

Walisongo Journal of Chemistry Vol. 8 Issue 1 (2025), 1-11 ISSN: 2621-5985 (online); 2549-385X (print) DOI: https://doi.org/10.21580/wjc.v8i1.22353

PROXIMATE ANALYSIS, MINERAL, AND PHYTOCHEMICAL CHARACTERIZATION OF RESIDUAL SEEDS AND PODS OF PIGMENT-EXTRACTED ANNATTO (*BIXA ORELLANA L*.)

Ebenezer Olanrewaju Aluko 1*, Abayomi Olagundoye Adetuyi 2, Ezekiel Omotoso 3

¹ Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, 220001, Nigeria

² Department of Chemistry, School of Science, Federal University of Technology, Akure, 340282, Nigeria

³ Department of Physics, Faculty of Science, Obafemi Awolowo University, Ile-Ife, 220001, Nigeria

*Corresponding author: ebenezeraluko5@gmail.com

Abstract

In natural dye extraction, plant residues are often discarded, raising environmental and sustainable practices concerns. This study therefore aimed to evaluate the nutrient, mineral, and phytochemical profiles of residual seeds and pods from Bixa orellana after pigment extraction for the purpose of waste management. Proximate analysis revealed significant differences between seeds and pods in moisture, ash, protein, crude fibre, and carbohydrate contents, while fat content showed no significant variation. The seeds contained moisture (6.32±0.10%), ash (4.10±0.29%), crude fat (4.10±0.08%), crude protein (16.31±0.04%), crude fibre (11.67±0.10%), and carbohydrates (57.48±0.50%), while the corresponding values for the pods were 9.05±0.03%, 3.40±0.21%, 4.35±0.11%, 10.52±0.04%, 41.63±0.20%, and 31.05±0.15%, respectively. Mineral analysis showed high concentrations of magnesium (12.003 mg/g) in seeds and calcium (52.011 mg/g)mg/g) in pods, while chromium was absent in seeds but detected in pods (0.002 mg/g). Phytochemical analysis revealed alkaloids as the most abundant compound in both seeds $(8.9\pm1.6$ mg/g) and pods (7.2±0.4 mg/g), while flavonoids were the least (1.8±0.1 mg/g and 1.3±0.1 mg/g), respectively). ANOVA revealed significant differences (p < 0.05) in the proximate, mineral and phytochemical compositions, highlighting the nutritional and bioactive potential of both seeds and pods residues. These findings suggest that residual seeds and pods of Bixa orellana are valuable, rich in nutrients and minerals resources with potential applications in the food and pharmaceutical industries, promoting waste valorization and sustainability.

Keywords: bioactive; environmental; natural dye; seeds and pods residues; sustainability; waste valorization

Introduction

Natural dyes are widely regarded as eco-friendly due to their extraction from renewable and biodegradable sources (Pizzicato et al., 2023). However, the processing of natural dyes, from harvesting to pigment extraction, often results in the indiscriminate disposal of plant parts such as seeds, pods, leaves, and roots. This situation has prompted research into the utilization of these residual plant components in dyeing processes to promote sustainability and improve resource efficiency. For instance, Lee & Kang (2014) examined the dyeing of cotton fabrics using residual parts of cultivated

Pteridium aquilinum, demonstrating the feasibility of using plant residuals in textile dyeing. In a similar study by Yang et al. (2012),waste roots from Rubia wallichiana were used in dyeing processes, indicating the potential of plant residuals as alternative dye sources. Nevertheless, studies examining the proximate composition, phytochemical content, and mineral profile of plant residues following dve extraction remain scarce.

The seeds of Bixa orellana are primarily used for dye extraction, while other parts of the plant, including roots, leaves, pods, and foliage, have traditionally been employed for their therapeutic properties (Aluko et al., 2024). However, there has been limited literature addressing the use of residual parts of this plant for dyeing or other applications.

