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Evaluation of In Vitro Antibacterial Activity of various extracts of Shigru

Manita Ahlawat[1], Prof. Pawankumar Godatwar[2]

1. Assistant Professor, Dept. of Roga Nidana & Vikriti Vijnana, NIA Jaipur, E mail: dr.ahlawatmanita16@gmail.com, Mob. +917976257964

2. Technical officer, SEARO WHO, New Delhi, E mail: pgodatwar@gmail.com, Mob. +919314502834

Abstract :

Introduction : A wide spectrum of infectious diseases are emerging with multi drug resistant pathogens. With the rising trends of antimicrobial resistance, the researchers are in constant search of new herbal agents with immense antimicrobial potential. Shigru one of the drugs indicated in krimighan mahakashaya by Acharya Charaka has been selected for the study to prove its antibacterial efficacy on scientific grounds.

Objective

To evaluate the antibacterial effect of aqueous and ethanolic extracts of Moringa oleifera Lam. Stems Bark on the growth of gram-positive and negative bacteria.

Methods

Aqueous and ethanolic extracts of Shigru with three concentrations 10%, 20%, 30% were prepared using Continuous extraction method by Soxhlet Apparatus. All extracts were tested against Staphylococcus aureus (ATCC 29213), Escherichia coli (ATCC 25922), and Pseudomonas aeruginosa (ATCC 27853). The susceptibility tests were performed using Agar ditch method. The antibacterial activity was assessed on the basis of Zone of inhibition and activity index.

Results

Aqueous extract and ethanolic extract of Shigru showed mean ZOI (in mm) 22.33, 27.33, 28.33 and 11.67, 14.3, and 23.33mm at 10%, 20 % and 30 % concentration respectively against S.aureus. In case of E.coli, at 10%, 20%, and 30% concentrations, the average ZOI (in mm) for Shigru was recorded as 0, 0 and 14.67 with aqueous extract

and 0, 12.67 and 23.67 with ethanolic extract respectively as compared to 27.33 and 26.67 mm ZOI in the corresponding positive controls. The average ZOI (in mm) was reported as 13.33, 18 and 22.33 with aqueous extract and 0, 13.67 and 23.33 with ethanolic extract of Shigru at 10%, 20%, and 30% concentrations respectively as compared to 25.33 and 26.33 mm ZOI in the positive control against P. aeruginosa.

Conclusion:

For both aqueous and ethanolic extracts of Shigru, the inhibitory effect was maximum in S.aureus followed by P.aeruginosa and least in E.Coli. The study indicates that Shigru possess a significant antimicrobial potential and may be used as alternate treatment for infections caused by tested strains.

Key words : Shigru, Antibacterial Activity, Zone Of Inhibition, Activity Index.

Introduction

The bacterial disease burden in India is among the highest in the world[i]. Though antibiotics have played an important role in controlling infectious diseases but in recent era all over the world, development of resistance to antibiotics is on the increase amongst pathogenic bacteria[ii],[iii]. However, to counter the situation, very few new antibiotics have come into use in the last three decades[iv]. There is immense need that we find alternative methods to combat this problem.

Shigru (Moringa oliefera) is a well-known drug in traditional system of medicine due to its medicinal and nutritional properties. Samhita[v] and Nighantus had specifically mentioned Krimighna activity of Shigru[vi],[vii]. Along with antimicrobial potential, it has antioxidant, anticancerous, antidiabetic, hepatoprotective, anti tubercular, anti fertility, antiobesity, anti-inflammatory, immunomodulatory, and cardioprotective actions[viii].

Multiple researches has been carried out to find antimicrobial activity of leaves of Shigru. In the present study stem bark of the plant was used to explore the antibacterial properties of its aqueous, and ethanolic extracts against some selected pathogenic bacteria.

