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IN-VITRO AND PHYTOCHEMICAL SCREENING OF *BIXA ORELLANA* L. AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

In the present investigation the antimicrobial activity of acetone, hexane, ethyl acetate and petroleum ether extracts of *Bixa orellana* L. pericarp and seed was evaluated with agar well diffusion and broth dilution method. Extract exhibited moderate inhibitory effect against the testes bacterial pathogens at concentration 1 mg/ml. the gram negative bacteria *P. aeruginosa and E. coli* were found to be most susceptible to the pericarp extract, while the least antibacterial activity expressed by against gram positive bacteria *S. aureus*. Further the phytochemical screening of the extracts was carried out for the detection of metabolites present in the plants extract. The purification and identification of the number of compounds present in the extracts was carried out by thin layer chromatography.

Key words: Metabolites, *Bixa orellana*, Antimicrobial, Zone Of Inhibition, Thin Layer Chromatography.

INTRODUCTION

Bixa orellana L. (family: Bixaceae) is a small sized tree cultivated in the tropical & subtropical regions of the world **[1].** In India, the Ayurveda practitioners used *Bixa Orellana* as an astringent and mild purgative because the whole plant of *Bixa orellana* 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 **[1,2,3].** Plants are a critical therapy in many countries and are the basis for drug development efforts **[4].** Emerging countries encourage the incorporation of traditional medicine mainly through herbal preparation into the local health system **[5].** Annatto is an allowed natural colorant used in food industries, textiles, cosmetics and pharmaceutical products **[6].** The colorant is extracted from *Bixa orellana* seeds, which are covered by a red, resinous pericarp containing the pigments **[7].** The main pigment is bixin (methyl hydrogen 9-cis 6,6'-diapocarotene 6,6' dioate)which is responsible for the orange red colour in the seeds (80% of total carotenoids) six smaller amounts of norbixin are also present **[8].** Literature analysis showed important biological activities from aqueous extracts of the leaves including antifungal activity, antibacterial activity, antioxidant activity, anti-inflammatory, anti-diarrheal, analgesic, anti-carcinogenic, neuro-pharmacological and gastrointestinal effects [**9].** Some researchers have studied the anti-inflammatory role of *Bixa orellana* leaves on in vivo models **[10,11].** The ethanol extract of leaves shows the antibiotic activity against gram's positive and Candida albicans **[12].** In many studies, antimicrobial activity of Annatto extracts (leaves, capsules, seeds) has been identified against several food spoilage and pathogenic bacteria and fungus such as *S. aureus, E. coli, B. subtilis, C. albicans,s C. utilis* and *A. niger* **[12, 13].** More recently it was suggested that bixin could have the property of reducing blood glucose levels **[14].**

The seed and the root bark were utilized for treating gonorrhea. The root bark is used as antiperiodic and antipyretic **[15].** The roots and leaves help in epilepsy, fever and jaundice **[16].**

The plant extracts shows activity against helminthes, protozoans and platelet anti-aggregation activity.

Extracts of leaves and branches have been too effective at neutralizing the effects of snake venoms **[17].** In carotenoids, bixin is one of the most effective biological compounds and contribute to the cell protection and tissue damage against free radicals. Among these, carotenoids are widely used in biological process such as vitamin A activity, cancer preventing agent's protective agents against cardiovascular diseases and decreasing effects of cataract & age related degeneration **[18].** Novel phytochemical substances of plant origin have been shown to inhibit the growth or destroy bacterial cells, which were resistant to existing synthetic agents. The differences in chemical structure of these phytochemicals compared with the existing synthetic agents indicate possible differences in mechanism of action **[19].** Twenty five compounds from the essential oil of *Bixa orellana* have been identified **[20].** The present study aims to determine the evaluation of the antimicrobial and phytochemical analysis of *Bixa orellana* L. pericarp extracts against gram positive and gram negative strains.

EXPERIMENTAL DESIGN

Sample collection:

Fresh plants were collected and then washed with tap water and distilled water, cut into small pieces and separate them in seeds and pericarp and kept for drying in sunlight. The dried pericarp and seeds were then grinding to powdered form using a mixture or grinder.

Extraction of secondary metabolites:

The powder of the samples were dipped in 1:10 ratio in respective polar and non-polar solvents, after that incubated at room temperature for 48 hours. Further filtered and evaporated the solvents to obtain metabolites. These metabolites were scratched in dimethyl sulphoxide and preserved for further use **[21]**.

