ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MICROBIOLOGY

ANTIBACTERIAL POTENTIAL OF THE AQUEOUS AND ORGANIC EXTRACTS OF *BIXA ORELLANA* L.

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ABSTRACT

Bixa orellana is valued for its food and medicinal uses. It is considered as a good remedy for treating dysentery and kidney diseases. The present study dealt with antibacterial potential of the crude aqueous and organic extracts from leaves, seeds and empty seed capsules of *Bixa orellana* against one Gram-positive and three Gram-negative bacteria by using agar diffusion method. The leaf extracts of methanol, dimethyl sulphoxide, ethanol and acetone exhibited potential and significant antibacterial activity against *Staphylococcus aureus* at 800, 1600; 1600 and 3200 µg/ml, respectively. However, none of the aqueous extracts showed antibacterial activity against the tested bacteria. The extracts of dimethyl sulphoxide and methanol from empty seed capsules also showed effective antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi* at 3200 and 800 & 3200 µg/ml, respectively. Various solvents and aqueous extracts of the seeds did not show any appreciable antibacterial activity except in dimethyl sulphoxide solvent extracts. Moderate growth inhibition zone of *Salmonella typhi* and *Staphylococcus aureus* in dimethyl sulphoxide was noticed at high concentrations of 6400µg/ml.

ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

KEY WORDS

Antibacterial potential, aqueous extracts, organic extracts, *Bixa orellana* L.

INTRODUCTION

Bixa orellana L. commonly known as annatto belonging to the family Bixaceae is valued for its food and medicinal uses. In developing countries people of native communities use this plant in folk medicine for the treatment of common infections in the form of decoctions, teas, juices etc.[1]. The plant grows equally well in low lands and mountainous regions or areas of higher elevations[2]. It is native to the tropical America and is found in large quantities from Mexico to Ecuador, Brazil and Bolivia. This plant is cultivated in warm regions of the world, such as India, Sri Lanka and Java mainly for the dye obtained from the seeds[3]. In India, the plant is cultivated and found wild especially in Western parts of the country.

Bixa orellana is a shrubby tree which ranges from 3-10 meters in height. Its glossy cordate acuminate leaves are ever green with reddish veins with a thin long petiole. The young twigs are covered with rust colored scales and became bare when older. Flowers are white or purplish white in color. Fruit is a capsule, reddish brown, soft and with bristly hairs. The two valved round fruits are approximately 4cm wide; appear in a variety of colors like scarlet, yellow, brownish yellow and bright red. When ripe, the fruits split open and reveal a numerous amount of small, fleshy seeds about 5mm in diameter and covered with red orange pulp[4].

The plant is used medicinally in Indo-China, the Philippines, Brazil, Guiana, Cambodia, North West Amazonia, Uruguay, West India, Central America and Venezuela. The dye obtained from the pulp of the seeds called bixin is used all over the world as a red orange dye for coloring rice, cheese, soft drinks, oil, butter and soup. The dye is also used in some regions to dye textiles and seeds are used as a condiment[5,6,7]. Various Indigenous groups paint their hair and bodies with the pulp to repel insects and protect from sunburn. The seeds are given to bulls to make them aggressive for bull fighters and are taken by Indians as an aphrodisiac[7].

In India, the plant is 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 antiperiodic and antipyretic. In Philippines, the leaf decoction is used to cure skin diseases and burns. The leaves are a popular febrifuge in Combodia. The infusion of leaves is prescribed as a purgative and in the treatment of dysentery. In Central America, the oil derived from seeds is used to cure leprosy and decoction is given to treat jaundice[8].

The traditional healers claim that some medicinal plants such as *Bixa* sps; are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants represents an alternative treatment for non severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant[9].

The present study is aimed at the evaluation of the antibacterial potential of *Bixa orellana* using various organic solvents, with the hope that such a study will create an interest among the people to search for new phytodrugs.

MATERIALS AND METHODS

Plant material

The plant materials used for the study were collected from the campus of Tamil University, Thanjavur, Tamil Nadu. Plant parts like leaves, empty seed capsules and seeds were shade dried for a week at room temperature (27ºC).

Preparation of plant extract

The dried leaves, empty seed capsules and seeds of *Bixa orellana* L. were powdered and sieved through a 40-mesh screen. The fine powder was kept in air tight containers and stored in the refrigerator.

The stock solution for each plant parts was made in to 20% extract and incubated for a week (200 mg powder was soaked in 1ml of different aqueous and organic solvents). The extract was filtered using membrane filter and stored in a refrigerator at 4°C until required for use[10].

Microorganisms

Pure isolates of *Escherichia coli* (521), *Klebsiella pneumoniae (*481), *Salmonella typhi (*378) and *Staphylococcus aureus (*377) were obtained from the Department of Clinical Microbiology, K.A.P.V. Government Medical College, Tamil Nadu, India and stored in a semisolid medium at 4°C until needed.

