Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Bacterial growth inhibition potential of annatto plant parts

Akshatha Venugopalan, Parvatam Giridhar*

Plant Cell Biotechnology Department, CSIR – Central Food Technological Research Institute Mysore– 570 020, India.

ARTICLE INFO

Article history:
Received 25 August 2012
Received in revised from 5 September 2012
Accepted 7 December 2012
Available online 28 December 2012

Keywords:
Antibacterial
Annatto
Bixa orellana L
Zone of inhibition

ABSTRACT

Objective: To study the antibacterial efficacy of *Bixa orellana* leaves and deseeded fruit capsule extracts against both Gram positive and Gram negative bacteria. **Methods:** The antibacterial activity of the ethanolic, methanolic, acetone and dimethyl sulphoxide extracts of *B. orellana* were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococus aureus* by disc diffusion method. **Results:** The antibacterial activity of leaf was more pronounced even at low concentrations and fruit extracts exhibited the same at relatively higher concentrations. Only DMSO extract of seeds showed growth inhibition of *S. aureus*, *B. subtilis*, *B. cereus*, and *P. aeruginosa*. **Conclusions:** The present study suggested that the leaves and deseeded capsule extracts of *B. orellana* possess significant antibacterial activity thereby providing substantial support for the ethanobotanical applications of this plant.

1. Introduction

Annatto plant (*Bixa orellana*) is known for its reddish orange dye produced on aril portion of its seeds, which is widely used for colouring dairy, confectionary and bakery products and also as cosmeceutical and for dying leather etc[1]. Bixa plant is a shrub to tree, native to Neotropics but widely distributed throughout the tropics[2]. Recent studies on Bixa has been more inclined towards improving its annatto pigment content through various biotechnological approaches[3-7].

Ethnobotanical significance of this plant has been reviewed recently especially for its use in traditional medicinal practices in various parts of world[8]. Though annatto dye of seed is commercially important, looking at other bioactives and useful compounds from this plant parts would substantiate its medicinal use as extracts from this plant. Leaves, roots and seeds have traditionally been used for medicinal purposes including for the treatment of wounds and to treat diarrhoea and asthma[9]. Seeds of this plant are reported to be purgative, anti-pruritic and for buccal tumors[10]. Screening for phytoconstituents from *B. orellana* leaves[11,12] was attributed to its antifungal[13] and antibacterial potential[14]. However, all these previous investigations were performed using either aqueous or ethanolic extracts of leaves. In the present study, various solvent extracts of leaves, seeds and capsule of this plant are investigated for their antibacterial potential.

2. Materials and methods

2.1 Collection of plant material and preparation of plant extracts

The plant materials viz., leaves, seeds and deseeded fruit capsules of 3 year old *Bixa orellana* L. (Bixaceae) were collected from standing crop of annatto field that was established in Plant Cell Biotechnology Department of CFTRI campus. Respective plant parts were dried for one week at room temperature before using the same for experiment.

The dried leaves, seeds and empty seed capsules of *B. orellana* were powdered and sieved through a 40-mesh screen. The fine powder was stored in air tight amber glass containers and preserved in the refrigerator. A known quantity (1 gm dry weight) of plant material was subjected to soxhlet extraction[15] and exhaustively extracted with respective solvents (100 ml) for about 24 hrs. The extracts were filtered and concentrated in vaccum under reduced

*Corresponding author: Dr. Parvatam Giridhar.Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute Mysore- 570 020, India.
Ph: 91-821-2516501; Fax: 91-821-2517233
e-mail: parvatamg@yahoo.com

pressure using rotary flash evaporator and dried in a desiccator. The same was stored in a refrigerator at 4℃until required for use.

2.2 Test microorganisms

Pure isolates of *Escherichia coli* (MTCC 40), *Klebsiella pneumoniae* (MTCC 39), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 430) were obtained from IMTECH, Chandigarh and stored in a semisolid medium at 4℃ until needed. The bacterial strain *Staphylococus aureus* was procured from stock culture maintenance of Food Microbiology Department of CFTRI, Mysore. Tetracycline (HiMedia, Mumbai) was used as reference standard for antibacterial activity of bacteria.