Screening of secondary metabolites:

The screenings of the metabolites were achieved by performing the antibiogram analysis. Agar well diffusion method was used for the analysis and the effective outcomes were observed on the basis of zone of inhibition **[22]**.

Phytochemical analysis:

The analysis was carried for the detection of presence and absence of secondary metabolites in plants extracts **[23,24]**.

Drug modification:

For enhancing the activity of the metabolites the modification in the drugs were carried out. The modification in the drug was achieved by adding different metals at different concentration with extracts **[25]**.

Minimum inhibitory concentration:

The identification of the effective dose of the drugs with respective to pathogens were carried out by using the broth dilution method **[26]**.

Purification of metabolites:

Thin-layer chromatography (TLC) is a very commonly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction **[27]**.

RESULT

Sample collection:

Table 1: the collected sample of *Bixa orellana*

Plant sample	Scientific name	Sample
Sindur	*Bixa orellana*	

Screening of metabolites:

The extracted metabolites were screened against three strains *P. aeruginosa*, *S. aureus* and *E. coli,* where the acetone extracts of *Bixa orellana* pericarp and *Bixa orellana* seed executed effective results against these bacterial pathogens. Hence this extract was selected for the modification.

Table 2: Antibiogram analysis of the extracts of *Bixa orellana* pericarp and *Bixa orellana* seed against pathogens.

Pathogen	Zone of Inhibition (mm)			
	A	H	E	P
***Bixa orellana* pericarp**				
Pa	17	0	16	18
Sa	15	0	13.5	0
Ec	16.5	0	16	0
***Bixa orellana* seed**				
Pa	16.5	18	20	0
Sa	14	12.5	13	0
Ec	0	0	13	12.5

where Pa = *P. aeruginosa*, Sa = *S. aureus* Ec = *E. coli,* A = acetone, H = hexane, E = ethyl acetate, P = petroleum ether**.**

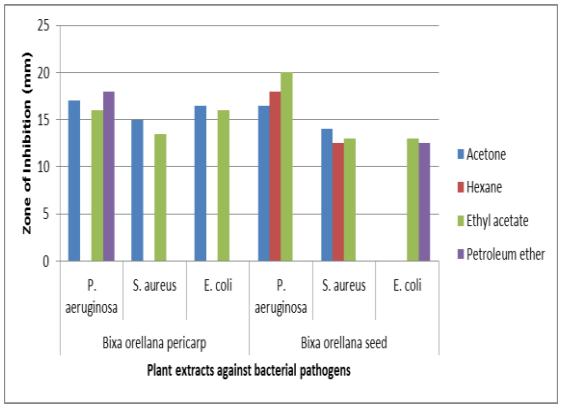

Figure 1: graphical representation of antibiogram analysis of the extracts of *Bixa orellana* pericarp and *Bixa orellana* seed against *P. aeruginosa*, *S. aureus* and *E. coli*.

Drug modification:

Modification is carried out by showing antibacterial activity of the different metals at different concentrations with acetone extract of *Bixa orellana* pericarp and *Bixa orellana* seed against the pathogenic microorganisms. Where 1.5% $ZnSO_4$ shows best antimicrobial properties with *B. orellana* pericarp acetone extract and 1.5% $MgSO_4$ in case of *B. orellana* pericarp acetone extract.

Table 3: Effects of metals and acetone extract of *Bixa orellana* pericarp and *Bixa orellana* seed against pathogenic microorganisms.

Metals	Zone of inhibitions (mm)		
	Pa	*Sa*	*Ec*
PC	12	15	19
NC	0	0	0
***B.orellana* pericarp**	13	15	15.9
$MgSO_4$	0	0	0
0.5% $MgSO_4$	15	11	12.5
1% $MgSO_4$	11	11	10
1.5% $MgSO_4$	15.5	14.6	18.3
$ZnSO_4$	10	9	10
0.5% $ZnSO_4$	12	16	11.5
1% $ZnSO_4$	16	18.5	15.5
1.5% $ZnSO_4$	20	20.5	18.5
$CaCl_2$	0	12	0
0.5% $CaCl_2$	0	0	12.5
1% $CaCl_2$	0	0	13
1.5% $CaCl_2$	0	0	13
$PbNO_3$	0	12	0
0.5% $PbNO_3$	0	0	12.5
1% $PbNO_3$	0	0	13
1.5% $PbNO_3$	0	0	0
***B. orellana* seed**	15	12	18
$MgSO_4$	0	0	0
0.5% $MgSO_4$	19	15	20
1% $MgSO_4$	15	19	18.6
1.5% $MgSO_4$	25	24	21
$ZnSO_4$	10	9	11
0.5% $ZnSO_4$	12	11.5	11
1% $ZnSO_4$	11.5	10.5	11
1.5% $ZnSO_4$	12	13	15
$CaCl_2$	0	0	0
0.5% $CaCl_2$	13	0	11.5
1% $CaCl_2$	0	0	0
1.5% $CaCl_2$	15	0	0
$PbNO_3$	0	0	0
0.5% $PbNO_3$	11.5	12	10.5
1% $PbNO_3$	12	12	13
1.5% $PbNO_3$	15	19	15