Materials and methods

1. **Preparation of the extracts of trial drugs** : Aqueous and ethanolic extracts of trial drugs with three concentrations 10%, 20%, 30% were prepared using sterile water and ethanol as diluents. The method for extraction was Continuous extraction method by Soxhlet Apparatus.
2. **Bacterial isolates:** following bacterial strains were selected for the antimicrobial study:

Pathogen		Bacteria	ATCC no.	Supplier Company
Gram positive	1.	B1 : *Staphylococcus aureus*	ATCC 29213	Hi media Laboratories

Gram negative	2.	B2 : Escherichia coli	ATCC 25922	Pvt. Ltd. Mumbai- 400086, India
	3.	B3 : Pseudomonas aeruginosa	ATCC 27853	

Culture media

N- broth (Himedia) was used for preparing the inoculums. N broth Composition:

Ingredients	Grams/ litre
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5

Final pH (at 25º C) 7.4 +/- 0.2

Mueller Hinton agar (Himedia) was used for the anti-microbial activity

Ingredients	Grams/ litre
Beef infusion	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.1

Method

Zone of inhibition by agar ditch method

The pathogenic strains of above bacteria were produced and anti-bacterial study was done at Microbiology Unit, Central laboratory, NIA, Jaipur.

Procedure

A 24 hours old young culture was prepared of the organisms Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa by inoculating isolated colony into 3 ml of sterile N- broth. The OD of each inoculated broth obtained was 0.6 when checked at 600 nm.

Agar ditch method Procedure

Mueller – Hinton (MH) agar plates were prepared by solidifying 20 ml of sterile MH agar into petri plates. In sterile conditions, 100 ul of test organisms were inoculated by spread plate method. With the help of sterile cup

borer, wells were prepared of 8 mm in every plate. 200 ul of different concentrations of the samples were added into the wells. The plates were incubated then at 37º C for 24 hours. Next day the plates were observed for the zone of inhibition.

Positive control

- Doxycycline 30 µg

Experiment was carried out in triplicate and the average diameter of zone of inhibition was measured in mm. with the help of a scale. Then the mean was calculated of the three readings taken and then activity index was calculated.

- Determination of the activity index[ix]

  The activity index of the test samples extract was calculated as

  $$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

  Each experiment was done in triplets. The average diameter of zone of inhibition was measured in mm. with the help of a scale. Then the mean was calculated of the three readings taken and then activity index was calculated.

Results

Table 1. Mean of ZOI (in mm) of aqueous extract at 10%, 20%, 30% concentration of Shigru against S.Aureus, E.Coli and P.aeruginosa with negative and positive control

Extract	Bacterial strain	Parameter	10% conc.	20% conc.	30% conc.	Positive Control	Negative Control
Aqueous	**S.Aureus**	Mean ZOI	22.33	27.33	28.33	54.33	0
		Activity index	0.41	0.50	0.52	-	-
	E.Coli	Mean ZOI	0	0	14.67	27.33	0
		Activity index	0	0	0.54	-	-
	P.aeruginosa	Mean ZOI	13.33	18	22.33	25.33	0
		Activity index	0.53	0.71	0.88	-	-

