

ISOLATION, EXTRACTION, ANTIOXIDANT ACTIVITY AND STANDARDIZATION OF NATURAL DYES FROM *BIXA ORELLANA* L. SEEDS AND THE LEAVES OF *LAWSONIA INERMIS* L.

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ABSTRACT

The antioxidant potential of different fractions of ethanol extracts of seeds of *Bixa orellana* L. (Bixaceae) and ethanolic extracts of leaves of *Lawsonia inermis* L. were investigated via 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity using ascorbic acid (Vitamin C) as a standard reference. The free radical scavenging activity of annatto extract ranged from 14.40% to 77.95% in *Bixa orellana* and 16.50% to 71.8 % in *Lawsonia inermis* relative to ascorbic acid (15% to 86%) at respective concentration between 10µm/ml and 50µm/ml. phytochemical analysis shows the presence of secondary metabolites such as alkaloids, saponin, tannin, terpenoids and phenolic groups. Hence, both the samples have potential source of new natural antioxidant. Another aim of this study is that the people to get the natural dyes instead of synthetic dye. Natural dyes are not only eco-friendly but also protect human skin. These two samples have both dyeing property and medicinal properties.

Key Words- Antioxidant, *Bixa orellana* L. *Lawsonia inermis* L. DPPH, Natural Dye.

INTRODUCTION

Plants have aided humanity since the dawn of time. In the present scenario, a great interest has been made towards the natural products derived from medicinal plants all over the world as they show to have enormous health care benefits such as antioxidative, antihypertensive, antimutagenic, skin disorders, antispasmodic and so on. Natural dyes have a big role in the world in different industries nowadays. For the utilization of synthetic dyes huge amount of water is requires, which resulting large quantity of effluent and it creates serious environmental pollution. Also, synthetic dyes have harmful effects on human life. According to the World Bank, 17-20% of industrial water pollution comes from synthetic textile dyes (Rashmi Srivastav and Neetu Sing, 2019).

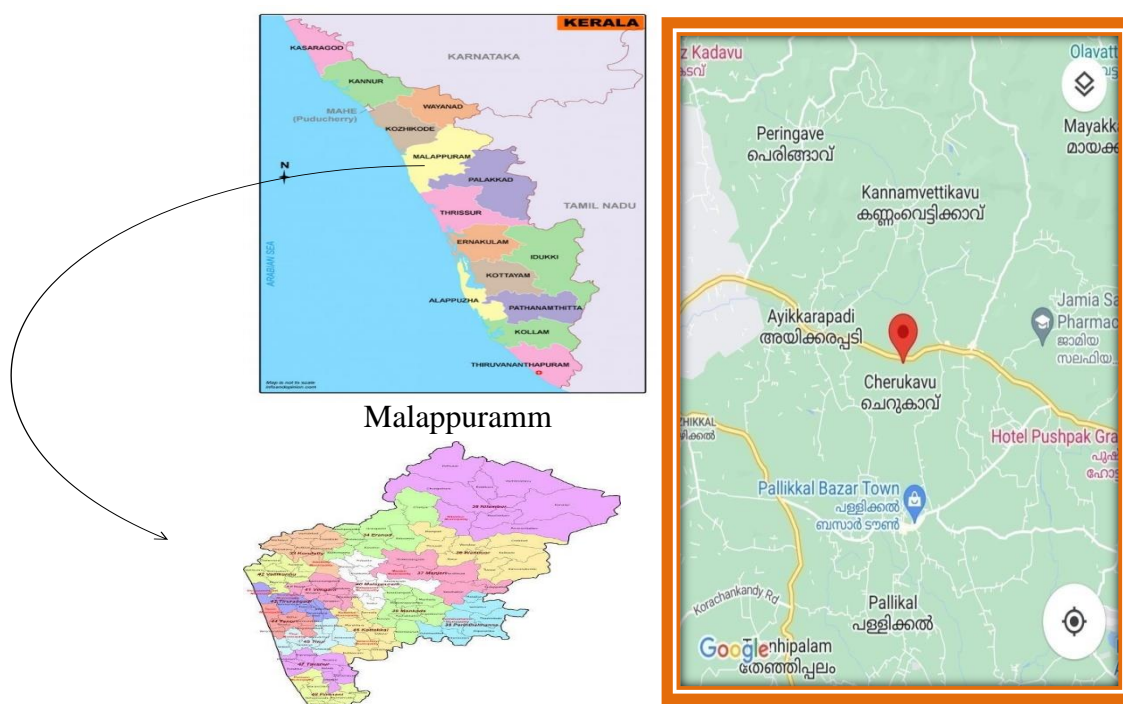
Natural dyes are derived from natural resources like plants, animals, minerals and microbial origins. Dyeing techniques included sticking plants to fabric otherwise rubbing crushed pigments into cloth for a long time. So that natural dyes were used for coloration of various textile materials. The textile industry of many countries enhancing their economy through using dyes. Therefore, the textile industry plays an important role in the economy of

