Extraction and evaluation of antimicrobial activity of Bixin from *Bixa Orellana L.* Seed

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Abstract: -The research work was focused to investigate antimicrobial activity of Bixin, extracted from *Bixa orellana L.* seed. Bixin can be used as a decoction or an infusion to treat bacterial infections, according to the literature. Soxhlet extraction was used to carry out extraction on dried seed powder. At concentrations ranging from 1000 ug/ml to 5000 ug/ml, B. orellana seed extract significantly inhibited the pathogens. The activity was done against various pathogenic bacteria such as E. coli, Streptococcus aurues, Pseudomonas aeruginosa, and others. The highest inhibition zone found against Pseudomonas aeruginosa was 21.2 mm. For all microorganism strains, the minimum inhibitory concentration was determined. Antimicrobial activity was investigated using the disc diffusion method. The zone of inhibition was estimated and the concentration of seed extract revealed substantial antibacterial action. Furthermore, it was shown that the methanolic seed extract of Bixa orellana exhibited superior antibacterial activity and that extracts comprising innovative drug delivery systems may prove to be successful treatments for microbial infections.

Keywords- B. Orellana seed, extraction, antimicrobial activity, zone of inhibition etc.

DOI: 10.48047/ecb/2023.12.8.742

1.INTRODUCTION

Bixa orellana seed is a member of the bixaceae family. It is a tiny tree that is grown in tropical and subtropical regions of the world. Because the Bixa orellana L. plant contains a reddish-colored natural dye in various places, it is also known as the Sindur tree or the Lipstick Tree [1-2]. The many parts, such as seeds, leaves, and fruit, are widely used for a variety of functions. Achiote, Annotta, Arnatta, Arnatto, Bija, and other common names for annatto seed. For the traditional uses it is widely used in Orissa, Andhra Pradesh, Maharashtra, kerala, Karnataka and Tamilnadu. Ayurvedic practitioners in India utilized Bixa orellana as an astringent and mild purgative because the entire plant possessed great therapeutic characteristics and is thought to be an effective treatment for diarrhea and renal problems. Bixa species, according to traditional healers, are more effective at treating infectious disorders than manufactured antibiotics [3]. The important component known as bixin can be found in varying concentrations in the seed, leaf, and fruit components [4-6]. According to published research, annatto seeds contain a significant amount of the carotenoid bixin. The seeds of this plant consist of a "inner seed" with a shelled kernel containing oils, waxy substances, mineral ash, and alkaloid substances, a peel made of cellulose and tannins, and an outer coating made of colors, moisture, and a trace quantity of oils.

For the extraction of annatto pigments from the seeds, a variety of methods have been used, including crushing the seeds into hot vegetable oil, diluted alkaline aqueous solutions, and solvents

[7-10]. For the effective extraction of important carotenoid pigments like Bixin and norBixin from seeds, numerous alternative technologies, such as ultra sound assisted extraction, microwave assisted extraction, and supercritical fluid extraction, have also recently been developed. Additionally, a number of analytical techniques for the separation and detection of Bixin have been reported. It is advised to use two-dimensional thin layer chromatography for the qualitative detection of Bixin [11,12].

The in-vitro antibacterial activity of an extract can be evaluated or screened using a variety of laboratory techniques. The disk diffusion and broth or agar dilution methods are the most well-known and fundamental techniques [13-16] Other techniques, such as the technique of poisoned food, are specifically employed for antifungal testing. Agar disk diffusion was used to carry out antimicrobial activity.

The objective of the study was to carry out extraction using an economical method without compromising the quality of the original material. The antibacterial activity of Bixin, which is derived from Bixa orellana seed, is further investigated and evaluated.

1. MATERIAL AND METHODS

Plant collection

The fresh and dried seeds received from Sree ratna enterprises from gokavaram andhrapradesh cultivated in same region. Other excipients and chemicals received from sigma Aldrich (MH, India).

Extraction method

The B. orellana seed was exposed to two days of sun drying before spending 72 hours at 50 °C in a hot air oven. The mechanical grinding of the dried B. orellana seed provided the powdered material needed for the extraction process. For identifying the extraction method with the best yield percentage, two alternative methods were used [17].

