ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

# Evaluation of Antihelminthic Activity of *Bixa orellana*

Swastiprava Das[1], Sugimani Marndi[1], Susanta Kumar Biswal[2], Tikendrajit Baro[3], Ramesh Singh[4], Rajkumari Supriya Devi[2], Rikina Choudhury[1], Sanjeet Kumar[1]*

## Abstract

Intestinal worms are common problems in remote and rural areas of India. The communities of remote areas use some wild plants to treat intestinal worms. Among them, *Bixa orellana* is a monogenic plant of family *Bixaceae*, popularly known as lipstick tree or Annatto and locally called as Sinduri. It is traditionally used to kill the intestinal worms, results revealed that seeds of *B. orellana* possess diverse secondary metabolites such as tannin and phenolic compounds which might be responsible to kill the worms. Antihelminthic activity showed that seed extracts (aqueous, acetone, methanol, and ethanol) were effective against *Eisenia fetida*. Keeping the traditional uses of Sinduri against stomach worm and to reduce the side effects of synthetic drugs, the present experiment has been designed.

**Keywords:** Antihelminthic activity, Intestinal worm, Secondary metabolites

*Asian Pac. J. Health Sci*., (2021); DOI: 10.21276/apjhs.2021.8.4.46

## Introduction

*Bixa orellana* (Annatto) is native to Brazil but grows on other parts of South and Central America. This plant is mostly cultivated in tropical regions such as East Africa, Kenya, Mexico, Peru, India, Indonesia, and Ecuador.[1] In India, it is well distributed in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Odisha, Gujarat, Maharashtra, Madhya Pradesh, and Chhattisgarh and also cultivated as an ornamental plant as well as commercial production of seeds.[2] On the basis of flower, color, and shape of fruits, *B. orellana* has three varieties: One with white flowers and green capsules, second with purple flowers and brownish red capsules, and third with pink flowers and red capsules.[3] It is a bushy shrub with a height ranging from 3 to 10 m. Bark is more or less smooth with many warty lenticels and fissured when old. It has taproot systems. Leaves are ovate with round heart-shaped base and acute or acuminate apex. Petioles are swollen at the base and the apex too. Leaves are arranged in spiral manner. Leaf blade is waxy in pink flower while rough in white flowered plant. Leaves of pink flowered plant show dark red venation and pale green in purple and white flowered plants.[3] Panicle type of inflorescence, flowers are actinomorphic, hermaphrodite, and hypogynous. It has five petals with numerous stamens emerging centripetally. Petals are ovate, distinct having imbricate aestivation and dotted due to the presence of color cells. The filaments are long, very thin, and white or pink in color with basifixed, dithecous, and horseshoe-shaped anthers depending on the petal color.[3-6]

The Annatto seed extract comprises many color pigments, among them bixin and norbixin are vital. Bixin is responsible for redness and norbixin for yellow color.[7] Annatto colors have huge significance in the food industry and it is used as a natural edible dye. It is also used as economically important natural food colorants.[8,9] Its non-food application includes textile color,[10-12] fabrics and weapons,[13,14] body paint, insect repellent, sunscreen and heart burn, and stomach distress. It was observed that the plant has been used in treating microbial infection since ancient times.[15,16] In India, the bark is used to treat fever, gonorrhea phlegm, headache, etc.,[17] and there are evidences that the plant does not show any type of urogenital infections while treating gonorrhea.[18] In Central and South America, the plant is used to cure internal inflammation and in Malaysia for stomach problem and gastric ulcers.[19] The Amazon tribes of Brazil commonly used seed extracts to paint their body which also acts as insect repellent.[20] In Trinidad and Tobago, it is commonly used to treat jaundice, diabetes, and hypertension.[21]

Pharmaceutical companies use Annatto as colorant for ointments and plasters.[22] It is also used as tablet coating and oral liquid drugs.[23] Annatto oil has soothing effect and its high carotenoid content offers antioxidant benefits on body care products and aids rich sunny color to creams, lotions, and shampoos.[24] Antiapoptotic effect of T3 components of Annatto has been documented *in vitro* in human, mice, and tumor cell lines. Among the natural carotenoids, bixin is one of the more active biological singlet molecular oxygen quenchers and may contribute to the protection of cells tissues against harmful effects of free radicals.[25] Therefore, it is a sound antioxidant and inhibitor lipoxygenase.[26] It was noted that methylbixin has shown improvement in activity of gap junctional communication which is important in cancer prevention.[27] Methanol extract of *B. orellana* seeds proved hepatoprotective activity against liver damage made by carbon tetrachloride.[28] It results in decreased levels of serum makers, indicating the protection of hepatic cells. *B. orellana* has been used for the treatment of diabetes mellitus too. It also lowered

