COMPARATIVE STUDY ON THE EVALUATION OF PHYTOCHEMICAL COMPONENTS AND IRON-CHELATING ACTIVITIES OF NUTRACEUTICAL TABLET AND KOTATA BLACKBERRY (*RUBUS KOTATA*) EXTRACT

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Abstract
Although the body needs iron for blood formation and other biological processes, it lacks an excretion mechanism for excess iron released, which gets deposited on body parts such as the liver and causes chronic anemia. Antioxidants from fruits protect cells from oxidative stress caused by free radicals, and they are more optimal than synthesized chelators as they have a longer life span, are readily bioavailable, and rarely have side effects. In this study, the phytochemical and iron-chelating property of Kotata berry was compared to a nutraceutical tablet. Extracts of Kotata berry and the nutraceutical were prepared by soaking, maceration, and condensed to dryness using a rotary evaporator. Phytochemical analysis and assay of iron-chelating activity were carried out using standard methods. Results showed that tannins, saponins, steroids, and alkaloids were present in both nutraceutical and Kotata berry extracts. Results also showed that Kotata extracts had more quantities of tannins (26.00±0.49), steroids (30.15±0.57), and saponins (25.05±0.47), with significantly high iron-chelating activity (>99%) than the nutraceutical.

Keywords: Iron chelating, Phytochemical, Antioxidants, Kotata blackberry; Nutraceutical

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INTRODUCTION

Iron is a significant cofactor in oxygen handling biological machinery, particularly a central pathogenic mechanism where iron overload induces tissue damage resulting in lipid peroxidation of biological membranes [1–3]. During overloading states of iron, hereditary hemochromatosis, transfusional siderosis, and thalassemia major are some of the deleterious effects of excess liver iron deposits. These diseases are believed to occur when the propagation and initiation of reactive oxygen become associated with iron, resulting in damage of bio(macro)molecules via oxidation leading to total cellular dysfunction, failure, or death [4].

Free radicals' generation above the body's antioxidant capacity has been shown to lead to oxidative stress; the underlying cause and mechanism of several disorders. While typical (iron) chelators – which are chemically synthesized – can confer protection on cells from oxidative stress by attacking and inhibiting free radicals, they have certain limitations such as poor bioavailability, side effects, and short half-life, which renders them suboptimal in action. This has prompted the search for and use of more effective antioxidants – molecules that are free radical scavengers – to protect the body's cells from oxidative stress and other associated damages caused by free radicals [5–7].

Phytochemicals, especially fruits-derived antioxidants, inhibit the activity of free radicals and protect cells from oxidative stress. In ancient civilization, plants and their constituent phytochemicals were highly regarded and used for the treatment of a wide range of ailments; and they have remained an important and reference source of treatment and management of diseases [7,8].

Kotata blackberry (*Rubus kotata*) was bred by the Oregon State University and the United States Department of Agriculture (USDA). It is a hybrid berry obtained by crossing OSC743 (Pacific and Boysen) and OSC877 (Jenner-1 and

Eldorado). It is a trailing berry, has larger berries with thorny canes, and is highly resistant to cane and leaf spot disease [9,10].
With the issues associated with nutraceuticals and iron chelators, the promising aspects of plants derived antioxidants, and the lack of data on Kotata berry; this study aimed to compare the phytochemical components and iron-chelating activities of Kotata berry and a nutraceutical.

MATERIAL AND METHODS

Sample collection and preparation

The Kotata blackberry was bought from Jos Main market, Plateau State, Nigeria, and authenticated at the Botany Department, Federal University of Lafia. The berries were washed with distilled water under a running tap. Prior to soaking and maceration in ethanol for 24 hours at room temperature (25 ± 2^0C) and subsequent filtration of extracts using Whatman No. 1 filter paper, the berries' fleshy part (pulp) were scraped and homogenized with a blender. The resulting filtrate was concentrated and condensed to dryness with a rotary evaporator with resulting fine powder collected and stored at 4^0C.
The nutraceutical (brand name omitted for ethical and legal reasons) tablet was obtained from a pharmaceutical store in Lafia, Nasarawa State, and transported to the laboratory. It was aseptically homogenized into fine powder form stored at 4^0C.
The analysis of both the Kotata fruits and nutraceuticals was done at the Chemistry Laboratory of the Department of Chemistry, Federal University of Lafia.

