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Research Article

Phytochemical Screening and Antimicrobial Activity of *Bixa Orellana Linn*

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ABSTRACT

Bixa orellana is a well known plant in Asia, including India, which has a wide range of medicinal importance. The drugs of this plant are used in India as a folk remedy in the form of decoctions and infusions to treat bacterial infections and it is also effective against different skin diseases. The present study is to examine the antibacterial properties of leaves and seed extracts of *Bixa orellana* with different solvents such as petroleum ether, aqueous, methanolic, ethanolic extracts on *staphylococcus aureus, Escherechia coli, and klebsiella pneumoniae, Pseudomonas aeuroginosa,* by disc diffusion method to study the presence of various phytochemicals in *Bixa orellana*. The different concentrations of leaf and seed extracts exhibited antibacterial activity and then the zone of inhibition was calculated. Among seeds and leaf extracts, methanolic seed extracts of *Bixa orellana* have shown better antimicrobial activity.

Keywords: *Bixa orellana*, Disc diffusion method, Antibacterial activity, Zone of inhibition.

INTRODUCTION

Bixa orellana L. (Family: Bixaceae) is a small sized tree cultivated in the tropical and subtropical regions of the world. It has been widely used in food, textile and pharmaceutical industries due to the presence of annatto dye in the seeds as a natural colorant[1,2,3]. It is widely used in India including areas such as Orissa, Andhra Pradesh, Maharashtra, kerala, Karnataka and Tamilnadu. In India, the ayurveda practitioners used *Bixa orellana* as an astringent and mild purgative because the whole plant of it showed valuable medicinal properties and it is considered as a good remedy for treating dysentery and kidney diseases. The traditional healers claim that *Bixa* species are more efficient to treat infectious diseases than synthetic antibiotics[1,2]. The seed and root bark were utilized for treating gonorrhea. The root bark is used as antiperiodic and antipyretic[4]. The leaves and roots help in epilepsy, dysentery, fever and jaundice[5]. The plant extracts show activity against helminthes, protozoans and platelet antiaggregant activity. Extracts of leaves and branches have shown to be effective at neutralizing the effects of snake venoms[6]. Previous phytochemical investigations have revealed the presence of several carotenoid compounds including bixin and norbixin some terpenoids, tocotrienols, and flavonoids in *Bixa orellana* seeds[7]. Among these, carotenoids are widely used in biological process such as vitamin A activity, cancer preventing agents, protective agent against cardiovascular diseases and decreasing effect of cataract and age related degeneration. Bixa seeds are used as purgative, anti pruritic and for buccal tumours. In carotenoids, bixin is one of the most effective biological compounds and contribute to the cell protection and tissue damage against free radicals. The present study determines the evaluation of the antibacterial, phytochemical activities of *Bixa orellana* using various organic compounds.

MATERIALS AND METHODS

Collection of plant materials

The leaves and seeds of *Bixa orellana. L* were collected from Tirumala Tirupathi Devasthanam(TTD) nursery of Tirupati. The leaves were plucked and separated and washed with water and allowed it to shade dry. The dried material was pulverized into a coarse powder by using a dry blender and passed through the sieve before being stored in a closed vessel for further use.

Preparation of Plant extracts

The powdered leaves and seeds of *Bixa orellana. L* was dissolved in a polar solvent system (methanol, ethanol, petroleum ether and water). The powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48 hours by using solvent system. The extract was concentrated under the vacuum in the rotary flash evaporator and successively in hot air oven till solid to semisolid mass.

Test Microorganisms

Staphylococcus aureus, Escherichia coli, Pseudomonas

Table 1: Antimicrobial Activity of Tetracycline (100µg/ml) Control.

Pathogens	Zone of Inhibition (mm)
Escherichia coli	12
Pseudomonas aeruginosa	10
Staphylococcus aureus	9
Klebsiella pneumoniae	8

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Table 2: Dose Dependent Antimicrobial Activity of Bixa orellana Seed Extracts.

