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ASSESSMENT OF PHYTOCHEMICALS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *Bixa orellana* LEAF

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ABSTRACT

Bixa orellana is a well known plant in Asia, including India, which has a wide range of medicinal importance. The drugs of this plant are used in India as a folk remedy in the form of decoctions and infusions to treat bacterial infections and it is also effective against different skin diseases. The present study is to examine the bioactive compounds, Antimicrobial properties and antioxidant activity of leaves extracts of *Bixa orellana* with ethanol solvent. Phytochemicals such as Alkaloids, Flavonoids, phenols, carbohydrates, cardiac glycosides and saponins were recorded positive result in ethanol extract. Antibacterial activity of ethanolic extract was tested against *Pseudomonas aeuroginosa,* by well diffusion method to study the presence of various phytochemicals in *Bixa orellana* then the zone of inhibition 12.7 mm was calculated. Antifungal activity of ethanolic extract were tested against *Collectotrichum acutatum, Pemicillium griseofulvum, Alternaria alternate* and *Rhizopus oryza.* The leaves showed significantly higher moisture, flavonoids, phenols and saponins content; hence to ensure sustainable management of the plant resources, the leaves should be the primary target of any phytochemicals extraction activities for *B. orellana*.

KEYWARDS: Phytochemical, Flavonoids, Antibacterial, Antifungal, *Bixa orellana.*

INTRODUCTION

Plants are an important treatment in many countries and are the basis of drug development efforts (Revathi and Rajeswari, 2015). Developing countries are promoting the inclusion of traditional medicine especially through remedial treatment in local health systems (Florence et al., 2015). In this context, the World Health Organization (WHO) has reported strategies to ensure the safety and effectiveness of herbal products (WHO, 2013). Therefore, it is important to find new herbal remedies that include those that contain many active metabolites such as alkaloids, glycosides, resins, essential oils, gums, tannins, coumarins, saponins, anthraquinones, flavonoids and phenolic compounds (Himesh et al., 2011; Revathi and Rajeswari, 2015; Sepúlveda et al., 2016).

The growing demand for plants in the cosmetics, food and pharmaceutical industries suggests that systematic studies of medicinal plants are becoming increasingly important in the campaign to find effective combinations for use. (Nostro A et al., 2001) Traditional medicine is an important component. of health systems in developing countries. (UNESCO 1997) Medicinal plants play a major role in health care worldwide and about 80 percent of Africans rely on phytomedicine. There is a growing need for plants that can be medicinal plants, especially in Africa, such as HIV / AIDS, high blood pressure, sickle cell anemia, diabetes, and malaria. (UNESCO 1998).

Bixa orellana L. (Bixaceae), also known as annatto in English and Sinduri in Sanskrit, is native to tropical America but is now widely grown in tropical countries, including India. (Kirtikar KR, Basu BD, 1935, Cooke T. 1967, Naik, VN, 1998) Annatto seeds inhibits taste, febrifuge in action and is used as a remedy for Gonorrhea. The pulp surrounding the seed is used as a mosquito repellent, hemostatic, anti-dysentric, diuretic and is useful in treating epilepsy, kidney and other skin diseases. It is often used as an aphrodisiac, to treat inflammatory conditions and parasitic diseases. A decoction of the leaves is used to prevent vomiting and nausea; to treat urinary and gastrointestinal problems. (Giorgi A et al., 2013) The non-toxic Annattol dye found in pulp is used to dye foods such as butter, ghee, margarine, cheese and chocolate. (Voeks RA, Leony A, 2004) The roots and root bark are sweaty and act as an antipyretic used as an antiperiodic and to control the Asthmatic Paroxysm(Chopra, R et al., 2002, Prajapati ND et al., 2003).

Bixa orellana - Annatto, the main use of bright red fruit (seedpods) as a natural color for food, fabrics, materials, body, hair and face hence the name "Lipstick tree". The

ancient Maya and the Aztecs considered the Annatto to be a symbolic plant. Ancient Maya texts were written with ink made of annatto juice and both civilizations adopted the juice instead of blood and thus called sacred meanings. Armerindians eat seeds to be brave and as an aphrodisiac. The whole plant has a long history as a valuable medicinal plant used to treat various ailments ranging from flu to cancer. (Von Carlowitz, 1991; Liogier HA, 1990) Leaf extraction, including seed color produces a beverage that helps relieve the discomfort associated with the female reproductive system. The juice prepared from the pulp of dried fruit is used as a remedy against prussic acid present in Manihot esculenta and other toxic tropical plants, such as *Jatropha curcas* and *Hura crepitans.* (Liogier HA, 1990).