Asia and other countries. Indigo, the blue dye still used in Jeans, is chemically the same as the dye extracted from the indigo plant (Mahabub Flasan *et al.*, 2015). There are many benefits of natural dyes. The preliminary screening and phytochemical analysis of henna plants can create wide information about the plant (Wasim Raja *et al.*, 2013). Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β -carotene, flavonoids and minerals Selenium are well known (Deepak *et al.*, 2015). Compounds from all parts of this plant exhibit a wide range of pharmacological activities that include antibacterial, antifungal, antioxidant, anti-inflammatory, neuropharmacological, anticancer, enhanced gastrointestinal motility, anticonvulsant, analgesic and antidiarrheal activities (Shahid *et al.*, 2016).

Materials and methods

Kerala is located in southern most part of India. Malappuram is a district in Kerala state. The district possesses 9.15% of the total area of the state. The district is located between 75° E -77° E longitude and 10° N -12° N latitude. Malappuram has a coastal area bounded by Arabian sea on the west, a midland at the center and a hilly area bounded by Western Ghats on the east. Cherukavu village is located between Kondotty and Ramanattukara. The average annual temperature is 31° C, humidity of 74.0% and precipitation here is about 1780 mm.

Plate-1: Study area and location map



Collection of the selected samples

The fresh samples were collected during the month of November 2021. The collected fruits of *Bixa orellana* L. plant were broken down and collected the seeds. The seeds were washed with running tap water twice to remove the impurities. Then it was spread on a blotting paper to remove excess water and the seeds were shade dried at room temperature. The dried seeds were made in to powder using Pulverizer. Likewise leaves of *Lawsonia* were collected, washed, dried and homogenized to made fine powder.

Sample -1: *Bixa orellana* L.**Systematic Position**

Kingdom : Plantae
 Division : Tracheophyta
 Class : Magnoliopsida
 Order : Malvales
 Family : Bixaceae
 Genus : *Bixa*
 Species : *orellana* L.

Morphological Description:

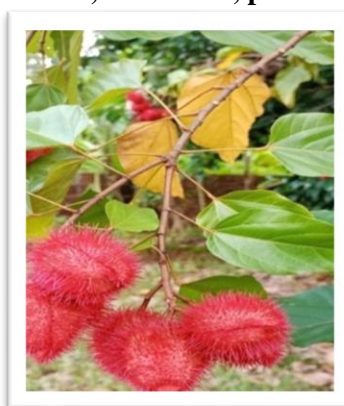
Bixa orellana L. is a shrubs or small evergreen tree. It is originally from Tropical America. About 2 to 9 m in height, the young branches may densely dark scaly, wood pale yellow and soft. Leaves are alternate, subcordate ovate at the base, having long-acuminate at apex. Pedicels are scaly and 7-10 mm long. Flowers are 3-5 cm long in across. Sepals are broadly ovate or suborbicular, concave. Sepals are scaly and purple in colour. Have many stamens and their filaments are slender. The common names are annatto and lipstick tree. It is also known as achiote. It is grown as ornamental plant. The shrub is most well known as the source of the red-orange annatto pigment.

Uses:

Bixa orellana L. seeds are often mixed with other seeds or spices to form a paste or powder for culinary uses. The seeds are heated in oil or lard to extract their dye and also flavor for use in dishes and processed foods like cheese, soup, gravy, butter and other items. The seeds impart a subtle flavor and also a yellow to reddish-orange color to food, used in traditional medicines. Also, the tree has been used in Ayurveda the folk medicine practices of India. Different parts of the plant are thought to be useful as therapy. The plant is valued for its stem fiber to make rope mats and also for the adhesive gum.

Plate-2: Habit, fresh and dried fruit, dried seed, powdered seeds of *Bixa orellana* L.

A



B



C



D



E

(A) Habit (B) Fruits (C) Dry fruits (D) Seeds (E) Seed Powder

Sample-2: *Lawsonia inermis* L.

Systematic Position

Kingdom : Plantae
 Division : Tracheophyta
 Class : Magnoliopsida
 Order : Myrtales
 Family : Lythraceae
 Genus : *Lawsonia*
 Species : *inermis* L.

