International Journal of Pharmaceutical and Healthcare InnovationVol III, Issue I ISSN: 2584-2781

https://ijphi.com

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL AND HEALTHCARE INNOVATION
journal homepage: www.ijphi.com

Review Article

OPEN ACCESS

A Review: Annatto *(Bixa orellana l.)* as a Natural Colorant and Bioactive Plant

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Article Info

Article history:

Manuscript ID:

IJPHI1312191223122025
Received: 13- DEC -2025
Revised : 19- DEC -2025
Accepted: 23- DEC -2025
Available online:
DEC - 2025

DOI: https://doi.org/10.62752/9svfjy16

Keywords:
Annatto; bixin; natural dye; Bixa orellana; sustainable colorants.

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Abstract

For centuries, industries ranging from food to cosmetics and pharmaceuticals have relied on natural compounds sourced from mineral, animal, and plant origins. While the modern market often frames the demand for "natural" products as a new trend, these materials have been integral to human civilization since the ancient Chinese and Egyptian eras. In fact, clinical analysis reveals that over half of current medications are derived from natural precursors. Today, safety and environmental concerns are driving a decisive shift away from synthetic dyes toward sustainable natural alternatives. A leading candidate in this transition is Annatto, a dye extracted from the resinous seeds of Bixa orellana L., a tropical shrub of immense agro-industrial value. Its primary pigments, bixin and norbixin, provide vibrant yellow-to-red hues and are widely used in food, medicine, and textile dyeing. To make Annatto a viable economic competitor to synthetics, however, we must optimize extraction yields and stability. This review explores the isolation and application of Annatto as a sustainable substitute for artificial colorants, evaluating extraction methodologies and analytical techniques. Current literature confirms that Annatto is not only environmentally superior to synthetic counterparts but also offers rapid and efficient extraction possibilities.

Introduction

Although synthetic dyes currently dominate the commercial landscape, environmental and health concerns are fueling a global transition toward safer, sustainable natural colorants [1]. Artificial agents such as tartrazine (E102), allura red (E129), and sunset yellow FCF (E110) face increasing scrutiny in developed nations, where studies have linked their unregulated consumption to degenerative conditions, including cancer [2]. Consequently, while natural alternatives like *Bixa orellana L.* extract (Annatto, E160b) gain popularity, several artificial dyes-such as carmoisine (E122) and Ponceau 4R (E124)-have already been banned in the United States and Europe [3]. While natural dyes can be categorized by source or application, structural classification is often superior as it uniquely links color properties to specific chemical groups [4, 5]. Major categories include indigoids, pyridine-based compounds, quinoids, flavonoids, betalains, tannins, and carotenoids [6, 4], with quinones, flavonoids, and carotenoids being the most prevalent [7].

Bixa orellana L., often called "achiote" or the "lipstick tree," is an ancestral plant historically used in Central and South America for body art. Though native to the Americas, it is now cultivated globally in regions including Peru, Mexico, Ecuador, Indonesia, India, and Kenya [8]. A key advantage of these plant-derived dyes is their superior biodegradability compared to synthetic options [9, 10].Annatto seeds are a premier source of natural pigment for food, cosmetics, and textiles, seeing increased demand following WHO restrictions on synthetic additives. WHO recommends Annatto as a non-toxic option that preserves food nutritional value [11]. Its primary carotenoid, bixin (9′Z-6,6′-diapocarotene-6,6′-dioate), represents 70-80% of the natural pigment market [12-14]. While historically used like paprika for cooking, modern research into its bioactive properties has expanded its application into pharmaceuticals and cosmetics, such as sunscreens [15, 16]. This review analyzes the extraction methods and chemical composition of *Bixa orellana L.* based oncurrent academic literature.

Botanical Description :

The annatto tree belongs to the *Bixa* genus within the Bixaceae family. While multiple species exist, *Bixa orellana L.* is the most globally widespread. The species derives its name from Francisco Orellana, the European explorer credited with the first navigation of the Amazon River [17]. Commercially, the plant is cultivated primarily for the natural dye obtained from its seed arils. The seed's outer layer, or pericarp, is of particular industrial interest as it contains the valuable pigments. Structurally, the seed is complex: it features a pigment-rich exterior containing moisture and oils, a peel consisting of cellulose and tannins, and an inner kernel ("inner seed") that houses waxy substances, mineral ash, alkaloid chemicals, and oils [18].

Annatto's Use and Industrial Application:

Annatto extract has established itself as a preferred natural colorant in the pharmaceutical, cosmetic, and food sectors due to its non-toxic profile and lack of flavor interference [19]. It is extensively utilized in food formulations to color products such as yogurt, cheese, sausages, margarine, and ice cream.Beyond food, the cosmetic and pharmaceutical industries leverage annatto for its rich carotenoid profile [20]. These high carotenoid levels are associated with significant medicinal properties, including antioxidant and hypoglycemic effects [21]. Ethnobotanical records highlight diverse traditional applications; for instance, the Amazonian Piura tribe utilizes tea brewed from young roots as an aphrodisiac, astringent, and remedy for hepatitis, fever, and skin ailments. In Colombia, the plant serves as an antivenin for snakebites, while the seeds are traditionally used as an expectorant to treat gonorrhea [22].

