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A review of *Bixa orellana* L. (Annatto) leaves as medicinal resource: Use in the population as complementary medicine, phytotherapeutic action and quality parameters

Sonia P. Molina-Romani [1], Pablo E. Bonilla-Rivera [1], Ricardo Diego D.G. de Albuquerque [2,*]

[1]Facultad de Farmácia, Universidad Nacional Mayor de San Marcos, Peru
[2]Laboratório de Tecnologia em Produtos Naturais, Universidade Federal Fluminense, Brazil

ABSTRACT: *Bixa orellana*, known as Annato or Achiote, is a species popularly used in various countries of America as a dye and medicine. Its leaves possess several biological activities and are commonly marketed as filters. However, despite some ethnomedicinal and pharmacological studies with *Bixa orellana* leaves having been published, there is a lack of review articles that collect this information. In addition, the few studies about the quality parameters of these filters must be addressed jointly so that the usefulness of existing information can be better addressed in the discussion of commercial regulation. In this way, this review aims to compile systematically useful information about the medicinal use and pharmacology of *B. orellana* leaves, in addition to channeling the studies on the quality parameters of filters, which in turn can define the quality, efficacy and safety of the marketed products. As a result of the review, the searches showed that the leaves of *B. orellana* are traditionally used for various purposes, including treatment of headache, bronchitis, and inflammation, in addition to using as a diuretic, analgesic, antiophidic, among others. These findings were corroborated by pharmacological studies, which also point to other biological activities such as antioxidant, antimicrobial, anti-inflammatory, neurodepressant and gastrointestinal modulator through the administration of leaf extracts. Finally, stability and extraction yield studies were also important in providing data for implementing quality control of *B. orellana* products.

1. INTRODUCTION

1.1. History and ethnobotanic use of *Bixa orellana*

The annato tree belongs to the Bixaceae family and *Bixa* genus. Despite the existence of various species, the most common in America is *Bixa orellana* L., named by Francisco Orellana, the first European to navigate the Amazon. This species is found in various countries of Central and South America, including Peru, Ecuador, Colombia, Venezuela, Brazil, Cuba, Mexico and others, and also in the Asian and African continents (Rahmatullah et al., 2009; Rojas et al., 2006; Villar et al., 2014). Annatto is a plant native to South America, more specifically to the Amazon region, and the Caribbean. The ethnobotanical use of its dyes by Native Americans was first described by Christopher Columbus (1492), after arriving on the island of San Salvador (Leal & Clavijo, 2010). The popular name "urucum" is originated from the Tupi word "ru-ku," which means "red." In Brazil, this plant is also commonly known as urucú. Other popular names in other countries are: atol'e, achiote, and bija, in Peru and Cuba; axiote (Mexico); achiote, annatto, annatto, bija, and santo-domingo (Puerto Rico); annatto (Guyana); analto (Honduras); guajachote (El Salvador); onotto and onotillo (Venezuela); achiote and urucu (Bolivia); urucu (Argentina); roucou (Trinidad); roucou and koessewee (Suriname); and annatto (United States). The wide spread of its use in these regions is related to the growing demand for natural dyes by the pharmaceutical, cosmetic, textile, and especially food industries, although it has been used for centuries by native peoples to color skin and objects, and also for its photoprotective function (Villar et al., 2014). Annatto dye is obtained from its seeds and its use has been stimulated by the prohibition of synthetic dyes in cosmetics and food, since it is one of the few accepted by the World Health Organization (WHO) for be non-toxic and do not appear to change the value of the food. It is estimated that 70% of all natural dyes consumed in the world are derived from *B. orellana* dye (Bastos

* Corresponding author.
E-mail address: ricardo-diego-cf@hotmail.com (Ricardo Diego D.G. de Albuquerque)

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et al., 1999).

