

Original Research Article

Comparative Phytochemical Analysis of Brown, Green and Red Propolis from Umudike, Abia State Nigeria

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ARTICLE INFO

Article history

Submitted: 2020-10-31

Revised: 2020-11-29

Accepted: 2020-12-03

Available online: 2020-01-03

Manuscript ID: [AJCB-2010-1068](#)

DOI: [10.22034/ajcb.2021.121910](https://doi.org/10.22034/ajcb.2021.121910)

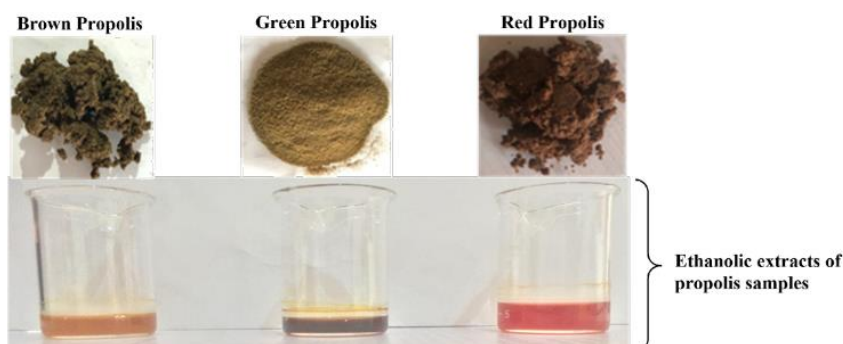
KEYWORDS

Propolis Color,
GC-FID,
Floral Marker,
Phytochemicals,
Therapeutic Properties

ABSTRACT

The phytochemical composition of brown, green and red propolis samples collected from an apiary in Umudike, Abia State, Nigeria, was analyzed using the GC-FID technique. The phytochemical analysis results revealed that anthocyanin was the predominant phytochemical, which occurred highest in red propolis (84.68 $\mu\text{g/g}$) followed by brown propolis (79.35 $\mu\text{g/g}$) but was not detected in green propolis. The brown propolis contained anthocyanin (79.35 $\mu\text{g/g}$), phenols (29.11 $\mu\text{g/g}$), flavanones (23.64 $\mu\text{g/g}$), naringenin (19.30 $\mu\text{g/g}$), flavan-3-ol (14.79 $\mu\text{g/g}$), proanthocyanins (12.79 $\mu\text{g/g}$) and steroids (10.35 $\mu\text{g/g}$) as major constituents while the green propolis showed significant presence of four phytochemicals such as proanthocyanins (60.63 $\mu\text{g/g}$), lunamarin (19.90 $\mu\text{g/g}$), phenols (14.68 $\mu\text{g/g}$) and naringenin (10.64 $\mu\text{g/g}$). Proanthocyanins (69.18 $\mu\text{g/g}$), anthocyanin (84.68 $\mu\text{g/g}$), spartein (13.57 $\mu\text{g/g}$), phenols (46.99 $\mu\text{g/g}$), catechin (13.49 $\mu\text{g/g}$), and quinine (21.09 $\mu\text{g/g}$) were most abundant in the red propolis. The quantities of flavonoids / phenolic revealed may therefore be regarded as an important tool for recognizing the propolis floral type. This study has given scientific backing for attributing propolis color as one of the determinants of its floral origin and therapeutic properties. Propolis color should therefore be incorporated in future standardization and at the same time influence its acceptability by the consumers.