2.3 Screening of extracts for antibacterial activity

Nutrient broth containing an overnight culture of test bacterial cells mixed with nutrient agar to give a final concentration of 10^6cfu/ml were poured into sterile plates and allowed to solidify. Discs of antibacterial agents were prepared from stock solution so that the final concentrations used for the study were 100, 250, 500, 750, 1500, 3000 and 6000 μg. The above mentioned concentrations were prepared by loading the required micro liters on sterile whatman paper disc of 6mm diameter[15]. The discs were allowed to dry and were stored in air tight sterile containers. Tetracycline was used as positive control at concentration of 25 μg. After placing the respective discs on medium containing plates (10 discs for each concentration per bacterial culture),the plates were incubated at 35±2℃ for 24 hr in upright position. The Zone of inhibition was measured in mm and the experiment was carried out in triplicates.

3. Results

Antibacterial potential of different plant parts of *B. orellana* on selected bacterial strains are given in Table 1 and 2. Aqueous extracts of respective plant parts did not show any antibacterial activity against the selected organisms. However, the degree of inhibition varied with respect to different concentrations of various solvents on the test organisms. Acetone extract of Leaf was found to be highly effective against (>14 mm diameter) *Staphylococcus aureus* from 750 μg to 6000 μg. In other concentrations (100 − 500 μg), moderate level (10−14 mm) of antibacterial activity was noticed. The bactericidal activity of acetone extract of leaves was markedly potential at 6000 μg and moderate between 750−3000 μg. Below 750 μg, the growth inhibition of bacteria was comparatively low. Inhibition of growth of *E. coli* and *S. aureus* was significantly high for DMSO extracts of leaves above 1500 μg concentrations. All the tested bacteria except

Table 1

Inhibition of bacterial growth by solvent extracts of *Bixa orellana* leaves.

Test	AC							DMSO							ET							ME							T
Organism	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	100 μg	250 μg	500 μg	750 μg	1500 μgg	3000 μg	6000 μg	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	25 μg
EC	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
KP	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
PA	–	A	A	A	A	A	A	–	–	A	A	A	A	A	–	–	–	A	A	A	A	–	–	–	A	A	A	A	A
BS	–	–	–	–	A	A	A	–	–	–	–	–	A	A	–	–	–	A	A	A	A	–	–	–	A	A	A	A	A
BC	–	–	–	–	A	A	A	–	–	–	–	–	A	A	–	–	–	A	A	A	A	–	–	–	A	A	A	A	B
SA	–	A	A	C	C	C	C	A	A	A	B	C	C	C	A	B	B	B	B	C	C	B	B	B	B	B	C	C	D

(–) = No activity, Inhibition zone A= 5−8 mm, B= 9−13mm, C= 14−18 mm, AC=acetone, DMSO= Dimethyl sulphoxide, ET=Ethanol, Me= methanol, EC= *Escherichia coli*, KP= *Klebsiella pneumoniae*, PA = *Pseudomonas aeruginosa*, BS = *Bacillus subtilis*, BC = *Bacillus cereus*, SA = *Staphylococcus aureus*, T = Tetracycline

Table 2

Inhibition of bacterial growth by solvent extracts of *Bixa orellana* deseeded capsule extract.

Test	DMSO							ET							ME							T
Organism	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	100 μg	250 μg	500 μg	750 μg	1500 μg	3000 μg	6000 μg	25 μg
EC	B	C	C	C	C	C	C	–	–	–	–	–	–	–	A	A	B	B	B	B	B	B
KP	B	B	B	B	C	C	C	–	–	–	–	–	–	–	–	–	A	A	B	B	B	A
PA	–	–	–	A	A	A	A	–	–	–	–	–	A	A	–	–	–	A	A	A	A	A
BS	–	–	–	–	A	A	A	–	–	–	–	–	–	A	–	–	–	A	A	A	B	A
BC	–	–	–	–	A	A	A	–	–	–	–	–	–	A	–	–	–	A	A	A	B	B
SA	B	B	C	C	C	C	C	–	–	–	–	–	–	–	B	B	B	B	B	C	C	D

(–) = No activity, Inhibition zone A = 5−8 mm, B = 9−13mm, C = 14−18 mm, AC = acetone, DMSO = Dimethyl sulphoxide, ET = Ethanol, Me = methanol, EC= *Escherichia coli*, KP= Klebsiella pneumonia, PA = *Pseudomonas aeruginosa*, BS = *Bacillus subtilis*, BC = *Bacillus cereus*, SA = Staphylococcus aureus, T = Tetracycline

Bacillus species were found to be sensitive to DMSO leaf extracts at 750 μg.

