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Comparative Qualitative And Antibacterial Study Of Bixa Orellana L. Leaves With Some Selected Solvent For Some Selected Bacteria

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Abstract-

A quantitative assessment of antimicrobial activities was carried out by determining the minimum inhibitory and microbicidal concentrations (MICs and MMCs) of the various solvent extracts like 70% ethanol, water , ethyl acetate and chloroform against two Gram negative bacteria - Escherichia coli and Pseudomonas aeruginosa and two Gram positive bacteria - Staphylococcus aureus and Bacillus cereus,. The testing was done by the Agar disc diffusion method. Zones of inhibition of extracts were compared with that of standard ofloxin and amoxicillin antibiotics for antibacterial activity. From our study, we can conclude that ethanol extract has more prevailing and sustainable antibiotic properties than other solvents extract. Preliminary phytochemical screening of Bixa Orellana showed the presence of alkaloids, glycosides, steroids, phenols, tannins, flavonoids and saponins in the crude drug.

Keyword- Antibacterial, Zones of inhibition, Alkaloids, Phytochemical; MICs and MMCs,

INTRODUCTION

Bixa orellana Linn., known as annatto in English, achiote in Spanish, yanzhimu in Chinese or urucum in Portuguese (Brazil), is a member of the family Bixaceae [1] *B. orellana* (Family: Bixaceae, common name: achiote, annatto in english,[2] sinduri in sanskrit, rangamala in kannada) belongs to a small sized tree that are grown in the tropical regions**.** To get a wide variety of drugs, medicinal plants are recommended by the World Health Organization.[3] Herbal medicines, which often contain phytochemicals obtained from medicinal plants, are used by about 80% of people in developed countries [4].

The brilliant red fruit (seedpods) known as annatto is used mostly as a natural colouring additive for food, fabrics, items, the human body (including the hair and skin), and cosmetics[5]. The

entire tree has a long history of use as a medicinal herb to cure a variety of ailments, from cancer to fevers [6] Two carotenoids are bixin and carotenoids a unique red colour in B. orellana.[7] Extensive research studies carried out in the last few decades have shown isolation of several different classes of phyto constituents including carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes and sesquiterpenes, triterpenoids, volatile oils and other miscellaneous compounds from all parts of this plant.[8]Annatto has been used for centuries in many parts of the world for the prevention and treatment of a number of heath disorders such as constipation, fevers, heartburn, asthma, scabies, ulcers, diarrhea, stomach upset, skin diseases[9] measles, anecdotal treatment of diabetes, allergy, leprosy, infectious diseases, burns, measles, gonorrhea, diarrhea, asthma, angina, tumors, skin problems, and urinary infections (oral and topic)[10] This phenomenon gives rise to the concept of using naturally derived compounds for their potential application to control pathogenic microorganisms.[11] Natural compounds may offer a new source of antibiotics more effective than synthetic antibiotics [12].

EXPERIMENTAL SECTION

Material and methods-The Bixa Orellana leaves were collected from the herbal garden of AKS university satna [M.P.] and authenticated in department of biotechnology of AKS university satna.

Selected bacterial species –

Gram-positive bacteria - *Bacillus cereus*, *Staphylococcus aureus* .
Gram-negative bacteria - *Escherichia coli*, Pseudomonas aeruginosa

Preparation of extract- The fresh leaves were washed with distilled water and air dried to constant weight for six days. The dried material was grinded and the bioactive components were extracted by soaking 12gm of leave powder in 100ml of each selected solvent at 25˚c. After three days extracts were filtered and filtrate were concentrated in vacuumed at 35˚c (Akerele.et al, 2008).

Test for phytochemical constituents – The following tests were carried out to determine the presence of various active phyto-constituents.

Carbohydrates: To 2-3 ml extract, few drops of *Molisch reagent* was added, mixed well and conc. H2SO4 was added from the sides of the test tube, violet ring formation at the junction of two liquids indicated the presence of carbohydrates.[13]

Amino acids: *Ninhydrin test* was conducted for amino acids in general and presence of cysteine was checked by adding 40% NaOH and 10% lead acetate solution to extract. Appearance of black lead sulphate precipitate after boiling confirmed the test.[14]

Proteins: Presence of proteins was determined by Biuret test. To 3 ml extract 4% NaOH and few drops of 1% CuSO4 solution was added and appearance of violet - pink colour indicated the presence of proteins in the extract.[15]

Steroids: *Libermann-Buchard test* was conducted for steroids. While performing test to 2 ml extract with chloroform.1-2 ml aceteic unhydride few drops cons. H2SO4 was added from the side of test tube. Steroids were indicated by reddish brown coloured ring at the junction of two layers.[16]

Glycosides: To 2 ml extract glacial acetic acid, few drops of 5% FeCl3 and conc. H2SO4 were added. Reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicating the presence of glycosides.[17]

Alkaloids: For the confirmation of alkaloids three tests were conducted.
Dragendroff's test confirmed the presence with formation of orange brown precipitate on adition of few drops of dragendroff‘s reagent onto the extract.

