WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 2715-2719. Research Article ISSN 2277– 7105

BIXA OREALLANA. L: A STUDY OF PHYTOCHEMICAL, CYTOTOXIC AND ANTIOXIDANT ACTIVITY PROFILE OF THE LEAF EXTRACTS OF THE PLANT.

Sunita Bhatnagar*, Sasmita Swain and Manasi Sahoo

Medicinal and Aromatic Plants *Regional Plant Resource Centre, Nayapalli,* Bhubaneswar 751015, India,

Article Received on
12 Aug 2015,

Revised on 04 Sept 2015,
Accepted on 27 Sept 2015

***Correspondence for Author**
Dr. Sunita Bhatnagar
Medicinal and Aromatic Plants Regional Plant Resource Centre, Nayapalli, Bhubaneswar 751015, India,

ABSTRACT

Solvent extracts of Bixa oreallana leaves were tested for phytochemical, antioxidant, and cytotoxic activity. In phytochemical analysis aqueous extract showed the presence of tannins, saponins, flavanoids, terpenoids, steroids and hydroquinone. Brine shrimp assay was done to study the cytotoxic activity followed by MTT reduction assay, a mild cytotoxic activity was observed in ethyl acetate extract. Antioxidant activity was conducted using TLC based DPPH assay, Chloroform and ether extracts showed significant antioxidant activity followed by acetone and methanol extracts with similar antioxidant profile. Extracts lacked cytotoxic potential as depicted by brine shrimp assay.

KEYWORDS: cytotoxic, antioxidant, extracts, phytochemical.

INTRODUCTION

Bixa Orellana is commonly known as annatto and is popular because its seeds produce a natural dye which is used as food colour.[1] Colouring agent isolated from the plant is a carotenoid known as bixin. This is the only certified naturally occurring food colour by FDI.[2] and its production does not need any license. The tincture, made from the fruit and seeds, is anti-fungal, anti-oxidant, anti-bacterial, anti-inflammatory. Based on the traditional usage a number of studies have been conducted for its antimicrobial potential.[3,4] As sited from the literature fruits and seeds have been reported to possess a number of medicinal properties, hence in this study medicinal potential of the leaves of the plant were explored for their phytochemical, cytotoxic and antoxidant potential.

MATERIALS AND METHODS

Collection and Processing Planting material

Leaves of Bixa orellana were collected from medicinal germplasm garden of Regional plant Resource Centre, Bhubaneswar. Leaves were washed thoroughly under running tap water, were dried in shade and pulverized in mechanical grinder of Lexus make. Weight of wet leaves and dried leaves were taken to study the moisture content of leaves.

Solvent extract preparation

Solvent extracts were prepared by cold maceration technique, 5 gms of leaf powder was macerated in solvent for 12hrs in refrigerator. After 12hrs sample was filtered and residue was again macerated in same solvent and for each solvent, process was repeated thrice

Aqueous extract was prepared separately by taking the powder in distilled water followed by filtration. Filtrate were dried and concentrated to get semisolid mass. All the phytochemical Assays were conducted using the same extracts.

Phytochemical analysis

Phytochemical tests were conducted using the standard protocols.[5]

Antioxidant test

Antioxidant assay was conducted using Thin layered chromatography based DPPH **(**2, 2diphenyl-1-picryl hydrazyl) assay. Chromatograms were developed in three solvents, these were as follows.

1. Ethyl acetate/methanol/water (40:5.4:4) [EMW] (polar neutral).
2. Chloroform/ ethylacetate/ formic acid (5:4:1) [CEF] (intermediate polarity/acidic).
3. Benzene/ethanol/ ammonium hydroxide (90:10:1) [BEA](Nonpolar/basic).[6] Cytotoxic activity**.**

Brine shrimp assay

Brine shrimp eggs were kept for hatching in 6% normal saline for 18hrs, extracts obtained by following the above protocols were subjected to motility assay.[7] Readings were taken every hour up to 4hrs and later at 24 hrs.Motility of graded as below.

4+ highly motile, 3+ motile, 2+ sluggish, 1+ slow, Nil no activity at all

RESULTS AND DISCUSSIONS

Phytochemical analysis

As can be observed from the Table 1, Aqueous extract was the richest amongst all the extracts, it contained tannins, anthroquinones, flavonoids, steroids, saponins and terpenoids. Methanol extract consisted of only tannins and glycosides whereas acetone extract showed the presence of terpenoids an important class of molecule. Terpenoids were earlier reported in the ether extract of Bixa orellana.[8] however same was not found in this study.

Antixoidant activity

Thin layered chromatography based DPPH assay gives a fairly good amount of antioxidant potential of various extracts. Number of yellow bands against purple background is considered as antioxidant bands. As can be seen from the Table 2, Benzene/ethanol/ ammonium hydroxide (90:10:1) BEA solvent showed the best separation of extracts with chloroform and ether extracts with maximum number of antioxidant potential.