Fig.1.a Aqueous extract of Shigru on Staph. aureus

Fig.1.b Aqueous extract of Shigru on E.Coli

Fig.1.c Aqueous extract of Shigru on P.aeruginosa

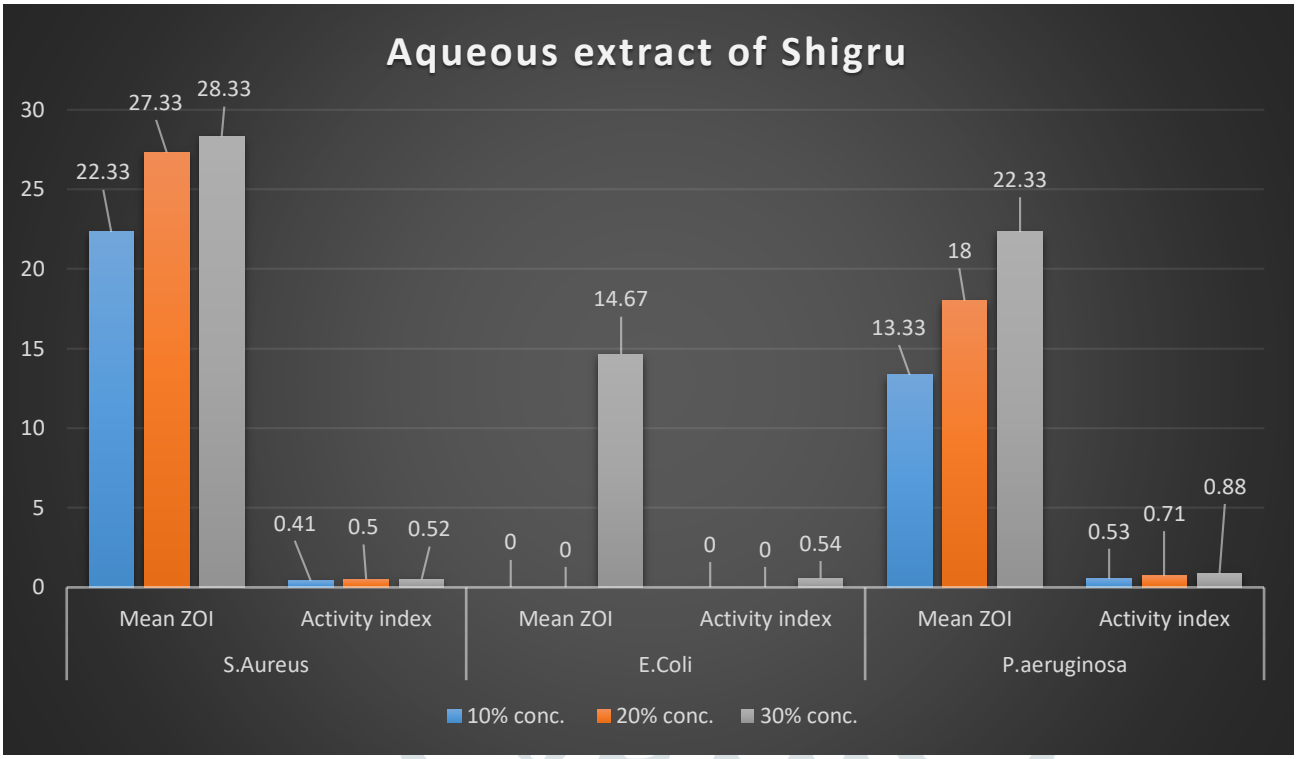


Table 2. Mean of ZOI (in mm) of ethanol extract at 10%, 20%, 30% concentration of Shigru against S.Aureus, E.Coli and P.aeruginosa with negative and positive control

Extract	Bacterial strain	Parameter	10% conc.	20% conc.	30% conc.	Positive Control	Negative Control
Ethanolic	**S.Aureus**	Mean ZOI	11.67	14	23.33	54.67	0
		Activity index	0.21	0.25	0.42	-	-
	E.Coli	Mean ZOI	0	12.67	23.67	26.67	0
		Activity index	0	0.47	0.89	-	-
	P.aeruginosa	Mean ZOI	0	13.67	23.33	26.33	0
		Activity index	0	0.52	0.89	-	-