Bixa orellana, commonly cultivated in tropical regions such as South America and Africa, is highly valued for its seeds, which yield a non-toxic dye known as annatto. It is renowned for its excellent dveing capabilities (Ventosa, 2018). Hence, annatto is extensively used in the food, cosmetics, and household industries due to its characteristic red-to-orange-yellow coloration and mild flavor (Aluko, 2024). Beyond its dyeing applications, various parts of the plant, including the seeds, have been used in traditional medicine to stimulate digestion, lower blood pressure, protect the liver, reduce inflammation, treat coughs, cleanse the blood, soothe mucous membranes, alleviate fevers, and assist in wound healing and snakebite treatment (Rather & Mohammad, 2016). Recent of plant-derived screenings bioactive compounds have identified novel medicinal substances with cytoprotective and therapeutic potential for treating various health conditions (Ndam et al., 2014). Thus, medicinal plants continue to play a significant role in global healthcare.

This study aims to enhance waste management in dyeing and dye extraction by exploring the proximate, mineral, nutrient, and phytochemical potentials of residual seeds and pods of pigment extracted annatto. These analyses will help to evaluate the potential applications of these residues in the food and pharmaceutical industries.

Methodology

Sample Preparation

Residual seeds and pods of Bixa orellana, previously used for pigment extraction, were collected in February 2024 during the dry season. The samples were from Alagbaka. sourced located approximately 12 km south of the Federal University of Technology, Akure, Nigeria. Nigeria, a tropical country in West Africa, experiences a warm climate with distinct wet and dry seasons, and abundant rainfall characterizes the southern regions. In contrast, the north is typically hot and dry. The collected materials were thoroughly washed with distilled water, air-dried, and finely blended in preparation for proximate and phytochemical analyses.

Proximate Analysis

Proximate analyses were conducted following the standard methods outlined by the AOAC (2006). All experiments were performed in triplicate.

Moisture Content

Moisture content was determined using the oven-drying method. Five grams of each sample were weighed and placed in a laboratory oven (Memmert, Germany, Model 1001) at 105°C for 4 hours until a constant weight (W_1) was obtained. After drying, the crucibles were cooled in a desiccator for 30 minutes before recording the final weight (W_2) Moisture content was calculated using Equation 1.

% Moisture =
$$\frac{W_2 - W_1}{W_1 - W_0} \times 100$$
(1)

Ash Content

Clean, empty crucibles were first heated in a muffle furnace (Carbolite, UK, Model ELF11/14) at 550°C for 1 hour, cooled in a desiccator, and weighed to obtain W_0 . One gram of each sample was placed in separate crucibles and weighed (W_1). The samples were incinerated at 550°C for 3 hours until grayish-white ash appeared, indicating complete oxidation of organic matter. After cooling, the final weight (W_2) was recorded. Ash content was calculated using Equation 2.

% Ash content = $\frac{W_2 - W_1}{W_1 - W_0} \times 100$ (2)

Crude Protein

Crude protein was measured using the Kjeldahl method. Each 0.5 g sample was digested in a Kjeldahl flask (Kjeltec, Foss, Denmark) with 10 ml of concentrated H_2SO_4 (99% w/w, Merck, Germany) and 8 g of a catalyst mixture (Na₂SO₄:CuSO₄ in an 8:1 ratio). The mixture was heated on a hot plate (Thermo Scientific, USA) until a clear blue-green digest was obtained. The digest was diluted to 100 ml with distilled water, and 10 ml of the solution was distilled using a Kieltec distillation apparatus. Ammonia was collected in 20 ml of 4% w/v boric acid containing a few drops of methyl red indicator and titrated with 0.1 N HCl to a pink endpoint. A blank was processed alongside. Nitrogen content was calculated and converted to crude protein using a factor of 6.25, as seen in Equation 3.

% Crude Protein = 6.25x %N (6.25 = Correction factor)(3)

Crude Fat

Two grams of moisture-free samples were subjected to Soxhlet extraction using petroleum ether (boiling range: 40–60°C, Sigma-Aldrich, USA). The extraction was carried out using a fat-free thimble and a pre-weighed receiving flask. After six cycles, the ether extract was transferred to a preweighed glass dish, evaporated in a water bath (Heidolph, Germany), dried at 105°C for 2 hours, cooled in a desiccator, and weighed. The crude fat content was calculated using Equation 4.

