JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Attributes of various parts of fabulous Annatto Tree (*Bixa Orellana* Linn.) for culinary purpose and cosmetics

Manoj Kumar[1], Chandrashekhar Meena[2], Lakha Ram[3], Jagdish Ram[3], Raaz K. Maheshwari[3]

1. Department of Chemistry, Govt College, Kota, Rajasthan, India
2. Department of Chemistry, Govt College, Gangapur City, Swai Madhopur, Rajasthan, India
3. Department of Chemistry, SBRM Govt PG College, Nagaur, Rajashan Rajasthan, India

Abstract

Annatto is a yellow-orange-red colorant derived from the resinous coating of the seeds of the plant *Bixa orellana* L., grown in Central and South America, India, and East Africa. Annatto has a long history of use and is available as oil or water solutions, suspensions, emulsions, encapsulated products, and spray-dried powders. Various parts of this tree are thus used for the treatment of conditions such as microbial infections, sunstroke, tonsilitis, burns, leprosy, pleurisy, apnoea, rectal discomfort, headache and for the treatment of small burns, poisoning, thinning hair, headaches, gonorrhea, skin diseases, nausea and vomiting, snake bites etc. This tree has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions. Previous phytochemical investigations have revealed the presence of several carotenoid compounds including bixin and norbixin some terpenoids, to cotrienols, and flavonoids in Bixa orellana, seeds. Among these, carotenoids are widely used in biological process such as vitamin A activity, cancer preventing agents, protective agent against cardiovascular diseases and decreasing effect of cataract and age related degeneration. Bixa seeds are used as purgative, antipruritic and for buccal tumors. In carotenoids, bixin is one of the most effective biological compounds and contribute to the cell protection and tissue damage against

free radicals. The present study pretends to explore to determines and evaluate the antibacterial, phytochemical activities of *Bixa orellana* L.

Keywords: Annatto extract, Bixin, Norbixin, Tocopherols, Tocotrienols, Body colors

Introduction

The Annatto *Bixa orellana* L., also known as " Lipstick tree", is a tree reaching 10 meters, and is cultivated in the tropical and subtropical regions of the world. Annatto is a native plant from South America, more specifically of the Amazon region. The popular name "urucum" comes from the Tupi word "ru-ku," which means "red." In Brazil, this plant is commonly known as urucum, urucu, açafrão, açafroa, and açafroeira-da-terra. It is known by other popular names in other countries: atolé, achiote, and bija (Peru and Cuba); axiote (Mexico); achiote, achote, anatto, bija, and santo-domingo (Puerto Rico); bixa (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). [1-3] Annatto first spread in the form of food coloring, also known as paprika, a condiment widely used in cooking to enhance the color of food. Today, however, its use has spread into many segments of industrial production. Thus, it is now applied on the skin—in the form of makeup and sunscreen—and there is research proving that its use brings health benefits, which makes producers thankful for cultivating it . The wide dissemination of its use in those regions is related to the growing demand for natural dyes by many pharmaceutical, cosmetic, textile, and especially food industries. It is also widely used in the printing industry and dye manufacturing.

Plant Flowers, leaves and seeds of Annato t ree

The species name of this plant is named after the Spanish scientist conquistador, Franscisco de Orellana. The Bixaceae family is one of the smallest plant families, consisting only of one genus, Bixa. There are only five species grouped under a single genus, and the most common species is Bixa orellana, an evergreen shrub grown not only because of its beautiful red flowers and ornamental red spiny fruits, but also for its economic value. Leaves are entire, ovate, 8 to 20 cm long, 5 to 12 cm wide, with a broad and heart-shaped base, and a pointed tip. [4-7]The fruits dry and harden to brown capsules. It has been widely used in food, textile and pharmaceutical industries due to the presence of annatto dye in the seeds as a natural colorant. It is widely used in India including areas such as Orissa, Andhra Pradesh, Maharashtra, kerala, Karnataka and Tamilnadu. In India, the ayurveda practitioners used Bixa orellana as an astringent and mild purgative because the whole plant of it showed valuable medicinal properties and it is considered as a good remedy for treating dysentery and kidney diseases. The traditional healers claim that Bixa species are more efficient to treat infectious diseases than synthetic antibiotics. The seed and root bark were utilized for treating gonorrhea. The root bark is used as antiperiodic and antipyretic. The leaves and roots help in epilepsy, dysentery, fever and jaundice. The plant extracts show activity against helminthes, protozoans and platelet anti aggregant activity. Extracts of leaves and branches have shown to be effective at neutralizing the effects of snake venoms.