***Mayer's test*:** Few drops of the Mayer‘s reagent was when added onto the extract, white - pale yellow precipitate was seen which indicated the presence of alkaloids. [18]

***Wagner's test*:** Extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner‘s reagent was added. A yellow or brown precipitate indicated the presence of alkaloids.

Tannins: *Lead acetate test* was performed for confirming the presence of tannins in the extract. To an aqueous solution of dried plant material, 5% lead acetate solution was added; appearance of white precipitate indicated the presence of tannins.[19]

Phenol: For the purpose of analysing the presence of phenol *FeCl3 test* and *Folin-ciocaltaue test* was carried out. [20]

FeCl3 Test: Small amount of extract of plant material was treated with neutral FeCl3. Formation of blue or green colour indicated the presence of phenol.

Folin-ciocaltaue reagent test: When the extract was treated with a little residue of ammonia solution, a blue or blue to gray colour is formed indicating the phenols are present.

The screening and quantitative analysis for phytoconstituent of *Bixa orellana* L. with different solvents extract should be represented below in a table no.2.1

Table 2.1- Qualitative tests of *Bixa orellana* L. with different solvents.

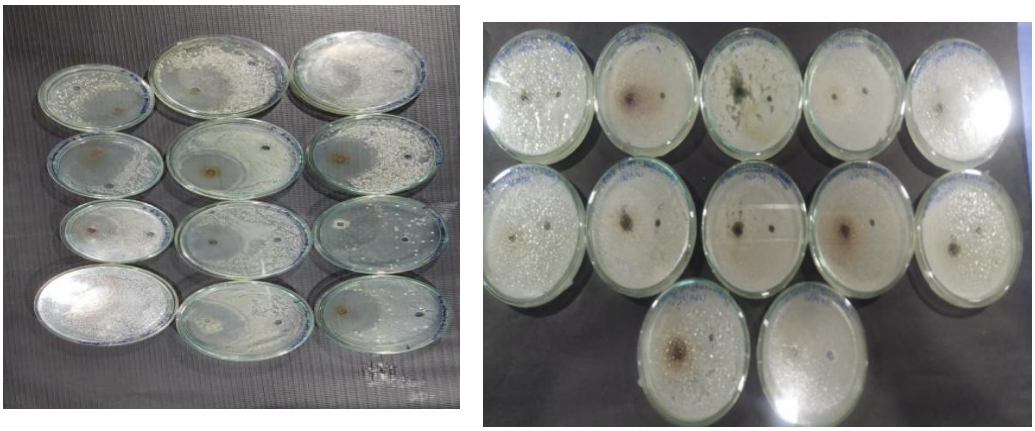
Test for phytochemical	Carbohydrates	Amino acids	Proteins:	Steroids:	Glycoside	Alkaloids:	Tannins	Phenol	Flavonoid
Aqueous extract	++	++	+	+	-	-	++	+	+
ethanol extract	-	+	+	-	+	-	+	+	+
Ethyl acetate extract	-	-	_	+	-	+	+	++	+
Chloroform extract	-	-	_	+	-	+	+	+	+

ANTIMICROBIAL ACTIVITY TEST

Antibacterial Susceptibility Test (AST) Microorganisms pure cultures of bacteria isolated from clinical specimen obtained from the Biotechnology Laboratory at AKS University. The organisms are Escherichia coli ATCC 25932, Staphylococcus aureus, ATCC 29213, Pseudomonas aeruginosa and Bacillus cereus ATCC 6633. The organisms were maintained on Nutrient agar slants at 4°C and sub-cultured for use in testing ofloxin, amoxicillin and ampicillin 10µg/mL were used as positive control for the sensitivity test against bacterial strains.

Determination of zone of inhibition (ZOI) method

In vitro *a*ntimicrobial activity testing was carried out by using Agar disc diffusion method. The zones of inhibition around the disks were measured after 24 h of incubation at 37°C for bacteria. Control experiments were carried out under similar condition by using Ampicillin and ofloxin for antibacterial activity as standard drugs.



Photograph 1- Antibacterial activity of Bixa Orellana extract against selected strain of bacteria by agar disc diffusion method.

