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# Ethanol-Based Extraction of Annatto (*Bixa Orellana L.*) and Characterization of the Bixin and Norbixin

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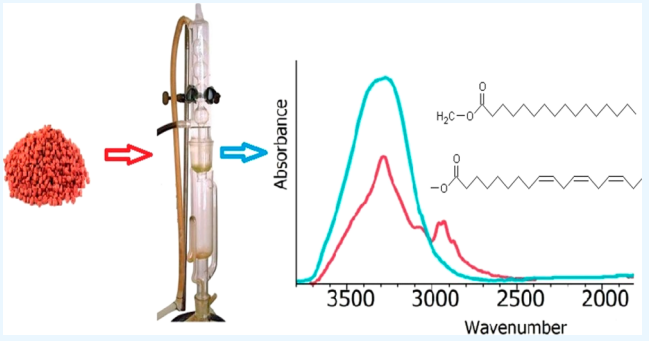
**ABSTRACT:** *Bixa orellana* dye was extracted from the seeds by the method of Soxhlet extraction using ethanol where a percentage yield of 31.4 was obtained. Recrystallization of the dye was performed using chloroform and ethanol to increase the degree of purity. Thin layer chromatography was used to separate the dye into Bixin and Norbixin with $R_f$ values of 0.56 and 0.42, respectively. The extracted dye was pH sensitive, insoluble in water but soluble in some organic solvents like ethanol and acetic acid. Characterization by UV−vis spectroscopy showed maximum absorption for Bixin and Norbixin at 457 and 453 nm, respectively. Fourier transform infrared spectra of Bixin and Norbixin showed bands confirming similar functional groups for both Bixin and Norbixin except for −CO− stretching and methylene vibration which was absent in Norbixin. With the yield, purity level, and other properties of the extracted dye, the comparative advantages of ethanol-based Soxhlet extraction of the dye were well established.




## 1. INTRODUCTION

Nontoxic plant extracts with high dying power and ample color are excellent for use in coloring food, products, cosmetics, pharmaceuticals, and textiles. One of these plants, annatto (*Bixa orellana*) which originated from Central America and is now widely grown throughout the tropical regions of the world, including West Africa, has its seeds as the raw material for pigment extraction.[1] The chemical constituents of the pigment include bixin which represents more than 80% of the total fat-soluble carotenoids, norbixin, and norbixinate, whose levels are variable according to seed maturation.[2] Bixin is responsible for imparting reddishness, and norbixin is responsible for imparting yellow.

The main product from *B. orellana* is an organic dye present in the seed coat called "annatto," lipid-soluble and widely used in the food industry for its red to orange-yellow colors (cheese, butter, oils, margarine, ice cream, pastries, soft drinks, soup, rice).[2,3] Next to caramel, it is the world's second most important food colorant. Besides providing an attractive color to meat and other dishes, it also imparts a subtle and distinctive flavor. In the cosmetic industry, it finds use in hair, nail, and soap products, and also in many household products like floor wax, shoe polish, russet leather, and wood stains.[4]

This research focuses on the alternative extraction of *Bixa orellana* dye other than the historical aqueous which is prevalent among local cultivators for commercial production.[5] Corresponding survey carried out within the Akure Township where the annatto seeds were harvested showed that the community had been applying conventional water maceration extraction. This research discusses ethanolic extraction of annatto, not only as an alternative with considerably high yield but also with high purity.[6]

Similarly, this study emphasizes the methodology involved in obtaining annatto dye in the dried powder form rather than the usual aqueous form employed by local dyers. The powder form is known to have longer shelf life for storage purposes.[7] It also helps to prepare the dye for wider applications: food, cosmetics, and nanoscience.[8]

**1.1. Bixin and Norbixin.** Bixin was isolated for the first time from the seeds of *Bixa orellana* in 1875 and in 1961. Its complete chemical structure and stereochemistry were determined by $^{1}H$ and $^{13}C$ NMR.[9] Bixin, as shown in Figure 1, belongs to the small class of natural apocarotenoids, whose formation occurs by the