Phytochemical Screening

Qualitative phytochemical components of both the Kotata blackberry and nutraceutical were analyzed as [11] described. The phytochemicals tested for were: tannins, steroids, triterpenes, saponins, alkaloids, terpenoids, and flavonoids.
The methods described by [12] were used to quantitatively analyze the phytochemicals found in both Kotata berry and nutraceuticals.

Iron-chelating activity assay

Assay of the iron-chelating activities of the extracts of Kotata berry and the nutraceutical were determined with the method described by [13]. Briefly, a 5mL final volume of the reaction mixture was prepared by mixing 2mL of each concentration of extracts (2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL and 10mg/mL) with 2mL of $FeCl_3$ and 1mL of O-phenanthroline, vortexed and allowed to stand (incubated) for 10 minutes at room temperature. The spectrophotometric absorbance reading of each reaction mixture was taken at an absorbance of 510nm. Each test was replicated thrice, and the iron-chelating activity percentage was calculated using the formula below:

$$\%Fe\ C.A = \frac{Test\ absorbance - Control}{Test\ absorbance}\ x\ 100$$

A control was prepared using ascorbic acid instead of the extracts in the reaction mixture.

Statistical analysis

The result was statistically analyzed, and a comparison between the percentage content of the phytochemical screening and the iron-chelating activity was performed using Correlation. Data are also expressed as Mean± Standard deviation (Mean ± SD) for the quantitative screening and considered significant at ($P>0.05$).

RESULTS AND DISCUSSION

Table 1 below shows the result of the qualitative phytochemical component of the standard nutraceutical tablet and *Rubus kotata* extract. The standard nutraceutical tablet contains more (6 out of 9) phytochemicals, including tannins, steroids, saponins, phenols, alkaloids, and flavonoids. *R. kotata* extract was observed to contain 4 out of the 9 tested phytochemicals, which include tannins, steroids, saponins and alkaloids. Terpenoids and terpenoids are absent in both nutraceutical tablets and *R. kotata* extract. Phenol and flavonoids are contained in nutraceutical tablets but are not detected in *R. kotata* extract.

Table 1. Phytochemical Screening of Nutraceutical Tablet and Kotata Blackberry (*Rubus kotata*)

Phytochemical	Standard Nutraceutical tablet	*R. kotata* Extract
Tannins	+	+
Steroids	+	+
Triterpenes	-	-
Saponins	+	+
Phenol	+	-
Alkaloids	+	+
Terpenoids	-	-
Flavonoids	+	-

(**+**) indicated Present and (**-**) indicated Not Detected.

Table 2. % Conc. (Mean ±SD) and Wavelength of Quantified Phytochemicals

Phytochemical	% Conc. (Mean ±SD)		Wavelength (nm)
	Standard tablet	Extract	
Tannins	25.45±0.47	26.00±0.49	395
Alkaloids	41.35±0.64	39.45±0.60	562
Saponins	20.75±0.39	25.05±0.47	490
Steroids	21.20±0.40	30.15±0.57	563

Rubus kotata extract has a greater percentage (%) concentration of tannins, saponins and steroids than the Nutraceutical tablet. Nutraceutical tablets had a higher concentration of alkaloids than Rubus kotata extract. This is as shown in Table 2.

The result in table 3 showed some variations in iron-chelating activity between the Nutraceutical tablet and *Rubus kotata*. The Iron-chelating activity of *R. kotata* extract was found to have a greater percentage concentration than Nutraceutical tablet in the various concentrations assayed. The highest percentage (99.95%) value was obtained at concentration of 10 mg/mL and the least percentage (99.60%) value at concentration of 2 mg/mL. The Iron-chelating activity of the Nutraceutical tablets had its highest percentage (84.94%) value at a concentration of 8 mg/mL and least percentage (56.96%) at a concentration of 2 mg/mL.