As can be seen from the figure chloroform and ether extracts showed very closely situated antioxidant bands about 14 and 16 anti oxidant bands respectively, suggesting a promising antioxidant potential of the two extracts. Polar extracts methanol and antioxidant also showed promising antioxidant bands (Fig 1).

Cytotoxic activity

Brine shrimp assay is well known for ascertaining toxicity of plant products[9] . Leaves of Bixa oreallana showed no toxicity in any of the extract, only ethyl acetate extract showed mild activity as can be seen from the Table 3. Thus leaves are totally safe as fodder for animals.

Table: 1 Presence of Phytochemicals in different extract

Class of compound	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

Table 2: Number of antioxidant bands in three different solvents.

Solvents	Hexane	Chloroform	Ether	Methanol	Aqueous	Acetone
BEA	4	14	16	5	0	7
CEF	0	3	3	streak	streak	streak
EMW	0	3	2	2	2	3

Table 3: Cytotoxicity activity of extracts of leaf of Bixa orellana against Brine shrimp assay and MTT reduction assay

Sample	10.30	11.30	12.30	1.30	2.30	after24hrs	MTT reduction assay
Control	4+	4+	4+	4+	4+	4+	
Hexane	4+	4+	4+	4+	4+	4+	No Inhibition
Ether	4+	4+	4+	4+	4+	4+	No Inhibition
Chloroform	4+	4+	4+	4+	4+	4+	No Inhibition
Ethyl acetate	4+	4+	4+	4+	4+	3+	29%
Acetone	4+	4+	4+	4+	4+	4+	No Inhibition
Methanol	4+	4+	4+	4+	4+	4+	No Inhibition

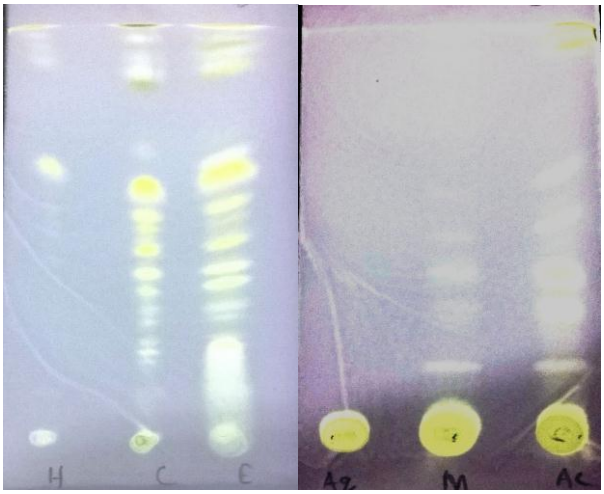

Fig 1: DPPH assay of solvent extracts of Bixa oreallana leaf extracts

H=hexane, C= chloroform, E= Ether, Aq= aqueous, M=methanol Ac= Acetone

CONCLUSION

Leaf extracts of Bixa oreallana possessed a number of phytochemical constituents, lacked cytotoxic activity but were rich in antioxidant potential.

ACKNOWLEDGEMENTS

Acknowledgements are due to Govt. of Orissa for providing funding for the project fellow Manasi Sahoo. Acknowledgements are also due to the Forest and Environment Department, Government of Orissa for the laboratory facilities.

REFERENCES

1. Srivastava A, Shukla YNM, Jain SP and Kumar S. Chemistry, pharmacology and uses of Bixa oreallana: A review. J Med Aro Plt Sci, 1999; 21: 1145-54.
2. Hallagen JB, Allen DC, Borxelleca JF. The safety and regulatory status food, drug and cosmetic color addictives exempt from certification. Food and Chem Toxicol, 1995; 33: 515-28.
3. 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 International Journal of Pharmaceutical & Biological Archives, 2011; 2(5): 1497-1501.
4. Fleisher TC, Ameade EPK, Mensah MLK, Sawer IK Antimicrobial activity of the leaves of Bixa orellana. Fitoterapia, 2003; 74(1): 136–138.
5. Harborn, JB. Phytochemical Methods: A guide to Modern Techniques of plants Analysis, Chapman & Hall. London, Ltd, 1973; 49-188.
6. Eloff, JN., Katerere, DR., McGaw, LJ. The biological activity and chemistry of the southern African Combretaceae. J Ethnopharmacology, 2008; 119: 686–99.
7. Bhatnagar S, Sahoo S, Mohapatra AK and Behera DR. Phytochemical analysis, Antioxidant and Cytotoxic activity of medicinal plant Combretum roxburghii (Family: Combretaceae). Int J of drug dev res, 2012; 4(1): 193-202.
8. Pino JA, Correa MT. Chemical composition of the essential oil from annatto (Bixa orellana L.) seeds. J Essent Oil Res, 2003; 15(2): 66–67.
9. Meyer BN, Ferrign RN, Putnam JE, Jacobson LB, Nicholas DE, and McLaughlin, JL. Brine shrimp: A convenient general bioassay for active plant Constiutuents. Planta medica, 2003; 45: 3134-39.