The achiote has long been used by American Indians to make body paint, especially for the lips, which is the origin of the plant's nickname, lipstick tree. Annatto used in food dye, body paint, treatment for heart burn and stomach distress, sunscreen and insect repellant. Its folk medicine are used in the form of decoctions, teas and juices for the treatment of common infections. The leaf decoction is used to cure skin diseases and burns. The leaves are a popular febrifuge in Cambodia. [8-9]The infusion of leaves is prescribed as a purgative and in the treatment of dysentery. In Central America, the oil derived from seeds is used to cure leprosy and decoction is given to treat jaundice. The species is used medicinally in various parts of the world & cultivated in warmer regions like India, Sri Lanka and Java, exclusively for the dye obtained from the seeds. In India, the plant is cultivated especially in western parts of the country. A colored compound obtained from the pulp of the seeds called ‘bixin’ is used all over the world as a red-orange dye, for coloring rice, cheese, soft drinks, oil, butter and soup. The dye is also used in some regions in textile industry and the seeds, as a condiment. Ayurveda practitioners in India use it as an astringent and mild purgative and are considered as a good remedy for treating dysentery and kidney diseases. The root bark is anti-parasitic and antipyretic. The traditional healers claim that Bixa sps are more efficient to treat infectious diseases than synthetic antibiotics. [6,7-10]

Annato Seeds, Seed Extract, Lipstick, paste, oil and Sauce

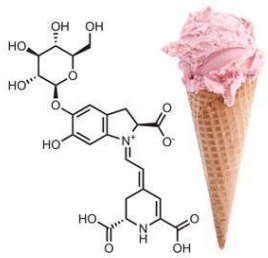
Phytochemical constituents and Pharmacological attributes

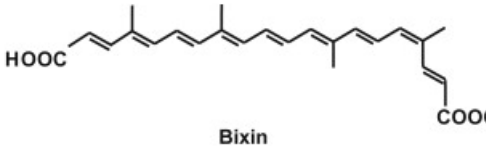
Phytochemical analysis of leaf and seed extracts suggested the presence of flavonoids, tannins, saponins, steroids. Alkaloids were detected only in the leaf and anthroquinones, in the seeds. This study on Bixa orellana L. expresses the well-characterized pharmacological actions and phytochemistry that may be considered relevant for the future development of an innovative therapeutic purposes. Annatto seeds have a high phosphorus and a low calcium content. Its protein contains adequate levels of tryptophan and lysine, but is low in methionine, isoleucine, leucine, phenylalanine and threonine. The color pigments in annatto seeds are apo-carotenoids namely bixin and nor-bixin, which extracted from the outer coating of the seeds. Bixin is a fat-soluble carotenoid which contributes for majority of (about 70-80%) pigment in the seeds. Besides bixin and norbixin, the other chemical substances that have been isolated from the seeds of annatto are isobixin, β-carotene, cryptoxanthin, lutein, zeaxanthin, orellin, bixein, bixol,

crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan, and phenylalanine. [11-15]

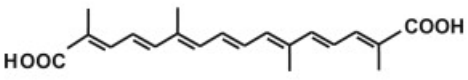
The principal pigment is bixin, the cis form of the mono-methyl ether of a carotenoid carboxylic acid (R=CH_3 – bixin; R = H – nor-bixin). Bixin is oil soluble compound and it is bas eof oil of oil soluble products. The carboxylic acid nor-bixin that is formed from the saponification of bixin is the main component in water soluble products. The best quality of sedd contains over 3 per cent bixin, but supplies often about 2 per cent. Phytochemical screening of seeds revealed gallic tannins, flavonoids, anthocyanins, leucoanthocyanins, saponins, triterpenoids, steroids, mucilage, reducing compounds, and C-heterosides. Nutritionally, the seeds contain protein (12.83%), fats (4.64%), Potassium (14.59 mg/g), Sodium (6.93 mg/g), Phosphorus (4.79 mg/g), Calcium (2.89 mg/g), Magnesium (1.04 mg/g), Manganese (0.19 mg/g), Copper (0.19 mg/g), and Iron (0.01 mg/g). In addition to being an excellent source of dyes usable for various purposes, Annatto is a potentially nutritious product. It is therefore of paramount importance in the improvement of the quality of traditional food.