[1]Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Odisha, India

[2]Department of Botany, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India

[3]Department of Biotechnology, Bodoland University, Assam, India

[4]Department of Botany, Hemvati Nandan Bahuguna Garhwal University, Uttarakhand, India

**Corresponding Author:** Sanjeet Kumar, Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Cuttack, Odisha. Email: sanjeet.biotech@gmail.com

**How to cite this article:** Das S, Marndi S, Biswal SK, Baro T, Singh R, Devi RS, Choudhury R, Kumar S. Evaluation of Antihelminthic Activity of *Bixa orellana*. Asian Pac. J. Health Sci., 2021;8(4):227-232.

**Source of support:** Nil

**Conflicts of interest:** None.

**Received:** 25/06/21 **Revised:** 07/08/21 Accepted:12/09/21

the blood glucose level by stimulating peripheral consumption of glucose.[29] It has antihistamine, anti-inflammatory, anticonvulsant, and antidiabetic activities.[30-32] It is antagonistic to chromosomal mutation made by radiation[33,34] and clastogenic effects of antitumor agents against radiations.[35] The wood of *B. orellana* is weak, lightweight, and not hard; therefore, it is used to start fires by friction. The pericarp of fruit is the by-product of annatto color extraction industries which is used as a potential source of fuel.[36]

Antimalarial activity of *B. orellana* has been found against *Plasmodium gallinaceum*, *Plasmodium lophurae*, *Plasmodium Falciparum,* and *Plasmodium berghei*.[37] *B. orellana* extracts possess antiprotozoal, anthelmintic, and platelet antiaggregant activity.[38,39] Helminths are soil transmitted intestinal worm which infects gastrointestinal tract. These are the endoparasites with many cells.[40] Most common groups of helminths that inhabit in human guts are Nematodes (roundworms), Cestodes (tapeworms), and Trematodes (flatworms).[41] Helminths are able to survive in their mammalian hosts for several years because they deploy hosts immune response by secreting immunomodulatory products.[40] Infections by helminths are known as helminthiasis or intestinal worm infections. Some common parasitic worms in human are *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale* and *Necator americanus* (hookworms), *Enterobius vermicularis* (pinworm), *Schistosoma mansoni* (blood fluke or bilharzia), *Schistosoma haematobium* (bladder fluke), *Wuchereria bancrofti* (filarial worm), *Onchocerca volvulus* (river blindness), *Giardia lamblia,* and *Entamoeba histolytica*.[42,43] Heavy worm burdens have been related with substantial illness and mortality. Individual suffering from helminthiasis faces hematuria associated with *Schistosoma haematobium* infection, complicated nephrosis and portal hypertension.[44] Anthelmintic drugs are used in expelling out the parasitic worms either by stunning them or by killing them. Some common important synthetic anthelmintic drugs are piperazine, benzimidazole, levamisole, pyrantel, paraherquamide, ivermectin, emodepside, and nitazoxanide.[45] The most frequently reported side effects caused by drugs used for the treatment for soil transmitted helminthiasis are epigastric or generalized abdominal pain, dizziness fever, headache, nausea, vomiting, anorexia, skin rash, drowsiness, allergic reaction (edema, rashes, and urticaria), and gastrointestinal trouble.[42] The dye obtained traditionally from fruits is also used as a food additive; therefore, if the fruits have antihelmintic activities, it could act as a nutraceutical. Some compounds such as thiabendazole, mebendazole, albendazole, and flubendazole presence of *B. orellana* also showed the anti-endoparasitic activities. However, less or no reports are available on antihelmintic activity of seeds of *B. orellana* against *Eisenia fetida*; show the novelty of the present study. Therefore, keeping all the importance of *B. orellana* and the occurrence of intestinal worm in remote areas of the country, an experiment has designed to evaluate the anthelmintic activity of its seeds to validate the tribal claims.