S. aureus was sensitive to ethanol extracts of leaves effectively at 3000 μg/ml and 6000 μg/ml and moderately to all the concentrations tested in the present study (100 − 1500 μg). A slightly different trend was observed with reference to the sensitivity of the bacterial strains to methanolic extracts of leaves (Table 1), wherein the bactericidal effect was highly significant on *E. coli*, *K. pneumoniae* and *S. aureus* at 6000 and 3200 μg concentrations. Even at lower concentrations methanol extract of leaves showed inhibition zone in the range of 10 − 14 mm diameter.

All the four test organisms were resistant to the acetone, aqueous and ethanol extract of deseeded capsule of *B. orellana* (Table 2). DMSO and methanol extracts showed growth inhibition at different degrees. The deseeded capsule's DMSO extract showed good inhibition of *E. coli* at all concentrations except above 100 μg and at 750 μg to 6000 μg against *S. aureus*. Similarly above 1500 μg, *K. pneumoniae* appears to be highly sensitive (>14 mm). All the four solvent extracts induced growth inhibition of *S. aureus* at 3000 and 6000 μg and at concentrations above 1500 μg methanol. Ethanol and acetone extracts exhibited growth inhibition of *P. aeruginosa*, *B. subtilis*, and *B. cereus*. The effect of methanolic extracts of deseeded capsules was moderate at 500 − 6000 μg wherein, 10−14 mm diameter was noticed (*E. coli* and *K. pneumoniae*). Only DMSO extract of seed showed some moderate effect on the growth of *Bacillus* species, *P. aeruginosa* and *S. aureus* at 6000 μg. At concentrations below 750 μg all tested bacteria were found to be resistant.

From the results of the present investigation, it is understood that the organic solvent extracts of the concentration of 1600 μg are bactericidal to all the four bacterial strains. The antibacterial effect of acetone and DMSO extracts was more compared to the methanol and ethanol extracts. Among the four strains selected, *S. aureus* was found to be highly sensitive to all the solvent extracts even at 100 μg followed by *E. coli*.

4. Discussion

In general, plant parts are a rich repository of bioactives and phytochemicals which contribute to the antimicrobial potential[15-17]. In this regard, various phytoconstituents such as saponins[18], alkaloids[19], flavonoids,[20] etc., were explored for their efficiency to combat microbial growth[21-23]. Similar phytoconstituents were observed in leaves and seeds of *B. orellana* by Fleischer et al[14] and the same can be attributed to antimicrobial potential of ethanolic extracts. Crude ethanolic extract from *B. orellana* leaves have shown antibacterial potential against *S. aureus* with minimal inhibitory concentration of 62.5 μg/ml[11]. Similarly ethanolic extract of Bixa seeds also proved to be more active against *E. coli* and *B. cereus* than gentamycin sulfate[24]. There was also a report on the antimicrobial activity of Bixa in vitro cultures[25]. In view of the significant response from leaf extracts[26], we have extended the study to capsules, which too showed moderate to good antibacterial activity. In our study, in addition to ethanolic extracts, we have demonstrated the bacterial growth inhibition potential of acetone, DMSO, methanol extracts of leaves, seeds, and deseeded capsule, wherein both acetone and DSMO extracts found to be efficient.

This study supports the ethnobotanical applications of various parts of the plant as reported[2]. This investigation also substantiates scientific backing to its antimicrobial uses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Department of Biotechnology, New Delhi, India for financial assistance (BT/PR7375/NDB/51/110/2006).

References

[1] Collins P. The role of annatto in food colouring. *Food Ingred and Process Internat* 1992; **23**: 23−27.

[2] Wealth of India. *Bixa orellana*. In: Raw Materials, Vol. 3. CSIR, India; 1990, p. 141−143.

[3] Giridhar P, Parimalan R. A biotechnological perspective towards improvement of annatto color production for value addition−the influence of biotic elicitors. *Asia Pac J of Mol Biol and Biotechnol* 2010; **18**: 77−79.

[4] Parimalan R, Giridhar P, Ravishankar GA. Enhanced shoot organogenesis in *Bixa orellana* L. in the presence of putrescine and silver nitrate. *Pl Cell Tiss and Org Cult* 2011a; **105**: 285−290.

[5] Parimalan R, Akshatha V, Giridhar P, Ravishankar GA. Somatic embryogenesis and Agrobacterium −mediated transformation in *Bixa orellana* L. *Pl Cell Tiss and Org Cult* 2011b; **105**: 317−328.

[6] Mahendranath G, Akshatha V, Parimalan R, Giridhar P, Ravishankar GA. Annatto pigment production in root cultures of Achiote (*Bixa orellana* L.). *Pl Cell Tiss and Org Cul* 2011a ; **106**: 419−424.