Annatto seeds are also rich source of organic volatile compounds such as bornyl acetate, copaene, cubebene, β-pinene, 4-methylpyridine, elemene, cyclosativene, geranyl phenylacetate, 1-heptanetiol, 3-methylpyridine, β-humulene, isoledene, d-selinene, etc. Adding to the known health benefits exerted by carotenoids, a bioactive sesquiterpene from achiote exhibited moderate anti-fungal activity. Extracts of the leaves of achiote possess antimicrobial activity against Gram positive microorganisms, with maximum activity against Bacillus pumilus. [16-18]

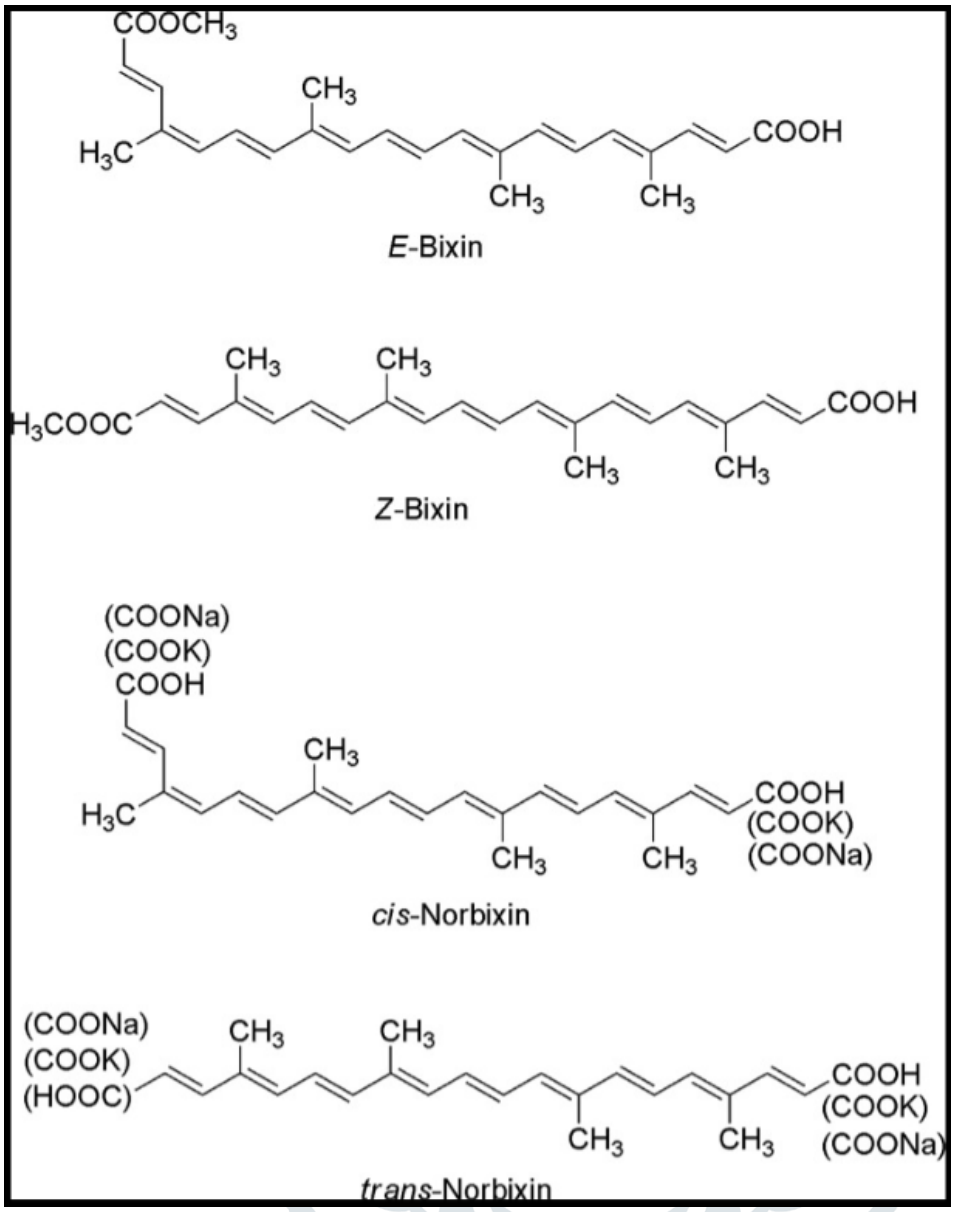




Bixin

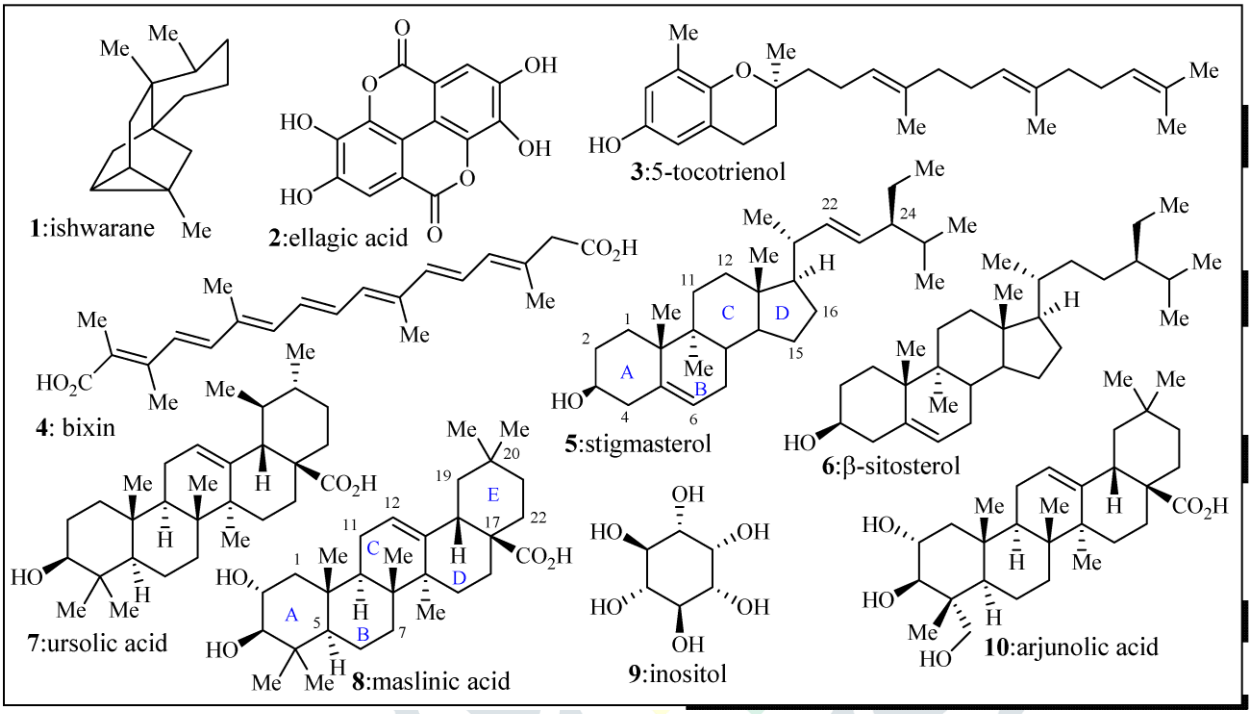


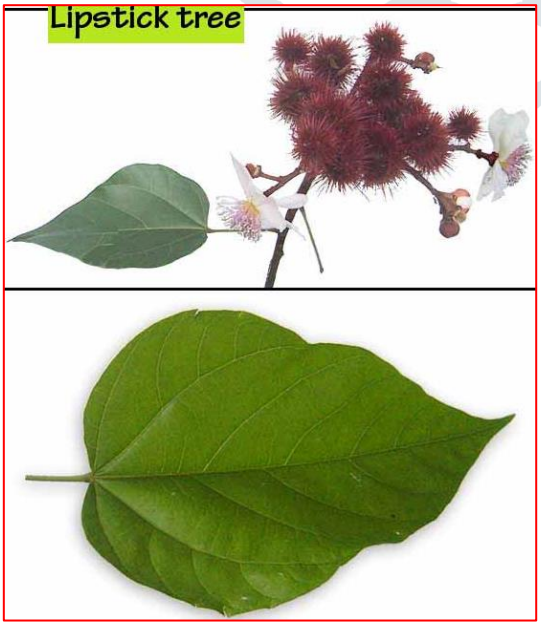
Crocetin



Achiote leaves have been employed to treat malaria and Leishmaniasis. The seeds urucum supplies seeds that have been used as a condiment as well as laxative, cardiotonic, hypotensive, expectorant, and antibiotic. In addition, it has anti-inflammatory activity for bruises and wounds and has been used for the treatment of bronchitis and for wound healing purposes. The infusion of the leaves has been shown to be effective against bronchitis, sore throat, and eye inflammation. It provides fibers for rough cordage, and the powder resulting from grinding the seeds has been used as an aphrodisiac. Finally, the infusion of cold buds serves to wash inflamed eyes, whereas the decoction of the leaves has been used for antiemetic therapy during pregnancy. Annatto extracts from leaves, roots and seeds have traditionally been used for medicinal purposes like asthma. Extracts of leaves, bark, and roots are reported to be antidotes for poisoning and antivenin for snakebites. The entire plant is used against fever and dysentery[19-21]. A decoction of the leaves is used to stop vomiting and nausea; treat heartburn, prostate and urinary difficulties, stomach problems and internal inflammation, arterial hypertension, high cholesterol, cystitis, obesity, renal insufficiency, and to eliminate uric acid and as a mild diuretic. It is used as an aphrodisiac and astringent and to treat skin problems and hepatitis as well as to avoid phlegm in newborn babies. The seeds are believed to be an expectorant, while the roots are thought to be a digestive aid and cough suppressant. Its efficiency against STD such as gonorrhea was reported.