%	Crude	fat	= <u>wt.</u>	of	ether	<u>extract</u>	Х	100	
	wt. of sample								
								. (4)	

Crude Fibre

Two grams of each sample were washed with ether and oven-dried for 1 hour. The dried samples were boiled in 150 ml of 0.128 M H_2SO_4 for 1 hour, washed, and then treated with 150 ml of 0.233 M NaOH. After rinsing and treatment with acetone, the residues were dried at 105°C for 1 hour and ashed at 550°C for 3 hours. The percentage of crude fibre was calculated using Equation 5.

% Crude =
$$\frac{W_2 - W_1}{W_0}$$
 x 100 %......(5)

Carbohydrate

Carbohydrate content was estimated by difference, subtracting the sum of moisture, crude protein, crude fat, crude fibre, and ash from 100 using Equation 6.

% CHO = 100 - % (Moisture + Crude protein+ Crude fat + Crude fibre + Ash.....(6)

Mineral Analysis

The mineral content of Bixa orellana L. seeds and pods was determined using an Atomic Absorption Spectrophotometer (AAS, Hitachi Model 170-10, Japan) at the Central Laboratory of the Federal University of Technology, Akure. The instrument was calibrated with standard solutions of each mineral before and during the analysis to ensure accuracy (Skoog et al., 2017). Specific electrode lamps for each mineral were used during the analysis (Skoog et al., 2017).

The analyzed minerals included iron (Fe), zinc (Zn), calcium (Ca), chromium (Cr), manganese (Mn), sodium (Na), potassium (K), and magnesium (Mg). The absorption measurements were recorded in parts per million (ppm) and subsequently converted to milligrams per gram (mg/g) using Equation 7.

<u>Absorbance (ppm) x dry wt. x D</u> (7) wt. of sample x 1000

Notes:

D = Dilution factor for the respective minerals.

Qualitative Phytochemical Screening

Oualitative phytochemical screening of the prepared Bixa orellana seed and pod samples was carried out using the procedures described by Sofowora (1993), El-Olemy et al. (1994), and Siddiqui and Ali (1997). Ethanol extracts were first prepared by soaking 20 g of each sample in 100 mL of 96% ethanol for 24 hours at room temperature. The mixtures were filtered using Whatman No. 1 filter paper, and the residues were washed with an additional 20 mL of ethanol to ensure complete extraction. The extracts were stored in a refrigerator at 4°C for subsequent analysis. A similar procedure was followed for aqueous extraction, using distilled water in place of ethanol as the solvent. For each phytochemical test, 2 mL of the ethanolic and aqueous extracts were used. A summary of the tests and their results is provided in Table 1.

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis of finely ground annatto seeds and pods was conducted using the methods described by Evans (2009), Sofowora (1993), El-Olemy et al. (1994), and Siddiqui and Ali (1997). All analyses were performed in triplicate.

Tannins

To determine tannin content, 0.5 g of each *Bixa orellana* pod and seed sample was soaked in 10 ml of 70% acetone for 8 hours. Subsequently, 0.2 ml of the supernatant was mixed with 1 ml of distilled water. To this mixture, 2 ml of 20% sodium carbonate (Na₂CO₃) and 0.5 ml of Folin-Ciocalteu reagent were subsequently added. The solution was incubated in a water bath at 40°C for 1 hour. UV absorbance was measured at 725 nm against a reagent blank. Tannin content was expressed as mg/g tannic acid equivalent, using the calibration curve: Y = 0.0593X - 0.0485, R² = 0.9826, where X is the absorbance, and Y is the tannic acid equivalent.

Flavonoids

For flavonoid estimation, 1.0 g of each sample (seeds and pods) was weighed into separate beakers, and 10 ml of 80% methanol was added. The mixtures were left to stand for 2 hours and subsequently filtered into pre-weighed petri dishes. The filtrates were evaporated to dryness in a water bath at 40°C. The petri dishes were reweighed to determine the weight of the dried residue. The percentage of flavonoids was calculated by the difference in weight and converted to mg/g based on the initial sample weight (1.0 g).

Phenols

For phenolic content analysis, 0.5 g of each sample was soaked in 10 ml of 70% acetone for 8 hours. A 0.2 ml aliquot of the supernatant was diluted to 3.0 ml with distilled water, followed by the addition of 0.5 ml of Folin-Ciocalteu reagent. After mixing, the solution was left to stand for 3 minutes before adding 2.0 ml of 20% sodium carbonate into the tubes. The mixture was incubated in a water bath at 40°C for 1 hour, then cooled. Absorbance was read at 650 nm against a blank. Total phenolic content was expressed as mg/g tannic acid equivalent (TE) using the calibration equation: Y = 0.1216X, $R^2 =$ 0.9365, where X is the absorbance, and Y is the tannic acid equivalent.