In addition to its coloring properties, annatto has a history as a culinary spice. Native American cultures frequently added ground *Bixa* seeds to cacao beverages to impart a saffron-like musky taste and a subtle red tint [23, 24]. Modern usage varies by region; specific applications and allowable quantities in products like smoked fish, snacks, sauces, and confectionery differ across nations due to distinct food laws and cultural dietary habits [25, 26].

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Dilleniidae
Order	: Malvales
Family	: Bixaceae
Genus	: *Bixa*
Species	: *Bixa orellana*

Fig1.plant of *bixa orellana L* Fig2. leave of *bixa orellana l* Fig3. fruit of *bixa orellana L*

DOI: https://doi.org/10.62752/9svfjy16

Use and Industrial Application

Due to its non-toxic nature and lack of flavor interference, annatto extract has become a staple natural colorant across pharmaceutical, cosmetic, and food sectors [19]. In the food industry, it imparts color to diverse products including yogurt, margarine, cheese, sausages, and ice cream.The pharmaceutical and cosmetic sectors also utilize it for its high carotenoid content [20]. These carotenoids are linked to therapeutic benefits, including antioxidant and hypoglycemic properties [21]. Ethnobotanical uses are extensive; the Piura tribe of the Amazon uses young root tea as an aphrodisiac and astringent, as well as a treatment for fever, hepatitis, and skin conditions. In Colombia, it serves as an antivenin for snakebites, while the seeds are used to treat gonorrhea [22].Historically, annatto also served as a spice. Native American cultures used ground bixa seeds to give cacao beverages a musky flavor and reddish tint, similar to saffron or paprika [23, 24]. Today, usage levels in food products like dressings, snacks, and smoked fish vary globally depending on local food laws and cultural preferences [25, 26].

Conventional Medicine

The Annatto plant (*Bixa orellana*) is a native of South America, with its roots deeply embedded in the Amazon region. Its name "urucum" actually comes from the Tupi word "ru-ku," which simply means "red"—a fitting description for such a vibrant plant. While "urucum" is the go-to term in Brazil, you will hear it called by many other names depending on where you are. For instance, it is known as *bixa* in Guyana, *axiote* in Mexico, and *roucou* in Trinidad. In places like Puerto Rico and Venezuela, names range from *achiote* and *onotto* to *santo-domingo*. This wide variety of names really highlights just how widespread its use is, especially now that industries from food to cosmetics are hunting for natural alternatives to synthetic dyes [57]. According to research by Correa [27], *Urucum* seeds are much more than just a kitchen spice. Traditionally, they have been used to treat a whole range of issues, acting as laxatives, expectorants, and even heart tonics (cardiotonics). The plant is also well-regarded for its anti-inflammatory abilities, often applied to help heal bruises and wounds. Interestingly, it is not just the seeds that are useful; infusions made from the leaves are a common remedy for bronchitis and sore throats, while the pulp around the seeds is used to bring down fevers or mixed into soft drinks.

From an industrial standpoint, the plant is valuable because it produces two key ingredients: bixin (red) and orellin (yellow). In the culinary world where it is often called "do Reino “these pigments are the secret behind the rich color of butter, margarine, cheese, sausages, and many baked goods. It is also heavily relied upon in the textile and printing industries. On a cultural level, Annatto plays a significant role for indigenous communities. The natural dye is frequently used to decorate pottery and other household items. More personally, many indigenous groups apply the dye directly to their skin. This serves a dual purpose: it acts as body paint for religious ceremonies and provides a practical shield against UV rays and mosquitoes in the forest [49]. Other parts of the plant are utilized as well; the pulverized seeds act as an aphrodisiac, the fibers are widely used for cordage, and leaf decoctions help alleviate nausea during pregnancy [27]. It is fascinating to see that, despite the distinct cultures across Central and South America, the traditional medical uses treating everything from fevers to insect bites are remarkably consistent.

Chemical Compounds and Composition:

The vibrant color of the annatto seed coat comes primarily from Trans-Bixin, which accounts for more than 80% of the total pigment content [36]. Historically, this compound was first isolated from Bixa orellana L. seeds back in 1875, though it wasn't until 1961 that researchers fully mapped its structure and stereochemistry using 1H and 13C-NMR technologies.From a chemical perspective, bixin ($C_{25}H_{30}O_4$, MW = 394.51) is an oil-soluble diapocarotenoid. It features two carboxylic acid groups, one of which is esterified. When this ester group undergoes hydrolysis, it transforms into norbixin. Both of these key pigments originate from the oxidative breakdown of larger C40 carotenoids [37].