The Annatto seed extract contains many color principles among all bixin, oil soluble and norbixin, water soluble principles are responsible for its dye characteristics. Bixin responsible for imparting reddishness and norbixin for yellow. [20-22]





Unique Health benefits of Annatto seeds

Although not much of calorific value, annatto seeds hold their role as natural, chemical-free food colorant at the same time not seem to change the food value. Its use as a safe food additive is also acknowledged by the US-FDA and WHO. These biological compounds in them have potential use as antiviral, antibacterial, anti-inflammatory, insect repellent, anti-malarial agents in the traditional medicines in Brazil, Mexico and Peru. The indigenous Amazonian people use the annatto as a condiment as well as to treat sunburn, wounds and bruises. Several in-vitro studies suggest that the extracts of Bixa orellana showed biological activities such as antioxidant, anti-allergic, and anti-tumor for carcinoma of the lung. It is used to treat bronchitis and heal wounds. The infusion made from leaves helps to treat sore throat, bronchitis and eye inflammation. It is used to treat tonsilitis, sunstroke, leprosy, burns, apnoea, pleurisy, recta discomfort, apnoea and headaches. [5,7,9,21-23] It is used as a wash for skin infections and cure for stomach and liver disorders. It is used as a repellant for flies and mosquitoes. Shoots and leaves possess febrifugal, astringent and aphrodisiac properties. It is used as a treatment for rashes, diarrhea, dysentery, sores and swellings. The decoction made from leaves is used as a gargle for throat and mouth infections. Annatto is used for fluid retention, malaria and hepatitis. It is also used as a bowel cleanser. It is used to