GRAPHICAL ABSTRACT



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Introduction

Plants, which mainly are multicellular and photosynthetic eukaryotes of the kingdom plantae, produce a huge amount of natural products (secondary metabolites) of important ecological and pharmaceutical functions. There has been an increase in the use of and search for plant-derived drugs in recent years. Scientists globally are in continuous research for bioactive phytoconstituents that could serve as a drug lead to treat various human ailments [1-3]. The honey bee, a type of insect in the Apidae family, has a wide variety of life-sustaining products such as pollen, royal jelly, wax, honey, and propolis. Propolis is a plant-derived natural product formed by bees in their hives. It is a resinous material composed of bee saliva, bee wax, and exudates gathered from tree buds, sap flowers, or other botanical sources [4-8]. Propolis was initially prepared by the honeybee to seal the cracks, smooth walls, and to keep moisture and temperature stable in the hive all year around. However, it has found application in traditional medicine for treating colds, wounds, ulcers, rheumatism, sprains, heart disease, diabetes, and dental caries due to some of its revealed biological properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, antiulcer, and anti-HIV activities. The increased use of propolis as a component of pharmaceuticals, cosmetics, and food supplements has drawn growing attention to its chemical composition to identify the components responsible for its activity [4, 6, 9-12]. Physically, propolis samples are hard and brittle at low temperatures while soft at high temperatures. They have been found to occur in different colors, mostly black and brown, sometimes red, green, and rarely yellow. Propolis samples can be classified based on their physicochemical properties (color, texture, and chemical composition) which vary depending on the location of the hives and local flora [8, 13]. The significant variation of local flora with respect to different geographical areas and

seasons and the preferences of bees towards particular plants, or plant materials determine the color and the chemical composition of propolis [5, 11, 14]. Therefore it is believed that propolis is a territory-linked natural product that can be used as characteristic biomarkers and can be chemically characterized according to each region of production and season of the world [15-17]. Several researchers have proved that the regional chemical composition of propolis can interfere in its biological properties, which indicates each regionally-based propolis could also be unique in its healing potential [18-20]. Nigerian Propolis, just like the ones in other tropic regions, such as Brazil, Cuba, Venezuela, and Chile, have not been studied extensively but have been attracting much attention in the last years due to its unique chemical profiles [9]. A survey of samples from different parts of the country has revealed samples from southern Nigeria as the most bioactive and capable of yielding new compounds [21]. Interestingly, a novel compound riverinol, which was isolated from a red propolis sample from River State, Nigeria and named after the State, was found to have good inhibiting abilities against *Trypanosoma brucei* [22]. Many studies on the variability of chemical composition and biological activities of different propolis samples have shown that the chemical composition of propolis is difficult to be standardized. This is why propolis from areas not yet studied seems to be a promising source of new bioactive molecules [23-24]. Studies on the composition and properties of propolis from apiaries in southeast Nigeria are scarce in literature. The present study evaluates bee propolis from Umudike, Umuahia, South-eastern Nigerian, for its chemical composition to bridge the knowledge gap.