## Materials and Methods

For the present experimental works, there is no need of any ethical clearance and plants were collected from an urban area with concern of owner of the land. The present work has been done between November 2019 and March 2020. The plant specimen was collected from Khandagiri Road, Bhubaneswar, India [Figure 1], and was identified following Flora Books for experimental works.[1,5,46]

**Figure 1:** Collection of *Bixa orellana* fruits from Bhubaneswar, India

### Preparation of Extracts

#### *Aqueous extract*

Decoction process was adopted to obtain aqueous extract. The fruits were dried at room temperature under the shade and the desiccated seeds were collected in an airtight container for experimental use. Three grams of seeds of *B. orellana* were boiled with 50 ml of distilled water for 1 h then it was allowed to cool at room temperature for 1 h. It was filtered through filter paper and the aqueous extract was then collected and stored in refrigerator for further experimental works.

#### *Seed extraction*

Maceration method was adopted to prepare different seed extracts. The seed extraction was carried out using four solvents as per polarity index, namely, n-hexane, methanol, acetone, and ethanol extracts were prepared by mixing 2 g of seeds in 20 ml of each chemical then the solutions were kept for 72 h in a refrigerator for future use.

### Phytochemical Screening

Phytochemical screening was carried out to evaluate the qualitative analysis of bioactive compounds using different solvents using standard procedure.[47-51]

#### *Test for saponin*

Three milliliters of plant extract were mixed with 1 ml of normal distilled water and shaken vigorously. The stable persistent forth was formed, indicated the presence of saponin.

#### *Test for tannin*

Three–5 drops of 0.1% ferric chloride solutions were added to 3 ml of solvent extract. The brownish-green or black coloration was obtained, indicated the presence of tannins.

### *Test for terpenoids*

Six milliliters of extracts were mixed with 2.5 ml of chloroform and then 3 ml of concentrated sulfuric acid was added. A reddish-brown coloration of interface indicated the presence of terpenoids.

### *Test for phenolic compounds*

Three milliliters of extract were treated with 3–5 drops of 1% ferric chloride solution formation of bluish-black coloration showed the presence of phenolic compounds.

### *Test for steroids*

Two milliliters of plant extract were dissolved in 5 ml chloroform and then 5 ml of concentrated sulfuric acid. Formation of two phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

## Evaluation of *In vitro* Anthelmintic Activity

### *Collection of worms*

All the experiments were carried out on red wiggler worm (*E. fetida)*. The worms were collected from Vermiculture division, Department of Soil Science, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha [Figure 2]. The worms were kept in soil compost. *E. fetida* is taken in the place of parasitic worms as a model which is responsible for diarrhea, nausea, fatigue, and abdominal pain.

### *Preparation of extracts*

The aqueous, methanol, and acetone extracts of seeds of *B. orellana* of different concentration (100 mg/ml, 200 mg/ml, and 300 mg/ml) were prepared and final volume was made to 1 ml by adding distilled water for respective concentration. Groups of approximately equal size worm consisting one earthworm in each group were released into 1 ml of desired concentration of drug and extract in the beaker. Distilled water used as a control.

## Experimental Work Plan

The collected worms were washed with water to remove all fecal matters. The anthelmintic activity was performed according to the standard method. *E. fetida* was placed in beakers containing three different concentrations (100 mg/ml, 200 mg/ml, and 300 mg/ml) of methanol, aqueous, and acetone extracts of seeds of *B. orellana*. The different concentrations were taken to know the exact initial value of paralysis time as per the concentration. For the evaluation of antihelmintic activity, time of paralysis and death of worms are taken. Each beaker was placed with one worm observed for paralysis or death. Time for paralysis was noted when no movement of any sort could be observed, except the worm was shaken vigorously. The death of worms recorded after observing no movement even shaking or giving external stimuli. The experiments were repeated 3 times and mean and standard deviation was calculated. The death of the worms was confirmed when the worms were unable to move and looking at the scar of white secretion and fading of their body color [Figure 3].[52]

## Statistical Analysis

The antihelmintic experiment is repeated 3 times. Paralysis time and death time are noted. The unaffected worms against used extracts were noted as +4.