treat vaginal infections. It is used to treat skin problems, dysentery, fevers and hepatitis. Roots are used as an aid for digestive health and cough suppressant. It is used to treat rashes, cuts and other ailments. The infusion made from bark and leaves are useful for stomachache, dysentery, nausea, vomiting, stomach problems and acid reflux. Apply the boiled leaved to the body and head for the treatment fever. For sore throat, use boiled leaves as a gargle. Mix the coconut oil and pounded leaves and apply it to forehead for the treatment of headaches. The poultice made from leaves is useful for the treatment of gonorrhea. Its leaves are useful for snake bites. The pulp of Annatto is useful to eliminate scars caused due to blisters and also stops bleeding. The seeds and bark are used for treating thinning hair. The branches and bark are used to promote menstrual flow and also alleviates menstrual pain. The decoction prepared from pulp of Annatto seeds are treatment for coughs. The decoction made from leaves is used to eliminate the symptoms of heat stroke. [20-24]

Therapeutic uses of Annatto/Achiote leaves, pulp, flowers, bark and seeds

Concoction of leaves are used to wash burn wounds to promote healing. The infusion of leaves are used as purgative in dysentery. Boiled leaves (shoots) are applied to the head and body to treat fever. Boiled leaves (young shoots) are used as gargle for sore throat. Pounded leaves mixed with coconut oil are applied to the forehead for headaches. Poultice of leaves are used to treat gonorrhea. Achiote leaves are used to treat snake bites. Extracts from Achiote leaves, roots and barks are used as antidote for cassava poisoning, Jatropha poisoning and allergy from sandbox tree. Decoction of leaves are used to treat nausea and vomiting. Boiled leaves are used to treat gastric and stomach ailments. Achiote pulp is used to prevent scars due to blisters. Achiote pulp is used to stop bleeding. Pulvorized pulp is used to treat fever. Achiote pulp is also used to treat constipation, dysentery and