Standardization of inoculum

Organisms from the semisolid nutrient medium were inoculated into peptone water. After 6 hours of inoculation, a loop full of inoculated peptone water was streaked on Muller Hinton Agar to check the purity. About 3-5 pure colonies of each organism were inoculated into normal saline and the turbidity was adjusted to the Mc Farlands scale (150×10^6 cfu/ml)[11].

Preparation of discs of antibacterial agents

Discs of antibacterial agents were prepared from stock solution (1ml = 200mg). The concentrations used for the study were 100, 200, 400, 800, 1600, 3200 and 6400µg/ml. The discs were prepared by loading the required micro liters on sterile Whatmann paper disc of 6mm diameter. The discs were allowed to dry and were stored in air tight sterile containers.

Antibacterial testing

The plant extracts were tested for antibacterial activity using disc diffusion assay[12]. Four bacterial strains namely *Escherichia coli, Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* were tested. The test organisms were inoculated. The disc containing different concentrations of the plant part extracts were placed over the solidified agar in such a way that there is no over lapping of zone of inhibition. The inoculated petri dishes were incubated at 37°C for 24 hours. The zone of inhibition produced by various concentrations of plant extracts on different organisms were measured and recorded by using a zone reader[13].

RESULTS AND DISCUSSION

Antibacterial activity of leaf extracts of *Bixa orellana* on 4 selected pathogenic bacteria is given in table 1. The degree of inhibition varied with respect to different concentrations of various solvents on the test organisms. Leaf extracts of acetone was found to be highly effective against (>15mm diameter) *Staphylococcus aureus* at concentration 800 to 6400µg/ml. At concentrations (100-400µg/ml), moderate level (11-15mm) of antibacterial activity was noticed (Table 1). This result is in agreement with the scientific report[14]. In other organisms tested in our study due to acetone extract of *Bixa orellana,* the bacterial activity was markedly potential at 6400 µg/ml and between 800–3200 µg/ml the activity was moderate. Marked growth inhibition of *E. coli* and *S. aureus* was recorded by concentration at 1600µg/ml or above of the crude DMSO extracts of the leaves of *Bixa orellana*. Where, *K. pneumoniae* and *S. typhi* were found to be

inhibited markedly by concentration at 3200µg/ml or above. All the four test organisms were found to be moderately sensitive to DMSO leaf extracts at 800µg/ml (the zone of inhibition was 11-15mm diameter). At 100 and 200µg/ml concentrations, *K. pneumoniae*, *S. typhi* and *S. aureus* showed scanty growth as the zone of inhibition recorded was only between 7-11mm diameter (Table 1).

There are studies on crude extract from leaves of *Bixa orellana* L. that showed antibacterial activity against both Gram-positive and Gram-negative bacteria where the crude ethanolic extract did not show any effect[15]. Whereas, a narrow spectrum of antibacterial activity of ethanolic extract of leaf was reported effective against only the Gram-positive bacteria[16]. These studies are dissimilar to our results. The preliminary pharmacological screening of *Bixa orellana* L. leaves revealed antibacterial activity against selected causative agents of diarrhoea and dysentery including *Shigella dysenteriae*[17]. Similar to our study, there were reports[16,18].

Ethanol leaf extracts of *Bixa orellana* was remarkably effective at 6400µg/ml on *S. typhi. B*actericidal effect of the concentrations at 1600 and 3200µg/ml was moderate and below 1600µg/ml, the potentiality to inhibit the growth of *S. typhi* was very low. Whereas, *S. aureus* was highly sensitive to ethanol extracts at 3200 and 6400µg/ml and exhibited moderate sensitivity to all the concentrations tested in the present study (100 - 1600µg/ml). Ethanol extracts of leaf at 100-800µg/ml concentrations was recorded to have minimal antibacterial effect on *E. coli* and *K. pneumoniae* (Table 1). Results of our study find supportive evidence from the antimicrobial investigations[8]. The experimental results of, ethanol extracts from leaf of *Bixa orellana* was found to be highly bactericidal on *S. aureus*[19].

A slightly different trend was observed with reference to the sensitivity of the bacterial strains to methanolic extracts of *Bixa orellana* leaves. The bactericidal effect of the extracts on *E. coli, K. pneumoniae* and *S. aureus* was highly significant at 6400 and 3200µg/ml concentrations. Effect of methanol leaf extract on different strains of bacteria of our study fall in line with other scientific findings[20].

The various solvent extracts of *Bixa orellana* empty seeded capsules against the tested pathogenic bacteria are presented in table 2. All the four test organisms were resistant to the extracts of acetone, aqueous and ethanol. DMSO and methanol extracts showed growth inhibition at different degrees. The empty seeded capsule extracted with DMSO showed marked growth inhibition on *E. coli* at all concentrations except at 100µg/ml. The same extracts (DMSO) revealed significant and potential growth inhibition of *S. aureus* from 1600 to 6400 µg/ml. Below 800µg/ml, there was minimal growth inhibition. Where as, *S. typhi* was found to be moderately sensitive to DMSO extracts from 100 to 800µg/ml. Above 1600µg/ml, *S. typhi* was registered to be highly sensitive (>15mm). The growth of *K. pneumoniae* was prevented from 1600 µg/ml onwards. Below 1600µg/ml the sensitivity was moderate (11-15mm).