[7] Mahendranath G, Akshatha V, Giridhar P, Ravishankar GA. Improvement of Annatto pigment Yield in Achiote through Laminarin Spray: An Ecofriendly Approach. *Int J of Agri and Environ Biotechnol* 2011b; **4**(2): 163−166.

[8] Akshatha V, Giridhar P, Ravishankar GA. Food, ethanobotanical and diversified applications of *Bixa orellana* L.: a scope for its improvement through biotechnological mediation. *Ind J of*

Fundament and Appl Life Sci 2011; **1**: 9-31

[9] Parrotta JA. Healing plants of Peninsular India. CAB International, Wallingford, UK; 2001, p. 944.

[10]Lauro GJ, Francis FJ. Natural Food Colorants. Chapter 6, Lauro GJ and Francis FJ Editor. New York: Marcel Dekker Inc; 2000.

[11]Ongsakul M, Jundarat A, Rojanaworant C. Antibacterial effect of crude Alcoholic and Aqueous extracts of six medicinal plants against *S. aureus* and *E. coli*. *J of Health Res* 2009; **23**(30): 153-156.

[12]Shilpi JA, Taufiq-Ur-RahmanMd, Uddin SJ, Alam MS, Sadhu SK, Seidel V. Preliminary pharmacological screening of *Bixa orellana* L. leaves. *J of Ethnopharmacol* 2006; **108**: 264-271.

[13]Freixa B, Vila R, Vargas L, Lozano N, Adzet T, Canigueral S. Screening for antifungal activity in nineteen Latin American plants. *Phytotherapy Res* 1998; **12**(6): 427-430.

[14]Fleischer TC, Ameade EPK, Mensah MLK,Sawer IK. Antimicrobial activity of the leaves and seeds of *Bixa orellana*. *Fitoterapia* 2003; **74**(1-2): 136-138.

[15]Vijayaramu D, Giridhar P, Ravishankar GA. Antimicrobial activity of *Mimosa pudica*. *Asian J of Microbiol and Biotechnol and Environ Sci* 2004; **6**: 551-552.

[16]Ayyanar M, Subash-Babuhttp://www.sciencedirect.com/science/article/pii/S2221169112600501 - aff2 P. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pac J of Trop Biomed* 2012; **2**: 240-246

[17]Viqar Khan A, Ahmedhttp://www.sciencedirect.com/science/article/pii/S2221169111600993 - aff2 QU, Ramzan Mir M, Indu Shukla I. Ali Khanhttp://www.sciencedirect.com/science/article/pii/S2221169111600993 - aff1 A. Antibacterial efficacy of the seed extracts of Melia azedarach against some hospital isolated human pathogenic bacterial strains. *Asian Pac J of Trop Biomed* 2011; **6**: 452-455.

[18]Desai SD, Desai DG, Kaur H. Saponins and their biological activities. *Pharma Times* 2009; **41**(3): 13-16.

[19]Okwu DE, Igara EC. Isolation, characterization and antibacterial activity of alkaloid from Datura metel Linn leaves. *Afr J of Pharma and Pharmacol* 2009; **3**(5): 277-281.

[20]Rattanachaikunsopon P, Phumkhachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of Psidium guajava. *J of Med Plants Res* 2010; **4**(5): 393-396.

[21]Viqar Khan A, Uddin Ahmedhttp://www.sciencedirect.com/science/article/pii/S2221169112600409 - aff2 Q, Indu Shukla I. Ali Khanhttp://www.sciencedirect.com/science/article/pii/S2221169112600409 - aff1 A. Antibacterial activity of leaves extracts of *Trifolium alexandrinum* Linn. against pathogenic bacteria causing tropical diseases. *Asian Pac J of Trop Biomed* 2012; **2**: 189-194

[22]Chew AL, James J, Jessicahttp://www.sciencedirect.com/science/article/pii/S2221169112600379 - aff2 A, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera* . *Asian Pac J of Trop Biomed* 2012; **2**: 176-180

[23]Prasadhttp://www.sciencedirect.com/science/article/pii/S2221169111600968 - aff1 TNVKV, Elumalai EK. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pac J of Trop Biomed* 2011; **1**:439-442

[24]Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Complement and Alternat Med* 2006; **6**: 2-7.

[25]Castello MC, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana* L. *Ind J of Exp Biol* 2002; **40**(12): 1378-1381.

[26]Tamil Selvi A, Dinesh MG, Satyan Rs, Chandrasekaran B, Rose C. Leaf and seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens. *J of Appl Pharm Sci* 2011; **1**(9): 116-120.