Alkaloids

To determine alkaloid content, 5 g of Bixa orellana seeds and pods were placed in separate 250 ml beakers containing 100 ml of 10% acetic acid in ethanol. The mixtures were covered and left to stand for 4 hours. After filtration. the filtrates were concentrated over a water bath to oneof their original volume. quarter Concentrated ammonium hvdroxide (NH₄OH) was added to each to induce precipitation. The precipitated alkaloids were collected on pre-weighed filter paper, washed with dilute NH₄OH, dried, and weighed. The alkaloid content was

			Seeds		Pods	
Phytochemical	Reagent	Confirmation	EtOH	H ₂ O	EtOH Extract	H ₂ O
Test	-		Extract	Extract		Extract
Phenol	Ferric chloride	Deep blue	+	+	+	+
		coloration				
Saponin	Foam	Foamy lather	+	+	+	+
Steroids	Acetic	Blue/green	+	+	+	+
	anhydride/Conc.	coloration				
	H_2SO_4					
Glycosides	Glacial acetic	Interfacial	+	+	+	+
	acid/FeCl ₃	brown ring				
		coloration				
Flavonoids	Ferric chloride	Blackish red	+	+	+	+
		coloration				
Tannins	Ferric chloride	Brownish	+	+	+	+
		green/blue-				
		black				
	_	coloration				
Resins	Aqueous HCl	Resinous	_	_	_	_
		precipitate				
Alkaloids	Dragendorff's	Red	_	+	+	+
	reagent	precipitate				
Carbohydrate	Conc. H ₂ SO ₄	Effervescence	+	+	+	+
		and				
		blackening				
Protein	Millon's reagent	Red	+	+	+	+
		precipitate				
Reducing	Benedict's	Red	+	+	+	+
Sugar	reagent	precipitate				

calculated based on the weight difference and converted to mg/g relative to the initial 5 g sample weight.

Saponins

For saponin determination, 5 g of each sample (W_0) was weighed into separate 250 ml conical flasks. Each was treated with 25 ml of 20% ethanol and heated in a water bath at 55°C for 4 hours with continuous stirring. After filtration, the residue was reextracted with an additional 50 ml of 20% ethanol. The combined extracts were concentrated to 40 ml in a water bath at approximately 90°C. This extract was transferred to a 250 ml separating funnel and mixed with 10 ml of petroleum ether. After vigorous shaking, the aqueous layer was retained, and the ether layer was discarded. Subsequently, 20 ml of n-butanol was added to the aqueous layer. The resulting n-butanol extract was washed twice with 10 ml of 5% NaCl solution. The final extract was collected in a pre-weighed petri dish (W_1), dried in an oven at 90°C to a constant weight (W_2), and the saponin content was calculated using Equation 8. The percentage was then converted to mg/g.

Results and Discussion

Proximate Analysis of Residual Seeds and Pods of *Bixa Orellana*

The proximate analysis, presented in Table 2. highlights the nutritional composition of residual seeds and pods of Bixa Orellana while Figure 1 portrays a line graph showing the comparison between proximate compositions of the residual seeds and pods. The seeds exhibited higher levels of protein and carbohydrates, while the pods demonstrated significantly greater fibre content. ANOVA results indicated significant differences (p < 0.05) in moisture, ash, protein, crude fibre, and carbohydrate contents, whereas no significant variation was observed in fat content.

The moisture content of the residual seeds was 6.32±0.10%, compared to a slightly higher value of 9.05±0.03% in the pods. These values fall within the typical range for plant materials, indicating that both residues were suitable for stable storage and further processing (Zheng et al., 2011). Notably, the seed moisture content of 6.32% exceeded the 4.89% reported by Dike et al. (2016) but was slightly lower than the 6.75% reported by Valério et al. (2015).

