7147.369	14				
	5	6722.839	14				
	6	13332.181	14				
	7	6813.858	14				
	8	6718.905	14				
	9	12783.725	14				
	A.ACID	7.549	14				

^{s}=Significant, ^{ns}=Not significant, *A.ACID=Ascorbic acid

Table 4. The Duncan Multiple Range Test separates the mean for the honey's scavenging ability on 2-2-Diphenyl-1-picrylhydrazyl radicals.

Conc. µg/g	S1	S2	S3	S4	S5	S6	S7	S8	S9
400.00	60.59^{g}	74.59^{c}	58.10^{h}	60.21^{f}	62.31^{e}	83.49^{a}	63.22^{d}	63.37^{d}	79.63^{b}

200.00	35.03^{g}	68.18^{a}	37.09^{f}	30.70^{i}	33.81^{h}	43.84^{c}	37.83^{e}	40.58^{d}	66.50^{b}
100.00	10.41^{h}	40.42^{a}	13.33^{g}	8.83^{i}	15.43^{e}	14.86^{f}	17.28^{d}	20.05^{c}	25.79^{b}
50.00	6.08^{g}	20.67^{a}	6.15^{g}	6.54^{f}	9.19^{d}	8.54^{e}	9.53^{c}	10.17^{c}	12.95^{b}
25.00	2.30^{f}	9.40^{a}	2.37^{f}	1.13^{g}	4.27^{d}	3.44^{e}	5.96^{c}	6.80^{b}	7.34^{b}

Mean values within rows with same superscript are not statistically different from each other.

DISCUSSION

The phytochemical contents of honey produced from Oyo, Osun and Ogun states were examined for their absorbance or scavenging potential on 2-2-Diphenyl-1-picrylhydrazyl (DPPH) radicals. The response of ascorbic acid to the DPPH reagent was compared with the responses of honey samples studied. It was observed in this study that honey consists of phytochemicals like saponin, tannin, flavonoid, alkaloid and phenol. This is consistent with the report of Fernandes *et al.* (2022) that honey has antimicrobial mechanisms such as phytochemicals and antimicrobial peptides among other components. The concentration of phytochemicals like total phenol was highest in Ibadan honey, implying greater scavenging ability. This agrees with Vazquez *et al*. (2021) who affirmed the presence of phenol in bee honey. This is also similar to the emphasis of Khalil *et al*. (2011) who stated that polyphenols in honey contain hydroxyl groups that increase the humectant properties of these components, which are also related to antioxidant activities.

More similar assays are mentioned by Huang *et al*. (2005), that the Trolox equivalent antioxidant capacity (TEAC) assay measures the antioxidant capacity of a given substance, as compared to the standard Trolox. The TEAC assay is considered an easy and accurate method for measuring the radical scavenging ability of honey by hydrogen-donation reactions (Alvarez-Suarez *et al*., 2009). Phenolic compounds are secondary metabolites of plants generally involved in their defence mechanism against ultraviolet radiation or pathogens infection and have been confirmed as the main component responsible for the antioxidant activity of honey (Jaganathan and Mandal, 2009; Khalil and Sulaiman, 2010; Hossen *et al*., 2017).

The Tannin phytochemical concentration was highest in Ilaro honey. This corroborated Ojo (2022) who reported that flowering plants like legumes, tea, coffee, cocoa and grapes are rich in tannins. These tannins end in honey when bees collect nectar from flowering plants. Also, Tamokou *et al*. (2017) reported that tannins are secondary metabolites, widely distributed in plants and are polymeric

phenolic substances with astringency properties. It was also observed that the highest phytochemical concentration of alkaloids was in Iwo honey. This is consistent with the findings of Kowalczyk and Kwiatek (2018) who reported that pyrrolizidine alkaloids are one of the most common groups of natural toxins found in foods of plant origin. Boppré (2011), emphasised that plant produces alkaloids as a chemical defence against herbivores.

The flavonoid phytochemical concentration was highest in Osogbo honey. This is consistent with the report of Pasupuleti and Arigela (2020), who affirmed that phenolic acids and flavonoids are the main classes of polyphenols in honey, and flavonoids can be categorised into several types like flavonols, flavanones, flavones, anthocyanidins and isoflavones due to their dietary significance. It was also observed that the phytochemical concentration of saponin was highest in Ilaro and Ijebu Ode's honey in equal proportions. This agrees with the claims of Faizal and Geelen (2013), that saponins are steroid and triterpenoid glycosides that display diverse biological activities in plants, and have a potential for pharmaceutical application which has led to saponin extraction and identification in numerous plant species.

CONCLUSION

All the screened phytochemicals were detected in the honey produced in the study area, and the concentration of alkaloids was highest. The revealed values of the honey's phytochemicals are evidence of their potential as antioxidant agents.

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