Seeds extract	Concentration	Pathogens			
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
Aqueous	50mg/ ml	-	-	-	-
	100 mg/ ml	-	-	-	-
	150 mg/ ml	-	-	-	1
	200 mg/ ml	-	-	-	2
Methanol	50 mg/ ml	3	-	1	2
	100 mg/ ml	6	-	2	4
	150 mg/ ml	9	-	5	6
	200 mg/ ml	10	-	6	8
Ethanol	50 mg/ ml	1	-	-	-
	100 mg/ ml	2	-	-	-
	150 mg/ ml	4	-	1	-
	200 mg/ ml	5	-	2	-
Acetic acid	50 mg/ ml	-	-	-	-
	100 mg/ ml	-	-	-	-
	150 mg/ ml	-	-	-	1
	200 mg/ ml	-	-	-	2
Petroleum ether	50 mg/ ml	-	1	-	-
	100 mg/ ml	-	2	-	-
	150 mg/ ml	1	3	-	-
	200 mg/ ml	2	4	-	-

Table 3: Dose Dependent Antimicrobial Activity of *Bixa orellana* Leaves Extracts.

Leaves extract	Concentration	Pathogens			
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
Aqueous	50 mg/ ml	-	3	-	-
	100 mg/ ml	-	5	-	-
	150 mg/ ml	-	-	-	1
	200 mg/ ml	-	-	-	2
Methanol	50 mg/ ml	2	-		1
	100 mg/ ml	4	-		3
	150 mg/ ml	6	-	2	5
	200 mg/ ml	9	-	4	7
Ethanol	50 mg/ ml	-	-	-	-
	100 mg/ ml	-	-	-	-
	150 mg/ ml	-	1	-	-
	200 mg/ ml	-	2	-	-
Acetic acid	50 mg/ ml	-	-	1	-
	100 mg/ ml	-	-	2	-
	150 mg/ ml	-	-	3	-
	200 mg/ ml	-	-	4	-
Petroleum ether	50 mg/ ml	-	-	2	-
	100 mg/ ml	-	-	4	-
	150 mg/ ml	1	-	-	-
	200 mg/ ml	2	-	-	-

aeruginosa and Klebsiella pneumoniae.

Culture preparation

A loop full of each tested bacterial strains was aseptically transferred into 5 ml of maintaining media and incubated at 37^0C for 18-24 hours before use. The optical density at 600 nm of each active culture was adjusted using fresh broth to obtain approximately 106 CFU/ml.

Antibacterial Bioassay

Agar disc diffusion bioassay

For bioassays, a suspension of approximately $1.5x10^8$ bacterial/ml in sterile normal saline was prepared as described by *Forbes et al*.1990[8] About 1.5 ml was uniformly spread on nutrient agar media in glass petri dishes & Kept aside for 15 mins an excess suspension was drained and discarded properly. Bioassay was determined by measuring Diameter of the Inhibition Zone (DIZ) in mm. The discs were placed on a surface of the nutrient agar media and the various concentrations of leaf