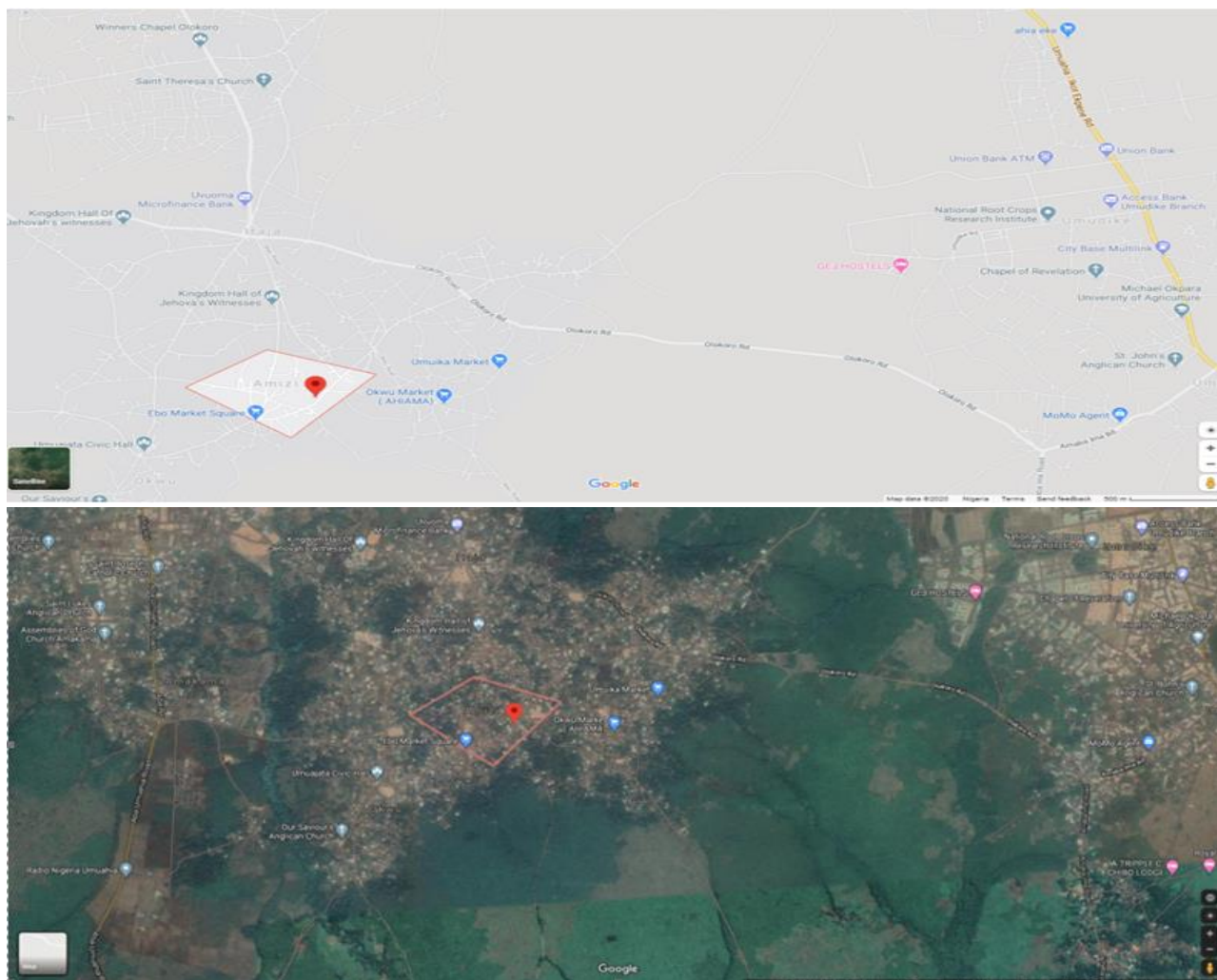


Fig. 1: Maps showing position of apiary (marked in red)

Methodology

Study area

The propolis samples were collected from a private apiary in Amizi, Ikwuano Local Government Area, Abia State Nigeria. It is a semi-urban settlement of about 10 km east of Umuahia, the State capital, and lies between the geographical coordinates $5^{\circ} 28' 1'' N$, $7^{\circ} 30' 16'' E$ and altitude 149 m above sea level. The area's average annual temperature is between $22 - 32^{\circ} C$, and relative humidity of $60 - 83\%$, with a mean yearly rainfall of about 2 400 mm. The vegetation is mainly lowland tropical rainforest, with two seasons: raining season (March – October) and dry season (November – February).

Sample collection and preparation

Samples of propolis were collected using hive scraping methods [25]. Large quantities of propolis were harvested in April 2020 from slats of wood with 4cm gaps introduced to the hive boxes. The large opening stimulates African derived bees to fill the slats with propolis. When the gap is filled with a thick layer of propolis, the wood slats are removed, and the propolis harvested using a knife to cut out the sheet (**Fig. 2**). The Propolis material was chopped into small pieces, air dried at room temperature for 2 weeks and stored in the freezer until further processing.



Fig. 2: Propolis on slats of wood introduced to the sides of the hive boxes

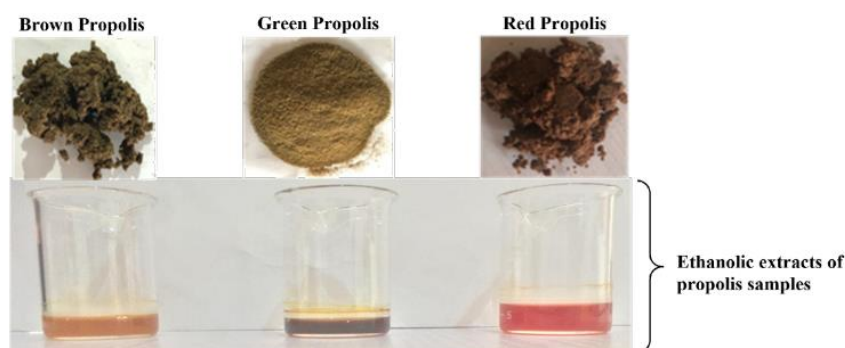


Fig. 3: Propolis samples and their ethanolic extracts

Propolis (10 g) was ground to powder and extracted with 100 mL of ethanol (**Fig. 3**). The alcoholic extract solution was then filtered using a Whatman filter paper to eliminate the residual mass and centrifuged for 10 min at 4000 rpm.