The ash content, representing the inorganic matter in the samples, was higher in seeds $(4.10\pm0.29\%)$ than in pods $(3.40\pm0.21\%)$. This result suggests that the seeds contained a slightly higher proportion of minerals. The elevated ash content in both residues confirmed the mineral richness of annatto waste, reinforcing its potential as a sustainable source of essential minerals. Indeed, the mineral content of Bixa orellana seeds is traditionally utilized in cuisines across the Caribbean, Latin America, the Philippines, and Mexico (Ulbricht et al., 2012).

Crude fat content was similar between the seeds $(4.10\pm0.08\%)$ and pods $_6$

(4.35±0.11%), with no statistically significant differences. These values fall within an acceptable range for plant-based indicating materials, potential for applications in oil extraction (Rahim et al., 2023), biofuel production (Osman et al., 2024), or cosmetics and nutraceuticals as sources of beneficial fatty acids (Rahim et al., 2023). While the fat content in seeds (4.10%) was lower than the 7.20% reported by Dike et al. (2016) and 6.3% by Prabhakara Rao et al. (2015), it remains notable for plant-derived oils.

The crude protein content in the pigment-extracted seeds was 16.67%, surpassing values reported by Prabhakara Rao et al. (2015) (11.2%) and Habboubi (2020) (9.1%). This higher protein level highlights the potential of Bixa orellana seed residues as a valuable protein source for food and animal feed industries.

Crude fibre content was significantly higher in pods (41.63±0.20%) than in seeds (11.67±0.10%). This suggests that residual pods might have greater utility in applications requiring high fibre content, such as dietary supplements or industrial materials. The seed fibre value of 11.67% closely aligned with the 12.55% reported by Dike et al. (2016), reinforcing their potential use in functional foods promoting digestive health.

Carbohydrate content was substantially higher in the seeds (57.48±0.50%) compared to the pods (31.05±0.15%), positioning the seeds as a energy-dense more option. The carbohydrate level in seeds exceeded the 42.2% observed by Melissa (2005) and Valério et al. (2015) but was lower than the 78.11% reported by Moraes (2007). These variations might result from genetic differences and growing conditions (Aluko, 2024). Given the importance of carbohydrates as an energy source, the relatively high carbohydrate content of the seeds enhances their value for energy-rich food formulations.

While research on the proximate composition of Bixa orellana pods remains limited, this study offers valuable insight into their nutritional potential. The pods exhibited higher levels of moisture, fat, and crude fibre than the seeds. Although less nutrient-dense, the pods may still be useful by-products for industrial applications. Seeds are better suited for food products requiring high protein and carbohydrate content, whereas pods hold promise as a lowest concentration in the pods (0.002 mg/g). Magnesium emerged as the most abundant mineral in the seeds (12.003 mg/g), while calcium dominated the pods (52.011 mg/g). These significantly high levels of magnesium and calcium are essential for numerous physiological

Table 2. Proximate Composition of Residual Seeds and Pods of Bixa orellana

Residue	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude Fibre (%)	Carbohydrate (%)
Seeds	6.32±0.10	4.10±0.29	4.10±0.08	16.31±0.04	11.67±0.10	57.48±0.50
Pods	9.05±0.03	3.40±0.21	4.35±0.11	10.52±0.04	41.63±0.20	31.05±0.15

*Mean ± standard deviation (SD) of three replicates. Significant differences were tested at p < 0.05.



Figure 1. Comparative Proximate Compositions between Residual Seeds and Pods of Bixa orellana

source of dietary fibre, an increasingly important component in functional food development.

Mineral Analysis of Residual Seeds and Pods of Bixa orellana

The mineral content of the residual seeds and pods of Bixa orellana, as presented in Table 3 shows significant variation in the concentrations of various minerals. Figure 2 presents a comparative illustration of the mineral analysis results for the residual seeds and pods of Bixa orellana, allowing for enhanced visual comparison of their respective mineral contents. Chromium was undetectable in the residual seeds but appeared at the functions, including bone health and enzyme regulation (Ciosek et al., 2021), indicating that both seeds and pods can serve as valuable sources of these key Other minerals. minerals. such as manganese (0.016 mg/g in seeds and 0.052)mg/g in pods), sodium (0.350 mg/g in seeds), potassium (10.000 mg/g in seeds and 19.500 mg/g in pods), and phosphorus (2.612 mg/g in seeds and 0.744 mg/g in)pods), showed significant differences between seeds and pods. Manganese and sodium were more concentrated in seeds, while potassium and calcium were more abundant in pods. The absence of significant differences in iron, zinc, and

magnesium suggests that these minerals are relatively stable across both residual materials. The mineral content and fibre fractions in Bixa orellana seeds closely resemble those found in cereals (Kumar et al., 2007).