Pigment Stability and Profile:

While bixin and norbixin are the stars of the show [38], the seeds also host a supporting cast of other carotenoids, including beta-carotene, cryptoxanthin, lutein, zeaxanthin, and methylbixin [21].Norbixin is particularly valuable in the industry because it is water-soluble (often functioning as a potassium or sodium salt in alkaline solutions), giving it high coloring power in water-based products.

Stability: Generally, antioxidant carotenoids are unstable when exposed to atmospheric oxygen. However, bixin and norbixin are outliers; they demonstrate surprisingly robust stability against light and heat during food processing compared to other carotenoids [26].

Terpenes and Other Components:

Beyond just color, Bixa orellana L. is a powerhouse of terpenes. In fact, it is recognized as the richest vegetable source of terpenes—specifically E-geranylgeraniol, which can represent up to 57% of the total terpene profile (or about 1% of the dry seed) [22,

DOI: https://doi.org/10.62752/9svfjy16

40].

The seed's nutritional profile is also dense, containing:
•Amino acids: Glutamate, aspartate, and leucine.
•Minerals: Phosphorus, iron, and zinc.
•Lipids: Linoleic acid, α-linolenicsacid, and oleic acid.

Volatile Compounds and Apocarotenoids

The chemical complexity of annatto is immense. Studies analyzing aqueous and organic extracts have detected over 100 volatile chemicals, with 50 specific compounds identified. These include bornyl acetate, α-caryophyllene, copaene, β-cubebene, (+)-cyclosativene, and spathulenol, among others [44]. Recent research [45, 46] has further isolated eight specific apocarotenoids from the seeds, including:

1.Methyl (7Z, 9Z, 9′Z)-apo-6′-lycopenoate
2.Methyl (9Z)-apo-8′-lycopenoate
3.Methyl 1(all-E)-apo-8′-lycopenoate
4.Methyl (all-E)-8-apo-beta-carotene-8′-oate
5.Methyl (all-E)-apo-6′-lycopenoate
6-geranylgeranyl-8′-methyl-6,8′-diapocaroten-6-8′-dioate
7.6′-geranylgeranyl-6′-methyl-(9Z)-6,6′-diapocaroten-6-6′-dioate
8.6-geranylgeranyl-6′-methyl-6-6′-diapocaroten-6-6′-dioate

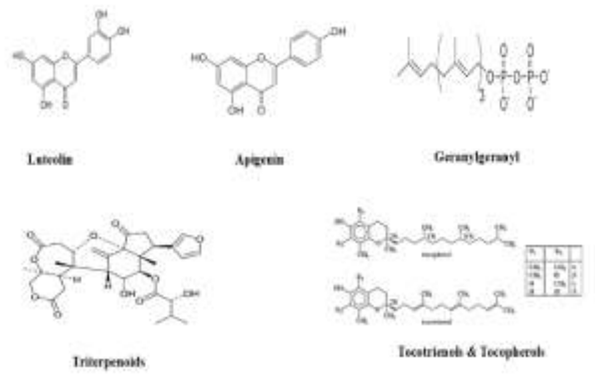

Figure 1

Extraction Method

Just like extracting compounds from any other plant, getting the most out of annatto seeds depends on a delicate balance of variables. Factors such as the solid-to-liquid ratio, temperature, extraction time, and the specific condition of the plant matrix all play a huge role. However, the most critical decision is choosing the right solvent. This choice essentially dictates the final product: using oil-based solvents yields **bixin**, while aqueous methods tend to produce water-soluble **norbixin** [19].

Commercial Techniques

Industrially, manufacturers typically rely on three main approaches to extract pigment from dried seeds [36]:

1. **Vegetable Oil Extraction:** This method uses oils (like corn or soybean) to extract the pigment.
2. **Organic Solvent Extraction:** Solvents like hexane, acetone, ethanol, or chloroform are used to produce the purest pigments.
3. **Aqueous Alkali Extraction:** Using solutions like NaOH or KOH, this method converts bixin into norbixin through saponification [47, 53].

Recent research has focused heavily on finding the perfect solvent mix to boost efficiency. While bixin is a non-polar substance meaning it loves polar solvents—studies suggest that **ethyl acetate** offers the best solubility for removing bixin from the seeds [39]. Other researchers have experimented with various combinations, such as mixing ethanol and chloroform (75:25 v/v) or using 1,2-dichloroethane [48, 49]. The goal is always the same: maximize the yield of bixin while keeping out unwanted byproducts that could ruin the stability or color intensity of the final extract [26].

Advanced Extraction Technologies

Beyond the standard commercial methods, scientists are pushing boundaries with newer technologies.