Phytochemical analysis

Qualitative and quantitative analysis of phytochemicals in the ethanolic extracts of the propolis samples were determined using a Buck M910 Gas Chromatography coupled with a flame ionization detector (GC-FID). The column used was a RESTEK 15 m MXT-1 (15 m × 250 μm × 0.15 μm) at an injector temperature of 280 °C, linear velocity of 30 cms⁻¹ with splitless injection of 2 μL of sample. The carrier gas used was Helium 5.0 Pa.s at a flow rate of 40 mL/min. The oven temperature was initially set at 80 °C and

then steadily increased to 330 °C at a rate of 5 °C/min, and maintained at this temperature for 5 minutes. The detector was operated at a temperature of 320 °C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals [26].

Results and Discussion

Phytochemical analysis

The gas chromatogram showing the phytochemicals in the different samples of propolis extracts is shown in **Fig. 4**. The concentrations of the identified phytochemicals in the different propolis samples are summarized in **Table 1**.

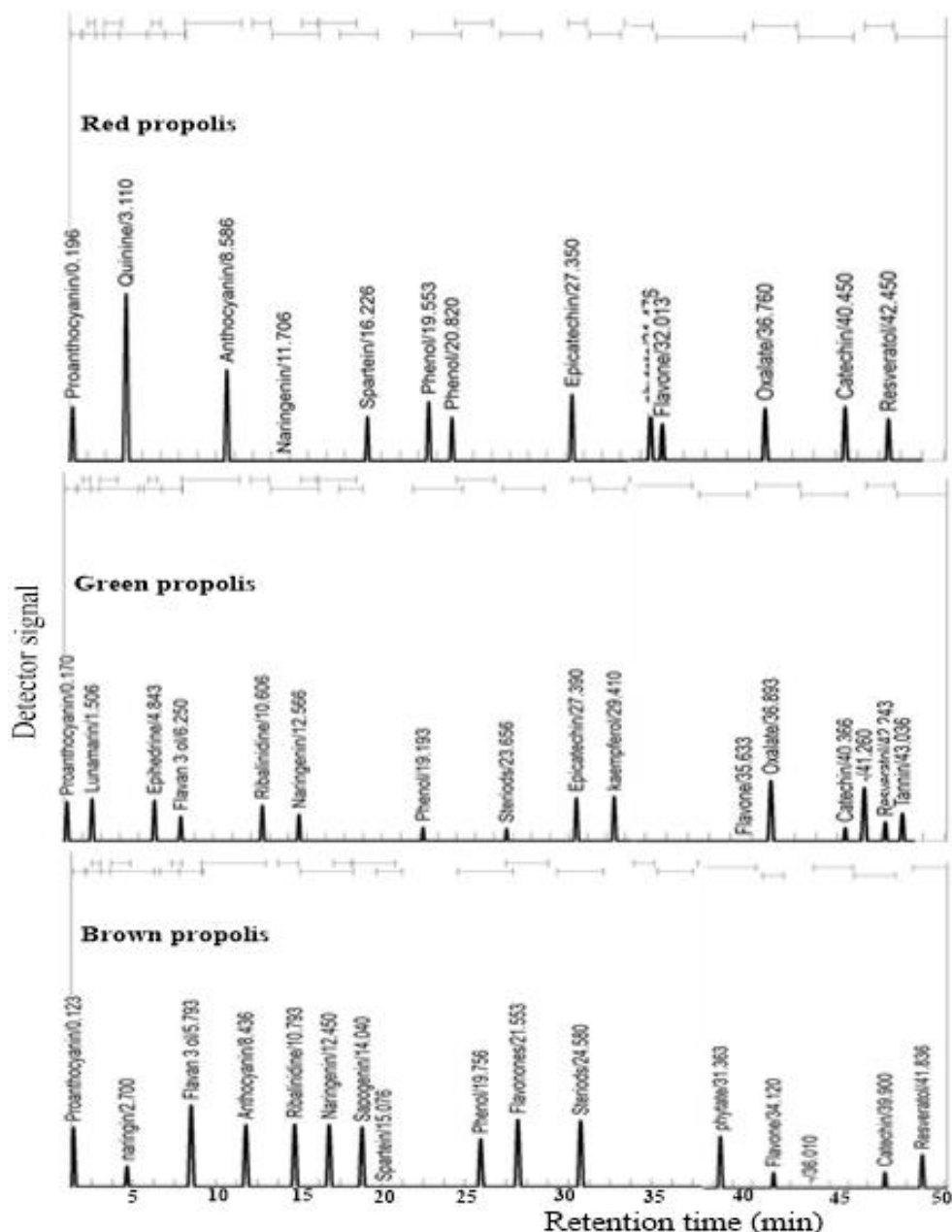


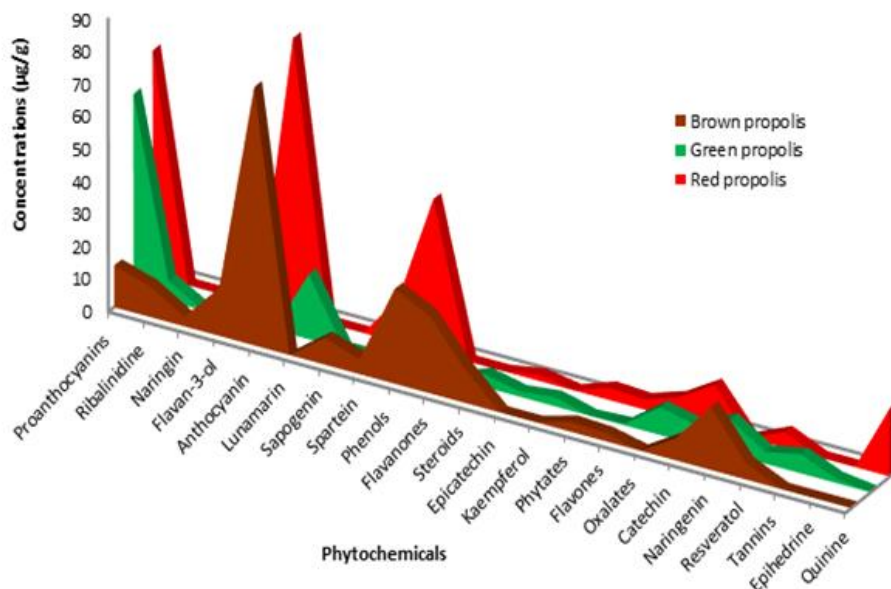
Fig. 4: Gas chromatogram of phytochemicals in propolis samples

The concentrations of the major phytochemicals in the propolis samples are as displayed in the 3D area distribution chart shown in **Fig. 5**. The propolis samples revealed various phytochemicals with the constituents and concentrations varying widely according to color type. A minimum of twelve (12) phytochemicals were identified in each propolis sample with seven (7), four (4), and six (6) major constituents occurring in brown, green, and red propolis,

respectively. The concentrations ($\mu\text{g/g}$) of the major phytochemicals identified in the brown, green and red propolis respectively are proanthocyanins (12.79, 60.63, and 69.18), anthocyanin (79.35, 0, and 84.68), Lunamarin (0, 19.9, and 0), spartein (4.51, 0, and 13.57), phenols (29.11, 14.68, and 46.99), steroids (10.35, 4.01, and 0), catechin (6.78, 6.07, and 13.49), naringenin (19.3, 10.64, and 0.06) and quinine (0, 0, and 21.09).