A different trend was noticed with reference to methanol extracts of *Bixa orellana*. Methanol extracts induced potential growth inhibition of *S. typhi* and *S. aureus* at 3200 and 6400µg/ml. From 1600µg/ml onwards, the growth inhibition of *S. typhi* was recorded to be moderate (11-15mm diameter). On the other hand, S. *aureus* was moderately inhibited at 800 and 1600µg/ml and below 800µg/ml, *S. aureus* was noticed to be resistant. Regarding *E. coli* and *K. pneumoniae*, the zone of inhibition recorded due to methanol extracts (400 - 6400µg/ml) of
Bixa orellana was between 11-15mm diameter

The bactericidal activity of the different solvent seed extracts on the selected test organisms showed an interesting trend. All the solvents used in the experiment except DMSO had no effect on the selected pathogenic organisms. *S. typhi* and *S. aureus* were

moderately inhibited by DMSO seed extracts at 6400µg/ml. From 800 to 3200µg/ml, inhibition zone was measured to be between 7-11mm diameter. Below 800µg/ml *S. typhi* and *S. aureus* were observed to be resistant.

From the results of the present investigation, it is understood that none of the aqueous plant extracts showed antimicrobial activity against the tested organisms. The bactericidal effect of leaf extracts of *B. orellana* due to methanol and ethanol was somewhat similar. With regard to acetone and DMSO the effect was highly significant. The antibacterial effect caused by acetone and DMSO extracts was more powerful when compared to the methanol and ethanol extracts. Of the four strains selected *S. aureus* was found to be highly sensitive to all the solvent leaf extracts even at 100µg/ml. It was followed by *E. coli*. The other two namely *S. typhi* and *K. pneumoniae* were noticed to be less sensitive to low concentrations. This study supports the medicinal usefulness of various parts of the plant against human pathogenic bacteria. This investigation also provides scientific backing to the use of *Bixa orellana* in traditional cures.

ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

Table 1

Antibacterial activity of Bixa orellana leaf extract against some pathogenic bacteria

TEST ORGANISM	Acetone							Aqueous							Dimethyl sulphoxide							Ethanol							Methanol							PC
	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	10mg
Escherichia coli	+	+	+	++	++	++	+++	-	-	-	-	-	-	-	+	++	++	++	+++	+++	+++	+	+	+	+	++	++	++	++	++	++	++	++	+++	+++	14mm
Klebsiella pneumoniae	+	+	+	++	++	++	+++	-	-	-	-	-	-	-	+	+	++	++	++	+++	+++	+	+	+	++	+	++	++	+	++	++	++	++	+++	+++	11mm
Salmonella typhi	+	+	++	++	++	++	++	-	-	-	-	-	-	-	+	+	+	++	++	+++	+++	+	+	+	+	++	++	+++	+	+	+	++	++	++	+++	22mm
Staphylococcus aureus	++	++	++	+++	+++	+++	+++	-	-	-	-	-	-	-	+	+	+	++	+++	+++	+++	+	++	++	++	++	+++	+++	++	++	++	++	++	+++	+++	30mm

+ = minimal growth inhibition (7-11); ++ = moderate growth inhibition (11-15); +++ = marked growth inhibition (>15);
- = No inhibition; PC = Positive control (Streptomycin).

Table 2

Antibacterial activity of Bixa orellana empty seed capsule extract against some pathogenic bacteria

TEST ORGANISM	Acetone							Aqueous							Dimethyl sulphoxide							Ethanol							Methanol							PC
	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	10mg
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	+	+	++	++	++	++	++	20mm
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	++	+++	+++	+++	-	-	-	-	-	-	-	-	-	++	++	++	++	++	13mm
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	+++	+++	-	-	-	-	-	-	-	+	++	++	++	++	+++	+++	21mm

Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	++	++	+++	+++	25mm

+ = minimal growth inhibition (7-11); ++ = moderate growth inhibition (11-15); +++ = marked growth inhibition (>15);
- = No inhibition; PC = Positive control (Streptomycin).

Table 3
Antibacterial activity of Bixa orellana seed extract against some pathogenic bacteria

TEST ORGANISM	Acetone							Aqueous							Dimethyl sulphoxide							Ethanol							Methanol							PC
	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	100 µg	200 µg	400 µg	800 µg	1600 µg	3200 µg	6400 µg	10 mg
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18mm
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19mm

+ = minimal growth inhibition (7-11); ++ = moderate growth inhibition (11-15); +++ = marked growth inhibition (>15);
- = No inhibition; PC = Positive control (Streptomycin).

CONCLUSION

All the different extracts of the plant showed varying degrees of antibacterial activity on the organisms tested. The antibacterial potential was more apparent in dimethyl sulphoxide followed by ethanol and methonal than the aqueous and petroleum ether extracts. This plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

ACKNOWLEDGMENTS

The authors thank UGC, New Delhi for providing financial assistance through Major Research Project and Dr. R. Parvathi, Head of the department of Botany for critically going through the manuscript.

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ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011