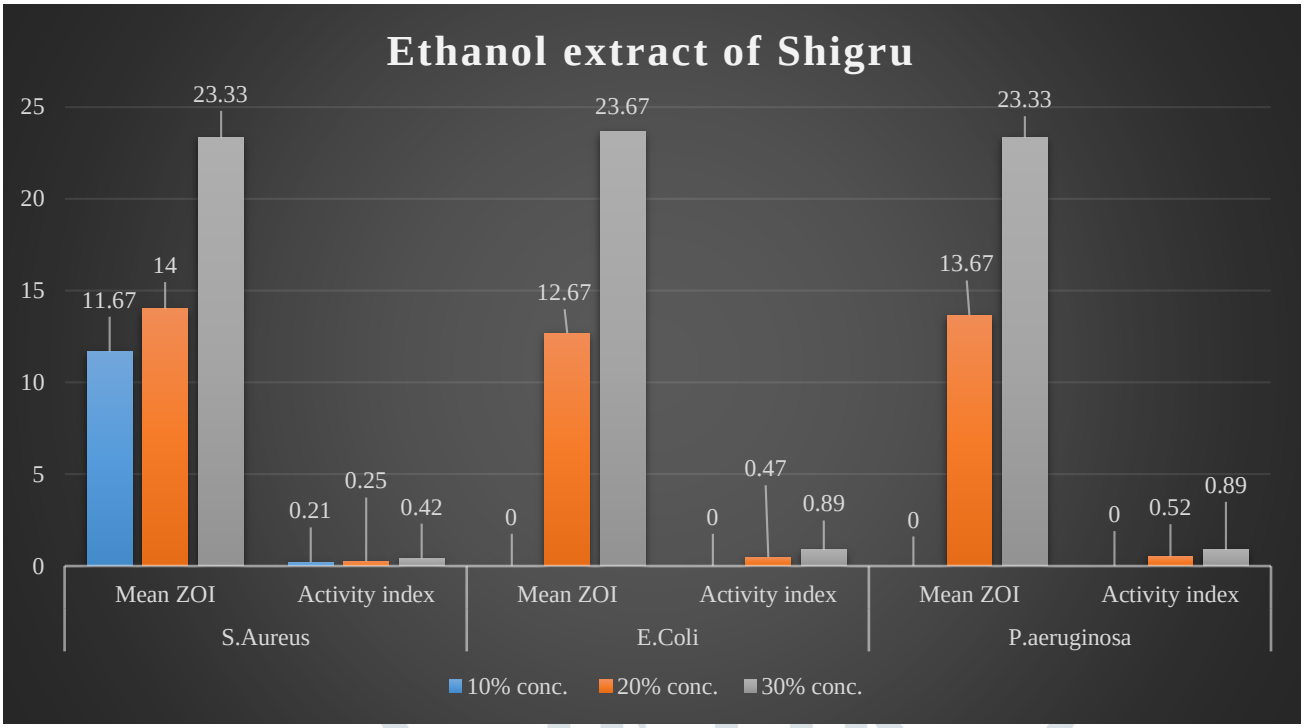


Fig.2.a Alcohol extract of Shigru on S.aureus

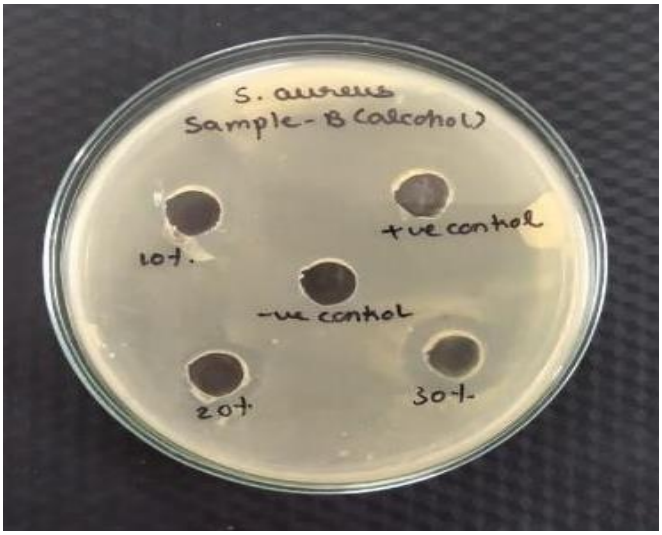
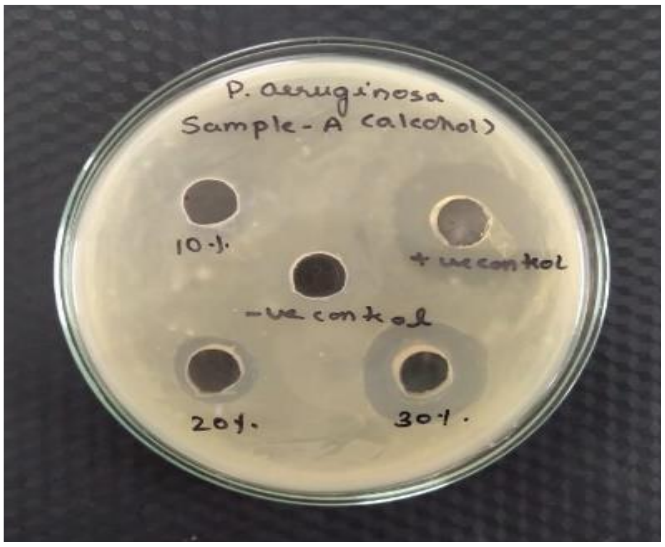