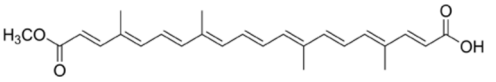



**Figure 1.** Bixin.

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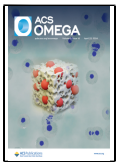

oxidative degradation of C40 carotenoids.[9] It is a polyunsaturated norcarotenoid, a red dye from the main fruit of annatto. Its seeds reduced to powder are widely used to color food and sunscreen.[10] The annatto seeds contain about 5% pigment, which consists of 70−80% of bixin. The bixin is soluble in fats but insoluble in water.[11] When exposed to alkalis, the methylester is hydrolyzed and produces norbixin dicarboxylic acid, a water-soluble derivative. Norbixin (Figure 2) is a

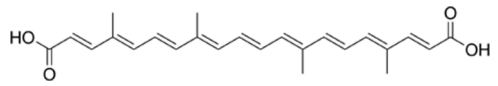


**Figure 2.** Norbixin.

demethylated derivative of bixin. Although it is a naturally occurring compound, it is almost always referred to as a saponification product of bixin.[11] Norbixin is a chemically unstable compound when isolated and is converted via isomerization into bixin trans-($\beta$-bixin), the cis-trans isomer of bixin. Carotenoids bixin and norbixin have two stereo configurations, i.e., cis and trans. In extracts, under normal conditions, the cis-bixin or cis-norbixin is more unstable. The cis-bixin or cis-norbixin solution under heating is partially converted into the trans-configuration, which is more stable and known as isobixin and isonorbixin.[10] In addition, in their respective concentrations, the following are found in the seeds of annatto: 40−45% cellulose, 3.5−5.5% sugars, 0.3−0.9% essential oils, 3% fixed oils, 1.0−4.5% pigments, and 13−16% proteins and alpha and beta-carotene, as well as tannins and saponins.[12]

## 2. MATERIALS AND METHODS

*Bixa orellana* seed and pod were harvested at Fiwasaye Girls' Grammar School, Akure. Identification was performed by the Biology Department of the Federal University of Technology, Akure. The samples were washed with distilled water to remove dust and air-dried for 4 days. The dried samples were blended and kept for analysis.

**2.1. Dye Extraction.** Two hundred grams of the seed sample were extracted for dye by the Soxhlet extraction method; 1000 mL of commercial ethanol degree at 96% ethanol was used for the extraction. The temperature of the Soxhlet Apparatus was maintained between 70 and 80 °C for about 2 h.[13] Several cycles of solvent were run so as to extract all of the dye present in the seed. The extract obtained was distilled with ethanol and concentrated.

**2.2. Defatting of Dye Extract.** Fifty milliliters of *n*-hexane were added to the dye concentrate with vigorous stirring for 30 min and left to settle for 4 h to remove fat, after which filtration was carried out. The dye crystals were obtained as the residue. The test sample was prepared for UV−visible spectroscopy, which was done at the Central Laboratory of The Federal University of Technology, Akure.

**2.3. Recrystallization of Dye.** Recrystallization of the crystal was done by using chloroform and ethanol. The dye crystals were dissolved in chloroform in a 250 mL beaker at room temperature and filtered when warmed. The filtrate was left to cool, and the crystallized dye was obtained by filtration as the residue.

Ethanol was also used to recrystallize some of the dye by dissolving in ethanol in a 250 mL beaker and heating on a water bath at 70 °C for 20 min. Filtration was carried out using Whatmann filter paper while hot. The filtrate was allowed to cool, and the dye crystallized out. A second filtration was then carried out where the dye crystals were obtained as residue. A test sample of the recrystallized dye was analyzed for UV−visible spectroscopy at the Central Laboratory of the Federal University of Technology, Akure while the Fourier transform infrared (FTIR) spectroscopy was done at Ladoke Akintola Akintola University of Technology, Ogbomoso.