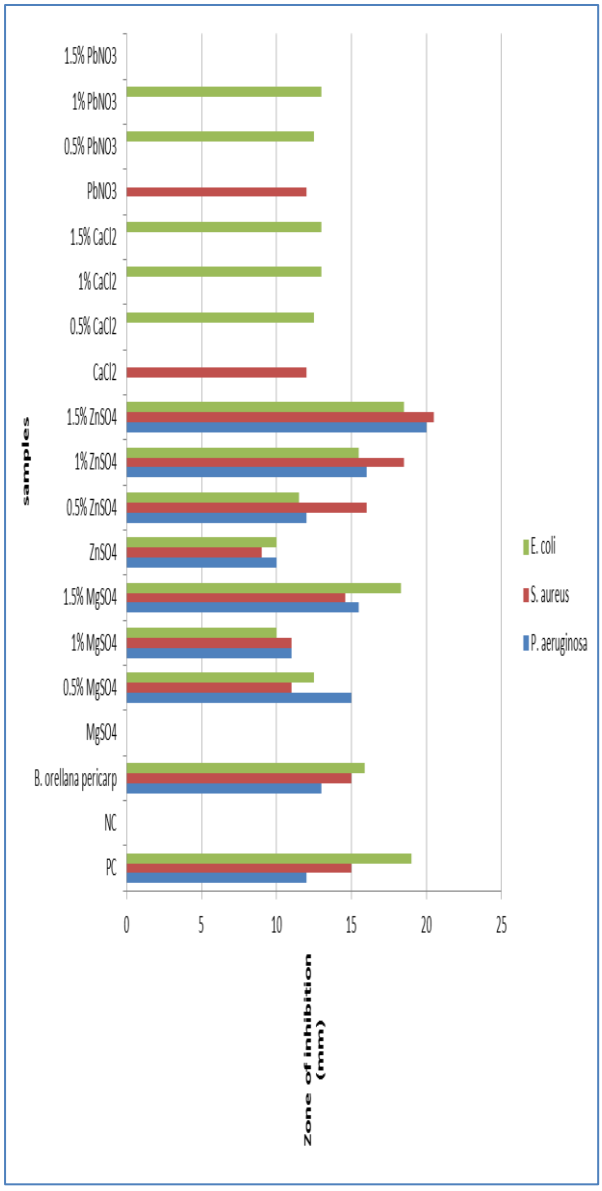

Figure 2: graphical analysis of the antibacterial activity of the different metals in combination with *B. orellana* pericarp acetone extract against *P. aeruginosa*, *S. aureus* and *E. coli*.