Hot extraction- 30 g dried powdered sample were taken in three soxhlet apparatus containing 200 ml of solvent. The extraction was carried out in a soxhlet apparatus for 12 hr with different solvents like methanol, ethanol, acetone, hexane at 60 ^{0}C, 70 ^{0}C, 50 ^{0}C and 60 ^{0}C respectively [2].

Cold extraction- 30 g dried powdered sample were taken in three different conical flask, containing 200 ml of solvent like methanol, ethanol, acetone and hexane. Extraction was carried out at 24 hr. For more than 8 hours, the flask was shaken at intervals of 0.30 hours to ensure that the solvent and seed sample was properly mixed. The reaction has been put aside for some time. After the filtration procedure, extract was used to collect the residue. Additionally, both extracts were retained to allow natural convection to evaporate the solvent as much as possible. The residue is stored at 40 ^{0}C for future study [18].

Phytochemical Screening from seed extract

According to the procedures described by Kokate C.K. in 1986, phytochemical screening of the plant extracts was done to check for the presence of various compounds [8,19]. The procedures were used to qualitatively analyze the freshly produced crude extracts for the presence of secondary metabolites like proteins, carbohydrates, alkaloids, glycosides, coumarins, tannins, phenols, flavonoids, and saponins.

Carbohydrate

Equal amounts of Fehling's A and B were added to test tubes containing one milliliter of each of the various extracts. For 10 to 15 minutes, the tubes were heated in a water bath at 65 °C. Carbohydrates were detected in the redbrick precipitate.

Alkaloids
Four to six drops of Wagner's reagent were added to one milliliter of extracts in test tubes. The presence of alkaloids was revealed by the radish-brown precipitate.
Glycoside
In test tubes, 1 mL of glacial acetic acid was added with 1 mL of extracts. Then a solution of 1% ferric chloride was added approximately 5–6 drops. Glycoside was present as shown by the brown color ring that formed at the top.
Tannins
Four to five drops of 1% ferric chloride were added after one milliliter of extracts had been mixed with one milliliter of distilled water. The presence of gallic tannin was indicated by the color blue, while cathecholic tannin was shown by the color greenish-black.
Phenols
To 1 mL of extracts, 1 mL of ethanol was added. Then, each tube had 6-7 drops of a 1% ferric chloride solution. Green, blue, or purple color formation suggested the presence of phenol.
Flavonoids
To 2 mL of extracts, three to four drops of a 20% NaOH solution were added. When 4-5 drops of diluted HCl were added, the bright yellow color formed and turned colorless. This suggested that flavonoids were present.
Saponins
To 1 mL of extracts, 2 mL of distilled water was added, and the mixture was agitated for 5 minutes. Saponin was detected by the 10-minute existence of 1-cm-thick foam.
Proteins
A few drops of concentrated HNO3 were added to one milliliter of the extract for treatment. Proteins were present as indicated by the development of a yellow color.

Antimicrobial activity methods
Disk diffusion assay: The antibacterial characteristics were confirmed using a disk diffusion study. Individual tests of B. orellana seed methanol extract against microorganisms were conducted. After being inoculated into tryptic soy agar, each bacterial species was then incubated there for a full day at 37 °C. The 6 mm diameter, sterile, empty disks were either placed on the inoculated agar or impregnated with 1 ml of extracts. As a control, standard antibiotic disks which were empty were used.[13] The inoculation plates underwent a 24-hour incubation period at 37 °C. Antibacterial activity was calculated by measuring the zone of inhibition in mm without taking the radius of the disk into account [20,21].
Minimum Inhibitory Concentration (MIC)
Selected extracts from plants were serially diluted in sterile nutritional broth medium from 1000 ug to 1.906 ug. The test was conducted using a 96-well titre plate, in which 20 ul of the culture organism and 20 ul of a chosen plant extract were loaded and incubated for 24 hours at 37 °C. The maximum dilution of a plant extract that still had an inhibitory effect and prevented a microbe from growing (causing turbidity) is recorded as the extract's MIC value. Parallel DMSO control was kept up [22,23].