The ethnomedicinal use of the species is also found in communities in various countries, such as antidiarrheal, antipyretic, antidiabetic, hypotensive, expectorant, and aphrodisiac activity, among others (Villar et al., 2014). In Trinidad and Tobago, popular veterinary use in dogs is also registered (Lans et al., 2000). Different parts of the plant can be used for this purpose, which includes the leaves, and it is common for the filters to be used to treat health problems, more specifically for skin problems, antipyretic activity, diuretic action, as well as it can also be used to treat pain, gastrointestinal, respiratory and liver disorders, sexually transmitted diseases and snake bites (Villar et al., 2014). In addition, other preparations such as infusions and decoctions can also be used medicinally, such as for ocular inflammation and antiemetic therapy during pregnancy (Ins, 2010).

1.2. Phytoconstituents of *Bixa orellana*

The chemical components of *Bixa orellana* L. can vary according to the part of the plant, location area of the species or even according to edaphic, climatic and environmental factors, in general. The biosynthetic routes of primary and secondary metabolites of plants can be altered by adaptation needs and physiological development (Ramakrishna & Ravishankar, 2011). Taking into account all the plant organs of *B. orellana*, the main compounds found are the pigments of the carotenoid class, which includes bixin, as well as some terpenoids, tocotrienols, and flavonoids (luteolin and apigenin, mainly) (Radhika, 2010; Ramírez, 2001). At the level of leaves and seeds of *B. orellana*, the phytochemical march has identified the low presence of alkaloids in leaves, a moderate amount of flavonoids, abundant leucoanthocyanidins and a moderate amount of tannins, not being found cardiotonic, saponins and quinones (Miranda, 2003). In seeds are found bixin (red dye), norbixin, cryptoxanthin, euxin, methylbixin, lutein and zeaxanthin, as well as amines, leucoanthocyanins, triterpenes, tannins and other polyphenols, such as the flavonoids apigenin-7-bisulfate, cosmosiin, hypoalectin-8 - bisulfate, luteolin-7-bisulfate, luteolin-7-O-b-D-glucoside and isoscutelarein, and the benzenoid gallic acid. Diterpenes are still important in seeds, mainly those found below: farnegeraniloctadecanoate, farnesylacetone, geranylgeraniol and geranylgeranil formate (Radhika, 2010), while specifically at the leaf level (Figure 1), flavonoids (apigenin, hypoalletin and cosmosiin), diterpenes (farnesylacetone, geranyl geraniol and geranyl formate), as well an alkaloids, steroids, phenols, pyrogallic tannins, anthraquinones, fixed coumarins, gallic acid and essential oil have been described. The essential oil is composed of mono and sesquiterpenes, among which are bixaganene and ishwaran. At the nutrient level, the leaves have a significant amount of vitamins (A, B, and C), proteins, sugars, fats, as well as calcium, iron, and phosphorus (Ramírez, 2001).

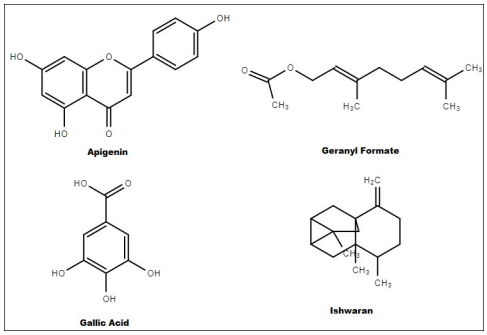

Figure 1. Main substances found in *Bixa orellana* leaves

1.3. Biological properties

The studies of biological properties from *Bixa orellana* were carried out taking account different parts of the plant, which in turn was collected in various parts of the world, in order to demonstrate a wide variety of activities, among which can be mentioned antibacterial, antimicrobial, antiviral, insecticidal, anti-inflammatory, hypoglycemic, antioxidant, cytotoxic and other activities (Villar et al., 2014). Especially on the leaves, the antibacterial, antifungal, antileishmanial, antioxidant, antidiarrheal, neurodepressant, and antivenin activities, among others, were described (15, 1). In addition, the mutagenicity and carcinogenicity tests concluded that the extracts of *B. orellana* do not cause mutations or the appearance of cancers, even in high concentrations (De Lima et al., 2003; Paumgartten et al., 2002). Finally, the toxicity studies also demonstrated the safety of the use of the extracts, so that these were related to the absence or low concentration of toxicological indicators, even though in the latter case, it was only possible to observe alterations in liver enzymes when overdoses were used (Hagiwara et al., 2003).