Fig.2.b Alcohol extract of Shigru on E.Coli

Fig.2.b Alcohol extract of Shigru on P.aeruginosa



Discussion

In the present study, Aqueous extract of Shigru at 10%, 20 % and 30 % concentration showed mean ZOI (in mm) 22.33, 27.33 and 28.33 respectively as compared to 54.33 mm ZOI in positive control against Staph.aureus. The extract was found biologically active against Staph.aureus at 20 % and 30 % concentration only with activity index of 0.50 and 0.52 respectively. The findings in the study are in accordance with previous researches also. A study reported dose dependent antibacterial activity of shigru patra against S.aureus and reported zone of inhibition of 13 mm for 75 µl, 10 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Staphylococcus aureus[x].

At 10%, 20%, and 30% concentrations respectively, the average ZOI (in mm) for Shigru was recorded as 0, 0 and 14.67 with aqueous extract as compared to 27.33 mm ZOI in the positive controls against E.Coli. Shigru was found biologically active against E.Coli at 30% concentration of the aqueous extract with activity index of 0.54.

The average ZOI (in mm) for Shigru at 10%, 20%, and 30% concentrations, respectively, was reported as 13.33, 18 and 22.33 with aqueous extract as compared to 25.33 mm ZOI in the positive control against P. aeruginosa. The drug was found biologically active against P. aeruginosa at all concentrations of aqueous extract with activity index >0.50.

In comparison to positive control's mean ZOI of 54.67 mm, the ethanolic extract of the Shigru showed mean ZOIs of 11.67, 14.3, and 23.33 at 10%, 20%, and 30% concentrations respectively. However, none of these concentrations of the extract were biologically active against Staph.aureus because their activity indices were below 0.50. In a previous research it was found that the aqueous, ethanol, and methanol extract of M. oleifera leaves exhibited antibacterial activity against Gram-positive bacteria S. aureus, Gram-negative bacteria E. coli, and Pseudomonas aeruginosa at the concentrations of 30, 60, 90, and 120 mg/ml[xi]. Another study on antibacterial activity of shigru leaves through disc diffusion method reported that the three concentrations 20%, 40% and 60% of methanolic extracts of Moringa oleifera had inhibitory effects of 16, 18, 18mm diameter on S. aureus[xii].

At 10%, 20%, and 30% concentrations of ethanolic extract of Shigru, the average ZOI (in mm) was recorded as 0, 12.67 and 23.67 against E.Coli as compared to 26.67 mm ZOI in the positive control. The drug was found biologically active against E.Coli at 30% concentration of ethanolic extract with activity index of 0.89.

The average ZOI (in mm) was observed as 0, 13.67, and 23.33 at 10%, 20%, and 30% concentrations of the ethanolic extract of Shigru respectively, as compared to 26.33 mm ZOI in the positive control against P. aeruginosa. With ethanolic extract, the drug was found biologically active against P. aeruginosa at concentrations of 20% (AI of 0.52) and 30% (AI of 0.89). The result is supported by a research study by Abhijeet D. Kumbhar et al, in which ZOI of 12 mm was reported by Shigru Patra at 75 µl concentration against P. aeruginosa.

Conclusion :

The drug showed significant antibacterial activity against S.aureus and P.aeruginosa at all conc. of aqueous extract but resistant in case of E.Coli at low conc. of 10 % and 20%. Significant antibacterial activity was reported with 20% and 30% ethanolic extract against all tested strains but E. coli and P. aeruginosa were found resistant at 10% concentration. Further clinical studies should be carried out to investigate in vivo antibacterial efficacy of the drug.

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