Table 1: Concentrations of phytochemicals in the propolis samples

Phytochemical	Concentrations ($\mu\text{g/g}$)		
	Brown	Green	Red
Proanthocyanins	12.79	60.63	69.18
Ribalinidine	9.56	6.16	-
Naringin	2.87	-	-
Flavan-3-ol	14.79	6.43	-
Anthocyanin	79.35	-	84.68
Lunamarin	-	19.90	-
Sapogenin	8.24	-	-
Sparteine	4.51	-	13.57
Phenols	29.11	14.68	46.99
Flavanones	23.64	-	-
Steroids	10.35	4.01	-
Epicatechin	-	1.63	1.92
Kaempferol	-	2.84	-
Phytates	3.03	-	3.78
Flavones	2.48	0.59	3.16
Oxalates	-	8.50	6.76
Catechin	6.78	6.07	13.49
Naringenin	19.30	10.64	0.06
Resveratol	5.04	3.61	4.82
Tannins	-	5.92	-
Epihedrine	-	1.42	-
Quinine	-	-	21.09

**Fig. 5:** 3D area distribution of the concentration of phytochemicals in the propolis samples

The seven major constituents of the brown propolis were anthocyanin (79.35 µg/g), phenols (29.11 µg/g), flavanones (23.64 µg/g), naringenin (19.30 µg/g), flavan-3-ol (14.79 µg/g), proanthocyanins (12.79 µg/g), and steroids (10.35 µg/g). The green propolis revealed significant presence of four (4) major phytochemicals which occurred in the order: proanthocyanins (60.63 µg/g) > lunamarin (19.90 µg/g) > phenols (14.68 µg/g) > naringenin (10.64 µg/g). The highest concentrations of some major phytochemicals such as proanthocyanins (69.18 µg/g), anthocyanin (84.68 µg/g), spartein (13.57 µg/g), phenols (46.99 µg/g), catechin (13.49 µg/g), and quinine (21.09 µg/g) were found in the red propolis. Anthocyanins, which are the main constituents in the brown propolis, alongside the other identified components, are majorly flavonoids typical of the poplar propolis type, characteristic of propolis from temperate regions [9]. They are phytonutrients which impart color and taste to numerous fruits and vegetables and protect their essential enzymes and vitamins, making them suitable for consumption [27]. Flavonoids are known to be significant propolis constituents, and their quantity is used as a criterion to evaluate the quality and the pharmacological activities of propolis [28]. Studies have shown that they possess anti-inflammatory, anti-oxidant, and other antimicrobial properties and have been reported to have cardiovascular disease and cancer-preventive properties [12, 29]. The flavonoids were reported to play an important role in the antioxidant activity of Brazilian propolis extracts, but other compounds could also be involved.

Proanthocyanins (also known as condensed tannins) are oligomers or polymers of polyhydroxy flavan-3-ol units. They were the most abundant in green propolis (60.63 µg/g) and also occurred significantly in the red propolis (69.18 µg/g). Scarce data exist about proanthocyanin or even tannins in propolis. They

are still known to be prevalent in some foods and dietary supplements, including several berries, cinnamon, baking chocolate, red grapes, and wines. Even though they are considered antinutrients in animal nutrition due to ability to bind several macronutrients, thus reducing their digestion and absorption [30], their specific advantages have been realized due to their unique chemical traits and are now being pursued [31, 32]. Various studies on proanthocyanins have reported them to have substantial antioxidant and radical scavenging activities, ability to improve glucose metabolism in type 2 diabetics, and may alter several reactions involved in cancer processes [30, 33]. Anthocyanins and proanthocyanidins from blueberry have been reported to be responsible for protecting the integrity of the capillaries in rats exposed to oxygen toxicity [34, 35]. Their consumption has been linked to myriad health benefits such as anti-inflammatory and anti-carcinogenic properties, diminution of cardiovascular disease incidence, obesity control, and diabetes mitigation effects. In vitro studies have also revealed certain potential cancer chemopreventive activities of anthocyanins [36]. Quantities of phenols revealed are 46.99 µg/g, 29.11 µg/g, and 14.68 µg/g in red, brown, and green propolis samples, respectively. Several studies have demonstrated the antimicrobial activity of phenols and phenolic extracts, making them promising alternatives to antibiotics and chemical preservatives. Lunamarin, a quinoline alkaloid was identified only in green propolis. It has been reported to have a radical scavenging function [37] and anti-amoebic activity [38]. Spartein and catechin were found to occur most in red propolis compared to other samples. Spartein is a quinolizidine alkaloid and has been reported to have bactericidal activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus thuringiensis* [39]. It has also been reported to induce uterine contraction as well as exhibiting diuretic and anti-inflammatory

activities [40]. The red propolis also revealed the presence of quinine, a naturally occurring alkaloid that has been reported to possess antiplasmodial effect [26, 41].