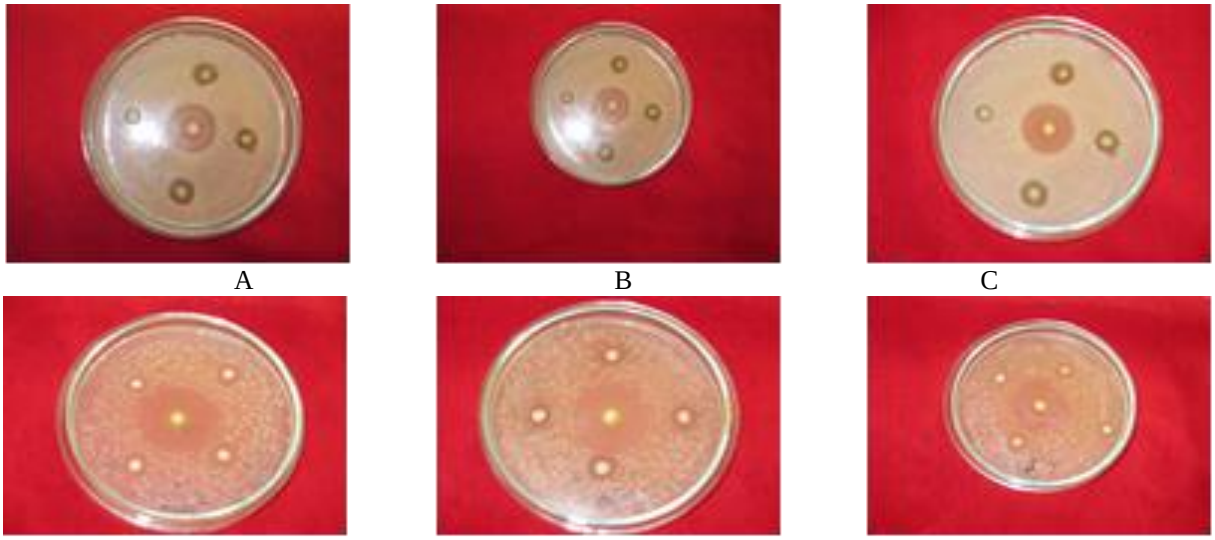

Figure 1: A) Highest antibacterial activity exhibited by methanol seed extracts against *Pseudomonas aeruginosa*
B) Highest antibacterial activity exhibited by methanol seed extract against *E. coli*
C) Highest antibacterial activity exhibited by methanol leaf extract against *staphylococcus aureus*
D) Highest antibacterial activity exhibited by methanol seed extract against *Klebsiella pneumonia*e.
E) Highest antibacterial activity exhibited by petroleum ether leaf extract against *staphylococcus aureus.*
F) Highest antibacterial activity exhibited by methanol leaf extract against *Klebsiella pneumoniae.*

Table 4: Results of Phytochemical Screening of *Bixa orellana* Seeds and Leaves.

Phytochemicals	Aqueous extract		Ethanolic Extract		Methanolic Extract		Acetic Acid extract		Petroleum ether extract	
	seeds	leaves	seeds	leaves	seeds	leaves	seeds	leaves	seeds	leaves
Alkaloids	+	-	+	+	+	+	+	-	+	+
Flavonoids	+	+	-	-	+	+	+	+	-	-
Steroids	+	+	+	+	-	-	+	-	+	+
Cardiac Glycosides	+	+	+	+	-	-	+	+	+	+
Saponins	+	+	-	-	+	+	-	-	+	+
Terpenoids	+	+	-	-	+	+	-	-	-	-
Amino acid	+	+-	-	-	-	-	+	-	+	+
Carbohydrates	+	+	+	+	-	-	-	-	+	+
Proteins	+	+	+	+	+	+	-	-	+	+
Phlobatinins	+	+	+	+	-	-	-	-	-	-

and seed extracts of *Bixa orellana. L* were taken from the range of 50, 100, 150 and 200 µg/ml was carried out using agar disc diffusion method. Each experiment was done in triplicates and the DIZ was calculated. The standard drug tetracycline (100µg/ml) was used to compare with the soxhlet extracts.

Determination of Minimal Inhibitory Concentration (MIC)

Bacterial cultures were added into the broth at the concentration of 106 CFU/ml and culture at appropriated temperature. The samples were taken for every 4 hrs microbial count and to evaluate the mode of actions, bacteriostatic and bactericidal action.

Phytochemical screening of the extracts

The ethanol, methanol, petroleum ether and water extracts were used for preliminary screening of phytochemicals such as alkaloids, flavonoids, saponins, cardiac glycosides, carbohydrates, protein, aminoacid, terpenoids and phlobatinins followed by the methods of various researchers[9].

Dose Dependent antimicrobial activity test

The different solvent extraction were conducted for dose dependent studies of seeds and leaves of *Bixa orellana* on microorganisms showing positive results for antimicrobial activity. Different doses of 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml of each extract was tested for their antimicrobial activity. The dose dependent antimicrobial activity was carried out using agar disc diffusion method. The plates for antimicrobial activity were incubated at 37ºC for 24hrs. After 24 hours the plates were observed for zone of inhibition and compared with the Tetracycline control. The zone was measured in mm (Table 1). The methanol extract of seed and leaves, showed higher antibacterial activity against all four tested strains. Petroleum ether extract of seed and leaves, have not shown the antibacterial effect against klebsiella

(Table-2,3). In general, the toxic effect to the bacterial membrane and function, because the lipophilic membrane has been used to explain the antimicrobial effect of essential oils and extracts[10,11].