# Results

The results of the present study revealed that *B. orellana* seeds possess secondary metabolites having -OH functional group such as tannin and phenolic compounds were detected in used solvents extract. In ethanol and acetone extract, tannin was detected. It was noted that only tannin was detected in methanol extract while phenolic compounds and tannin were detected in aqueous extract [Table 1]. The results of antihelmintic activity of seeds of *B. orellana* also showed excellent activity against *E. fetida*. It was noted that there was no paralysis or death of *E. fetida* observed in aqueous extract with 100–300 mg/ml concentration [Table 2]. In methanol extract, it was noted that no death with 100 mg/ml after 6 h but paralysis was observed in 200 mg/ml and death was observed in 300 mg/ml [Table 3] whereas death of *E. fetida* was observed in acetone extract with 200 mg/ml and 300 mg/ml [Table 4]. Therefore, it was confirmed that seeds of *B. orellana* have antihelmintic activity.

# Discussion

In this study, authors collected seeds of *B. orellana* for the evaluation of medicinal values against worm and the results revealed that seeds might be a good agent to formulate an herbal drug. The presence of phenolic compounds in aqueous extract

**Figure 2:** Vermiculture of *Eisenia fetida*

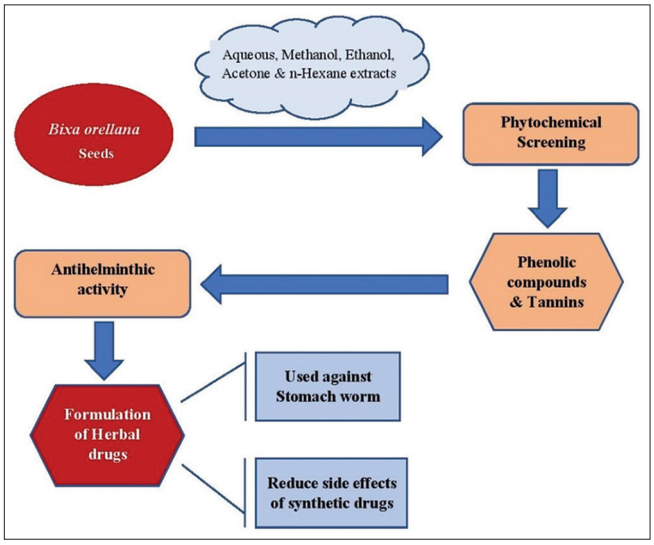


**Figure 3:** Future work aspects of the present study

**Table 1:** Phytochemical screening of *B. orellena* seeds

| *Bioactive compounds* | *Extract* | | | | |
|---|---|---|---|---|---|
| | *n-hexane* | *Ethanol* | *Acetone* | *Methanol* | *Aqueous* |
| Saponin | -ve | -ve | -ve | -ve | -ve |
| Tannin | -ve | +ve | +++ve | +ve | +ve |
| Terpenoids | -ve | -ve | -ve | -ve | -ve |
| Phenolic Compounds | -ve | -ve | -ve | -ve | +ve |
| Steroid | -ve | -ve | -ve | -ve | -ve |

(-ve: absent; +ve: present; +++: high)

**Table 2:** Anthelmintic potential of aqueous extract of *B. orellana* (Seeds)

| *Concentration* | *After 10 mins.* | *After 20 mins.* | *After 30 mins.* | *After 1hr.* | *After 2hrs.* | *After 3hrs.* | *After 4hrs.* | *After 5hrs* | *After 6 hrs.* |
|---|---|---|---|---|---|---|---|---|---|
| 100 mg/ml | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |
| 200 mg/ml | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |
| 300 mg/ml | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |
| 1 ml Distilled water | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |

(+4: No death or paralysis)

**Table 3:** Anthelmintic potential of methanol extract of *B. orellana* (Seeds)

| *Concentration* | *After 10 mins.* | *After 20 mins.* | *After30 mins.* | *After 1hr.* | *After 2hrs.* | *After 3hrs.* | *After 4hrs.* | *After 5hrs* | *After 6 hrs.* |
|---|---|---|---|---|---|---|---|---|---|
| 100 mg/ml | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |
| 200 mg/ml | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| 300 mg/ml | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead |
| 1 ml Distilled water | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |

(+4: No death or paralysis)

**Table 4:** Anthelmintic potential of acetone extract of *B. orellana* (Seeds)

| *Concentration* | *After 10 mins.* | *After 20 mins.* | *After30 mins.* | *After 1hr.* | *After 2hrs.* | *After 3hrs.* | *After 4hrs.* | *After 5hrs* | *After 6 hrs.* |
|---|---|---|---|---|---|---|---|---|---|
| 100 mg/ml | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |
| 200 mg/ml | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Dead | Dead | Dead |
| 300 mg/ml | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead | Dead |
| 1ml Distilled water | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 | +4 |