**2.4. Percentage Yield.** The percentage yield of the dye was calculated at the end of the recrystallization using the equation below:

$$\%\ \text{Yield} = \frac{\text{mass of dried dye} \times 100}{\text{mass of seed sample (200 g)}} \quad (1)$$

**2.5. Conversion to Norbixin.** To 2 g of the dye extract was added 20 mL of 0.1 M NaOH and the mixture was heated in a water bath to hydrolyze the coloring matter and cooled. The aqueous solution was filtered and acidified with concentrated hydrochloric acid, and a few drops slowly from the sides to precipitate norbixin. The precipitate was filtered, washed, dried, and milled to give a granular powder.[14] The norbixin was prepared for characterization by UV−vis spectroscopy and FTIR. The UV−vis spectroscopy was done at the Central Laboratory of the Federal University of Technology, Akure, while the FTIR was carried out at Ladoke Akintola University of Technology, Ogbomoso.

**2.6. Thin Layer Chromatography Characterization of Bixin and Norbixin.** A thin layer chromatography plate was coated with 500 $\mu$m thick silica gel film and kept in a hot air oven for one h at 110 °C. A 10% solution of the powder dye extract was dissolved in 95% ethanol and applied to the plate to dry. The chromatography was developed using a solvent comprising a mixture of benzene, ethyl acetate methanol in the ratio 3:4:2 by volume until the solvent front has ascended about 10 cm.

**2.7. Solubility and Color Change Test.** The solubility test and color change characteristics were carried out on the dye with water, acetone, acetic acid, hexane, carbonated soft drink, thinner, palm wine, ethanol, sodium hydroxide, and hydrochloric acid. 0.1 g of dye was used with 10 mL of each of the test reagents at room temperature.

## 3. RESULTS AND DISCUSSION

**3.1. Availability of Test Samples.** *Bixa* orellana was well-grown at the chosen site, making its seeds readily available. *Bixa orellana* is a plant that thrives well in tropical regions having originated from South and Central America and widely grown in Africa over the years.[9] Evidently, the favorable soil profile and fertility of Akure and the surrounding towns in Ondo state encourage the cultivation of *Bixa orellana*, primarily for ornamental purposes.[15] This cultivation culture has made the plant predominantly available for harvesting and extended cultivation by dyers.

**3.2. Physical Properties of Extracted *Bixa orellana* Dye.** *Bixa orellana* dye obtained as a solid is a red powder with a melting point of 190 °C and is rarely soluble in water as shown in Table 1. The poor solubility in water of the pigment is due to the small proportion of norbixin (less than 20%), the water-soluble constituent in comparison with bixin (about 80%) which is fat soluble.[11] The available structures for the two compounds revealed that norbixin has two hydroxyl end-groups, while bixin has just one of its hydroxyls methylated. A percentage yield of **31.4%** was obtained by the Soxhlet extraction used.


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**Table 1. Physical Properties of Extracted *Bixa orellana* Dye**

| dye | *Bixa orellana* |
|---|---|
| appearance | red powder |
| solubility in water | slightly |
| melting point | 190 °C |
| yield | **31.4%** |

Comparatively, Silva et al.[14] reported that Supercritical $CO_2$ extraction of bixin makes it easier to separate the organic solvent from the solute, leaving a pure final product with no organic solvent residues while Nobre et al.[16] reported a 30% extraction yield using Supercritical $CO_2$ with ethanol extraction.

Forty-four percent yield was obtained from acetone in an extraction carried out by Chuyen and Eun[17] while Okorie et al.[15] reported 40% for the same solvent. Cyclohexane: acetone (60:40) mixture was employed by Winda et al.[18] as a solvent for the extraction of bixin with a 48% yield recorded. Other extraction methods that have been employed in the literature include soybean oil with a 30.2% yield.[17] Okorie et al.[15] further reported that extraction in ethanol, xylene, water, and ammonia of bixin recorded percentage yields of 22.97, 16, 19, and 39% respectively.

Comparison between the solvents shows that *Bixa orellana* seed powder has significant extractive values in different solvents.[19] With a range of 16−48% reported for different solvents and extraction methods, the percentage yield of bixin is not only solvent-dependent but also edaphic−climatic (temperature, illumination, rainfall, and soil) and genetic (cultivar)-dependent.[20]

The extraction yield of 31.4% obtained in this study is fairly significant, higher than the previously reported for aqueous, xylene, and soybean extractions.[15] It is slightly higher than the reported 30.2% for supercritical carbon dioxide too.