In this context, the objective of this review work is to collect scientifically-based information that covers popular use, pharmacology, and studies of quality parameters for the production of filter filters from *Bixa orellana* L. leaves and, in this way, the standardization of these products can allow their rational and safe use as useful phytotherapeutics in the treatment of different diseases.

2. METHODOLOGY

Searches for scientific publications were carried out in the Scopus, Google Scholar, PubMed and Science Direct databases, using the combined words "*Bixa orellana*", "hojas" and "ethnomedicinal" or "popular use" for subseccion 3.1; "*Bixa orellana*", "leaves" and "activity" for subseccion 3.2; and "*Bixa orellana*" and "leaf filters" for subsection 3.3., in Spanish, Portuguese and English languages.

3. TRADITIONAL KNOWLEDGE OF *BIXA ORELLANA*

According to traditional knowledge, *B. orellana* has been used by various native peoples of the Americas for body painting and material purposes, as well as food coloring, or for use in religious and spiritual ceremonies and medicinal purposes (Leal

& Clavijo, 2010). Many Aboriginal people use *B. orellana* for dyeing, in which the dye is obtained naturally as a mixture and is used to color pottery and other household vessels. Additionally, in addition to all the previously mentioned, many people also use *B. orellana* to protect themselves from mosquitoes present in the forests and ultraviolet radiation, while the phloem provides fibers for rough twine. The pulp, including the seed, is used as febrifuge and for soft drinks. In addition, it can generate valuable dyeing substances such as yellow (orellin) and red (bixin), which in turn constitutes a crystallized active product. In the food industry, *B. orellana* is used to color sauces, bakery products, cheese, macaroni, as well as juices, ice creams, soups, margarine, butter, mayonnaise, mustard and sausage, where it is commonly called "do reino" (from the kingdom), from the Netherlands (Savithramma et al., 2014).

The medicinal use includes the treatment of various disorders of the human and veterinary organism, so that different parts of the plant can be used. The seeds have been used as a condiment, as well as a hypotensive, laxative, expectorant, antibiotic and cardiotonic. Furthermore, it possesses anti-inflammatory activity for wounds and bruises, besides to be used for the treatment of wound healings and bronchitis (Savithramma et al., 2014). The seeds are also used as an antidote for poisoning by Manihot esculenta (yucca, yuca brava, bitter yucca, sour yucca) and to prevent scars left by smallpox on the epidermis (Revilla, 2000; Yong et al., 2013). In Brazil, the seeds can be used to treat anemia, bronchitis and control cholesterol levels (Manganelli et al., 2018). Traditionally, the pulp of the fruits is applied to burns to prevent the formation of blisters and sores, while the powder resulting from crushing the seeds has been used as an aphrodisiac (Ins, 2010). Other properties related to the seeds are the following: antipyretic, laxative, antimalarial, antidiabetic, antidiarrheal, analgesic, and treatment of respiratory problems. For the roots, antidiarrheal, antigonorrheal, anthelmintic activities and for the treatment of hepatic and respiratory disorders were reported. The fruits, in turn, are used for purposes of astringent, diuretic, antidiarrheal action and as aphrodisiacs (Villar et al., 2014).

As for the leaves, its infusions have proven to be effective against bronchitis, sore throats and inflammation of the eyes, in addition to being used as a poultice to relieve headaches, while the decoction is useful for the treatment of sore throats and for antiemetic therapy during pregnancy (Ins, 2010). In Bangladesh, pills made from a mixture of leaves and fruits (3 times a day for a week) are used as appetite stimulants, digestive and also against weakness (Rahmatullah et al., 2009). Still, a recent study carried out in Brazil mentioned the usefulness of the leaves for the treatment of cough and flu and abdominal pain (Manganelli et al., 2018). Other ethnomedicinal studies report the use of the leaves for skin problems, antipyretic activity, diuretic action, treatment of pain, gastrointestinal and respiratory disorders, hepatic disorders, treatment of gonorrhea, and snake bites (Villar et al., 2014). And in general, the species is also used for the treatment of cystitis, renal failure, uric acid elimination, prostate disorders, as well as internal inflammations, arterial hypertension, high cholesterol and obesity (Ins, 2010). The veterinary ethnomedicinal use of the species in Trinidad and Tobago is also reported, specifically for the treatment of demodetic mange and other skin parasitism in dogs (Lans et al., 2000).