Morphological Description

Lawsonia inermis L. is a woody shrub. The bark is ashy grey or brown. It is smooth and the branchlets are ending in spines. Leaves are 2.5-4.5 x 12 cm in length. The shape of the leaf is elliptic or oblong to oblanceolate. The base is attenuate and apex acute or rounded. Inflorescence is terminal cymose panicles. The pedicels are 2-4 mm long. Flowers are creamy white in colour and are 4-merous. Calyx tube is 2 mm long and cupular. Lobes are 4 and 2-3 mm long. Fruits are purplish green in colour and 4-6 x 5-7 mm in length, globose, which dehiscing irregularly. Seeds are 2.5 mm in length.

Uses: It is an astringent herb that controls bleeding and is antibacterial. Leaf extracts have an astringent effect on the skin and making it somewhat hydrophobic. A decoction of the plant bark is used as an emmenagogue, and also to treat liver problems and nervous symptoms. The crushed leaves are used to prepare a very fast reddish or yellowish natural dye. It is used for dyeing cloth and hair. Basket's production is possible using fibers of the branches and the stem bark.

Plate- 3: Habit, flower, fresh leaves, dried leaves and powdered leaves of *Lawsonia inermis* L.



A



B



C



D



E

(A) Habit (B) Flower (C) Leaves (D) Dry Leaves (E) Leaves Powder

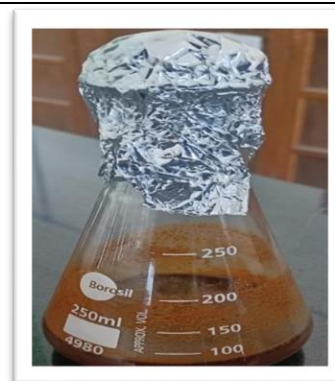
I-Extraction of natural dye from leaves of *Lawsonia inermis* L. -Maceration method:

A total of 100g of *Lawsonia inermis* L. dried leaves powder was macerated in 70% ethanol for 48 hours at room temperature, (Papita Das Saha *et al.*, 2012). The solution was filtered using Whatman filter paper. The filtrate was then concentrated using an evaporator. The obtained natural dye applied on cotton and observed.

Plate -4: Extracted dyes from the Sample



4-a. *Bixa orellana* L. from seed



4-b. *Lawsonia inermis* L. from leaves

II- Phytochemical analysis:

Preliminary Phytochemical analysis of the selected samples (Harborne 1998)

The major secondary metabolites like alkaloids, flavonoids, saponin, terpenoids, steroids, glycosides, tannins, Anthraquinones and primary metabolites such as proteins, amino acids and carbohydrates were analyzed as per standard procedure. The data were represented by table 1 & 2.

III- Estimation of antioxidant potential activity of the selected samples:

The free radical scavenging activity of methanolic extract of plant extract was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca *et al.*, 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.5 to 2.5 μ l of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolourization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes. Scavenging activity can be calculated by using the following equation:

$$\text{Scavenging activity \%} = \frac{A_{518}(\text{control}) - A_{518}(\text{sample})}{A_{518}(\text{control})} \times 100$$

RESULTS AND DISCUSSIONS

Application of dyes on cotton cloths

Natural dyes are obtained from natural sources, which having no harmful effects. They have significant role in the textile industry.

Table-1: Different colours of dye extraction

Samples	Parts used	Solvent	Colour of the dye	Colour of dye while applying on cotton cloth
<i>Bixa orellana</i> L.	Seeds	Ethanol	Red	Red
<i>Lawsonia inermis</i> L.	Leaves	Ethanol	Brown	Orange

The red and brown colour dyes were extracted by using maceration method with the solvent ethanol from *Bixa orellana* L. seeds and *Lawsonia inermis* L. leaves respectively within three to four days. The obtained natural red and brown colour dyes were taken in a petridish and a piece of cotton cloth; about 5cm × 5 cm sizes were dipped on the petridish separately. The petridish were kept in sunlight for 24 hours. After applying the obtained dye on cotton cloth, it shows red colour (Plate-5(b)) and brown colour (Plate-6(b)) respectively.

