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Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *Artemia salina* L.

S. Kumar, P. K. Tripathy and P. K. Jena[Ψ]

Department of Botany, Ravenshaw University, Cuttack- 753003, India

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ABSTRACT

Bixa orellana L. (Kum Kum) belonging to the family Bixaceae is a indegineous plant in Odisha. It is also cultivated at some places. The leaves and fruits of the plant are used as dye and as natural lipstick. They are also used as dye for colouring the vegetables and dishes. The plant and its parts are used in treatment of different diseases by the rural and some peripheral tribal communities of Odisha, such as those residing in Simlipal Biosphere Reserve Forest. Experiments were designed to study the ethnobotanical use of the plant among tribal groups of SBR, Odisha and the bioactive compounds presents in the plant and or its leaves and also whether the leaf extracts posses any toxic effect or not. The results revealed the potent bioactive compounds in the leaves of *Bixa orellana* L. The study further exhibited that the leaf extracts posses no toxic action against *Artemia salina* L., an experimental arthropod. Thus the studies emphasize upon the potent bioactive compounds present in the leaves of *Bixa orellana* L. without any toxic effect.

Medicinal plants are of great importance to the health of individuals and communities as well. The medicinal value of plants lies in some biochemical substances present in them that produce a definite physiological action on the human body (Okwu, 2001).The use of herbal medicine for the treatment of diseases and infections is an age old practice. The World Health Organization supports the use of traditional medicines provided they are proven to be efficacious and safe. In developing countries, a large number of people live in extreme poverty and many of them are suffering and dying for want of safe water and medicine, they have no alternative for primary health care (WHO, 1985).Therefore, the need to use medicinal plants as alternatives to medicines in the absence of primary healthcare cannot be avoided. More so herbal medicines have received much attention as sources of bioactive compounds since they are considered as time tested and relatively safe for human use and are also environment friendly (Fazly *et al*., 2005) Therefore, there is the need to search for more and more herbal medicinal plants with an aim to validate the ethno-medicinal use and subsequently the isolation and characterization of compounds in them which can be added to the potential list of drugs. *Bixa orellana* L. is a well studied plant of the family Bixaceae which is either naturally grown or cultivated in Odisha. *Bixa orellana* L. is a small tree with a round head, generally grown as an ornamental plant in rural areas because of its lovely flowers of various colors, such as dark red, light red and shiny red. The height ranges from 3-10 meters, leaves glossy ovate and ever green with reddish veins; heart shaped base and a pointed tip. The plant is with a thin, long stem and the leaves are between 8-12 cm long and 5-14 cm wide. Fruits round, with dense soft bristle or a smooth surface. Seed numerous, brown or black. Its seeds produce a natural dye used for colouring by the tribal people of Odisha in Simlipal Biosphere Reserve Forest and other forest block. It is used for colouring foods and cosmetics. It is a native of tropical America but is now distributed in most tropical countries in both wild as well as cultivated forms. In India, it is found mainly in Odisha, Andhra Pradesh and Maharashtra and to

[Ψ] *Corresponding author; Email:* padan_jena@rediffmail.com

some extent in Kerala, Karanataka and Tamilnadu (Venugopalan *et al.,* 2011) .It is native to Tropical America because it requires full sunlight and protection from wind. It grows equally well in lowlands as well as mountainous regions and areas of higher elevation. It is believed to come from Brazil and was carried through Central and South America to India. Indians used its color as body paint and also as lipstick. In India, the plant is also used by Ayurveda practitioners as an astringent and mild purgative and is considered by them as a good remedy for treating dysentery and kidney diseases. The root bark is antipyretic too. In Philippines, the leaf decoction is used to cure skin diseases and burns. The leaves are a popular febrifuge in Cambodia. The infusion of leaves is prescribed as a purgative and is used in the treatment of dysentery. In Central America, the oil derived from seeds is used to cure leprosy and decoction is utilized to treat jaundice (Metta *et al.,* 2009).

Bixa orellana L. leaves were collected from Medicinal Germplasm Garden of Regional Plant Resource Centre, Bhubaneswar. The collected plant materials were washed thoroughly by tap water followed by distilled water twice, and were oven dried. The dried materials were crushed to powder with mechanical devise and were kept in an air tight container for qualitative analysis of bioactive compounds. Qualitative phytochemical analysis were done using percolation method for crude extract (Harborne, 1973; Trease and Evans, 1989). The Brine shrimp assay was carried out according to the principle described by Meyer *et al.* (1982) and Krishnaraju *et al.* (2005).