gonorrhoea. Infusion of flowers used as expectorant. The seeds, ground and boiled, also used for burns. Fresh seeds when moistened produce a reddish colored juice that is applied to red rashes. [18,21,24] The seeds are used as antidote for cassava and J. urcas poisoning. Fine powder that covers the seeds is used as hemostatic, and internally as stomachic. Seed oil used for leprosy. The achuete dye used with lime for the treatment of erysipelas. In Uruguay seeds are ground and boiled and used on burns. In West Indian folk medicine, used for diabetes mellitus. In Guatemala used for treatment of gonorrhea. Female aphrodisiac in the Amazonia. Decoction of bark used for febrile catarrh. Also used for wound healing, regulation of heavy menses, and thinning hair. Mucilage produced from infusion of fresh branches used a emollient in the Antilles. In traditional Peruvian medicine, used to treat heartburn and stomach distress caused by spicy foods; also, as a vaginal antiseptic and cicatrizant. Achiote is among those herbs used in Colombian folk medicine to treat infections of microbial origin. [17,19,22-23] Many Aborigines use annatto for dyeing, where the dye is naturally obtained as a mixture and used to color ceramics and other vases for domestic use. In addition, most endogenous people use this dye on their skin to beautify themselves during religious rituals and mainly to protect themselves from ultraviolet radiation and from mosquitoes that infest forests.

Synthetic Sindoor/Kumkum and its importance

There are various forms of Sindhoor or Kumkum mentioned in Ayurveda. Traditional sindhoor is made from natural ingredients used for facial makeup (cosmetics). Most widely used traditional Sindoor is made from turmeric and lime juice. Other ingredients include Ghee, and slaked lime. Sindoor is also made from red sandal powder, saffron etc. The coloured powders sold as substitutes are traditionally not considered sindhoor. Traditional sindoor was made with turmeric and alum or lime, or from other herbal ingredients. Unlike red lead and vermilion, these are not poisonous. Modern material being sold as sindoor mainly uses vermilion, an orange-red pigment, the purified and powdered form of cinnabar [cinnabar is mercury sulfide (HgS), a toxic mineral. As with other compounds of mercury, sindoor is toxic and must be handled carefully. Sometimes, red lead (lead tetroxide, also known as minium) is added to sindoor. Red lead is toxic. Since the beginning of civilizations, Sindoor has got a great significance. In Sanatan Hindu culture, it is used as a symbol of auspiciousness and sacredness. Typically all Hindus, especially the married women, apply Sindoor on their forehead. Sindoor is also used in the worships, hawans, and Abhisheks. In fact every sacred ritual starts with offering the Sindoor to God and by putting sindoor tilak on the foreheads of the involved people. It is considered to be a form of Shakti. Scholars also consider red to be the colour of power and energy. Ancient shastras recommend offering Sindoor to the deity during the worship or hawans as it is believed that it gives the divine energy of the deity to the worshipper. Maa Shakti passes on glory, longevity, vigor, and strength to us when we offer Sindoor to the Yantra, Statue / Idol or Picture and then apply it on our forehead. Typically, the Sindoor is applied in the area between the eyebrows. This area is where

the Third Eye Chakra is situated. This chakra helps us in acquiring intuitive knowledge through concentration and it is also the chakra of compassion and forgiveness. Sindoor, when applied on this chakra, activates it and that helps in concentration. It is believed that the Sindoor, when applied on the forehead, also helps in preventing the energy loss which happens because of various worries and tensions. While the shastras have referred to several benefits of Sindoor, the shashtras have referred to the sindoor obtained from the Sindoori plant only.


The most commonly available sindoor in the market is not the 100% natural sindoor. It typically made by mixing Turmeric with Lime Water or Mercuric sulphide with Alum ($K_2SO_4 \cdot Al_2SO_4.24H_2O$) or by Red Lead powder. According to ancient shastra Tantraraj Tantram, lead is a highly negative element for any divine purpose. Mercury is easily obtained from Cinnabar Ore (commonly known as Vermillion). This is used for making red lacquer in China and dyes in India which is highly toxic element in nature. Because of continuous use of this, a false belief system has established among people that mercury is good for our health. However, the toxic effects of Mercury are well proven scientifically. Sindoor made from chemical substances such as Red Lead (Pb_3O_4), Mercuric sulphide (HgS) , and Alum or Lime Water [Calcium Hydroxide ($Ca(OH)_2$)] do not give positive benefits of using Sindoor for worship or for applying on forehead. Apart from these, there are also a lot of negative effects of such toxic sindoor on human body. It causes roughness and irritation to the skin It can have adverse effects on the vision in case it goes in the eyes If eaten mistakenly, it can have an adverse effect on the body. Such toxic sindoor also negatively affects the environment. When used on idols and Yantras, it erodes the metal surface.