The present study focuses on the screening of phytochemicals present in leaves of *Bixa orellana* L. for identifying their chemical constituents. This study is also evaluated the antimicrobial activity.

MATERIAL AND METHODOLOGY

Plant Sample Collection and Extract Preparation

The leaves of *B. orellana* L. were collected from Medicinal plant supplier. Leaves were shade dried and pulverized. 20 g of the powder was subjected to cold extraction with 100 ml of Ethanol.

Test Organisms

The microorganisms used for the Antimicrobial activity were purchased from the Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh. The Gram-ve bacteria *Pseudomonas aeroginosa* MTCC 2582 and *Collectotrichum acutatum* 3960, *Pemicillium griseofulvum* ATCC 2216, *Alternaria alternate* MTCC 960 and *Rhizopus oryza* MTCC 201 were tested. Fungal strains were maintained in Potato dextrose agar (Merck) and Sabouraud's glucose agar (Merck)

Preparation of Inoculum

Microbial cultures for experiments were prepared through transferring a loopful of culture from the stock cultures to Sabouraud Dextrose Broth (SDB) for fungi were incubated for 24 hours at 25°C and Mueller- Hinton broth (MHB) for bacteria and were incubated for 24 hours at 37°C. (Irobi et al., 1996).

Preliminary Phytochemical Screening

Phytochemical screening of the Ethanol leaves extract was carried out method given by Kokate C.K 1986 and Mohammed et al., 2010. Alkaloids, carbohydrates, Flavonoids, phenols, amino acid and protein, saponins, tannins, terpenoids, quinons, resins and coumarins were tested of leaves extracts.

Antibacterial Activity

In vitro antibacterial assay was performed by Well Diffusion (Kirby-Bauer) method. The Mueller- Hinton agar plates were prepared by pouring 20ml media into sterile petriplates. The plate was allowed to solidify and 100µl inoculum suspension of tested organisms was spread over the media. 250 µg of the extracts was loaded on 5 mm well & thoroughly dried in air draft to remove traces of the solvent. Negative control was prepared using respective solvent. Streptomycin (10 µg) was used as positive control. The plate was incubated at 37°C for 24 hr. Inhibition zones formed around the well were measured with transparent ruler (in millimeters) (Kumaraswamy et al ., 2002).

Antifungal Activity

Antifungal activity was performed by the standard procedure (Hari Babu *et al.*, 2011). 1 ml of ethanol extract of leaves of *B. orellana* was added to 100 ml of Potato Dextrose Agar medium and poured into sterile petriplates. After solidification, a loop full of culture was placed on the centre of the plate. Controls were maintained with DMSO. The plates were incubated at 27ºC Growth was monitored for 72 hours. The growths of treated samples were compared with their respective control plates.

Antioxidant Activity

The reaction mixture consisted of 100µl deoxyribose (3mM), 50µl $FeCl_3$, 50 µl EDTA (0.1mM), 100 µl H_2O_2 (1mM) in required amount of PBS. 100 µl of extract was then added to this mixture and make a final volume of 1ml in each test tube. The reaction mixture was kept for 1 hr. at room temperature in dark. The mixture was then incubated for 20 minutes in a boiling water bath with 0.5 ml of 5% TCA & 0.5ml of TBA. The absorbance was taken at 532 nm in an UV Spectrophotometer. The test tube with PBS was considered as BLANK & DMSO was used as positive control. The results were expressed as % Inhibition of TBARS. Ascorbic acid was used as a standard.

$$\% \text{ TBARS Inhibition} = \frac{\text{Blank} - \text{Ascorbic Acid or Drug}}{\text{Blank}} \times 100$$

RESULT AND DISCUSSION

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of crude ethanol leaves extract indicated the presence of Alkaloids, Flavonoids, phenols, carbohydrates, cardiac glycosides, saponins and tannins, terpenoids, quinons and coumarins in both leaf extracts of *B. orellana*. (Table 1)

Table 1: Phytochemical analysis of *B. orellana* leaf extracts.