Solvent extract was obtained from 5g of leaf powder which was macerated in solvent for 12 h in refrigerator. After 12 h sample was filtered and residue was again macerated in same solvent and for each solvent the process was repeated thrice. Solvent extraction was started with methanol followed by acetone and chloroform. Aqueous extract was prepared separately by taking the powder in distiled water followed by filtration. Filtrates were dried and concentrated to get semisolid mass. All the phytochemical assays were conducted using the same extracts. The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which might provide an indication of possible cytotoxic properties of the test materials. Brine shrimp nauplii have been previously utilized in various bioassay systems (McLaughlin *et al.,* 1991). It is a typical primitive arthropod with a segmented body to which is attached broad leaf-like appendages. The body usually consists of 19 segments, the first 11 of which have pairs of appendages, the next two which are often fused together carry the reproductive organs, and the last segments lead to the tail. The total length about 4 mm (Criel and Macrae, 2002). It is found suitable as a "standard" organism in toxicological assays, despite the recognition that it is too robust an organism to be a sensitive indicator species (Criel and Macrae, 2002). Shrimp eggs were kept for hatching in 6 % normal saline for 18 h. Plant extract obtained following the above protocol were subjected to motility assay. Readings were taken every hour up to 4 h and later at 24 h. Motility parameters (Table 1) such as, +4 indicate highly motile, +3 indicate motile, +2 indicate sluggish and +1 indicates slow.

Field survey has indicated sound ethnobotanical uses of Kumkum, particularly leaves among the aboriginals of SBR forest. Leaf juice is used against snakebites among the tribal communities of Kukurvuka village of Ghatkumar range of SBR forest. Leaf juice is used to treat gonorrhoea among the **Lohar** community of Sanuski village of Gurguria range of SBR forest. Leaves paste is applied to cure skin infections among the **Kolhoo** and **Khadia** tribe of SBR forest. This plant was classified by the Food and drug Administration of the U.S.A. as a "Color additive exempt of certification" (Paula *et al.,* 2009). The qualitative analysis of bioactive compounds of the leaves of *Bixa orellana* L. in different organic solvents and in aqueous extract were excellent, In aqueous extract tannis, anthraquinone, flavonoids, saponin, terpenoids and steroids were presents (Table 2). Flavonoids are known to have anti-fungal activities (Havsteen, 1983) which justifies the tribal claim of use of leaf paste is used against skin infections. Tannins were present in aqueous and methanol extracts which is also reported to have anti-fungal activities (Doss *et al.,* 2009). Terpenoids were present in aqueous and acetone extract which is known to be good against cancer (Tsuyoshi *et al.,* 2010). Glycosides were present in methanol, acetone, hexane and ether extracts, which is effective for heart problems (Schoner and Scheines, 2007). Steroids were found only in aqueous extract (Table 2). The study revealed that the leaf of *Bixa orellana* L. is rich in bioactive compounds. In order to asses the toxicity of the extracts BSLA was carried out. The Brine shrimp assay indicated no death of *Artemia salina* L. by organic and aqueous extract of leaves of *Bixa orellana* L. (Table 1).

The results of present study revealed the phytochemical value of *Bixa orellana* L. Qualitative investigation of bioactive compounds of leaves indicates the presence of tannins, glycosides, anthraqunine, flavonoids and saponins. The above observations support the presence of bioactive compounds in *Bixa orellana* L. and justify the usefulness of this plant in the treatment of various diseases. In addition to prepare as medicine, it can also be safely used as a dye or coloring materials due to its

Table 1
Toxicity of different leaf extracts of *Bixa orellana* L. against *Artemia salina* L.

Sample 5µl/ ml	10:30 AM	11:30 AM	12:30 PM	1:30 PM	2:30 PM	After 24 h
Control	4+	4+	4+	4+	4+	4+
Hexane ext.	4+	4+	4+	4+	4+	4+
Ether ext.	4+	4+	4+	4+	4+	3+
Chloroform ext.	4+	4+	4+	4+	4+	4+
Acetone ext.	4+	4+	4+	4+	4+	4+
Meth. ext.	4+	4+	4+	4+	3+	3+
Aqueous ext.	4+	4+	4+	4+	4+	4+

(+4 indicates highly motile, +3 indicates motile, +2 indicates sluggish and +1 indicates slow, ext-extract)

Table 2
Variations of bioactive compounds in the different leaf extracts of *Bixa orellana* L.

Bioactive compounds	Aqueous extract	Methanol Extract	Acetone extract	Chloroform extract	Hexane extract	Ether extract
Tannins	+ve	+ve	- ve	- ve	- ve	- ve
Anthraquinone	+ ve	- ve	- ve	- ve	- ve	- ve
Flavonoids	+ ve	- ve	- ve	- ve	- ve	- ve
Saponin	+ ve	- ve	- ve	- ve	- ve	- ve
Phlobatanin	- ve	- ve	- ve	- ve	- ve	- ve
Terpenoids	+ ve	- ve	+ ve	- ve	- ve	- ve
Glycosides	- ve	+ ve	+ ve	- ve	+ ve	+ ve
Steroids	+ ve	- ve	- ve	- ve	- ve	- ve

(+ve – Present, -ve – Absent)

non-toxic effect. Further work can be carried out to evaluate the specific bioactive compounds against specific pathogens and successful use of these compounds to preparation of medicines.

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