2. RESULT AND DISCUSSION
Phytochemical Screening from seed extract
Plants synthesize phytochemicals, which are chemically active substances that serve a variety of purposes. The extracts' secondary metabolites and bioactive elements have been defined

by qualitative phytochemical inspection. Methanol, ethanol, acetone, and hexane extracts were subjected to preliminary phytochemical screening in order to check for the presence of various phytochemicals such as carbohydrates, alkaloids, glycosides, tannins, phenol, flavonoids, saponins, and others. When compared to other solvent extracts, the methanolic extract of the seed exhibits the presence of all phytochemicals. Every phytochemical was concentrated to a great degree in the methanol extract. Acetone extract exhibits a lack of some phytochemicals, which could be a negative sign. From the results observed after phytochemical study the ratio of obtaining different compounds from solvent was higher in methanol than ethanol, acetone and hexane.[15,20] All the results were tabulated in table. 1

Table 1. Phytochemical analysis of seed extract in different solvent

Sr. no.	Phytochemical	Methanol extract	Ethanol extract	Acetone extract	Hexane Extract
1	Carbohydrate	++	++	++	++
2	Alkaloid	++	++	-	+
3	Glycoside	++	++	++	++
4	Tannin	++	++	-	++
5	Phenol	+++	++	++	+
6	Flavonoids	+++	+++	++	++
7	Saponins	++	++	+	++
8	Proteins	+++	+++	++	++

In- Vitro Antimicrobial activity

On three separate bacterial test organisms, antimicrobial assays were conducted, and the results were compared to the control group. An antibacterial effect of Bixa orellana leen plant seed extract on several infections was dose dependant. In hot extraction techniques compared to cold extraction, E. coli and P. aeruginosa species were found to be more sensitive. For every pathogen, all readings were compared to the streptomycin control group. By using an antibacterial assay, it has been proven that extract prepared using a hot extraction technique performs better than extract produced using a cold extraction method. It was further found that there was to be a change in antibacterial growth as the concentration of extract inoculation increased. All results were tabulated in Table no. 2

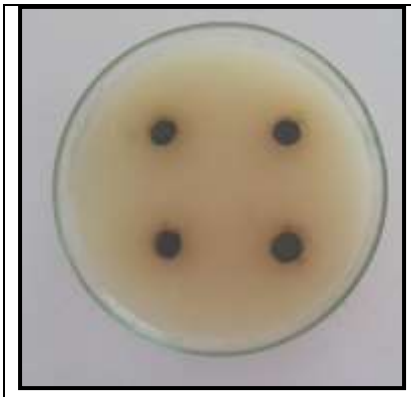
Table 2. Antimicrobial activity of seed extract by hot and cold extraction

Sr. no	Organism	Zone of inhibition of extract carried out by hot extraction in mm					Zone of inhibition of extract carried out by cold extraction in mm					Strept. Control
		1000 ug/ ml	2000 ug/ ml	3000 ug/ ml	4000 ug/ ml	5000 ug/ ml	1000 ug/ ml	2000 ug/ ml	3000 ug/ ml	4000 ug/ ml	5000 ug/ ml	5000 ug/ml
1	*E. coli*	8.2	11.3	14.4	17.8	19.4	8.2	11.4	12.5	13.9	15.2	24.2
2	*S. aurues*	6.5	9.7	11.6	13.0	14.7	9.1	12.3	14.0	15.1	16.5	19.5
3	*P. aeruginosa*	8.0	10.2	15.3	19.8	21.2	6.7	9.5	11.2	11.9	12.3	26.4

The findings of an antimicrobial investigation show that extracts made with the hot extraction approach produce superior outcomes than extracts produced using the cold extraction technique due to the presence of various components in higher concentrations. According to the study, using heat to the extraction process gives results that are more effective than those produced by any other method. Both gram-positive (Streptococcus aurues) and gram negative (Eschericha coli and Pseudomonas aeruginosa) bacteria were subjected to antimicrobial action. All of the extracts were found to inhibit bacterial growth in both bacterial species. The methanolic extract of seed was found to have the maximum zone of inhibition (21.2 mm) for Gram-negative bacteria P. aeruginosa and the lowest zone of inhibition (19.4 mm) for E. coli in the methanolic extract at a concentration of 5000 ug/mL extract. The methanolic extract at a concentration of 5000ug/mL had the largest zone of inhibition (14.7 mm) for gram positive bacteria against S. aurues. All results were tabulated in table 3 and figure 1 and 2 indicates zone of inhibition all three strains by two different extraction techniques.