Supercritical CO2 Extraction Using supercritical CO2—sometimes modified with chemicals like methanol or chloroform—has proven to be highly effective [56–59]. This method is often superior to traditional techniques because it requires less heat (preserving the product quality), uses less energy for solvent recovery, and offers excellent selectivity.

Microwave-Assisted Extraction (MAE) The rise of microwave technology has led to several innovative variations, such as Solvent-Free Microwave Extraction (SFME) and Vacuum Microwave Hydrodistillation (VMHD) [60–63]. These methods are designed to be faster and more efficient, often yielding better results than conventional approaches.

Pressure and Efficiency When comparing techniques, pressure matters. One study [38] pitted Pressurized Liquid Extraction (PLE) against Low-Pressure Solvent Extraction (LPSE). The results showed that PLE produced the highest mass of bixin when ethanol was used. However, LPSE was still effective, yielding about 4 g/kg at lower temperatures (323 K) and up to 6 g/kg at slightly higher temperatures (333 K).

Purity and Commercial Viability

Ultimately, any extraction method needs to be economically viable to survive in the market. Solvent extraction is particularly powerful here because it can produce **microcrystalline bixin** that is 80–97% pure. In terms of raw material, the bixin content in seeds can range anywhere from 1% to 6% depending on the specific cultivar and growing conditions [1]. For

DOI: https://doi.org/10.62752/9svfjy16

international export, meeting a specific purity threshold is crucial, with many markets requiring bixin levels above 2.7% or even 4% [42, 54, 55].

Whether analyzing cheese, margarine, or boiled sweets, ensuring these pigments are extracted and measured correctly—often using HPLC or spectrophotometry—remains a top priority for food scientists [67, 68].

Analysis of Annatto:

The chemical makeup of an annatto extract isn't static; it changes depending on the method and temperature used to extract it. This is critical because the specific ratio of bixin to norbixin is what actually dictates the final hue and tone of the food product [19].Before these pigments can be used, we have to verify what is actually in the extract. Typically, once the pigment is purified and concentrated, Thin Layer Chromatography (TLC) is the first step to confirm the presence of bixin and norbixin. For a deeper dive—to identify and quantify exactly how much is there—scientists rely on a suite of powerful tools: High-Pressure Liquid Chromatography (HPLC), UV-VIS spectrophotometry, Nuclear Magnetic Resonance (NMR), and Mass Spectrometry.

UV-VIS Spectrometry:

This is the classic approach and has been well-documented over the years [71-73]. Historically, researchers measure bixin using chloroform and norbixin using dilute sodium hydroxide (about 0.1M). It is relatively straightforward to identify bixin this way because it consistently hits absorption peaks at 470 and 500 nm.

HPLC Systems:

High-Performance Liquid Chromatography (HPLC) takes things a step further. Unlike basic spectroscopy, HPLC allows us to physically separate the different isomers of bixin and norbixin.

- **The Method:** Most labs use "reversed-phase" (RP) chromatography with C18 columns.
- **The Advantage:** When paired with a Photodiode Array (PDA) detector, HPLC provides much better-quality data than UV-VIS. It can even identify the thermal degradation products—the compounds formed when the pigment gets too hot—which is crucial for quality control in commercial formulations [76, 77].
- **Variability:** It is worth noting that results can vary. The elution time (how long it takes the compound to travel through the column) shifts depending on the column type, length, and the solvent mix used.

Spectrometry of Mass:

Mass Spectrometry helps us understand the molecular backbone of the pigment. Like other carotenoids, bixin and norbixin show a specific fragmentation pattern—essentially breaking apart in a predictable way, losing xylene and toluene from their polyene chains. Advanced techniques, such as Matrix-Assisted Laser Desorption Ionization (MALDI) Time-of-Flight (TOF) spectrometry, have been used to confirm the exact structures of purified bixin isomers [79].

Gas Chromatography (GC) Evaluation

Gas Chromatography is primarily used for the volatile stuff. It’s the go-to method for detecting thermal degradation products (specifically aromatic hydrocarbons) that might appear in food coloring [80]. It is also used to analyze the oil-soluble and water-soluble volatile compounds in the extract [81], as well as the lipid fraction of the seeds, where it can identify components like tocotrienols [82].

NMR examination:

Nuclear Magnetic Resonance (NMR) was the tool that finally unlocked the 3D shape (stereochemistry) of bixin. By looking at how specific protons shifted in the magnetic field, scientists were able to distinguish between the different isomers (cis- vs. trans-). Later research combined NMR with X-ray diffraction to fully map out the crystal structures of the bixin family [78, 84, 85].

Biological Activity:

Pharmacological studies have tested various plant parts—leaves, seeds, roots—for bioactivity. The results are categorized below:

Antifungal Activity: Extracts have shown effectiveness against *Trichophyton mentagrophytes* in studies conducted in Ecuador, though results against other strains in Guatemala were less conclusive [70, 82].