(+4: No death or paralysis)

revealed its medicinal potentials [Table 1]. Phenolic compounds are one of the most widely occurring phytochemicals and have significant physiological and morphological actions. Phenolic compound shows wide range of physiological properties such as antiallergic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective activity, and antithrombotic and exerts vasodilatory effects.[53-57]

The presence of tannin in all extracts except n-hexane shows that seeds of *B. orellana* could use against many infectious diseases. They are closely related with plant defense mechanisms against mammalian herbivores, birds, and insects.[58,59] Many researchers have experimentally demonstrated that tannins have antimicrobial, anthelmintic actions and have the ability to make complexes with proteins.[60-63] Therefore, seeds of *B. orellana* might be useful against microbial infections having nutraceutical potential.

Many researchers have reported the bioactive compounds present in the vegetative parts of *B. orellana*.[64-66] Prathima *et al*.[67] documented the bioactive compounds available in seeds and reported the presence of saponin, steroid, alkaloid, and flavonoid in aqueous extract and acetic acid extract, whereas the present study showed only presence of tannin and phenolic compounds ($n$ = 3). Tamil *et al*.[68,69] reported only tannin, saponin, steroid, and flavonoid from seeds of *B. orellana* and showed absence of alkaloids.

In the present study, all extracts showed the activity against *E. fetida* except aqueous [Table 2]. In 2016, Padhi and Panda documented the antihelmintic activity of leaf extracts of *B. orellana* and showed that petroleum ether, ethyl acetate, methanol, and ethanol extracts have antihelmintic activity against *Pheretima posthuma*.[70]

The present study has some limitation, *B. orellana* is not cultivated and naturalized in many regions and those regions have to find out the other option of it having antihelmintic activity along with less or no side effects. The acetone and methanol extracts showed its potential activity against worm due to the presence of responsible secondary metabolites like tannin.

## Conclusion

Problems related to stomach worm are very common throughout the world. The present study gives a baseline data for formulation of future herbal and synthetic drugs. The phytochemical investigation showed the richness of tannin in seeds of *B. orellana*, could be used against endoparasite and antibacterial infections. The results of antihelmintic show the potential of extracts of seeds

of *B. orellana* and it might be useful to make an herbal drug against stomach worm. However, it is a preliminary works and needs more advance works for formulation of drugs against stomach worm using seeds of *B. orellana* against different types of appropriate animal models.

## Acknowledgments

Authors are thankful to Dean, School of Applied Sciences and HOD, Botany Department, Centurion University of Technology and Management, Odisha, India. Authors are also thankful to local community of study areas and authorities of Orissa University of Agriculture and Technology, Odisha, for providing animal model for the present works.