Plate -5(a): Showing the colour of dye from *Bixa orellana* L. seeds



Plate- 5(b): Showing the colour of dye from *Bixa orellana* L. seeds when applying on cotton cloth



Plate- 6(a): Showing the colour of dye from *Lawsonia inermis* L. Leaves

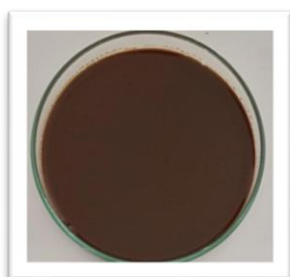


Plate-6(b): Showing the colour of dye from *Lawsonia inermis* L. Leaves when applying on cotton cloth



II -Phytochemical analysis

Phytochemicals are present naturally in plants. They have an important role in the therapeutic field. Identification of phytochemicals in plants implies the identification of new medicines for a disease. Phytochemicals can be considered as the gift of nature to the humans. The phytochemicals derived from plant serves as prototype to develop less toxic and more effective medicine. Through this study, we have also done the phytochemical studies of *Bixa orellana* and *Lawsonia inermis* for future medicinal uses.

Table- 2: Qualitative analysis of phytochemicals present in *Bixa orellana* L.seeds and *Lawsonia inermis* L. Leaves

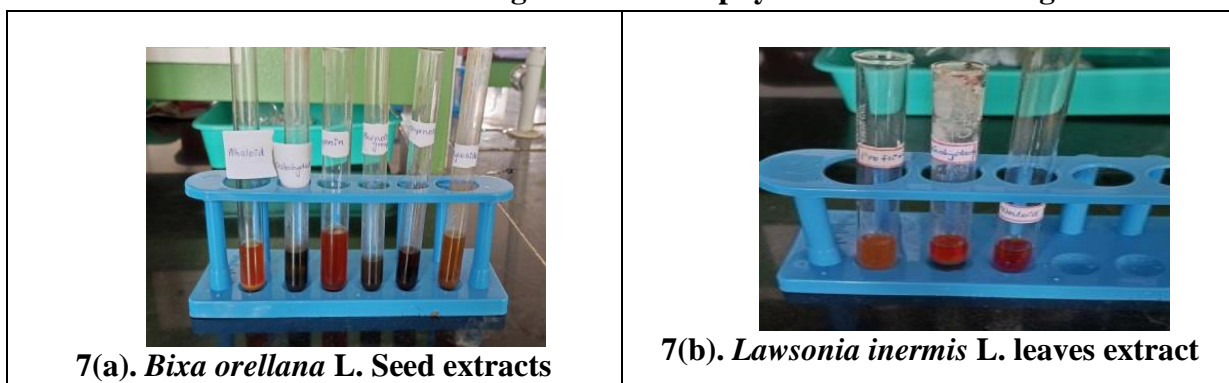
S.No	Phytochemicals	Inference of the <i>Bixa orellana</i> L. seeds extract in ethanol	Inference of the <i>Lawsonia inermis</i> L. leaves extract in ethanol
1	Alkaloids	+	+
2	Anthraquinone	-	-
3	Flavonoids	-	-
4	Carbohydrates	+	+
5	Glycosides	-	+
6	Protein	+	+
7	Saponins	-	+
8	Tannins	-	+
9	Phenolic groups	-	+
10	Terpenoids	-	+

* Key + Present, - Absent

*

The preliminary phytochemical screening was done in *Bixa orellana* L. seed extract prepared in ethanol solution. The test of carbohydrate, protein, alkaloid, anthraquinone, flavonoids, glycoside, saponin, tannin, phenolic groups and terpenoids were done in the sample. The sample showed the positive results in alkaloid, carbohydrate and protein and negative results in anthraquinone, flavanoid, glycoside, saponin, tannin, phenolic group and terpenoids (Plate:-7(a)). The preliminary phytochemical screening was done in *Lawsonia inermis* L. leaf extract prepared in ethanol solution (plate:-7-b). The sample showed positive result in alkaloid, carbohydrate, protein, saponin, tannin, phenolic groups, terpenoid and negative results in anthraquinone and flavanoid.

Plate -7: Showing the results of phytochemical screening



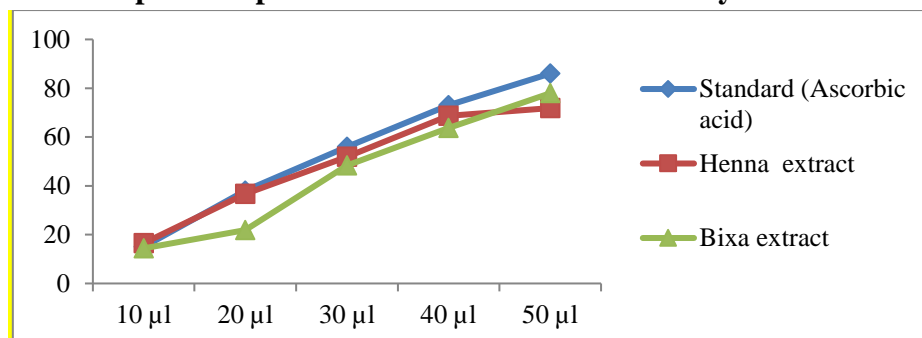
III- Antioxidant activity

Table: 3 Antioxidant activities of *Bixa orellana* L. seeds and *Lawsonia inermis* L. leaves