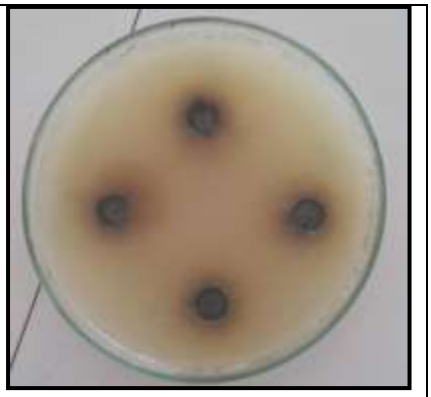
Table 3. In vitro antimicrobial activity of different species at highest concentration

Test strains	**Zone of inhibition (mm) Dose: 5000ug/ml (Extract Carried out by hot extraction)**	**Zone of inhibition (mm) Dose: 5000ug/ml (Extract carried out by cold extraction)**
Eschericha coli	19.4 mm	15.2 mm
Streptococcus aurues	14.7 mm	16.5 mm
Pseudomonas aeruginosa	21.2 mm	12.3 mm



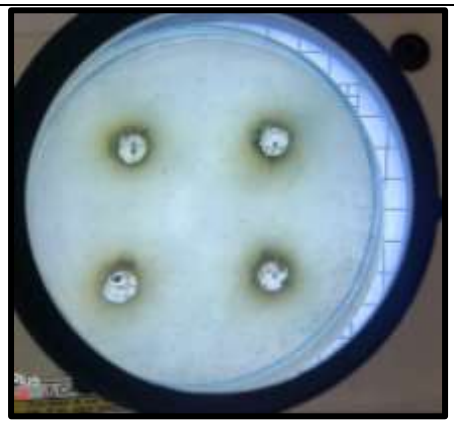
E. coli 19.4mm ZOI

S. aurues 14.7mm ZOI

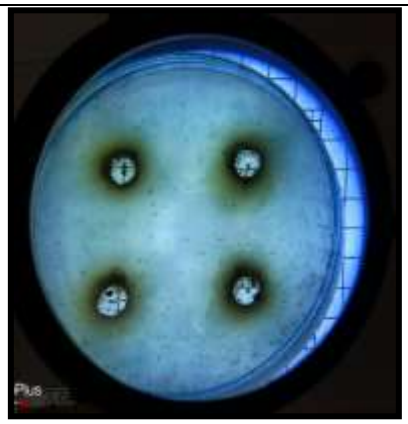


P. aurues 21.2mm ZOI

Fig. 1: Zone of inhibition (ZOI) of bacterial strains of extract by hot extraction



E. coli 15.2 mm ZOI



S. aurues 16.5 mm ZOI

P. aurues 12.3 mm ZOI

Fig. 2: Zone of inhibition (ZOI) of bacterial strains of extract by cold extraction

Minimum Inhibitory Concentration (MIC)

It was significant to find out that the MIC values for Escherichia coli, Streptococcus aurues, and Pseudomonas aeruginosa were 62.00 ug/ml, 60.00 ug/ml, and 59.00 ug/ml, respectively. Comparatively all the microbial strains show MIC at same or nearly equal level.

Test strains	Minimum inhibitory concentration (ug/ml)
Eschericha coli	62.00
Streptococcus aurues	60.00
Pseudomonas aeruginosa	59.00

3. CONCLUSION

The results of the current investigation make it clear that hot extraction, compared to cold extraction, performs better in terms of phytochemical presence and antimicrobial strain resistance. The outcomes of the phytochemical screening studies revealed that temperature has an impact on the extraction process. The presence of flavonoids in B. orellana seed extract may be responsible for its inhibitory effects. Flavonoids can form complexes with extracellular and soluble proteins, as well as bacterial cell walls. Lipophilic flavonoids have the potential to disrupt bacterial membranes as well. Flavonoid-rich extracts of seeds revealed inhibitory activity against *Eschericha coli, Streptococcus aurues, Pseudomonas aeruginosa.* Gram –ve bacteria which are responsible for a large number of infectious diseases have a unique outer membrane that contains lipopolysaccharides which render them impermeable to certain antibacterial compounds. *B. orellana* exerts notable activity against serious pathogens including *Eschericha coli* (19.4 mm) and *Pseudomonas aeruginosa* (21.2 mm). By, considering the results obtained from the study it was conclude that extract containing Bixin with novel drug delivery can be an effective treatment against microbial infection in future.

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