## References

1. Saxena HO, Brahmam M. The Flora of Orissa. Vol. 1. Bhubaneshwar: Regional Research Laboratory, Orissa Forest Development Corporation; 1994. p. 437-9.
2. Biswas SJ, Giri SK, Saha NC, Banerjee P, Raha S, Pandey A. Phytochemical evaluation acute toxicity studies and antimicrobial efficacy of seed extract of *Bixa orellana*: A plant growth in wild in Purulia district. J Pharmacogn Phytochem 2018;7:2065-71.
3. Jaime A, Silva TD, Dobranszki J, Madrid RR. The biotechnology (genetic transformation and molecular biology) of *Bixa orellana* L. (achiote). Planta 2018;248:267-77.
4. Pandey S, Sharma A, Panika G, Kumar M. Morphological studies, traditional and industrial uses of *Bixa orellana* a review. Curr Sci Int 2019;8:70-4.
5. Aktary N, Sultana S, Hossain ML. Assessment of analgesic and neuropharmacological activity of leaves of *Bixa orellana* (Family: Bixaceae). Int J Sci Rep 2020;6:13-20.
6. Srineeraja P. Pharmacological properties of *Bixa orellana* a review. Int J Sci Res 2017;6:751-5.
7. Husa NN, Hamzah F, Said HM. Characterization and storage stability study of Bixin extracted from *Bixa orellana* using organic solvent. IOP Conf Ser Mater Sci Eng 2018;358:012035.
8. Perotti GF, Silva FF, Couta RA, Lima FC, Petrilli HM, Leroux F, *et al*. Intercalation of Apocarotenoids from Annatto (*Bixa orellana* L.) into layered double hydroxides. J Braz Chem Soc 2020;31:2175.
9. Islam SU, Rather LJ, Mohammad F. Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications a review. J Adv Res 2016;7:499-514.
10. Hipolito GB. Awareness of households to the benefits of Achuete (*Bixa orellana*) in Central Luzon. Int J Adv Eng Manag Sci 2020;6:160-6.
11. Vallejo JV, Ventura JA, Wallis A, Cagle R, Handy SM, Davis J, *et al*. Using full chloroplast genomes of 'red' and 'yellow' *Bixa orellana* (achiote) for kmer based identification and phylogenetic inference. BMC Genom 2020;21:544.
12. Prabhavathi R, Devi AS, Anitha D, Padma A. Crocking fastness properties of Annatto (*Bixa orellana*) dye on cotton mordanted with eco-friendly mordants and post-treated with various dye fixing agents. Int J Curr Microbiol Appl Sci 2018;7:3009-20.
13. Mota DR, Flores LJ, Carrari F, Espinoza JA, Sanchez FD, Lopez LL, *et al*. Achiote (*Bixa orellana* L.): A natural source of pigment and Vitamin E. J Food Sci Technol 2017;54:1729-41.
14. Caldas DC, Diniz RC, Carmo LH, Batista MC, Vilanova CM. Influence of the incorporation of annatto oil *Bixa orellana* L. in the sun protection factor by visible spectroscopy of photoprotective formulations. Int J Dev Res 2020;10:33285-92.
15. Melka B, Bisrat D, Neelaiah BG. Isolation, characterization and biological activities of food colorants from *Bixa orellana*. J Pharmacovigil 2017;5:1-6.
16. Nathan VK, Rani ME, Rathinasamy G, Dhiraviam KN. Antioxidant and Antimicrobial Potential of Natural Coloring Pigment Derived from *Bixa orellana* L. Seed Aril. Vol. 89. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 2019. p. 137-43.
17. Gupta P, *Bixa orellana*: A review on its phytochemistry, traditional and pharmacological uses. World J Pharm Sci 2016;4:500-10.
18. Madrid RR, Espinosa MA, Conejo YC, Caligaris LE. Carotenoid derivates in achiote (*Bixa orellana*) seeds: Synthesis and health promoting properties. Front Plant Sci 2016;7:1-7.
