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Review Article

Annatto (*Bixa Orellana* L.): Tocotrienol Supplement Benefits

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Abstract

Annatto (*Bixa orellana* L.), a native Brazilian plant, is acknowledged for containing the natural pigments found in the seeds mostly used by food industries worldwide. The species domestication is attributed to the Brazilian Amazon population. Although the commodity is extensively valued, annatto (*Bixa orellana* L.) has long been used as home remedy treatment of an array of disturbances. The biological activities and phyto chemical composition from different parts of this species are abundantly reported in scientific literature. Herein, the authors sought to correlate a relationship of the pharmacological activities with the plant's chemical composition. According to published data annatto presents a wide range of biological activities, which corroborates the use of this commodity in pharmaceutical products .Among substances with biological activities, the Tocotrienol group is highlighted due to their great importance for human health. This study gives a review on the benefits of Tocotrienols from Annatto (*Bixa orellana* L.).

Keywords: *Bixa orellana* L.; urucum; annatto; tocotrienols

Introduction

Annatto is an arboreal plant, classified as *Bixa orellana* L. (Bixaceae), originally from Tropical America. The species is a rustic, perennial plant, which can reach up to 6 m in height [1]. The word annatto comes from the Tupi-Guarani language transliterated "uruku" meaning "red". The scientific name, "*Bixa orellana* L", was given in honor of Francisco de Orellana (1490-1546), a member of the expedition of Francisco Pizarro, who was the first Spanish explorer to navigate the Amazon River. The wide geographic distribution of this plant gave riseto several common names. In Brazil, the plant is also known by names such as annatto, annatto, annatto-uva, annatto-bravo, saffron, and bixa, in addition to indigenous names suchas ahitê, nukirê, bixe, and bixa. In Spanish America, annatto is known as achiote, anoto, Achote, onotto, onotillo, roekoe, schirabaeli, koessewee, koesowe, bija, cacicuto, uruca, achiotillo, arnotto, arnolta, hoarse, chancaguarica, kuxub, achitihuti, a Huantura, Atta, Santo Domingo, Analto, and Guajachote [2].

The first reference to annatto can be attributed to Pero Vaz de Caminha in his letter to the King of Portugal Dom Manuel informing him of the discovery of Brazil. Therein says in an excerpt of the letter: "Some brought green hedgehogs, from trees, which, in color, wanted to look like chestnut trees, although smaller, and they were full of small red grains, which, on crushing them between the fingers, made a very red tincture, from which they were red. And the more they got wet, the redder they became". Historical data indicate that the Aztecs used annatto pigments to give the consistency and appearance of blood to a drink made from cocoa. This drink was then used in their rituals, simulating human blood. South American natives used oils, resins, waxes, and fats extracted from plants or animals, for the preparation of annatto dyes. The use of these materials reinforced the protective action of the annatto against insects. Indians from Peru and southern Ecuador used the fat obtained from the guácharo, a nocturnal bird, to obtain this dye. Brazilian natives used fish fat, capybaras, and alligators to produce a kind of ointment that they used for body painting. The Tagnanis Indians of Mato Grosso mixed a perfumed resin obtained from the almecegueira, Protium heptaphyllum (capybaras and alligators) to produce a kind of ointment that they used for body painting [2].

The use of animal fat for pigment extraction reveals indigenous knowledge of the lipid solubility of bixin. The art of painting with the extracts of this plant was called embijado, which consisted of mixing hot fat with the seeds to extract the dye. In addition to the fats, the indigenous people used animal fat or oils from alligators, turtle eggs or iguanas. Referring to the term embijado, Fernández de Oviedo y Valdés named this species for the first time with the name bixa or bija [3]. The Xavantes, on the other hand, produce the dye by cooking in water [4].

Archaeological records can provide evidence of the presence of annatto in ancient human settlements. These records reveal that annatto was used among the Indians of the Peruaçu valley, in the state of Minas Gerais, between 500 and 1,000 years ago. Carbonized seeds up to 1,300 years old have been excavated in Colombia. Linguistic studies show that the pre-Mayan name for annatto was used in Central America 2,400 years ago. Evidence of the pigment has been found in prehistoric settlements in central Peru dating back 3,000 years. But the oldest evidence of the use of annatto comes from an archaeological site occupied 3,600 years ago on the small island of Saba, a Dutch colony in the Caribbean Sea [5, 6].