Phytochemical Analysis of Residual Seeds and Pods of Bixa orellana

As previously highlighted in Table 1, all of the tested phytochemicals were present in both the seed and pod extracts, except for resins. Alkaloids were specifically

Table 3. Mineral Content of Residual Seeds and Pods of *Bixa orellana*

Residue	Composition (mg/g)									
	Mn	Fe	Са	Cr	Zn	Mg	Na	К	Р	
Seeds	0.016±0.003	0.030±0.010	0.866±0.072	-	0.019±0.005	12.003±2.500	0.350±0.010	10.000±1.500	2.612±0.600	
Pods	0.052±0.012	0.688±0.520	52.011±4.203	0.002±0.000	0.026±0.008	8.002±1.620	0.040±0.002	19.500±3.552	0.744±0.162	

*Mean \pm standard deviation (SD) of three replicates. Significant differences were tested at p < 0.05.





Figure 2. Comparative Mineral Content between Residual Seeds and Pods of Bixa orellana

Table 4. Quantitative Phytochemical Analysis of Residual Seeds and Pods of Bixa orellana

Residue	Tannins (mg/g)	Phenols (mg/g)	Flavonoids (mg/g)	Alkaloids (mg/g)	Saponins (mg/g)
Seeds	0.34±0.01	0.22±0.01	1.8±0.1	8.9±1.6	5.7±1.
Pods	0.53±0.04	0.46±0.01	1.3±0.1	7.2±0.4	4.3±0.5



Figure 3. Comparative Phytochemical Results between Residual Seeds and Pods of Bixa orellana

8

detected exclusively in the aqueous extract. This suggests that alkaloids have a higher affinity for water than for ethanol, making ethanol less suitable as a solvent for extracting alkaloids from Bixa orellana. Tannins are known to protect cells from oxidative damage caused by free radicals. Therefore. the anticarcinogenic and antimutagenic potentials of tannins can be attributed to their antioxidative properties (Fraga-Corral et al., 2021), which help mitigate cellular oxidative processes such as lipid peroxidation Dike et al. (2016). Saponins anti-inflammatory are with expectorant effect. Saponins also act as adjuvant in enhancing immune response in the body (Skene & Sutton, 2006).

Phytochemical screening as presented in Table 4 and compared in Figure 3 revealed that alkaloids were the most abundant phytochemicals in both seeds (8.9±1.6 mg/g) and pods (7.2±0.4 mg/g), followed by tannins, phenols, and saponins. Tannin and phenol content was higher in pods $(0.53\pm0.04 \text{ mg/g})$ and 0.46±0.01 mg/g, respectively) than in seeds (0.34±0.01 mg/g and 0.22±0.01 mg/g, respectively). Flavonoids were the least abundant in both samples, with seeds containing 1.8 ± 0.1 mg/g and pods 1.3 ± 0.1 The ANOVA results indicated mg/g. significant differences in tannin, phenol, and flavonoid contents between the residual seeds and pods, but no significant differences in alkaloids or saponins at p < 0.05.

Historically, alkaloids have been utilized in medicine for their pharmacological effects. Examples include morphine and codeine for pain relief, quinine as an anti-malarial agent, and ephedrine as a decongestant (Heinrich & Amirkia, 2021). In foods, alkaloids with medicinal properties may be intentionally consumed for their perceived health benefits (WHO, 2021). The presence of saponins, flavonoids, and phenols in the seeds and pods of *Bixa orellana* contributes to their potential anticarcinogenic and antioxidant properties (Raju et al., 2022; Chen et al., 2023). Plants believed to be rich in a variety of secondary metabolites such

as alkaloids, flavonoids, terpenoids and saponins have therapeutic values (Agidew. 2022). These metabolites have received attention as active agents for the management of several disease conditions.