19. Yong YK, Zakaria ZA, Kadir AA, Somchit MN, Lian GE, Ahmad Z. Chemical constituents and antihistamine activity of *Bixa orellana* leaf extract. BMC Complement Altern Med 2013;13:32.
20. 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;2013:409826.
21. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed 2006;2:45.
22. Rusdi S, Yogaswara H, Prabowo WT, Chafidz A. Extraction of natural dues from kesumba keeling (*Bixa orellana*) seed and secang (*Caesalpinia sappan* Linn.) wood for coloring fabrics. Mater Sci Forum 2020;981:179-84.
23. Kapoor VP, Katiyar K, Pushpangadan P, Singh N. Development of natural dye based sindoor. Nat Prod Radiance 2008;7:22-9.
24. Dequigiovanni G, Ramos SL, Lopes MT, Clement CR, Rodrigues DP, Fabri EG. New microsatellite loci for annatto (*Bixa orellana*), a source of natural dyes from Brazilian Amazonia. Crop Breed Appl Biotechnol 2018;18:116-22.
25. Quiroz JQ, Velazquez V, Garcia LL, Torres JD, Delgado E, Ciro G, Rojas J. Use of plant proteins as microencapsulating agents of bioactive compounds extracted from Annatto seeds (*Bixa orellana* L.). Antioxidants 2020;9:310.
26. Faria DV, Correia ND, Souza MV, Ríos AM, Vital CE, Batista DS, *et al*. Irradiance and light quality affect two annatto (*Bixa orellana* L.) cultivars with contrasting bixin production. J Photochem Photobiol B Biol 2019;197:1-9.
27. Junior RG, Bonnet A, Braconnier E, Groult H, Prunier G, Beaugeard L, *et al*. Bixin, an apocarotenoid isolated from *Bixa orellana* L., senitizes human melanoma cells to decarbazine induced apotopsis through ROS mediated cytotoxicity. Food Chem Toxicol 2019;125:549-61.
28. Lopez CP, Sumalapao DE, Villarante NR. Hepatoprotective activity of aqueous and ethanolic *Bixa orellana* L. leaf extracts against carbon tetrachloride induced hepatotoxicity. Natl J Physiol Pharm Pharmacol 2017;7:972-6.
29. Russel KR, Omoruyi FO, Pascoe KO, Morrison EY. Hypoglycaemic activity of *Bixa orellana* extract in the dog. Methods Find Exp Clin Pharm 2008;30:301-5.
30. Stohs SJ. Safety and efficacy of Bixa orellana (Achiote, Annatto) leaf extract. Phytotherapy Research (2013). Doi: 10.1002/ptr.5088.
31. Patnaik S, Mishra SR, Choudhury GB, Panda SK, Behera M. Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of *Bixa orellana* Linn. Int J Pharm Biol Arch 2011;2:1497-501.
32. Garcia AL, Duch ES, Mota DR, Glory LF, Lopez LL, Cabrera FR, *et al*. Pharmacological, phytochemical and morphological study of three Mayan accessions of *Bixa orellana* L. leaves. Emir J Food Agric 2017;29:163-9.
33. Thresiamma KC, George J, Kuttan R. Protective effect of curcumim, ellagic acid and bixin on radiation induced genotoxicity. J Exp Clin Cancer Res 1998;17:431-4.
34. Karchuli MS, Ganesh N. Protective effect of *Bixa orellana* L. against radiation induced chromosomal aberration in Swiss albino mice. Int J Phytomed 2009;1:18-21.
35. Basting RT, Abreu PM, Sousa IM, Carvalho JE, Carvalho PR, Fogilo MA. *Bixa orellana* L. by products fractions from an industrial process: Antiproliferative activity on tumor cells and chemical profile. Nat Prod Res 2020;4:1-7.
36. Parimalan R, Giridhar P, Rajasekaran T, Ravishankar GA. Annatto