Culinary and Cosmetic Uses

Annatto is commonly used as a coloring agent for pharmaceutical ointments and plasters. It has been used in direct compression tablet coating and oral liquid drugs. The pulp, which includes the seed, is used for soft drinks and febrifuge. Moreover, it can provide valuable dyeing materials such as yellow (orellin) and red (bixin) substances, with the latter constituting a crystallized active ingredient. In the food industry, it is used to color butter, margarine, mayonnaise, sauces, mustard, sausage, soup, juice, ice cream, bakery products, macaroni, and cheese. Annatto imparts a wonderful color to oil when fried for a few minutes. The seeds are infused in hot water until the desired intensity of the color is achieved. This colorant then added to various dishes as a substitute for saffron. Annatto has several industrial applications. [6,8,11,20-22]The famous Jamaican national dish of saltfish (cod) and ackee is served in a vibrant sauce colored with

annatto. The Spanish introduced this spice to Filipinos, who use it extensively in their cuisine. Consumption of dishes prepared using an annatto colorant is safe, and allergic reactions are rare to occur. Annatto color imparts yellow to red with varied hue index as it possesses high tinctorial value, hence have significance in the food industry as a natural food grade colour, and stands second in rank among economically important natural food colourants, apart from its wide use in some regions of the world for non-food applications viz., to color textiles, fabrics and weapons. Isolated pure bixin and norbixin were subjected for color intensity stability. Annatto used in food dye, body paint, treatment for heart burn and stomach distress, sunscreen and insect repellent. The main actions of annatto color are it kills bacteria, parasites, germs, increases urination, stimulates digestion, lowers blood pressure, mildly laxative, and protects liver. The other actions of annatto color includes it reduces inflammation, cough, cleanses blood, soothes membrane, reduces fever, blood sugar, heals wounds. Annatto is being used increasingly in body care products. Annatto oil is an emollient, and its high carotenoid content provides antioxidant benefits on body care products, while adding a rich, sunny colour to creams, lotions, and shampoos. Protection from ultraviolet rays of sunlight, thereby protecting the skin from excessive sunburn. Dyes for lipstick are also obtained from Bixaorellana L hence, the name lipstick tree. It is also used as a colouring agent for the preparation of sindoor. []22-24

Conclusion

Bixa orellana L has been widely used in food, textile and pharmaceutical industries due to the presence of annatto dye in the seeds as a natural colorant. In India, the ayurveda practitioners used Bixa orellana as an astringent and mild purgative because the whole plant of it showed valuable medicinal properties and it is considered as a good remedy for treating dysentery and kidney diseases. The traditional healers claim that Bixa species are more efficient to treat infectious diseases than synthetic antibiotics. The seed and root bark were utilized for treating gonorrhea. The root bark is used as antiperiodic and antipyretic4. The leaves and roots help inepilepsy, dysentery, fever and jaundice. The plant extracts show activity against helminthes, protozoans and platelet anti aggregant activity. Extracts of leaves and branches have shown to be effective at neutralizing the effects of snake venoms. Previous phytochemical investigations have revealed the presence of several carotenoid compounds including bixin and norbixin some terpenoids, to cotrienols, and flavonoids in Bixa orellana seeds. Among these, carotenoids are widely used in biological process such as vitamin A activity, cancer preventing agents, protective agent against cardiovascular diseases and decreasing effect of cataract and age related degeneration. Bixa seeds are used as purgative, antipruritic and for buccal tumours. In carotenoids, bixin is one of the most effective biological compounds and contribute to the cell protection and tissue damage against free radicals.

References

1. Vilar DDA, Vilar MSDA, Moura TFAL, Raffin FN, Oliveira MRD, Franco CFDO, Filho PFDA, Diniz MFFM, Filho JMB. Traditional Uses, Chemical Constituents, and Biological Activities of Bixa orellana L.: A Review. 2014; https://doi.org/10.1155/2014/857292
2. Bressani R, Barneón FPED, Braham JE, Elías LG, Brenes RG. Chemical composition, amino acid content and nutritive value of the protein of the annatto seed (Bixa orellana, L.)]. Archivos Latinoamericanos de Nutricion. 1983;33(2):356-376.