**3.3. Solubility and Color Change Characteristics of Extracted *Bixa orellana* Dye.** As given in Table 2 below, the

**Table 2. Solubility and Colour Change Characteristics of Extracted *Bixa orellana* Dye**

| s/n | reagent/solvent | solubility/color |
|---|---|---|
| 1 | water | slightly soluble |
| 2 | ethanol | pale yellow |
| 3 | *n*-hexane | insoluble |
| 4 | palm wine | insoluble |
| 5 | thinner | soluble |
| 6 | carbonated drink | insoluble |
| 7 | aq. sodium hydroxide | pale yellow |
| 8 | hydrochloric acid | greenish yellow |
| 9 | acetic acid | intense yellow |

dye was soluble in only ethanol, thinner, acid, and alkaline solutions. Upon introduction of the dye into the polar solvents, partial ionization occurs due to the presence of the carboxyl groups (−COOH) in the bixin and norbixin. Electrostatic interactions therefore occur between the solute and solvent molecules. This electrostatic attraction disrupts the intermolecular forces within the solute, facilitating its dissolution in the solvent. The hydroxyl in the carboxylic acid of the dye is responsible for its solubility in ethanol via hydrogen bonding and dipole−dipole interactions. The excellent solubility of the dye in ethanol accounts for the latter's suitability as a solvent for the former's extraction and dissolution. The color variation in different pH media suggests that the dye is pH-sensitive and can function well as an acid−base indicator. The insolubility of the dye in carbonated drink and palm wine provides for predissolution in a food-grade soluble medium in the application of the dye as a food colorant while solubility in paint thinner suggests that paint thinner can be used to thin or clean up surfaces stained with the dye.

**3.4. UV-Spectroscopy Analyses of Extracted *Bixa orellana* Dye and Norbixin.** As shown in Figure 3, the

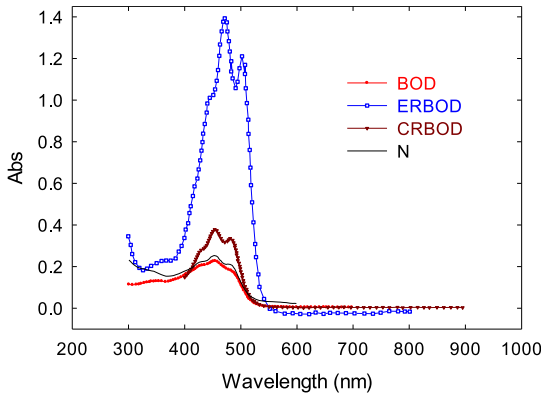


**Figure 3.** UV−vis spectra of the *Bixa orellana* dye and Norbixin. Legend: BOD = *Bixa orellana* Dye (Raw); ERBOD = Ethanol Recrystallized *Bixa orellana* Dye; CRBOD = Chloroform Recrystallized *Bixa orellana* Dye; N = Norbixin.

maximum absorption of the *Bixa orellana* dye extracted occurs at wavelength 454 nm. Adetuyi[9] recorded a similar maximum absorption wavelength of 454 nm. The ethanol recrystallized dye has maximum absorptions at 471 and 503 nm in agreement with previous work done in the literature studies.[7,21,22] The chloroform recrystallized has maximum absorptions at 457 and 484 nm as seen in Figure 3. The close maximum wavelengths of 471 and 484 nm with ethanol and chloroform recrystallized respectively revealed that either solvent can be used to purify the dye sample. Maximum absorption of the Norbixin occurs at 453 nm as shown in the figure. This value, close to the 457 nm of the pure dye, shows that Norbixin is a significant constituent of the color offered by the predominantly bixin *Bixa orellana* dye.[23]

**3.5. TLC Characterization of Bixin and Norbixin.** Two distinct yellow spots were seen on the plate (Figure 4). The two spots correspond to the bixin and norbixin, which are the pigment constituents of *Bixa orellana*. The different elution of the constituents on the plate is a result of the variations in their polarities. The $R_f$ values of about 0.52 and 0.46, respectively, corresponding to bixin and norbixin[24,25] were calculated, as shown in Table 3. The different $R_f$ values further prove the existence of Bixin and Norbixin as the major pigment constituents of *Bixa orellana*. The benzene−ethyl acetate−methanol solvents developed for the chromatography are able to elute the bixin higher than the norbixin because the bixin is less polar than the norbixin.