Table 3. Iron-chelating Activity

Concentration (mg/mL)	% Fe C.A of Nutraceutical Tablet	% Fe C. A. of Extract
2	56.96	99.60
4	73.75	99.74
6	80.75	99.94
8	84.94	99.94
10	83.98	99.95

The ethanol extracts of both *Rubus kotata* and Nutraceutical tablet contained tannin, steroids, alkaloids and saponins, while phenols and flavonoids were also present in the Nutraceutical tablet only. However, terpenoids and triterpenes were not detected in both tablet and extract. These phytochemicals are reported to confer a host of pharmacological properties to the plant extracts. Phytochemicals have been reported to have beneficial and positive effects on human and animal health, including heart and psychotic diseases, muscular degeneration, ulcer, inflammation and infection, diabetes and hypertension [14,15]. Phenolics have been reported to exhibit tumor inhibited activity and inactivate carcinogens and mutagens [16,17] while alkaloids are anaesthetics, stimulants and anticancer agents [18]. Saponins are cytotoxic and cholesterol-lowering agents and have also been reported to possess antimicrobial, insecticidal, molluscicidal, antiviral, antifungal, membrane-permeabilizing, immunostimulant, hypocholesterolemic and anticarcinogenic properties [19,20]. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and animals with wasting illnesses [21,22]. Triterpenes have been reported to possess antimicrobial activity [23,24]. Tannins possess antioxidant and antimicrobial properties; they are also involved in inhibiting the activity of viral reverse transcriptase and are found to contain both protein-precipitating and iron-binding activities [25,26]. It is also attributed to analgesic and anti-inflammatory activities. Apart from this, tannins promote the growth and healing of wounds and inflamed mucous membranes with the proper astringency [27]. These chemical compounds might have been responsible for the fruits and tablets' acclaimed and reported properties such as wound healing, antibacterial, antifungal, radical scavenging, antioxidant and cytotoxic properties [28].

Rubus kotata fruit contained more tannins, saponins and steroids than the Nutraceutical tablet. Only alkaloid is higher in Nutraceutical tablets than the *Rubus kotata*. Eating plant fruits high in sterol is known to help lower the cholesterol level in the blood [29]. Hence, consuming the fruit and nutraceutical tablets could effectively regulate cholesterol levels in the blood. Although it was reported that diarrhea and dysentery characterized gastroenteritis manifests with high saponin levels [30], Umaru et al. (2007) did show that saponin aids in the reduction of cholesterol levels. Polyphenols such as tannins have also been reported to be medically important antioxidants, helping prevent and reduce oxidative stress that could cause cancer, inflammatory and heart diseases [32]. Consumption of some alkaloid containing foods above 20mg/100g may trigger toxicological manifestation [33].

Iron is vital in some biological processes such as blood formation and cofactor in oxygen handling biological machinery. However, the body has no mechanism to eliminate the irons released from these processes. Therefore, high accumulation of iron in the body, especially the liver, causes anemia such as thalassemia, hemochromatosis, heart diseases, tissue damage, and other health challenges [34–37].

This study further showed that the extracts of Kotata blackberry had a high percentage of iron-chelating activity (%Fe C.A). The high chelating activity of the Kotata berry extract implies that the berry can confer protection on DNA, inhibit lipid peroxidation and prevent oxidative stress [28] more than the Nutraceutical tablet extract. Furthermore, the percentage of iron-chelating activity of the Kotata berries was higher than those of *Ribes nigrum* [12] and some Iranian medicinal plants [7]. The iron-chelating activity of the Kotataberry was also observed to be slightly dose-dependent and was statistically significant ($p<0.05$) when compared to the Nutraceutical tablet, which was entirely dose-dependent with iron-chelating activity increasing with an increase in concentration.

Although this study couldn't ascertain if it is due to the phytochemicals present, it could be presumed that having higher quantities of tannins, saponins, and steroids could be observed for the observed iron-chelating activity potential. This implies that the extract of *Rubus kotata* possesses iron-chelating

activity that can counteract free radicals' action– causing them to be more stable and should be further investigated as an alternate iron-chelating source.

CONCLUSION

This study's findings showed that Kotata berry has great health benefits due to its quantities of phytochemicals. It also showed that Kotata berry is a good source of antioxidants and a good iron chelator, making it a potential alternative to existing iron chelators.

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