Table 2.2.-Antibacterial activity of bixa orellana extract without mixing with antibiotics-

	Zone of inhibition in (mm)				
Species of Bacterial strain	Dose of extract	ethyl acetate extract	Chloroform extract	ethanol extract	Aqueous extract
Bacillus cereus	100 mg	10mm	11mm	12mm	13mm
staphylococcus aureus	100mg	11mm	12mm	19mm	12mm
Escherichia coli	100mg	11mm	10mm	14mm	13mm
Pseudomonas aeruginosa	100mg	8mm	10mm	11mm	10mm

Graph- 2.1 Graphical representation of Antibacterial activity of Bixa Orellana extract without mixing with antibiotics-

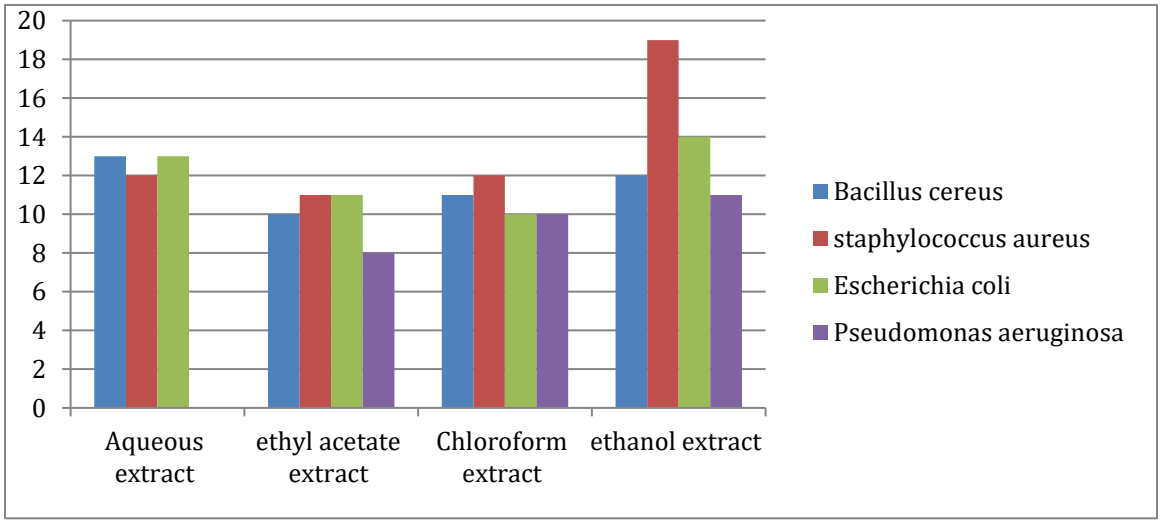


Table 2.3Antibacterial activity of bixa orellana extracts mixing with antibiotics-

Zone of inhibition in (mm)

Species of Bacterial strain	Dose of extract	ethyl acetate extract	Chloroform extract	ethanol extract	Aqueous extract
Bacillus cereus	100mg	31mm	28mm	33mm	30mm
staphylococcus aureus	100mg	35mm	29mm	37mm	36mm
Escherichia coli	100mg	36mm	32mm	38mm	37mm
Pseudomonas aeruginosa	100mg	40mm	30mm	34mm	31mm

Graph-2.2 Graphical representation of Antibacterial activity of bixa orellana extracts mixing with antibiotics