Figure 2. Leaves of *Bixa orellana*. Source: Universidade Federal de Santa Catarina.

4. PHARMACOLOGY APPROACHES OF *BIXA ORELLANA* LEAVES

According to the diversity of the chemical constituents mentioned above, mainly in relation to the presence of bixin, flavonoids and terpenoids, pharmacological studies of the use of *B. orellana* leaves have also shown a wide variety of activities (Table 1) such as: antibacterial, antioxidant, neuroderpessant (Shilpi et al., 2006), antifungal (Singh & Vidyasagar, 2017), antileishmanial (Almeida et al., 2012), anti-venom (Otero et al., 2000), antidiarrheal (M.A. Tagne et al., 2019), analgesic (Betancourt et al., 2006), anti-inflammatory (Moraes-Neto et al., 2022), antiulcer (Huamán et al., 2009), hepatoprotective (Huamán et al., 2013), healing (Milagros & Nieves, 2019), antihistamine (Yong et al., 2013), diuretic (Rahmatullah et al., 2009) and for the treatment of prostatitis (Cisneros-Hilario et al., 2014).

The antibacterial effect was observed in several studies with *B. orellana* leaves, which includes activities against different species and bacterial strains. Its methanolic extract was active against *Staphylococcus aureus* ATCC25923 at a concentration of 62.5 ug/mL and also against *Bacillus pumilus*, with a minimum zone of inhibition of 16 mg/mL (Pillai et al., 2018). The study by Shilpi et al. (2006) has shown activity of this extract against intestinal bacteria that cause dysentery, including *Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae*, presenting an inhibition zone of 15, 10 and 11 mm, using a concentration of 500 ug/disc (Shilpi et al., 2006). It was also possible to observe the antibacterial activity of the methanolic extract against dental caries bacteria, *Streptococcus mutans* (inhibition zone = 20mm, 62.5 ug/mL) and *S. sanguinis* (inhibition zone = 20mm, 125 ug/mL). (Medina-Flores et al., 2016). Furthermore, the work of Kar et al. (2021) reported the activity of the crude ethanolic extract (5 mg/mL) against resistant strains of *Vibrio cholerae* (halo = 16mm), *E. coli* (13mm), *Shigella flexineria* (21mm), *Salmonella enterica* serover typhi (25mm), *Acinetobacter* sp. (13 mm) and *Shigella boydii* (23 mm), also presenting a synergistic effect when administered together with ampicillin (Kar et al., 2022). The ethyl acetate extract produced

an antimicrobial (Minimum inhibitiory concentration (MIC) = 1.56 mg/mL) and bacteriostatic (MIC = 6.25 mg/mL) effect against *Mycobacterium abscessus* subsp. massiliense (Mabs) (Moraes-Neto et al., 2022). Finally, the methanolic extract presented activity against *Pseudomonas aeruginosa*, with maximum inhibition halo activity of 17.33 mm (Singh & Vidyasagar, 2017).

The antifungal activity was observed for the dichloromethane and methanolic extracts against *Cladosporium cladosporioides* CECT 2111 (halos = 14 and 15 mm, for concentrations of the dichloromethane extract 5 and 10 mg/disc), *Microsporum gypseum* CECT 2908 (halos = 14 and 15 mm, for concentrations of the methanolic extract 5 and 10 mg/disc) and *Tricophyton mentagrophytes* CECT 2795 (halos = 17 and 18 mm, for concentrations of the dichloromethane extract 5 and 10 mg/disc; 15 mm, for concentration of the methanolic extract 10 mg/ disc) (Freixa et al., 1998). The activity against *Neisseria gonorrhoeae* was observed for the ethanolic extract (50 mg), presenting a zone of inhibition of 17.4 mm (Cáceres et al., 1995). In another study with methanolic extract, the highest antidermatophytic activity was observed against *Candida albicans* (20 mm) followed by *T. rubrum* (12.6 mm), *Microsporum gypseum* (10.33 mm), T. tonsurans (9, 33 mm) and *T. mentagrophytes* (9 mm) (Singh & Vidyasagar, 2017). In addition, the chloroform extract was active against two species of *Aspergillus*, *A. flavus* and *A. niger*, presenting 10-40 % inhibition of biomass, using a concentration of 1 mg/mL (Jena & Bhatnagar, 2021).