**3.6. FTIR Analyses of Extracted *Bixa orellana* Dye and Norbixin.** The FTIR spectrum of the annatto extract (Figure 5) shows the following bands: at 3331 $cm^{-1}$ the −O−H stretching vibration is observed, at 2974 and 2887 $cm^{-1}$ the H−C−H bending vibration, at 1651 $cm^{-1}$ the carboxylic C═O group, at 1450 $cm^{-1}$ the alkene C═C stretching, at 1381 $cm^{-1}$ the C−H bending of the methyl groups, at 1274 $cm^{-1}$ the C═O


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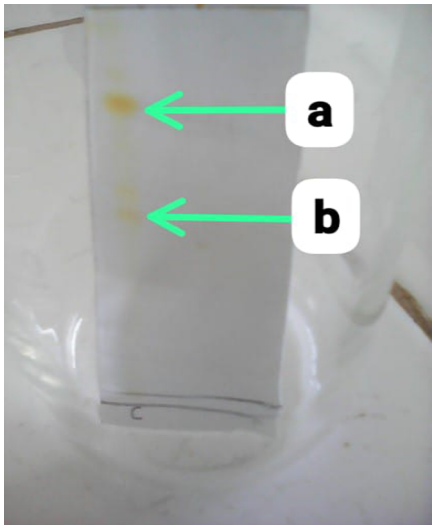


**Figure 4.** TLC for extracted *Bixa orellana* dye (a): Bixin spot (b): Norbixin.

**Table 3. $R_f$ Values for Bixin and Norbixin**

| $R_f$ value | inference |
|---|---|
| 0.56 | bixin |
| 0.42 | norbixin |

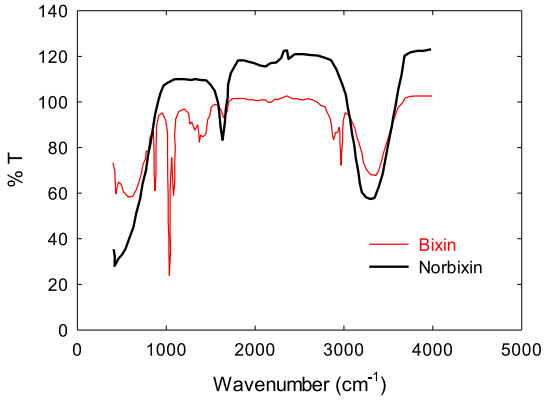


**Figure 5.** FTIR spectra of Bixin and Norbixin.

stretching, at 1085 and 1043 $cm^{-1}$ symmetric and asymmetric vibrations of the C−O−C ester group, 879 $cm^{-1}$ the methylene rocking vibration of *trans*-carotenoid, at 846 $cm^{-1}$ the coupling of the −C−O stretching vibrations, and at 798 $cm^{-1}$ the methylene rocking vibration of *cis*-carotenoid.

The FTIR spectrum of norbixin (Figure 5) shows the following bands: at 3 302 $cm^{-1}$ the −O−H stretching vibration is observed, at 2391 and 2193 $cm^{-1}$ the H−C−H bending vibration, at 1635 $cm^{-1}$ the carboxylic C═O group, at 1396 $cm^{-1}$ the alkene C═C stretching, at 1396 $cm^{-1}$ the C−H bending of the methyl groups, at 1396 $cm^{-1}$ the C═O stretching.