These quantitative phytochemical results indicate that the propolis samples are rich mainly in alkaloids and flavonoids (phenolic compounds). The presence of alkaloids in propolis has been reported to be responsible for its observed muscle relaxant property, antimicrobial, and anti-diabetic activities. Flavonoids and phenolic compounds are important components of propolis, which are responsible for the propolis quality, color, sensory and bioactive properties. Both substances have proven their ability to remove (or deactivate) free radicals, protect lipids and vitamin C from being destroyed in the oxidative process [42, 43], and have also been reported to be the most important phytochemical responsible for anti-cancer, anti-oxidant, antibacterial and antifungal activities of propolis [44, 45].

Considerable variation was found in phenolic compositions of the different propolis samples (Table 1); the red propolis sample had the highest concentration of phenolic compounds, followed by the green propolis samples. On the other hand, the brown propolis samples had the lowest concentration of phenolic compounds, a result in agreement to a previous work on a brownish colored southern Brazilian propolis samples which had low phenolic composition and, in turn, lower bioactivity [14]. Based on the quantification of the main bioactive constituents and in line with the fact that a good propolis quality means a high phenolic content [14]; the red propolis in this study can be referred to as the best followed by the green since previous studies have correlated the quality (bioactive potentials) of propolis samples to the concentrations of flavonoids (phenolic compounds) in the sample [46, 47]. These complexities in the composition of different

colors of propolis samples revealed in this study are in line with previously published works [48-50]. Different researchers have confirmed the superiority of red propolis over other varieties for efficacy on healing, antiparasitic, antibacterial, or cytotoxic properties [51, 52] while some other workers further explained that the superiority of red propolis in medical applications could be due to the more significant number of compounds, mainly flavonoids, and their synergistic effects with phenolic acids in modulating human physiology [53].

Propolis color, therefore, should be considered as one of the factors determining its acceptability as this study, in accordance to previous reports has revealed most of its floral markers to be its flavonoids / phenolic compounds which come from the nectar or pollen of specific plants [54]. Identifying these compounds can be an essential tool for recognizing the propolis floral type and therapeutic activities.

Conclusions

The phytochemical compositions of brown, green and red colored propolis samples from an apiary in Amizi, Abia State, Nigeria were studied. The red propolis was found to have highest amount of phenolic compounds eventhough the sample showed less number of constituents. The brown and green propolis both contained fifteen (15) different types of phytochemicals with the brown revealing more of flavonoids. Most of the alkaloid constituents were identified in green and red propolis with quinine being found only in the red sample. The presence of these phytochemicals implied that the propolis samples could have therapeutic properties like antimicrobial, anticancer, antioxidant, diuretic, and anti-inflammatory activities with the red sample having more potential to exhibit more pharmacological responses due to its unique composition

The extensive characterization of propolis from different regions of the country is therefore

relevant for its future standardization, the chemical composition being the best indicator for the floral origin. This study has revealed most of its floral markers to be its flavonoids/phenolic compounds that come from the nectar or pollen of specific plants. Identifying these compounds can be an essential tool for recognizing the propolis floral type and its therapeutic activities. This study may contribute to consumers' knowledge of choosing propolis with medicinal properties, as indicated by its color.

Acknowledgments

The author is grateful to Mr. Kingsely Nwaogu, CEO of Interglobal Green Blossom Limited, for making his apiary available for this study.

Funding

No funding was received for this work.

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HOW TO CITE THIS ARTICLE

Ijeoma Akunna Duru, Comparative Phytochemical Analysis of Brown, Green and Red Propolis from Umudike, Abia State Nigeria, Ad. J. Chem. B, 3 (2021) 86-97

DOI: [10.22034/ajcb.2021.121910](https://doi.org/10.22034/ajcb.2021.121910)

URL: http://www.ajchem-b.com/article_121910.html