Other interesting pharmacological activities were verified for extracts of *B. orellana* leaves. The *in vitro* antiprotozoal activity against *Leishmania amazonensis* was observed for the ethanolic extract, using concentrations in a range between 0.12–2.5 mg/mL (Almeida et al., 2012), while the anti-venom activity of this extract against snake venom *Bothrops atrax* presented an Lethal dosis 50% (LD_{50})> 260 ug/animal in rats (Otero et al., 2000). On the other hand, various studies evaluated the antioxidant potential of extracts from *B. orellana* leaves. The study conducted by Shilpi et al. (2006) demonstrated that the methanolic extract presented an IC_{50} of 22.36g/mL in the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, while the reducing potential over hydrogen peroxide was 70 %, using a concentration of 1 % of the ethanolic extract (Guevara-Cholón & Rosales-Azabache, 2021). In another study, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) value of the ethanolic extract was between 58.40 and 121.67 TEAC (Trolox equivalent antioxidant capacity) (Zarza-García et al., 2017). The antidiarrheal activity induced by castor oil was evaluated by M.A. Tagne et al. (2019). The ethanolic extract (50 mg/kg) produced significant decreases of up to 87.80 % in the severity of diarrhea in Swiss mice. The extract at 100 and 200 mg/kg bw showed a significant decrease in enteropooling induced by castor oil (61.08% and 65.41%), and only the 200 mg/kg bw extract showed a significant reduction in intestinal transit (24.46%). compared to the standard drug (M.A. Tagne et al., 2019). Furthermore, ethanolic extract of *B. orellana* (100-400 mg/mL) protected against acetic acid-induced ulcerative colitis in rats, which in turn is associated with the inhibition of free radicals production, tissue damage and hematologic disorders (M.A.F. Tagne et al., 2022).

Neuropharmacological activities were also evaluated for different extracts of *B. orellana* leaves. The methanolic extract (500 mg/kg) was able to reduce the onset time and duration of sleep induced by pentobarbitone in Swiss mice, in addition to causing a neurodepressant effect by reducing the amount of movements and convulsions induced by strychnine, and also presenting an analgesic effect by decreasing the number of abdominal contractions induced by acetic acid (Shilpi et al., 2006). Analgesic activity was also observed for the aqueous extract (150 mg/kg), increasing the time spent on the "hot plate" and reducing the number of abdominal writhings (Betancourt et al., 2006). In turn, the nociceptive effect of the methanolic extract was described by Aktary et al. (2020), so that the response time of the group treated with 200 mg/kg of the extract was 5.7 seconds (Aktary et al., 2020).

Another pharmacological activity considerably related to the effect of the *B. orellana* leaves is the anti-inflammatory activity, so that the aqueous extract at 500 ppm was able to inhibit the inflammation induced by carrageenan in a range of values statistically comparable to indomethacin. (Zarza-García et al., 2017). In a similar trial, the ethyl acetate extract (50 mg/kg) also reduced paw edema by 40%, being comparable to the efficiency of diclofenac (Moraes-Neto et al., 2022). Another work investigated the possible inflammatory mediators inhibited by the aqueous extract of the leaves, and the acute inflammation model was induced in Sprague-Dawley rats through the administration of carrageenan, histamine, serotonin and bradykinin in the hind legs, in addition to verifying to regression of granulomas in the model of chronic inflammation, so that in all models, the extract led to the reduction of inflammation, using doses between 50 and 150 mg/kg (Zuraini et al., 2007).