The functional groups mentioned in both the dye sample and norbixin component except for the −CO− stretching and methylene vibration, which were absent in the norbixin. The absence of these functional groups in the norbixin is a result of the conversion of the carboxylic acid in the bixin of the dye to norbixin by saponification with caustic alkali. This further helps in elucidating the available structure for the bixin and norbixin components of *Bixa orellana* dye.

## 4. CONCLUSIONS

The physical properties of *Bixa orellana* dye and the spectroscopies of the bixin and its alkali-hydrolyzed pair, norbixin, have been properly elucidated in this research. *Bixa orellana* dye is a vegetable dye with fascinating physicochemical properties, making it suitable for use as food dye on a wide range of substrates.

Ethanolic extraction of annatto is an alternative and better method than the traditional water maceration employed for commercial production in many local communities in that the yield is higher and of excellent purity. The comparative advantages of ethanol over other methods previously reported in literature include less environmental toxicity; cheapness, and availability. Commercially and locally, ethanol can be produced by fermentation of sugar-containing food such as corn, guinea corn, and palm juice. Repeated washing in ethanol under heat, similar to the Soxhlet extraction technique by local dyers, would help increase the yield of the dye.

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### Notes

The author declares no competing financial interest.

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## REFERENCES

(1) Tamil, S. A.; Dinesh, M. G.; Satyan, R. S.; Chandrasekaran, B.; Rose, C. Leaf and Seed Extracts of Bixa Orellana L. Exert Anti-Microbial Activity Against Bacterial Pathogens. *J. Appl. Pharm. Sci.* **2011**, *1*, 116−120.

(2) Valerio, M. A.; et al. Annatto Seed Residue (*Bixa orellana L.*): Nutritional Quality. *Food Sci. Technol.* **2015**, *35*, 326−330, DOI: 10.1590/1678-457X.6539.

(3) Akula, S.; Pamidighantam, P. R.; Rao, D. G. Chemistry, Processing and Toxicology of Annatto (*Bixa orellana L.*). *J. Food Sci. Technol.* **2003**, *40* (2), 131−141.

(4) Pillai, S.; Soni, S.; Dhulap, S.; Hirwani, R. R. Pharmacological and Cosmeceutical Applications of *Bixa orellana*: A Review of the Scientific and Patent Literature. *Indian J. Nat. Prod. Resour.* **2018**, *9*, 12972 DOI: 10.56042/ijnpr.v9i4.12972.

(5) Heuzé, V.; Tran, G.; Lebas, F. Annatto (*Bixa orellana*). In *Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO*; 2021, https://www.feedipedia.org/node/180.

(6) Thiago, T.; Fernando, A. C.; Marcos, A. S. Extraction of bixin from annatto seeds using combined technologies. *J. Supercrit. Fluids* **2015**, *100*, 175 DOI: 10.1016/j.supflu.2015.02.006.

(7) Adetuyi, A. O. Extraction, Spectroscopic Studies and Uses of Some Natural Dyes, Ph.D. Thesis; 2004.