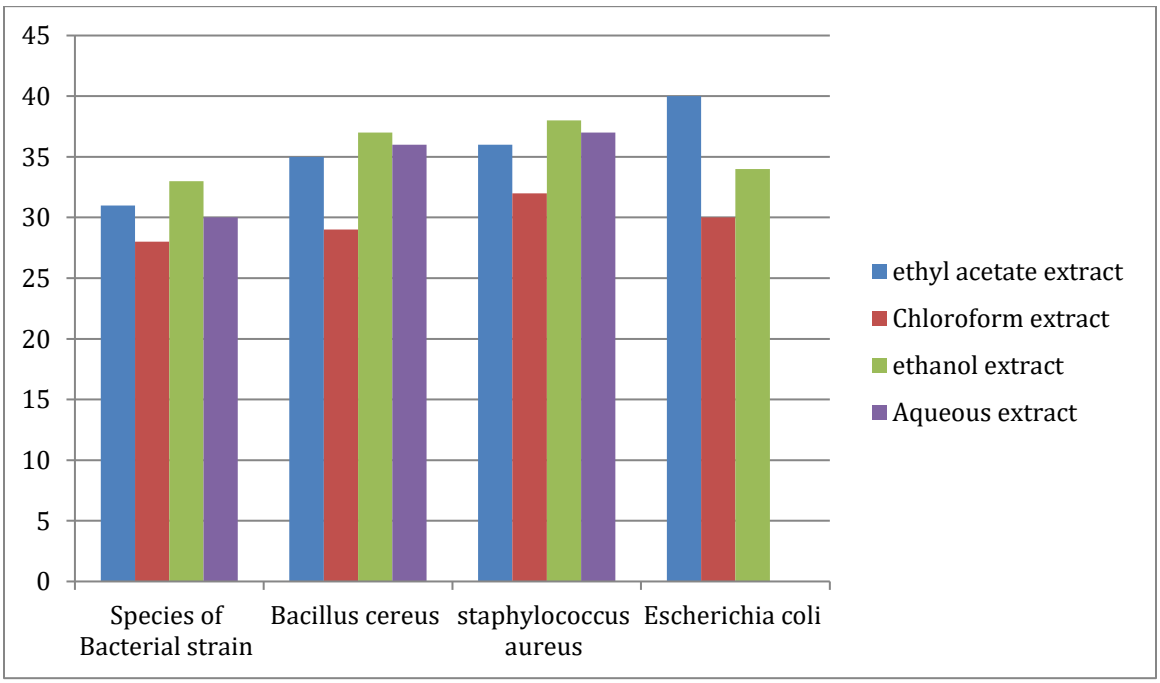
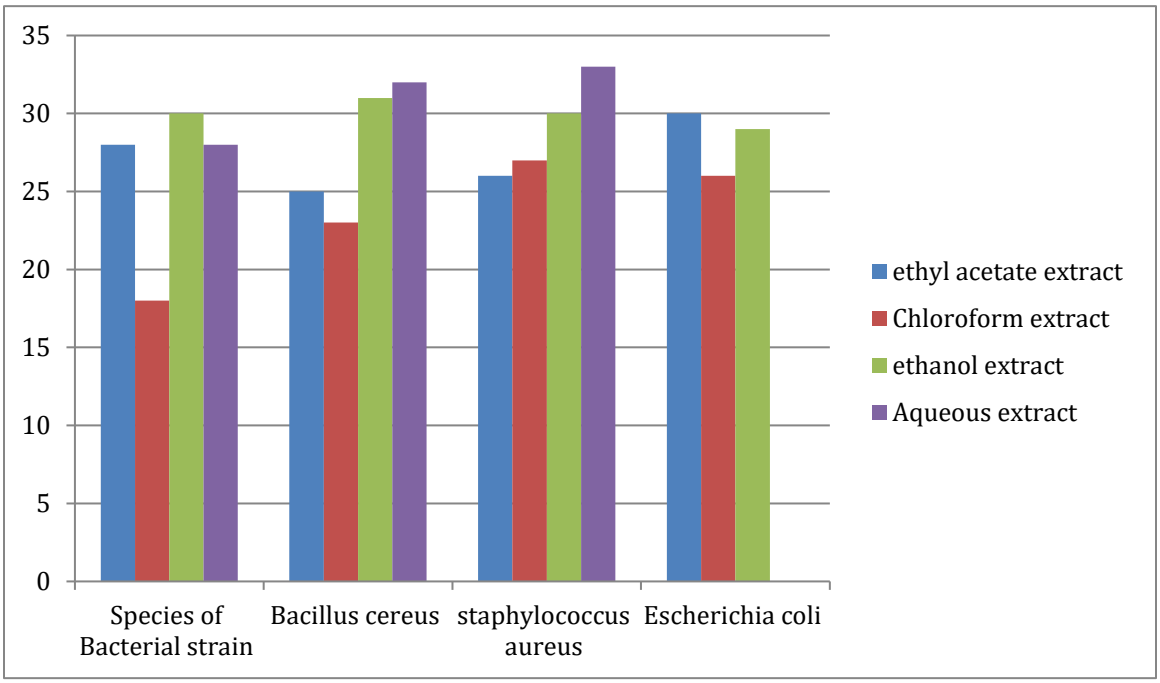


Table 2.4 Antibacterial activity of oflaxin and amoxicillin antibiotics-

Zone of inhibition in (mm)

Species of Bacterial strain	Dose of extract	ethyl acetate extract	Chloroform extract	ethanol extract	Aqueous extract
Bacillus cereus	100mg	28mm	18mm	30mm	28mm
staphylococcus aureus	100mg	25mm	23mm	31mm	32mm
Escherichia coli	100mg	26mm	27mm	30mm	33mm
Pseudomonas aeruginosa	100mg	30mm	26mm	29mm	29mm

Graph- 2.3 Graphical representation of Antibacterial activity of oflaxin antibiotics-



RESULT AND DISCUSSION

Results indicated that Bixa orellana (leaves) ethanol extract exhibited a maximal zone of inhibition (ZOI) against Staphylococcus aureus of around 19mm. The antibacterial activity of antibiotics appears to be enhanced by plant leaf extract. Bixa orellana has potent antibacterial and inhibitory effects on a few resistant types of microorganisms.

CONCLUSION

Thus, it is concluded that *in-vitro* experiments with different antibiotics, their combinations, as well as antibiotics and plant extracts, should be conducted to control a particular disease so that the proper combination could be formulated to cure the patient for an early and safe recovery from a specific disease. Since not all combinations result in a synergistic impact, a thorough screening of potential combinations is necessary.

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