The gastroprotective action was also evaluated by different studies with extracts of *B. orellana* leaves. Treatment with the ethanolic extract produced an inhibition of gastric lesions in 21.7% and 28.3%, using doses of 200 and 400 mg/kg, respectively. In addition, in the histological study, greater protection and less migration of proinflammatory cells were found in the groups that received the extract (Huamán et al., 2009). Another study showed that the aqueous extract (100 mg/mL) had a cytoprotective effect in gastric ulcers induced by indomethacin, maintaining the gastric pH value and decreasing the amount of malondialdehyde acid (MDA) (Ancheta-Henríquez & Guzmán-Santamaría, 2011). On the other hand, the aqueous and ethanolic extract, both in the concentration of 500 mg/kg, demonstrated to have hepatoprotective activity against the toxic effects of paracetamol. Treatment with the extracts decreased total and indirect bilirubin, while there was a decrease in liver mass (13.2% and 9.37%, respectively (Huamán et al., 2013). The healing effect was observed with the ethanolic extract in the concentration of

1 g/mL, which was capable of reducing the size of the wound by 56 % in a period of 7 days, in a healing model in albino rats (Milagros & Nieves, 2019).

The antihistaminic effects of *B. orellana* leaves were investigated by Yong et al. (2013), so that the aqueous extract, in concentrations in the range between 50 - 150 mg/mL, produced a significant inhibition of histamine-induced paw edema from the 60-minute time point, with a percentage maximum inhibition (60.25%) achieved with a dose of 150 mg/kg at 60 min. Up to 99% of the increase in peritoneal vascular permeability produced by histamine was successfully suppressed, and the expression of biochemical mediators of vascular permeability, nitric oxide (NO) and vascular endothelial growth fator (VEGF), was decreased in the treated group (Yong et al., 2013). The mechanism of antihistamine action of the aqueous extract has also been shown to involve other mediators, according to the work by Yong et al. (2015). The histamine-induced increased permeability of Human umbilical vein endothelial cells (HUVEC) was significantly attenuated by extract pretreatment, in a time- and concentration-dependent manner. Pretreatment suppressed the upregulation of phospholipase C activity, caused by histamine in HUVEC, in addition to also blocking histamine-induced intracellular calcium production. On the other hand, the extract suppressed the NO-cGMP (nitric oxide - cyclic guanosine monophosphate) signaling cascade when HUVECs were challenged with histamine and significantly abolished PKC (protein kinase C) activity (Yong et al., 2015).

Finally, the use of the medicinal and commercial product of *B. orellana* leaves is aimed at purifying the urinary tract, in addition to optimizing renal function and aiding in the treatment of prostatitis (Cisneros-Hilario et al., 2014). These effects can be explained by their diuretic activities and on the benign growth of the prostate, in addition to the previously mentioned activities, such as antioxidant, antimicrobial and anti-inflammatory (Radhika et al., 2010b;) (Shilpi et al., 2006; Zarza-García et al., 2017). Regarding the diuretic activity, the methanolic extract of the leaves presented significant effect at a dose of 500 mg/kg, increasing the total volume of urine, as well as its sodium, potassium and chloride ion levels, when compared with furosemide in a Wistar rats model (Radhika, 2010b). On the other hand, the effect of *B. orellana* leaves on the benign growth of the prostate is observed by both ethanolic and aqueous extracts, although the mechanisms of action seem to be independent. The ethanol extract (500 mg/kg) lowered prostate specific antigen (PSA) levels by 53.44%, when compared to the group treated only with testosterone, although it did not influence prostate volume and weight (Cisneros-Hilario et al., 2014). On the other hand, the aqueous extract, in the concentration range between 50-400 mg/kg, was able to reduce prostate growth, while the levels of PSA and thiobarbituric acid reactive species (BARS) were not interfered when compared to control groups (Huamán et al., 2012). One possible explanation for the difference in activity between the extracts is that the ethanolic extract concentrates more nonpolar substances, such as phytosteroids, which can act as inhibitors of active testosterone synthesis or testosterone receptor antagonists, while the aqueous extract concentrates polar substances, such as polyphenols, which have recognized anti-inflammatory and antioxidant activity (Kao et al., 2014;) (González et al., 2011). These last two activities are important for the treatment of inflammations in the urinary and prostatic tracts, since the various compounds present in the leaves have shown different ways of inhibiting the inflammatory process and antioxidant mechanisms, which in turn are important in the first stages of inflammation (Guevara-Cholón & Rosales-Azabache, 2021; M.A. Tagne et al., 2019; Zarza-García et al., 2017; Zuraini et al., 2007). Added to this, the antibacterial activity against *E. coli* is also an important contribution for the treatment of urinary disorders, since this bacterium is the main cause of urinary infections in humans (Kar et al., 2022; Shilpi et al., 2006).