Antibacterial Activity: Testing against strains like *Bacillus subtilis*, *E. coli*, and *Staphylococcus epidermidis* generally showed no significant activity in annatto leaf extracts.

Antimalarial Activity: *Bixa orellana* seed extracts have demonstrated modest efficacy against *Plasmodium berghei* and *Plasmodium falciparum* [92].

Cytotoxic & Mutagenic Activity: While mutagenic activity was not found in US and Brazilian studies [67, 93], cytotoxic activity against human lung cancer cells has been observed in ethanolic extracts at values lower than 100 g/mL [81].

Conclusions

Bixin and norbixin are the key active components in annatto, responsible for providing the red-to-yellow pigmentation essential to the cosmetic, food, and pharmaceutical industries. To ensure sustainability

DOI: https://doi.org/10.62752/9svfjy16

and effectively replace synthetic dyes, the industry must adopt optimized extraction techniques and rigorous analytical methods. The literature confirms that annatto offers a safe, environmentally friendly alternative with established methods for rapid analysis and extraction, positioning it as a viable substitute for artificial colorants in the global market.

Submission Declaration:

This manuscript has not been published previously and is not under consideration for publication elsewhere. The authors confirm that the work is original and have read and approved the final manuscript for submission.

Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Declaration Of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics Statement:

This review paper, "**Holistic and Conventional Approaches in Alzheimer's Therapy: Exploring Herbal, Synthetic, and Alternative Interventions**", involves no experimental research, human subjects, or animal studies that need ethical approval; instead, it is based entirely on publicly available literature. For academic openness and integrity, all acknowledged sources were appropriately referenced. I have done all in my power to provide an objective, accurate, and thorough literature review free from any conflicts of interest that could affect how the data are interpreted. The development of this study did not involve any instances of scientific misconduct, data manipulation, or plagiarism. Let me know if you need refinement.

Acknowledgments

The authors would like to thank Chhatrapati Shahu Ji Maharaj University, Kanpur, India, for access to electronic support and Mrs. Amrita Singh for their support and contributions to this work. Special thanks to the research teams and collaborators, whose efforts made this review possible.

Funding No: funding was received for conducting this study.

References

1. Zuccarini, C., Thomas, E. P., Colvin, M., Rosen, S. B., and Petzer, S. (2005). Tech. Rep., Medical Research Council, Buffalo, NY, USA "HIV prevalence study and costing analysis undertaken for the development of an HIV/AIDS workplace strategy for Buffalo City Municipality."
2. Yamashita, F., Gozzo, F.C., Chiste, R.C., and Mercadante, A.Z. Bixin and phenolic chemicals from annatto seeds are simultaneously extracted and analyzed using high performance liquid chromatography connected to diode array and mass spectrometric detectors. J Chromatog A, 1218 (1), 57-63 (2011)
3. Giorgi, A., Granelli, G., Chiesa, L. M., De Marinis, P., and Panseri, S. **Bixa orellana L L l.** from the Brazilian Amazon region: secondary metabolite profile, antioxidant capacity, and mosquito repellent efficacy, Journal of Chemistry, 2013, pp.
4. Silva, S. N. S., Amaral, C. L. F., and Reboucas, T. N. H. Adoption of conservation methods on farms and variety selection by annatto farmers in Vitoria da Conquista-BA, Revista Brasileira de Agroecologica, 2010, 5, pp. 106–113.
5. Ribeiro, J.A., Oliveira, D.T., Passos, M.L., and Barazzo, M.A.S. The equilibrium moisture equations for **Bixa orellana L L l.** seeds are distinguished using nonlinearity measurements. J. Food Eng., 66 (1), 63-68 (2005).
6. Smith, J. Annatto extracts: technical and chemical evaluation. Chem Tech Assess Manual, pages 1–21, 2006
7. Application of annatto dye formulation in baking and extruded food items Rao, P.P., Satyanarayana, A., Balaswamy, K., Jyothirmayi, T., Nagender, A., and Rao, D.G. Foodserv. J. 2007, 18, 53–58.
8. Research on the mutagenicity and antimutagenicity of a natural food coloring (annatto) in mouse bone marrow cells by De Lima, R.A., Azevedo, L., Ribeiro, L.R., and Salvadori, D.M.F. Food and Chemical Toxicology, 41(2), pp. 189-192, 2003
9. Venugopalan, P. A., Giridhar, G. A., and Ravishankar, A. G. "Food, Ethanobotanical and diverse applications of **Bixa orellana L L l.** L.: a potential for its enhancement through biotechnological mediation," Indian Journal of Fundamental and Applied Life Sciences, 2011, 1, pp. 9–31
10. Elsesser, K.J., Wuebber, I.A., Bruhns, K.O., Miksicek, C.H., and Hammond, N. Rethinking Ramon: an analysis of the lowland Maya subsistence of Reina and Hill. American Antiquity, 46(4), pp. 916-919, 1981.
11. Norton, M. Tasting empire: chocolate and the

assimilation of Mesoamerican aesthetics in Europe. The American Historical Review, 111(3), pp. 660-691, 2006