Conclusion

The proximate. mineral. and phytochemical analyses collectively highlight the significant nutritional, mineral, and bioactive potential of the residual seeds and pods of Bixa orellana. The proximate analysis indicates that the seeds are rich in protein and carbohydrates, making them suitable for food applications, while the pods offer a high fibre content, which can be used for dietary supplementation. The mineral analysis shows that both byproducts are excellent sources of essential minerals, including magnesium, calcium, and potassium. Furthermore, the phytochemical analysis identifies valuable bioactive compounds like alkaloids, tannins, and phenols. These findings suggest that instead of being discarded as waste, the residual seeds and pods of Bixa orellana could be effectively utilized in the food, pharmaceutical. and nutraceutical industries, contributing to waste valorization and promoting sustainability.

Acknowledgments

The authors acknowledge the Department of Chemistry, the School of Science, and the School of Postgraduate Studies at The Federal University of Technology, Akure, for their support in the success of this work.

References

- AC, A. (2006). Association of official analytical chemists. *Official methods of analysis of AOAC International*.
- Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bulletin of the National Research Centre, 46(1), 87.

https://doi.org/10.1186/s42269-022-00770-8

- Aluko, E. (2024). Ethanol-Based Extraction of Annatto (Bixa orellana L.) and Characterization of the Bixin and Norbixin. ACS omega, 9(16), 18273-18277.
- Aluko, E. O., Adetuyi, A. O., & Omotoso, E. (2024). Antibacterial Activity of Residual Seeds and Pods of Annatto (Bixa orellana L.) After Pigment Extraction. Walisongo Journal of Chemistry, 7(2), 221-230. https://doi.org/10.21580/wjc.v7i2.2235 4
- Chen, K., Wang, N., Zhang, X., Wang, M., Liu, Y., & Shi, Y. (2023). Potentials of saponins-based adjuvants for nasal vaccines. Frontiers in Immunology, 14, 1153042.
- Ciosek, Ż., Kot, K., Kosik-Bogacka, D., Łanocha-Arendarczyk, N., & Rotter, I. (2021). The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue. Biomolecules, 11(4), 506. doi: 10.3390/biom11040506.
- de Moraes, SA (2007). Agroindustrial byproducts and external indicators of apparent digestibility in goats
- Dike, I. P., Ibojo, O. O., Daramola, F. Y., & Omonhinmin, A. C. (2016). Phytochemical and proximate analysis of foliage and seed of Bixa orellana linn. Int. J. Pharm. Sci. Rev. Res, 36(2), 247-251.
- El-Olemy, M. M., Al-Muhtadi, F. J., & Afifi, A. F. A. (1994). Experimental phytochemistry: A laboratory manual. King Saud University Press.
- Evans, W. C. (2009). Trease and Evans' pharmacognosy. Elsevier Health Sciences.
- Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., ... & Prieto, M. A. (2021). By-products of agrifood industry as tannin-rich sources: A review of tannins' biological activities

10

and their potential for valorization. Foods, 10(1), 137.

- Habboubi, B. (2020). Bixa orellana L. pods and seeds: nutritional and chemical characterization, bioactivity studies, and development of a carotenoid-based food colorant (Master's thesis, Instituto Politecnico de Braganca (Portugal)).
- Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. Molecules, 26(7), 1836. doi: 10.3390/molecules26071836.
- Kumar, P. S., Reddy, Y. R., Ramesh, S., Gobinath, S., & Ramana, D. B. V. (2007).
 Evaluation of Pigment Extracted Annatto Seed (Bixa orellana) By Chemical, In-Vitroand In-Sacco Techniques In Buffaloes. Buffalo Bulleitn Ibic, Kasetsart University, Po Box 1084 Bangkok 10903, Thailand, 26(1), 5
- Lee, H., & Kang, E. (2014). Dyeing of cotton fabrics using residual parts of cultivated Pteridium aquilinum. Textile Coloration and Finishing, 26(1), 53-62. doi: 10.5764/TCF.2014.26.1.53
- Melissa, A.V., Maria I. L.R., José Antônio, B. N. and Maria L. R. M. (2005). Food Science Technology (Campinas), vol.35 no.2. Campinas.
- Ndam, L. M., Mih, A. M., Fongod, A. G. N., Tening, A. S., Tonjock, R. K., Enang, J. E., & Fujii, Y. (2014). Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants. Int J Curr Microbiol App Sci, 3(12), 768-778.
- Osman, A. I., Nasr, M., Farghali, M., Rashwan, A. K., Abdelkader, A., Al-Muhtaseb, A. A. H., ... & Rooney, D. W. (2024). Optimizing biodiesel production from waste with computational chemistry, machine learning and policy insights: a review. Environmental Chemistry