5. STUDIES OF IMPORTANT QUALITY PARAMETERS IN THE ELABORATION OF *BIXA ORELLANA* FILTERS

The study conducted by Tuesta-Gómez (2020) evaluated the effect of drying temperature and other parameters in obtaining leaf filter from the *B. orellana* leaves, so that the following conclusions could be made: a) the treatment at 30 °C it offers the best results in terms of the antioxidant content present in the filter, with a DPPH value equal to 0.42 mg/mL TE; b) Regarding the content of total polyphenols, the treatment at 30 ºC was the one that showed the best behavior in the filter, with a result in gallic acid equivalent of 0.08 mg/mL; c) The filtering infusion with the best sensory characteristic was the one obtained with the treatment at 30 ºC, according to the opinion of the panelists; d) Regarding yield, the best treatment was at 30 ºC, presenting 38.36 % of total yield; e) wrapping with trilaminate paper has allowed better conservation of polyphenols and antioxidants for the filter (Tuesta-Gómez, 2020).

In another study, it was possible to determine the process conditions that most favor the extraction of phenolic compounds from the leaves of *B. orellana*: extraction time of 60 h and solvent/leaf ratio (v/p) of 4/1, using 95 ethanol. %. The parameters evaluated were the content of total phenols and antioxidant activity evaluation methodologies (Sepúlveda-Rincón et al., 2016). Another recent work evaluated the effect of soluble solids and temperature on the antioxidant activity of *B. orellana* leaf extracts and the degradation rate of their phenolic compounds. Temperatures were studied including the range of typical food processes (70-90 °C) and food storage (−20-37 °C). The results highlighted that the thermal degradation of the phenolic compounds has followed first-order kinetics, so that the rate of degradation depends on the temperature, pH and the amount of soluble solids. The same occurred with the loss of antioxidant activity, so the half-life times of total phenols, under different storage conditions, were included within the range of 40.72 - 202.47 days, while for the ABTS and FRAP (ferric reducing antioxidant power) methods, the times means were 55.87 - 68.83 days and 57.85 - 107.03 days,