12. Selected natural colorants in foods and beverages, Ames, J.M. and Hofmann, T. (2001).
13. Scotter MJ. An overview of the chemistry and analysis of annatto food coloring. 2009, 26(8): 1123-1145 Food Add Contam.
14. Brandao, M. G. L., Grandi, T. S. M., Rocha, E. M. M., Sawyer, D. R., and Krettli, A. U. Survey of Amazonian medicinal plants used as antimalarials, Journal of Ethnopharmacology, 1992, 36(2), pp. 175–182
15. Hirschmann, G. S., and de Arias, A. R. "A survey of medicinal plants of Minas gerais, Brazil," Journal of Ethnopharmacology, 29(2), pp. 159–172, 1990
16. Amazonian Ethnobotanical Dictionary, Duke, J. A., and Martinez, R. V., CRC Press, Boca Raton, Fla., USA, 1994.
17. Villar, R., Calleja, J. M., Morales, C., and Caceres, A. Analyzing 17 Guatemalan medicinal plants for Platelet antiaggregant action, Phytotherapy Research, 11, pp. 441–445, 1997.
18. Giron, L. M., Freire, V., Alonzo, A., and Caceres, A. Ethnobotanical evaluation of the use of medicinal herbs by the Caribs of Guatemala, Journal of Ethnopharmacology, 1991, 34(2-3), pp.173–187.
19. D. L. Lentz, "Economic Botany, 1991, 47(4), Medicinal and other economic plants of the Paya of Honduras" 358–370 pages
20. Lentz, D. L., Clark, A. M., and Hufford, C. D. "Antimicrobial properties of Honduran medicinal plants," Journal of Ethnopharmacology, 1998, 63(3), pp. 253–263.
21. Morrison, E. Y., and West, M. E. "**Bixa orellana L L l.** (Annatto) and its effects on blood sugar levels in anesthetized dogs," West Indian Medical Journal, 1985, 34(1), pp. 38–42
22. Ramirez, V.R., Mostacero, L.J., Garcia, A.E., Mejia, C.F., Pelaez, P.F., Medina, C.D., and Miranda, C.H. Vegetables used in traditional Norperuana medicine. NACL Univ Trujillo & Banco Agrario del Peru, Peru, 1988, 54.
23. Preston, H.D., and Rickard, M.D. Annatto extraction and chemistry. Food Chemistry, 1980, 5(1), 47–56
24. Production of cheese color from annatto seeds by Ramamurthy MK and Krishna Rao S. Ind Dairyman, 1982, 34: 263-264
25. Bixin extraction from defatted annatto seeds by Rodrigues, L. M., Alcazar-Alay, S. C., Petenate, A. J., and Meireles, M. A. A. Comptes Rendus Chimie, 2014, 17(3): 268-283.
26. Characterization of several annatto extracts based on antioxidant and color properties by Cardarelli, R.C., Benassi, M.T., and Mercadante, A. Z. LWT Food Science and Technology 2008, 41, 1689-1693
27. Terpenoids and an apocarotenoid from **Bixa orellana L L l.** seeds Jondiko, I.J.O., and Pattenden, G. Phytochemistry 28, 3159–3162, 1989. (89) 80298-5 https://doi.org/10.1016/0031-9422 Lauro G, Francis J. Science and technology of natural food coloring. New York: CRC Press,2000
28. Giridhar, P. and Parimalan, R.A. A biotechnological perspective towards improvement of annatto color production for value addition - the influence of biotic elicitors. *Asia Pac J Mol Biol Biotechnol*. **2010**, *18*, 77-79
29. Chuyen HV, Hoi NT, Eun JB. Improvement of bixin extraction yield and extraction quality from annatto seed by modification and combination of different extraction methods. *Int J Food Sci Tech* **2012**, 47:1333– 1338. doi: 10.1111/j.1365-2621.2012.02977.x.
30. Galindo-Cuspinera, V., Lubran, M. B., and Rankin, S. A. Comparison of volatile compounds in water and oil-soluble annatto (***Bixa orellana L L l.*** L.) extract, *Journal of Agricultural and Food Chemistry*, **2002**, 50, 2010–2015.
31. Mercadante, A.Z., Steck, A. and Pfander, H. Isolation and structure elucidation of minor carotenoids from annatto (***Bixa orellana L L l.*** L.) seeds, *Phytochemistry*, **1997**, *46(8)* pp. 1379–1383.
32. Mercadante, A.Z., Steck, A. and Pfander, H. Three minor carotenoids from annatto (***Bixa orellana L L l.***) seeds," *Phytochemistry*, **1999**, *52(1),* pp. 135–139
33. Mortenson, M.A., Vickers, Z.M. and Reineccius, G.A. Flavor of whey protein concentrates and isolates. *International dairy journal*, **2008**, *18*(6), pp.649-657.
34. Ramamurthy, M.K., Krishna, Rao, S. Manufacture of cheese colour from annatto seeds. Ind Dairyman. 1982; 34: 263-264
35. Bhalkar, S.V, Dubash, P.J. Methods of

DOI: https://doi.org/10.62752/9svfjy16

extraction of annatto from the seds of ***Bixa orellana L L l.***. *Ind J Dairy Sci*; **1983,** *36***,** 157-161.