3. Selvi AT, Dinesh MG, Satyan RS, Chandrasekaran B, Rose C. Leaf and Seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens. J Appl Pharmaceut Sci. 2011;01 (09): 116-120.
4. Fleischer TC, Ameade EPK, Mensah MLK, Sawer IK. Antimicrobial activity of the leaves and seeds of Bixa orellana. Fitoterapia. 2003; 74:136–138.
5. Irobi ON., Young M, Anderson WA. Antimicrobial activity of Annatto (Bixa orellana) extracts. J Pharmaceutl Biol. 1996;34 (2): 87-90.
6. Shilpi JA, Rahman TUMD, Uddin SJ, Alam MSD, Sadhu SK, Seidel V. Preliminary pharmacological screening of Bisa orellana L. leaves. Braz J Med Biol Res. 2003;36:113-118.
7. Shilpi JA, Rahman TUMD, Uddin SJ, Alam SMD, Sadhu SK, Seidel V. Preliminary Pharmacological screening of Bixa orellana L. leaves. J. Ethnopharmacol. 2006; 2 (24): 264-271.
8. Padhi S, Panda SK. (2016). Phytochemical investigation and anthelmintic activity of various leaf extracts of Bixa orellana Linn. J Innov Appl Pharmaceut Sci. 2016;1(3), 41-45.
9. Patnaik S, Mishra SR, Choudhury GB. Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of Bixa orellana Linn. Int J Pharmaceut Biol Arch. 2011; 2(5):1497–1501.
10. Selvi AT, Dinesh MG, Satyanet RS, Chandrasekaran B, Rose C. Leaf and seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens. J Appl Pharmaceut Sci. 2011; 1(9):116–120.
11. Martos VM, Gomez CG, Navajas RY. In vitro antioxidant and antibacterial activities of extracts from annatto (Bixaorellana L.) leaves and seeds. J Food Safety. 2012; 32(4):399–406.
12. Giorgi A, De Marinis P, Granelli G, Chiesa LM, Panseri S. Secondary Metabolite Profile, Antioxidant Capacity, and Mosquito Repellent Activity of Bixa orellana from Brazilian Amazon Region. J Chem. 2013; 1-10.
13. Castello MC, Phatak A, Chandra N, Saron M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixaorellana L. Ind J Exp Biol. 2002; 40:1378-1381.
14. Parekh J, Nair R, Chanda S. Preliminary screening ofsome folklore medicinal plants from western India forpotential antimicrobial activity. Indian J Pharmacol2005; 37:408-409.2.
15. Ongsakul M, Jindarat A, Rojanaworarit C. Antibacterial effect of crude alcoholic and aqueous extracts of six medicinal plants against Staphylococcus aureus and Escherichia coli. J Health Res. 2009; 23:153-156.
16. Akshatha V, Giridhar P, Ravishankar GA. Morphological diversity in Bixa orellana L. andvariations in annatto pigment yield. J Horti Sci Biotechnol. 2011;86(4):319-324 .
17. Prathima D, Sujitha A, Usha R. Phytochemical Screening and Antimicrobial Activity of Bixa OrellanaLinn. Int J Pharmacog Phytochem Res. 2016; 8(7); 1078-1082.

18. Mota DR, Flores LJP, Fernando Carrari F, Espinoza JAME, Sánchez FDDL, López LLP, Hernández GG, FR· Achiote (Bixa orellana L.): a natural source of pigment and vitamin E. J Food Sci Technol. 2017; 54(6): 1729–1741. doi: 10.1007/s13197-017-2579-7

19. Giridhar P, Parimalan RA. 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.

20. Giuliano G, Rosati C, Bramley PM. To dye or not to dye: biochemistry of annatto unveiled. Trends Biotechnol. 2003;21:513–516. doi: 10.1016/j.tibtech.2003.10.001.

21. Akakpo E, Badoussi ME, Gnacadja CK, Houngbo H, Dossou A, Gbaguidi F, Azokpota P. Ethnobotanical, phytochemical and nutritional characterization of Bixa Orellana Linn. seeds of Benin Ecology Int. J. Biosci. 17(1), 46-56, July 2020. doi: http://dx.doi.org/10.12692/ijb/17.1.46-56

22. Srineeraja P. Pharmacological Properties of Bixa Orellana – A Review Int J Sci Res. 2017;6(7):751-755.

23. Radhika B, Begum N, Srisailam K, Reddy VM. Diuretic activity of Bixa orellana Linn. Leaf extracts. Ind J Nat Prod Resour. 2010;1(3):353-355.

24. Srivastava A, Shukla YNM, Jain SP, Kumar S. Chemistry, pharmacology and uses of Bisa arellana: A review. J Med Arom Plant Sci. 1999;21:1145-1154.