Table 1
Pharmacological activities of *Bixa orellana* leaves

Activity	Extract	Details	References
Antibacterial	Methanolic	Against *Staphylococcus aureus* ATCC25923 and *Bacillus pumilus*	Pillai et al. (2018)
	Methanolic	Against *Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae*	Shilpi et al. (2006)
	Methanolic	Against *Streptococcus mutans* and *S. sanguinis*	Medina-Flores et al. (2016)
	Methanolic	Against *Pseudomonas aeruginosa*	Singh and Vidyasagar (2017)
	Ethanolic	Against *Vibrio cholerae*, *E. coli*, *Shigella flexineria*, *Salmonella enterica* serover typhi, *Acinetobacter* sp. and *Shigella boydii*	Kar et al. (2022)
	Ethyl acetate	Against *Mycobacterium abscessus* subsp. massiliense (Mabs)	Moraes-Neto et al. (2022)
Antifungal	Dichloromethanic	Against *Cladosporium cladosporioides* CECT 2111 and *Tricophyton mentagrophytes* CECT 2795	Freixa et al. (1998)
	Methanolic	Against *Microsporum gypseum* CECT 2908 and *Tricophyton mentagrophytes* CECT 2795	Freixa et al. (1998)
	Methanolic	Against *Candida albicans*, *T. rubrum*, *Microsporum gypseum*, *T. tonsurans* and *T. mentagrophytes*	Singh and Vidyasagar (2017)
	Ethanolic	Against *Neisseria gonorrhoeae*	Cáceres et al. (1995)
	Chloroformic	Against *A. flavus* and *A. niger*	Jena and Bhatnagar (2021)
Antiprotozoal	Ethanolic	Against *Leishmania amazonensis*	Almeida et al. (2012)
Anti-venom	Ethanolic	Against *Bothrops atrax* venom	Otero et al. (2000)
Antioxidant	Methanolic	DPPH assay	Shilpi et al. (2006)
	Ethanolic	Hydrogen peroxide reducing potential	Guevara-Cholón and Rosales-Azabache (2021)
	Ethanolic	ABTS assay	Zarza-García et al. (2017)
Antidiarrheal	Ethanolic	Castor oil induced assay	M.A. Tagne et al. (2019)
Antiulcerative colitis	Ethanolic	Acetic acid induced assay	M.A.F. Tagne et al. (2022)
Sleeptime regulator	Methanolic	Pentobarbitone induced assay	Shilpi et al. (2006)
Anticonvulsivant	Methanolic	Strychnine induced assay	Shilpi et al. (2006)
Analgesic	Methanolic	Acetic acid induced assay	Shilpi et al. (2006)
	Aqueous	Hot plate and abdominal writhings assays	Betancourt et al. (2006)
Nociceptive	Methanolic	Response time evaluation	Aktary et al. (2020)
	Aqueous	Carrageenan induced assay	Zarza-García et al. (2017)
Anti-inflammatory	Aqueous	Carrageenan, histamine, serotonin and bradykinin induced assays and chronic model	Zuraini et al. (2007)
	Ethyl acetate	Paw edema assay	Moraes-Neto et al. (2022)
Gastroprotective	Ethanolic	Inhibition of gastric lesions by protective action and inhibition of inflammatory cells migration	Huamán et al. (2009)
	Aqueous	Cytoprotective effect in gastric ulcers induced by indomethacin	Ancheta-Henríquez and Guzmán-Santamaría (2011)
Hepatoprotective	Ethanolic	Paracetamol toxicity inhibition	Huamán et al. (2013)
Wound healing	Ethanolic	Healing model in Albino rats	Milagros and Nieves (2019)
Antihistaminic	Aqueous	Histamine-induced paw edema assay	Yong et al. (2013)
	Aqueous	Mechanism investigation	Yong et al. (2015)
Anti-prostatitis	Ethanolic	Prostatic hiperplasia inhibition	Cisneros-Hilario et al. (2014)
	Aqueous	Prostatic hiperplasia inhibition	Huamán et al. (2012)
Diuretic	Methanolic	Furosemide induced assay	Radhika et al. (2010)

respectively (Sepúlveda-Rincón et al., 2014).

Based on these data, it is possible to define parameters for the quality control of the production of *B. orellana* leaf filters, which involves obtaining the raw material, its production processes and the quality of the finished product, in addition to suggest the best way to preserve the products so that they can have higher stability. In short, the definition of quality parameters leads to greater reliability in the marketing of the product, so that the safety, efficacy and other qualities inherent to the products can be fully contemplated by consumers.

6. CONCLUSION

The ethnobotanical and pharmacological studies described in the review report the importance of the *Bixa orellana* leaves as an important natural resource for the treatment of various health problems, including respiratory, gastrointestinal and urogenital disorders, among others In addition, studies on quality parameters for products containing *B. orellana* provide important information for their commercial control, which would imply greater safety, quality and efficacy of these products

Figure 3. Example of product for infusion based on *Bixa orellana* "Achiote" leaves marketed in Peru.

when offered to the consumers.

CONFLICTS OF INTEREST

The aurhors has no conflict of interest to declare.

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ORCID

Sonia P. Molina-Romani 0000-0002-2049-7232
Pablo E. Bonilla-Rivera 0000-0002-7286-6810
Ricardo Diego D.G. de Albuquerque 0000-0002-8442-3849

AUTHOR CONTRIBUTIONS

SPMR, PEBR - Research concept and design, RDA - Collection and/or assembly of data, SPMR, RDA - Writing the article, PEBR, RDA - Critical revision of the article, PEBR - Final approval of the article.

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