fruit pericarp: Newer source as a potential fuel. Energy Fuels 2007;21:1181-2.
37. Viana JI, Zagmignan A, Lobato LF, Abreu AG, Silva LC, Sa JC, *et al*. Hydroalcoholic extract and ethyl acetate fraction of *Bixa orellana* leaves decreases the inflammatory response to *Mycobacterium abscessus* Subsp. *massiliense*. Evid Based Complement Altern Med 2018;2018:6091934.
38. Villar R, Calleja JM, Morales C, Cáceres A. Screening of 17 Guatemalan medicinal plants for platelet antiaggregant activity. Phytother Res 1997;11:441-5.
39. Karmakar UK, Sultana S, Nishi S, Biswas NN, Hossain L, Sheikh S. Antioxidant, analgesic, antimicrobial and anthelmintic activity of the dried seeds of *Bixa orellana* L. Int J Pharm 2018;8:150-63.
40. Castro GA. Helminths: Structure, Classification, Growth and Development. Galveston, TX: University of Texas Medical Branch at Galveston; 1996.
41. Haque R. Human intestinal parasites. J Health Popul Nutr 2007;25:387.
42. Utzinger J, Keiser J. Schistosomiasis and soil-transmitted helminthiasis: Common drugs for treatment and control. Exp Opin Pharm 2004;5:263-85.
43. Kucik CJ, Martin GL, Sortor BV. Common intestinal parasites. Am Family Phys 2004;69:1161-8.
44. Van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, *et al*. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop 2003;86:125-39.
45. Yadav P, Singh R. A review on anthelmintic drugs and their future scope. Int J Pharm Pharm Sci 2011;3:17-21.
46. Haines HH. The botany of Bihar and Orissa. Dehradun, India: Bishen Singh Mahendra Pal Singh; 2006.
47. Harborne JB. Phytochemicals Methods. London. Chapman and Hall Ltd.; 1973. p. 49-188.
48. Trease GE, Evans WC. Pharmacognosy. Philadelphia, PA: WB Scanders Company Ltd.; 1989. p. 89-300.
49. Sofowora A. Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa. Ibadan: Spectrum Books Ltd.; 1993.
50. Raaman N. Qualitative Phytochemical Screening and Phytochemical Techniques. New Delhi: New Delhi Publishing; 2006.
51. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Plant Med 1982;45:31-4.
52. Agarwal K, Singh H. An *in-vitro* evaluation of anthelmintic activity of *Morus alba* bark. MIT Int J Pharm Sci 2016;2:35-7.
53. Akakpo E, Badoussi ME, Gnacadja CK, Houngbo H, Dossou A, Gbaguidi F, *et al*. Ethnobotanical phytochemical and nutritional characterization of *Bixa orellana* Linn. seeds of Benin ecology. Int J Biosci 2020;17:46-56.
54. Goszcz K, Duthie GG, Stewart D, Lesilie SJ, Megson IL. Bioactive polyphenols and cardiovascular disease: Chemical antagonists, pharmacological agents or xenobiotics that drive an adaptive response? Br J Pharmacol 2017;174:1209-25.
55. Flores DM, Urizar GU, Colarossi RC, Garcia SC, Tovalino FM, Mendoza JD. Antibacterial activity of *Bixa orellana* L. (achiote) against *Streptococcus mutans* and *Streptococcus sanguinis*. Asian Pac J Trop Biomedicine 2016;4:400-3.
56. Cuong TV, Chin KB. Effects of annatto (*Bixa orellana* L.) seeds powder on physicochemical properties, antioxidant and antimicrobial activities of pork patties during refrigerated storage. Korean J Food Sci Anim Resour 2016;36:476-86.
57. Ahmed S, Moni BM, Ahmed S, Gomes DJ, Shohael AM. Comparative phytochemical, antioxidant, and antibacterial study of different parts of Doigota plants (*Bixa orellana* L.). Bull Natl Res Centre 2020;44:95.
58. Hagerman AE, Buttler LG. The specificity of proanthocyanidin-protein interactions. J Bio Chem 1981;256:4494-7.
59. Hassanpour S, Sadaghian M, Maherisis N, Eshratkhah B, Semsari MC. Effect of condensed tannin on controlling faecal protein excretion in nematode-infected sheep: *In vivo* study. J Am Sci 2011;7:896-900.
60. Goncalves ML, Mota AC, Deana AM, Cavalcante LA, Horliana AC, Pavani C, *et al*. Antimicrobial photodynamic therapy with *Bixa orellana* extract and blue LED in the reduction of halitosis a randomized, controlled clinical trial. Photodiagnosis Photodynamic Ther 2020;30:101751.
61. Ijeoma DP, Olaseike IO, Conrad DF. Phytochemical and proximate analysis of foliage and seed of *Bixa orellana* Linn. Int J Pharm Sci Rev Res 2016;36:247-51.
62. Alim S, Bairagi N, Shahriyar S, Kabir MM, Rahman MH. *In vitro* antibacterial potential of *Bixa orellana* L. against some pathogenic bacteria and comparative investigation on some standard antibiotics. J Pharmacogn Phytochem 2016;5:178-81.
63. Quiroz JQ, Duran AM, Garcia MS, Gomez GL, Camargo JJ. Ultrasound-assisted extraction of bioactive compounds from Annatto seeds, evaluation of their antimicrobial and antioxidant activity, and identification of main compounds by LC/ESI-MS analysis. Int J Food Sci 2019;2019:3721828.
64. Vera JSD, Castro EG, Dulay RM. Phytochemical constituents and teratogenic effect of lyophilized extracts of *Bixa orellana* L. (Achute) and *Piper betle* L. (Ikmo) leaves in Danio rerio embryos. Pharm Chem 2016;8:432-7.
65. Pande J, Chanda S. Mini reviews: Screening of Antioxidant Properties of Some Medicinal Plants. Proceedings of the National Conference on Innovations in Biological Sciences; 2020. p. 514-29.
66. Quiroz JQ, Torres AC, Ramirez LM, Garcia MS, Gomez GC, Rojas J. Optimization of the microwave- assisted extraction process of bioactive compounds from Annatto seeds (*Bixa orellana* L.). Antioxidants 2019;8):37.
67. Prathima D, Sujitha A, Usha R, Phytochemical screening and antimicrobial activity of *Bixa orellana* Linn. Int J Pharm Phytochem Res 2016;8:1078-82.
68. Tamil SA, 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 Pharm Sci 2011;1:116-20.
69. Ntungwe EN, Martin EM, Roberto A, Tavares J, Isca VM, Pereira P, *et al*. *Artemia* species: An important tool to screen general toxicity samples. Curr Pharm Des 2020;26:2892-908.
70. Padhi S, Panda SK. Phytochemical investigation and anthelmintic activity of various leaf extracts of *Bixa orellana* Linn. J Innov Appl Pharm Sci 2016;1:41-5.