36. Veggi, P.C., Cavalcanti, R.N., Meireles, M.A.A. Modifier effects on Supercritical Fluid Extraction of some Brazilian plants: Antioxidant activity and Economical evaluation. *Procedia Food Sci*, **2011,** 1, 1717-1724.
37. Wang, L., Weller, C.L. Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci Tech*, **2006***, 17(6*), 300-312.
38. Mercadante, A. Z. Steck, A. Rodriguez-Amaya, D. Pfander, H. and Britton, G. Isolation of methyl 9′Z-APO-6′ -lycopenoate from ***Bixa orellana L L l.***," *Phytochemistry*, **1996**, 41(4) pp. 1201– 1203.
39. Giuliano, G., Rosati, C. and Bramley, P.M. (2003). To dye or not to dye: biochemistry of annatto unveiled. *TRENDS in Biotechnology*, *21*(12), pp.513-516.
40. Fabri, E.G. and Teramoto, J.R.S. Urucum: fonte de corantes naturais. *Horticultura brasileira* **2015**, *33*,pp140-140.
41. Dias, N.O., Rebouças, T.N., São José, A.R. and Amaral, C.L. Morpho-agronomic characterization and estimates of genetic parameters in annatto plant. *Horticultura Brasileira*, **2017**, *35*, pp.242-246
42. Degnan, A.J, Elbe, J.H, Hartel, R.W. Extraction of Annatto Seed Pigment by Supercritical Carbon Dioxide. *J Food Sci*; **1991**, *56(6*): 1655.
43. Chao, R.R, Mulvaney, S.J, Sansom, D.R, Hsieh, F.H, Tempesta, M.S. Supercritical CO2 extraction of annatto (***Bixa orellana L L l.***) pigments and some characteristics of the color extracts. *J Food Sci*; **1991**, 56**:** 80-83
44. Silva, G.F, Gamarra, F.M.C, Oliveira, A.L, Cabral, F.A. Extraction of bixin from annatto seeds using supercritical carbon dioxide. *Br J Chem Eng*; **2008**, 25: 419–426.
45. Anderson SG, Nair MG, Chandra A, Morrison E. Supercritical fluid carbon dioxide extraction of annatto seeds and quantification of *trans*-bixin by high pressure liquid chromatography. *Phytochem Anal.* **1997***; 8*: 247-249
46. Sinha, K., Chowdhury, S., Saha, P.D. and Datta, S. Modeling of microwave-assisted extraction of natural dye from seeds of **Bixa orellana L L l.** (Annatto) using response surface methodology (RSM) and artificial neural network. *Industrial Crops and Products*, **2013**, *41*, 165-171. https://doi.org/10.1016/j.indcrop.2012.04.004;
47. Quintero Quiroz, J., Celis Torres, A., Muñoz Ramirez, L., Silva Garcia, M., Ciro Gomez, G. and Rojas Camargo, J. Optimization of the Microwave-Assisted Extraction Process of Bioactive Compounds from Annatto Seeds (***Bixa orellana L L l.*** L.). *Antioxidants*, **2019**, 8(2), 37. https://doi.org/10.3390/antiox8020037 and ultrasound-assisted extraction
48. Yolmeh, M., Habibi Najafi, M.B. and Farhoosh, R. Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chemistry*, **2014**, *155*, 319-324. https://doi.org/10.1016/j.foodchem.2014.01.059
49. Cardoso-ugarte, G.A, Juarez-Becerra, G.P, Sosa-Morales, M.E and López-Malo, A. Microwave-assisted extraction of essential oils from herbs, *Journal of microwave power and electromagnetic energy* **2013***, 47,* 63-72.
50. Nobre, B. P., Pessoa, F. L. P., Coelho, J. P., Palavra, A. F. Mendes, R. L. Supercritical carbon dioxide extraction of bixin from annatto seeds", *International Society for Advancement of Supercritical Fluids*,**1999**, pp.6.
51. Leal, P.F., Kfouri, M.B., Alexandre, F.C., Fagundes, F.H.R., Prado, J.M., Toyama, M.H., Meireles, M.A.A. Brazilian Ginseng extraction via LPSE and SFE: Global yields, extraction kinetics, chemical composition and antioxidant activity. *J Supercrit Fluid*, **2010**, *54(1*), 38-45.
52. Santos, D.T., Veggi, P.C., Meireles, M.A.A. Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. *J Food Eng*, **2012,** *108(3*), 444-452.
53. Smith PR, Blake CJ, Porter DC. Determination of added natural colours in foods. III. Annatto. Leatherhead Food R.A. Research Report No.431. Leatherhead: British Food Manufacturing Industries Research Association.1983.
54. Luf, W., and Brandl E. The detection of the annatto dyestuff norbixin / bixin in cheese using derivative spectroscopy and high-performance liquid chromatography. Zeitschrift für Lebensmittel-Untersuchung und