(8) Figueiredo, A. T.; Dos Anjos, F.; Brito, F.; Viana, V. G.; Valenca, S. S.; Lanzetti, M.; Finotelli, P. V. Bixin Loaded on Polymeric Nnanoparticles: Synthesis, Characterization and Antioxidant Applications in a Biological System. *Appl. Nanosci.* **2021**, *11*, 63−78, DOI: 10.1007/s13204-020-01555-z.
(9) de Araújo Vilar, D.; et al. Traditional Uses, Chemical Constituents, and Biological Activities of Bixa orellana L.: A Review. *Sci. World J.* **2014**, *2014*, No. 857292, DOI: 10.1155/2014/857292.
(10) Aline, I. A.; Afonso, M. R.; Paulo, S., *Carotenoids: Food Sources, Production and Health Benefits*; 2013.
(11) da Fonseca, A. M.; et al. Combined Study of Docking and Molecular Dynamics Against DNV-3 SN1 Protein By Bixinoids. *J. Biomol. Struct. Dyn.* **2023**, *41*, 4549−4559, DOI: 10.1080/07391102.2022.2070282.
(12) Hirwani, R. R.; et al. Pharmacological and Cosmeceutical Applications of Bixa Orellana L: A review of the scientific and patent Literature. *Indian J. Nat. Prod. Resour.* **2019**, *9*, 12972 DOI: 10.56042/ijnpr.v9i4.12972.
(13) Handayani, R.; Setyawati, R. Evaluation of Types of Solvent on the Extraction of Bixa Orellana and Application of Extract on a Chicken Sauage Product as A Natural Colour and Antioxidant Sources. *IOP Conf. Ser.: Earth Environ. Sci.* **2019**, *443*, No. 012048, DOI: 10.1088/1755-1315/443/1/012048.
(14) Silva, G. F.; Gamarra, F. M. C.; Oliveira, A. L.; Cabral, F. A. Extraction of Bixin from Annatto Seeds Using Supercritical Carbon Dioxide. *B J. Chem. Eng.* **2008**, *25*, 419−426.
(15) Okorie, N.; Fasogbon, S. A.; Mohammed, U.; Abubakar, U.; Avwioro, O. G.; Tsamiya, R.; Mohammed, M. O.; Tangaza, A. T. Study of Phytochemical Compositions and Separation of Colours Using Thin Layer Column Chromatography for Bixa orellana (Annatto) Seed Extracts, 2020.
(16) Nobre, B. P.; Mendes, R. L.; Queiroz, E. M.; Pessoa, F. L. P.; Coelho, J. P.; Palavra, A. F. Supercritical Carbon Dioxide Extraction of Pigments from Bixa orellana Seeds (Experiments and Modeling). *Braz. J. Chem. Eng.* **2006**, *23* (2), 251.
(17) Chuyen, H. V.; Eun, J. B. Effects of Different Extraction Methods on the Extraction Yield, Degradation of Bixin and Formation of Harmful Volatile Compounds in the Extracts from Annatto Seeds. *Food Res.* **2021**, *5*, 42−48, DOI: 10.26656/fr.2017.5(6).017.
(18) Winda, R.; Jean-François, F.; Zéphirin, M. Effects of Cyclohexane/Acetone Ratio on Bixin Extraction Yield by Accelerated Solvent Extraction Method. *Procedia Chem.* **2015**, *14*, 455−464, DOI: 10.1016/j.proche.2015.03.061.
(19) Sangeeta, S.; Manasi, M.; Chetna, S.; Suresh, K.; Murthy, S. N. Comparative phytochemical screening of qualitative and quantitative parameters of Bixa orellana. *World J. Pharm. Sci.* **2015**, *4* (12), 1001−1017.
(20) Shuhama, I. K.; Aguiar, M. L.; Oliveira, W. P.; Freitas, L. A. Experimental production of annatto powders in spouted bed dryer. *Journal of Food Engineering* **2003**, *59*, 93−97.
(21) Adetuyi, A. O.; Lajide, L.; Popoola, A. V. Spectroscopic and Dyeing Characteristics of the Yellow Dye from Morinda lucida (Brimstone Tree). *Pak. J. Sci. Ind. Res.* **2005**, *48* (6), 430.
(22) Dolores, J. Y.; María, T. D.; Ivan, L. V.; Antonio, F. B.; Sofia, V.; Laura, F. Characterization of Colouring Compounds In Annatto (Bixa Orellana L.) Used In Historic Textiles By Means Of UV-Vis Spectrophotometry and FT-IR Spectroscopy, 2008.
(23) Marta, G. D.; Adão, L. G.; Enieluce, S. B.; Paulo, R. N., *The Annatto Carotenoids and the Norbixin Absorption Coefficient Carotenoides*; 2017.
(24) Amusa, S. A.; et al. In vitro Antioxidant Activity of Bixa orellana (Annatto) Seed Extract. *J. Appl. Pharm. Sci.* **2014**, *4*, 101−106, DOI: 10.7324/JAPS.2014.40216.
(25) FAO-JECFA. Monographs 3. Compendium of food additive specifications. In *Sixty-seventh meeting*. FAO JECFA Monograph: Rome, 2006.


https://doi.org/10.1021/acsomega.3c10120
*ACS Omega* 2024, 9, 18273−18277