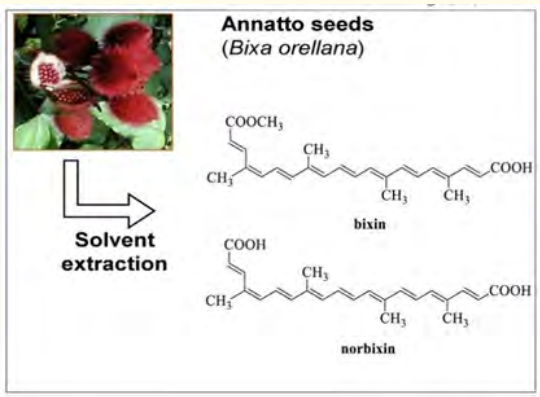

Figure 1: Adapted from [7].

The dye, in powder form found in the thin layer of aril, is known in Brazil as paprika or colorific, without aroma or flavor, that is widely used in cooking to enhance the color of food. Bixin, which is the coloring of annatto, acts as a color fixer in commercial products. The dye is also used for industrial purposes, in the formulation of beverages, in baking, and in other pastas, in dairy products (such as cheese), sausages, cosmetics, paints, as a sunscreen against ultraviolet rays, among others. The orange or reddish color of cheeses manufactured in Brazil indicates, for example, the presence of annatto dye [1].

In Brazil, annatto is one of the main sources of natural dyes. The pigment is extracted from the outer layer of seeds, being the source of the carotenoid. Bixin is indexed in the Color Index, an international body of dye nomenclature, as CI n°75120, but the best-known name is that of the European Community, as ECC n°E160b [8].

The annual world production of annatto seeds is approximately 14,500 tons (dry weight). Two thirds of this production is marketed as dry seeds and the rest as dye. Latin America produces 60% of the worldwide production, followed by Africa (27%), and Asia (12%). The main producers in Latin America are Peru, Brazil, and Mexico [9].

All this production is directed towards obtaining bixin and norbixin dyes for industrial use. However, the dye purification process gives rise by-products, such as a vitamin E enriched fraction, composed practically of tocotrienols, with high medicinal potential.

Tocotrienols

Tocotrienols and tocopherols are stereo isomers of vitamin E, one of the fat-soluble antioxidants. Historically, vitamin E is recognized for the biological activity of alpha-tocopherol, nevertheless activities have been reported for isomers of that class of compounds of tocotrienol sother than of alpha-tocopherol. Although alpha-tocopherol was the first vitamin E isomer to be identified, eight chemically distinct isomers were identified, consisting of alpha (α), beta (β), gamma (γ), and delta (δ)-tocopherols and α, β, γ, and δ-tocotrienols (T3), all of which are referred to as vitamin E [10]. Tocopherols and tocotrienols have a similar chemical structure, a chromanol ring with a hydroxyl group that can donate a hydrogen atom to reduce free radicals. The difference between tocopherols and tocotrienols is the presence of three double bonds in the hydrophobic side chain of tocotrienols, which provide greater antioxidant potency [11, 12]. While tocopherols are present in the seeds and leaves of most plants, tocotrienols are found in a small fraction of them [13].

Tocotrienols are absorbed in the same way as other vitamin E compounds, along with fat, in the small intestine after being cleaved by esterase enzyme, located in the lining of the stomach. Bile salts are required for absorption, which are then transported by chylomicrons in the lymphatic system. In the bloodstream, tocotrienols are exposed to oxidative free radicals and therefore perform most of their antioxidant activity. Tissue uptake occurs with the help of lipoprotein lipases, digesting lipoprotein constituents, or by lipoprotein receptor-mediated endocytosis. Lipoprotein lipase degrades lipoproteins into remaining particles, which are then absorbed by the liver or peripheral tissues by receptor-mediated endocytosis. Tocotrienols enter a variety of tissues, being found at higher levels in the adipose and adrenal glands. Fat-soluble vitamins can be stored in these tissues for long periods because of their excessively slow replacement rate. Tocotrienols are oxidized, after having performed their antioxidant function, being converted into their hydroquinone, by the P450 system, before being eliminated. In the form of hydroquinone, they bind to glucuronic acid and mix with bile for excretion through the feces [10].