–Forschung **1988**, *186*: 327-332

55. Cunha, F. G., Santos, K. G., Ataíde, C. H., Epstein, N., Barrozo, M. A. S. Annatto powder production in a spouted bed: An experimental and CFD study", *Industrial and Engineering Chemistry Research*, **2009**, *48(*2) pp.976-982.
56. Balaswamy, K., Prabhakara Rao, P. G., Satyanarayana, A., Ra, D. G. Stability of bixin in annatto oleoresin and dye powder during storage, LWT - Food Science and Technology, **2006**,*39(8)* pp.952-956.
57. McKeown GG, Mark E. The composition of oil-soluble annatto food colors. *J Assoc Official Anal Chem*; **1962**,
45: 761-766
58. Reith, J.F. Gielen JW. Properties of bixin and norbixin and the composition of annatto extracts. *J Food Sci*;
1971, 36: 861-864.
59. Zechmeister L. (1960). Cis-trans isomeric carotenoid pigments. *Progress in the chemistry of natural products*;
1960, 18: 223-349.
60. Eder, R. Pigments. In: Food analysis by HPLC, 2nd Edition revised. L.M.L. Nollet (ed). New York: Marcel Dekker Inc. **2000**, pp825-880.
61. Nishizawa M, Chonan, T, Sekijo I, Sugii, Y. Studies on the analysis of natural dyes: Analysis of annatto extract and gardenia yellow dye in foods and natural dye preparations. Hokkaidoritsu Eisei Kenkyusho; **1983,** 33: 32-34.
62. Scotter, M.J, Thorpe, S.A, Reynolds SL, Wilson LA. Strutt PR. Characterization of the Principal Col-ouring Components of Annatto Using High Performance Liquid Chromatography with Photodiode-Array Detection. *Food Add Contam* **1994**, 119 (3): 301-315.
63. Scotter MJ, Wilson LA, Appleton GP. Castle L. Analysis of annatto (***Bixa orellana L L l.***) food coloring formulations 1. Determination of coloring components and colored thermal degradation products by high-performance liquid chromatography with photodiode array detection. *J Agri Food Chem* **1998**, 46: 1031–1038.9.
64. Kelly DR, Edwards, AA, Parkinson JA, Olovsson G, Trotter J, Jones S, Abdul Malik, KM, Hursthouse MB, Hibbs DE. NMR, MS and X-ray crystal structure determination of the bixin family of apocarotenoids. J Chem Res (M); **1996**, 2640- 2697
65. Guaratini T, Vessechi RL, Lavarda FC, Maia Campos PMBG, Naal Gates PH, Lopes NP. New chemical evidence for the ability to generate radical molecular ions of polyenes from ESI and HR-MALDI mass spectrometry. *Analys*t; **2004***, 129*: 1223-1226.
66. Scotter MJ, Wilson LA, Appleton, GP. Castle L. Analysis of annatto (***Bixa orellana L L l.***) food coloring formulations 2. Determination of aromatic hydrocarbon thermal degradation products by gas chromatography. *J Agri Food Chem* **2000**, 48: 484–488.
67. Galindo-Cuspinera, V., Lubran, M. B., and Rankin, S. A. Comparison of volatile compounds in water- and oil-soluble annatto (***Bixa orellana L L l.*** L.) extract," *Journal of Agricultural and Food Chemistry*, **2002** vol. 50, no. 7,
pp. 2010–2015
68. Frega N, Mozzon M, Bocci F. Identification and estimation of tocotrienols in the annatto lipid fraction by gas chromatography mass spectrometry. *J Amer Oil Chem Soci*; **1998**, 75**,** 1723–1727.
69. Pino JA, Correa MT. Chemical composition of the essential oil from annatto (***Bixa orellana L L l.*** L.) seeds. *J Ess Oil Res*; **2003,** 15: 66–67
70. Barber MS, Hardisson A, Jackman LM, Weedon BCL. Studies in nuclear magnetic resonance. Part IV. Stereochemistry of the bixins. *J Chemi Soc*; **1961**, 16
71. Hirko B, Getu A. Bixa orellana (Annatto Bixa) A review on use, structure, extraction methods and analysis. Journal of Agronomy, Technology and Engineering Management.2022;5

DOI: https://doi.org/10.62752/9svfjy16