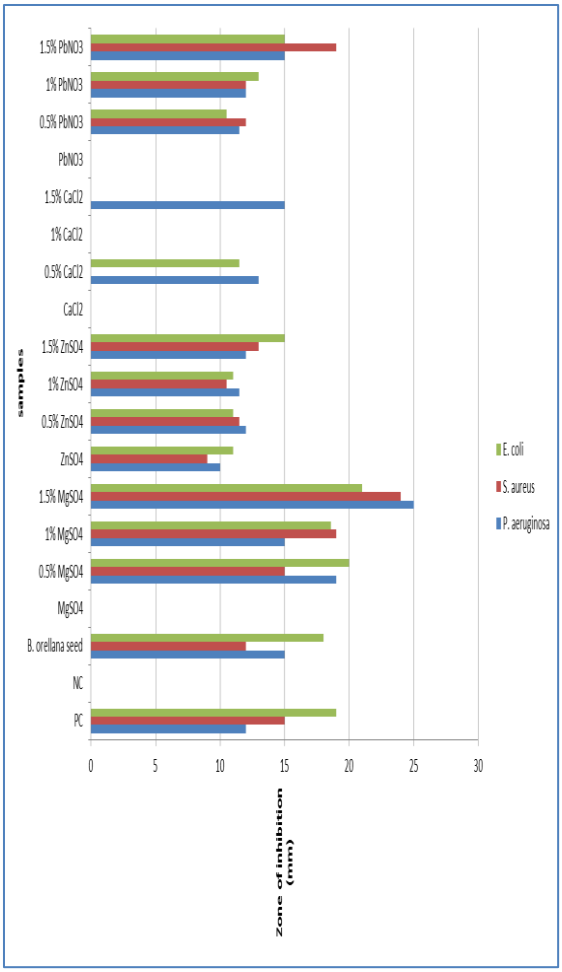

Figure 3: graphical analysis of the antibacterial activity of the different metals in combination with *B. orellana* seed acetone extract against *P. aeruginosa*, *S. aureus* and *E. coli*.

Phytochemical analysis:

Table 4: Phytochemical screening of acetone extract of *B. orellana* pericarp and *B. orellana* seed.

Secondary metabolties	*Bixa orellana* pericarp	*Bixa orellana* seed
Carbohydrates	-	+
Alkaloids	+	++
Steroids	+	++
Saponins	+	+
Tanins	+	++
Flavonoids	+	+++
Starch	+	++

Minimum Inhibitory Concentration test:

Table 5: Minimum inhibitory concentration of the acetone extract of *B. orellana* pericarp and *B. orellana* seed against *P. aeruginosa*, *S. aureus* and *E. coli*.

Pathogens	MIC value (mg/ml)	
	Bixa orellana pericarp	*Bixa orellana* seed
P. aeruginosa	7.5	2
S. aureus	3.5	9.5
E. coli	7.5	8.2

Thin layer chromatography

Table 6: Tabular representation of the Thin Layer Chromatography of Bixa orellana pericarp.

Fraction	No. Of spots	Compound colour	Retention factor
0.5% methanol and chloroform			
Actone	2	Brown Green	B=0.92 G=0.31
1% methanol and chloroform			
Acetone	1	Light green	LG=0.95
5% methanol and chloroform			
Acetone	2	Dark green Yellow	DG=0.38 Y=0.85
8% methanol and chloroform			
Acetone	3	Light Green Brown Yellow	LG=0.44 B=0.92 Y=0.51
10% methanol and chloroform			
Acetone	1	Brown	B=0.9

Table 7: Tabular representation of the Thin Layer Chromatography of *Bixa orellana* seed.

Fraction	No. Of spots	Compound colour	Retention factor
0.5% methanol and chloroform			
Acetone	1	Brown	B=0.76
1% methanol and chloroform			
Acetone	1	Light green	LG=0.92
5% methanol and chloroform			
Acetone	1	Light green	LG=0.92
8% methanol and chloroform			
Acetone	2	Brown Yellow	B=0.77 Y=0.73
10% methanol and chloroform			
Acetone	1	Dark Green	DG=0.73

DISCUSSION AND CONCLUSION:

The results for the antimicrobial sensitivity tests zone of inhibition diameters 9mm) for he extracts at 1 mg, standard antimicrobial doses, tetracycline 1mg against Gram negative and Gram positive bacteria are described in Table 2. The solvents used for the extraction of the extract were not able to show any antibacterial activity at the volume used. The crude acetone extract was the most active among the four extracts showing activity against all pathogenic isolates. The highest activity was against P. aeruginosa by the acetone fraction. The activities of all extracts against the pathogenic microorganisms, were in descending order viz; acetone extract > ethyl acetate fraction > petroleum ether > hexane fraction.

The minimum inhibitory concentrations (MICs) of the extracts and fractions are shown in Table 4. This varied between 2 and 9.5 mg/ml against all the bacterial strains used in this study. The broad spectrum of activity displayed by the acetone extract in this study would appear to justify and explain the scientific basis for some of the uses of the other parts of *Bixa orellana* such as its use in the treatment of skin infection by some native folks in Benin City. There is as yet no literature report on the antimicrobial activity neither of the *Bixa orellana* pericarp extract, nor of either the aqueous or chloroform fractions. The earlier antimicrobial reports were on dried root decoction in the treatment of tooth decay and gonorrhea. Reports have shown that *Bixa orellana* possessed alkaloids, flavonoids, saponins tanins, steroids, starch, carbohydrate **[24]**. By performing the thin layer chromatography it was found that number of different components were present in the acetone extracts defined in table 6 and 7. Further studies on the isolation and further characterization of the bioactive substances in the crude ethanol extract and concomitant aqueous and chloroform fractions would be undertaken.

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