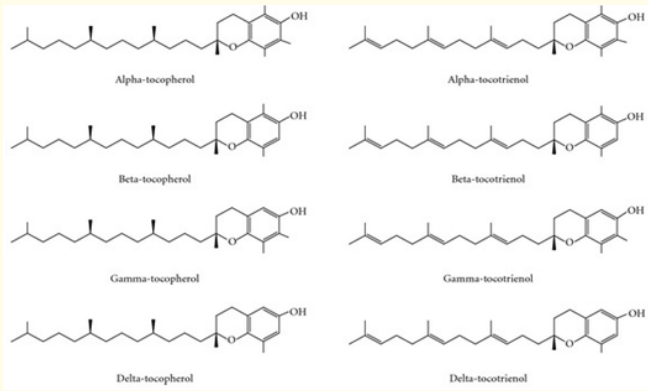

Figure 2: Adapted from [14].

Some of the main sources with the highest tocotrienols / tocopherols ratio are rice bran oil (50/50), palm oil (75/25), and annatto seeds (99.9/0.1), the latter being the vegetable sources with higher amounts of tocotrienols. [9, 15, 16].

Studies on the toxicity of tocotrienols

Over the years, a significant body of experimental evidence has emerged that can provide guidance on the safety of tocotrienols. Genotoxicity data do not raise safety concerns [17]. On the contrary, there are studies showing protection of tocotrienols against genotoxic effects, including a clinical study [18].

Nakamura [19], using a palm oil extract containing 70% tocotrienols, performed an oral toxicity study during 13 weeks in rats, at 0, 119, 474 and 2130mg/kg concentrations in the diet of male rats, and 0, 130, 491 and 2,047mg in the diet of females. The study established levels without adverse events (NOAEL) of 1.9g/kg in the diet, which corresponds to 120mg/kg/day for males and 130mg/kg/day for females, since adverse events were only observed in the two highest dose groups, including increased liver and adrenal weight in all treated males and ovarian and uterine weight in females at higher doses [19].

Chronic study of 52 weeks with Wistar Hannover rats of both sexes were treated with a pure fraction of tocotrienols at concentrations of 0, 0.08, 0.4 or 2% of the powdered basal diet. Of these groups, only the one that received the 2% diet showed liver toxicity and death of six males due to hemorrhage. In male and female rats that received 0.4% or less, no toxicological changes were observed in any of the examined parameters. Based on these results, which demonstrate nodular liver damage only at the highest dose, the authors concluded that the level of no observed adverse effect (NOAEL) is 0.4%, which corresponds to 303mg/kg/day for males. and 472mg/kg/day for females [20].

Several studies in humans with doses ranging from 50 to 400mg/day (equivalent to up to 6.7mg/kg for a 60kg individual) for periods of 2 weeks to 18 months (56 days for the 400mg/day study) with no adverse effects were reported, except occasional transient effects [21].

The study using the highest dose (400 mg/day) was a double-blind, placebo-controlled clinical trial with 108 healthy volunteers randomly assigned to receive a placebo supplement (control group) or 400mg tocotrienols (group of study) daily. During the 2-month study period, volunteers were invited to attend three clinical sessions (on days 0, 28 and 56) and blood samples were taken from the volunteers during follow-up. The results showed that supplementation with tocotrienols significantly increased the total plasma vitamin E level of the supplemented volunteers compared to the placebo group. The work does not report adverse effects in the group supplemented with tocotrienols [22]. Another study, with healthy volunteers, determined the plasma levels of tocotrienols after treatment duringixty days with doses of 80, 160, and 320mg/day, where no adverse effects requiring intervention were observed [23]. A study conducted by Radha krishnan [24], also with healthy volunteers, did not observe adverse effects after eight weeks of treatment at a dose of 250mg/day. In healthy volunteers aged 50 to 55 years, daily supplementation of 150mg per day of tocotrienols produced no adverse effects after 6 months of supplementation [25].