Concentration	Standard (Ascorbic acid)	<i>Lawsonia inermis</i> extract	<i>Bixa</i> extract
10 μ l	15 %	16.50 %	14.40 %
20 μ l	38 %	36.67 %	21.84 %
30 μ l	56 %	51.90 %	48.38 %
40 μ l	73 %	68.73 %	63.75 %
50 μ l	86 %	71.85 %	77.95 %

Extracts were subjected for the evaluation of antioxidant activity. DPPH radical scavenging activity was observed in both extracts. The *Bixa orellana* L. seed extract shows dominant activity followed by *Lawsonia inermis* L. leaves. Proton radical scavenging action was an important attribute of antioxidants which was measured by DPPH radical scavenging assay. Hydrogen donating ability of the antioxidant molecules contribute to its free radical scavenging nature. These two extracts show antioxidant activity in concentration dependent manner. The result shows that *Bixa orellana* L. seeds have high antioxidant activity than *Lawsonia inermis* L. leaves.

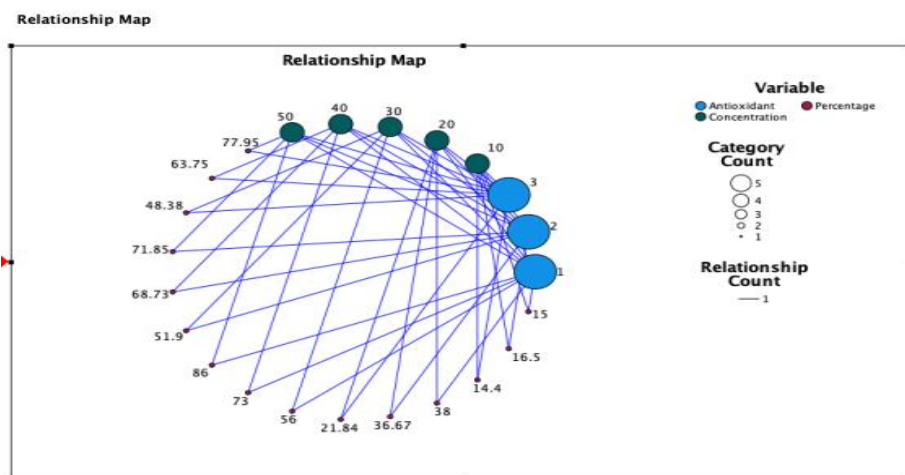
Chart:- 1 Graphical representation of antioxidant activity of selected samples



The relationship between the Factor (Concentration) and variables

The diagram shows the relationship between the presence of the Antioxidants activity standard (Ascorbic acid), *Lawsonia* Extract, and *Bixa* Extract. In this analysis, Antioxidants, 1 represents Standard (Ascorbic acid), 2 represents the *Lawsonia* Extract, and 3 represents the *Bixa* Extract, using this diagram we can find the individual relationship between the dependent and independent variables. This Diagram helps us to understand the percentage of antioxidants Activity is present in the selected 2 samples.

Chart-2 Antioxidant relationships between two samples



CONCLUSION

Natural dyes are obtained from natural sources like plants, invertebrates or mineral. They have significant role in textile industry. When using natural dyes there is no problem for our health and also having some benefits to health. Phytochemicals are substances made principally by plants and these substances have biological within the pharmaceutical industry. Plants represent the most supply to get varied active ingredients. They exhibit medicinal effects applicable to degenerative diseases and prevent or slowdown the damage to cells caused by free radicles. Though natural dyes are eco-friendly and protective to skin. Research shows antioxidant rich in the plant extract serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slowdown the degenerative diseases. So, the present study explores the antioxidant activity of commercially available *Bixa orellana* L. seed extract and *Lawsonia inermis* L. leaves extracts, and the study aims for the society to get the natural dyes instead of synthetic dyes. The extraction and application of natural dyes will lead to several developments. With the help of this knowledge people can completely avoid the use of synthetic dyes and come back to natural dyes.

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