Letters, 22(3), 1005-1071. doi: 10.1007/s10311-024-01700-y

- Pizzicato, B., Pacifico, S., Cayuela, D., Mijas,
 G., & Riba-Moliner, M. (2023).
 Advancements in sustainable natural
 dyes for textile applications: A
 review. Molecules, 28(16), 5954.doi:
 10.3390/molecules28165954
- Prabhakara Rao, P., Narsing Rao, G., Jyothirmayi, T., Satyanarayana, A., Karuna, M. S. L., & Prasad, R. B. N. (2015). Characterisation of Seed Lipids from Bixa orellana and Trachyspermum copticum. Journal of the American Oil Chemists' Society, 92, 1483-1490.
- Rahim MA, Ayub H, Sehrish A, Ambreen S, Khan FA, Itrat N, Nazir A, Shoukat A, Shoukat A, Ejaz A, Özogul F, Bartkiene E, Rocha JM. Essential Components from Plant Source Oils: A Review on Extraction, Detection, Identification, and Quantification. Molecules. 2023 Sep 29;28(19):6881. doi: 10.2290/molecules28196881

10.3390/molecules28196881.

- S. K., Chandrasekar, Raju, S., Vengadhajalapathy, P., Sundaram, R., Periyasamy, S., Chinnaraj, T., ... & Kumar, S. (2022). Review on phytochemical composition pharmacological and activities of Bixa orellana L. Journal of Pharmaceutical and Biological Sciences, 10(2), 57-67.
- Rather, L. J., & Mohammad, F. (2016).
 Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications–A review. Journal of advanced research, 7(3), 499-514.
- Siddiqui, A. A., & Ali, M. (1997). Practical pharmaceutical chemistry. CBS Publishers & Distributors.
- Skene, C. D., & Sutton, P. (2006). Saponinadjuvanted particulate vaccines for clinical use. Methods, 40(1), 53-59.

- Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of Instrumental Analysis (7th Ed.). Cengage Learning.
- Sofowora, A. (1993). Medicinal plants and medicine in Africa. John Willey Spectrum, Ibadan Nigeria, 281-285.
- Ulbricht, C., Windsor, R. C., Brigham, A., Bryan, J. K., Conquer, J., Costa, D., ... & Weissner, W. (2012). An evidence-based systematic review of annatto (Bixa orellana L.) by the Natural Standard Research Collaboration. Journal of Dietary Supplements, 9(1), 57-77.
- Valério, M. A., Ramos, M. I. L., Braga, J. A., & Macedo, M. L. R. (2015). Annatto seed residue (Bixa orellana L.): nutritional quality. Food Science and Technology (Campinas), 35(2), 326-330. doi: 10.1590/1678-457X.6539.
- Ventosa, E. (2018). Bixa orellana (annatto). CABI. org. https://www. cabi. org/isc/datasheet/9242. Accessed March, 22, 2024.
- World Health Organization (WHO). (2021). WHO Model List of Essential Medicines -21st List. Retrieved from https://www.who.int/publications/i/ite m/WHOMVPEMPIAU2019.06 on 28/06/2024
- Yang, R., Zhang, Y., Ranjitkar, S., Li, M., Guo, Y., Yan, X., ... & Yang, L. (2021). Reusing wasteroot of Rubia wallichiana dyeing from Monpa of Tibet in China. Scientific Reports, 11(1), 14331. doi:10.1038/s41598-021-93848-8
- Zheng, Y., Yates, M., Aung, H., Cheng, Y. S., Yu,
 C., Guo, H., ... & Jenkins, B. M. (2011).
 Influence of moisture content on microbial activity and silage quality during ensilage of food processing residues. Bioprocess and biosystems engineering, 34, 987-995.