SN	Test	Ethanol Extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Phenols	+
4.	Terpenoids	+
5.	Quinons	+
6.	Carbohydrates	+
7.	Cardiac glycosides	+
8.	Saponins	+
9.	Tannins	+
11.	Resins	-
12.	Coumarins	+

Antimicrobial Assay

Leaf extracts of *B. orellana* exhibited dose dependent antimicrobial activity against the tested pathogens. Ethanol leaf extract showed 18±0.1 against *P. aeruginosa* compared to Streptomycin control 21.8±0.3 mm on average. (Table 2.). The blind control (DMSO) exhibited 'nil' inhibition. Ethanol and acetone extract of leaf was found to be the most active against the tested fungi *Collectotrichum acutatum, Pemicillium griseofulvum, Alternaria alternate* and *Rhizopus oryza* and result were presented in Table 3.

Table 2: Antibacterial Activity of Ethanol and Acetone leaf extract against *P. aeruginosa.*

SN	Ethanol Extract	Streptomycin
1.	12.7 mm	28.8 mm

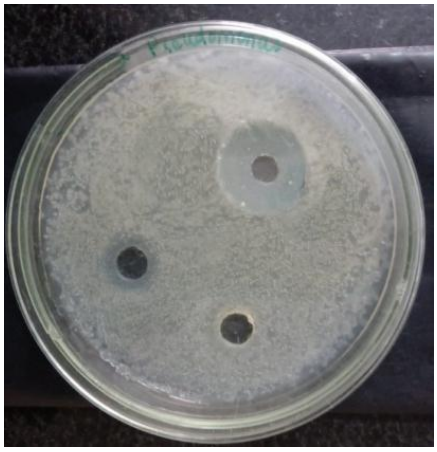

Fig. 1: Antibacterial Activity.

Table 3: Antifungal Activity of Ethanol leaf extract against *P. aeruginosa.*

Organism Name	25%	50%	75%	100%
Collectotrichum acutatum	5±0.2	15±0.1	10±0.1	15±0.1
Pemicillium griseofulvum	5±0.2	5±0.2	20±0.1	10±0.1
Alternaria alternate	15±0.1	15±0.1	20±0.1	10±0.1
Rhizopus oryza	10±0.1	10±0.1	20±0.1	20±0.1

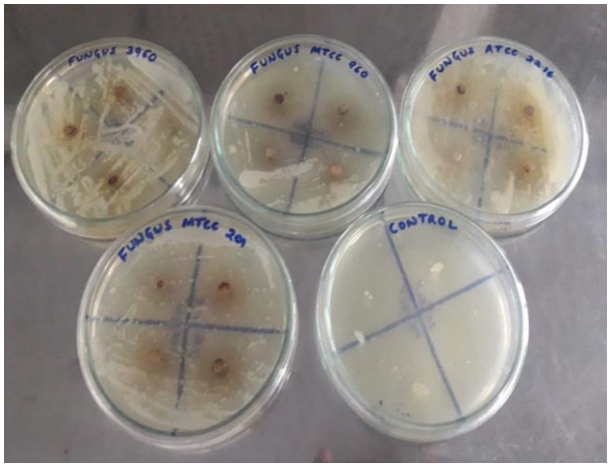

Fig. 2 Antifungal Activity.

Antioxidant Assay

The high amount of phenolic compounds recorded indicates that *Bixa orellana* possess high antioxidant potentials. Antioxidant activity of Ethanol leaf extract of *Bixa orellana* 68.5% was recorded in percentage of Inhibition of TBARS.

CONCLUSION

Bixa orellana provides a range of food intake once pharmaceutical industries, beyond the present role established as color and food additive as well feed. Its phytochemical elements and content show a plant that forms a good source of phytochemicals tannins, glycosides, phenols, saponins and flavonoids. The leaves constitute the primary target for sourcing such phytochemicals in *B. orellana*. Seeds may find other apps in preparing food for it content of low antinutritional properties. Versatility as well the firmness of the plant, so that it can grow in almost all of them soil types mean that Annatto can be grown in high altitude volume with minimal input. This will slow down the harmful effects of a full-blown harvest plants by indigenous peoples, researchers as well such bioprospectors are chemicals.

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