Many studies have also been carried out in groups of patients with different diseases, such as in diabetics at a dose of 3mg/kg under treatment duringixty days [26]; patients with hyperlipidemia, and aortic stenosis at doses of 240mg/day for two years [27], with no reports of adverse effects. With patients with more severe conditions, such as diabetic nephropathy, Tan [28], evaluated the activity of tocotrienols in type 2 diabetic patients who had renal impairment. In this study, patients were treated with two daily doses of 200mg of tocotrienols for eight weeks. According to the authors, the dose of 400mg is the maximum approved by the Food and Drug Administration (FDA). Among the results acquired, there were no reports of side effects or changes in plasma biochemistry parameters. Recently, Pervez et al. [29] evaluated the activity of tocotrienols (90% delta-tocotrienol, and 10% gamma-tocotrienol), obtained from annatto, in patients with hepatic steatosis, with two daily doses of 300 mg. During the 24 weeks of treatment, in addition to the improvement in plasma biochemical markers, no adverse effects were observed in the group treated with tocotrienols [29]. In healthy volunteers, alpha-tocotrienol doses between 200 and 3200 mg/day for 14 days produced no adverse effects [30] with two daily doses

of 300 mg. During the 24 weeks of treatment, in addition to the improvement in plasma biochemical markers, no adverse effects were observed in the group treated with tocotrienols [29].

In sum given the pharmacokinetics of tocotrienols, including their short half-life, consumption of 3–5 mg/kg/day should not cause adverse effects, which agrees with the conclusions reached by the EFSA expert panel (European Food Safety Authority) and one for GRAS (Generally Recognized as Safe). Tocotrienols are initially beta-oxidized by cytochrome P450 enzymes, then conjugated and excreted. As all these mechanisms are very well regulated, the potential for adverse effects is limited [21].

In addition to drug use, the safety profile of tocotrienols allows the development of foods with functional and/or health properties. In this regard, the Food and Drug Administration (FDA) has recently recognized the status of "Generally Recognized as Safe (GRAS)" for the addition of up to 40mg of tocotrienols per kilogram of food [31].

Also, a recent study [32] conducted with γ-tocotrienol in nanoemulsion demonstrated that "the nanoemulsified α-tocopherol and γ-tocotrienol showed a higher skin penetration and retention than the pure vitamin E. The authors declare that "The present study explores its potential use in topical therapeutic accumulation. The mechanisms of action of microwave in skin therapeutic delivery requires further in vitro/in vivo investigation with respect to its biological effects, in addition to physicochemical attributes prior human trials." It identifies that tocotrienol is a great ingredient to apply in cosmetic product and must be conduct more studies in order to expand the skin application.

Pharmacological activity and clinical studies

Over time, hundreds of publications have reported the pharmacological activities of tocotrienols in experimental models using laboratory animals. Furthermore, several clinical studies have been carried out tocorroborate in humans the effects observed in laboratory animals. Experiments conducted with mice and humans have shown potential health benefits with tocotrienol (T3) supplementation, including a distinct and effective anti-inflammatory activity. Many studies have shown a lipid-lowering effect and a superior anti-inflammatory and antioxidant activity, compared to tocopherols, in cardiovascular diseases. The anti-inflammatory activity of T3 has also been proposed as a mechanism of action, explaining the improvement in conditions related to diet-induced metabolic syndrome in rats. The anti-inflammatory activity of T3 has also been proposed to protect against neurodegenerative diseases, including Alzheimer's disease (AD) and alcohol-induced cognitive impairment in rats. Suppression of inflammation is also among the mechanisms by which T3 can neutralize the ability of cancer cells to proliferate, metastasize, escape apoptotic signals, and develop chemoresistance. Finally, low intake and serum levels of tocopherols and tocotrienols have been associated with several age-related pathologies, including osteoporosis, sarcopenia, and cognitive impairment [33]. Suppression of inflammation is also among the mechanisms by which T3 can neutralize the ability of cancer cells to proliferate, metastasize, escape apoptotic signals, and develop chemoresistance.

Contributions

A systematic review evaluated the potential effect of ingestion of tocotrienols or circulating levels of tocotrienols (epidemiological studies) on parameters associated with aging, considering cognitive function, osteoporosis, and DNA damage. The results suggest that, in middle-aged and elderly individuals, tocotrienols have potential antiaging action with respect to cognitive impairment and DNA damage. However, more robust clinical trials are needed to elucidate these effects [34].

Conflict of Interest statement

The authors do not have any conflicts of interest.

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Conflict-of-interest disclosure

The authors declare no competing financial interests.

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