RESULTS AND DISCUSSION

Regarding the antibacterial activity, seed and leaf extracts inhibited the growth of all four tested strains (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa*) (Table-1). The results showed that methanolic extract, ethanolic extracts of seeds & leaves of *Bixa orellana* possessed maximum antibacterial activity when compared with aqueous, acetic acid & petroleum ether extracts. The ethanolic extract of *bixa orellana* seeds & leaves showed antibacterial activity against *Klebsiella pneumoniae & Staphylococcus aureus* but not *Pseudomonas aeruginosa & Escherichia coli. Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* showed more sensitive against methanolic extract of seeds & leaves of *bixa orellana* while *Klebsiella pneumoniae* does not showed any activity. There is no antibacterial activity against *Escherichia coli* with aqueous, ethanolic, acetic acid & petroleum ether extracts when compared to methanolic extracts. *Pseudomonas aeruginosa* showed antibacterial activity with methanolic extract but not with aqueous, ethanolic, acetic acid & petroleum ether extracts. *Klebsiella pneumoniae* showed antimicrobial activity with ethanolic extracts but not with aqueous, methanolic, acetic acid & petroleum ether extracts. The zone of inhibition tetracycline is used as a control for all the organisms (Table-2). *Pseudomonas aeruginosa showed* zone of inhibition with petroleum ether extract of *bixa orellana* seeds and no activity with other organisms. *Klebsiella pneumoniae* & *Staphylococcus aureus* showed zone of inhibition with ethanolic extract of *bixa orellana* seeds when compared to other organisms. *Escherichia coli, Pseudomonas aeruginosa* & *Staphylococcus aureus* showed zone of inhibition with methanolic extract of seeds & leaves of *Bixa orellana* and no activity with ethanol, petroleum ether & acetic acid.

Phytochemical screening of the extracts

The present study has revealed the presence of medicinally active metabolites. The phytochemical characters of *Bixa orellana* are summarized in the below table(Table-4). The aqueous extract has shown to contain amino acids, carbohydrates, steroids, proteins, flavonoids, phlobatanins, terpenoids, cardiac glycosides and saponins. Ethanolic extract contained alkaloids, amino acids, carbohydrates, cardiac glycosides, steroids, proteins, and phlobatanins. Petroleum ether showed the presence of carbohydrates, proteins, amino acids, steroids, alkaloids, cardiac glycosides, saponins. Methanolic extract contained alkaloids,glycosides, saponins, flavonoids, proteins and terpenoids. The chemical prospection of *Bixa orellana* leaves and seed extracts have shown the presence of various secondary metabolites that are known to present different therapeutic applications, for example, flavonoids (anticarcinogenic, antiviral, antihemorrhagic and antioxidant)[12,13,14]. The phytochemical analysis showed that the plant leaves were rich in carbohydrates, proteins, amino acids, steroids in all the extracts. Some extracts showed presence of alkaloids and also flavonoids. Steroids were found to be present in all the extracts except methanolic extract. It should be noted that steroids have biological functions and certain steroids (such as cholesterol) are important components of cell membranes which alter membrane fluidity and steroids are signaling molecules which activate the hormone receptors. The presence of cardiac glycosides have been reported, by other researchers in *Bixa orellana*, and this plant is widely used in Indian medicinal system. The plant studied can be seen as a potential source of useful drugs. Further studies are going on, in this plant to identify further more uses in the field of medicine.

CONCLUSION

In the present study, the antimicrobial activity of *Bixa orellana* seeds and leaf extracts against pathogenic organisms are reported and was observed that active components in the plant material was identified in polar and non-polar solvent systems. Hence, antimicrobial activity of extract using polar solvent is higher than non-polar solvent. The study showed that pharmacological importance of seeds and leaves of *Bixa orellana*, whereas it provides further study the phytochemical analysis for antimicrobial activity against tested pathogenic bacteria. However, the study provides a direction for investigation of different plants to discover the new molecules having antimicrobial properties.

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