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ANTI MICROBIAL ACTIVITY OF *Ehretia Laevis Roxb*. (KHANDU CHAKKA) PLANT

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ABSTRACT

Local peoples from Vidarbha(MS) India are using paste of leaves of this plant for wound healing since many years and known as Khandu Chakka. Study is planned to assess the antimicrobial activity of leaves of this plant against gram positive and gram negative organism responsible for wound infection. Which will be helpful to provide the cheap convenient and alternative medicine for wound healing. Plant is confirmed by taxonomist. Ethanolic and water extracts are done by soxhlet extractor. Crude extract is collected in all aseptic precaution. Nutrient agar medium for bacterial strains will be used for the antimicrobial activity. Amoxicillin drug will be used as a standard for antimicrobial activity. Antibacterial Activity is done by Agar well diffusion method. Size of zones (in mm) will be observed for different concentration. (in µg/ml) i. e. 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 10 µg/ml. Amoxicillin, the standard compound have shown the maximum zone of inhibition against both the selected species of microorganism i.e. S. aureus and E. coli. at all the concentrations. Ethanolic extract was found to be ineffective against E. coli at all concentrations. The zone of inhibition produced by the Ethanolic extract on S. aureus was significant at lower concentration but did increase significantly with increase in the concentration of extract. The moderate antimicrobial activity was produced by crude and water extract at all concentrations against E. coli when compared with the Amoxicillin. Conclusion- Antimicrobial activity of Crude and water extract of Khandu Chakka of was found to be better in S. aureus as compared to E. coli. Antimicrobial activity of Crude and Water extract of Khandu Chakka was found to better on *S. aureus* as compared to ethanolic extract.No antimicrobial activity was found in Ethanolic extract on *E.coli.* Anti microbial activity of water extract of Khandu Chakka was found to be best amongst three extracts.

KEYWORDS: *Ehretia laevis Roxb.*; Anti Microbial activity; Khandu Chakka; Wound Healing: Folklore Medicine.

INTRODUCTION

In the present study, one such folk tribal herbal drug not mentioned in standard Ayurvedic text was found to be very effective in wound healing. It was routinely employed by tribal for wound management, with surprising output. The present study was hence under taken to evaluate the science behind such wonderful herb.

There are many wound healing problems like infections, old age, stress, diabetics, chemotherapy drugs, obesity, alcohol consumption, smoking, mal nourishment.
Lots of higher expensive antibiotics are used to treat wound infection. This is not affordable by rural population. Day by day resistances of higher antibiotics are increases in human. Patients have to face untoward effects of higher antibiotics.

This plant is commonly used in Wardha district for fractures, body ache by rural population. Local name of this plant is Khandu-Chakka.[1] Local peoples from Vidarbha are using paste Of this plant for wound healing since many years and this is also proved on scientific ground.[2] Hence this plant is taken for study to prove its antimicrobial properties on scientific ground and to provide cheap option for wound healing, to effectively adopt the therapeutic uses of this plant and to help needy patients. Also it would be one of the best options of crop cultivation for farmer in farmer suicidal belt like Vidarbha Maharashtra India.

The Main objectives of this study are to assess the antimicrobial activity of leaves of this plant against gram positive and gram negative organism responsible for wound infection, which will be helpful to provide the cheap convenient and alternative medicine for wound

healing. The previous study is done by Methanolic Extract for anti microbial activity.[3]

MATERIALS AND METHODS

- **Collection of study material**

The fresh leaves of identified plant Khandu Chakka were collected from Dhaga forest area of Wardha district. The leaves of plant were shade dried. After the complete dried the leaves were powdered separately and preserved in air tight container and used for its antimicrobial activity.

- **Extraction**

1. Crude extract

Leaves were washed with distilled water and then cleaned by absolute alcohol and then rinsed by distilled water three times. Leaves were crushed in autoclaved mortar and pestle. Crude extract collected by sterile cloth in sterile bottle with all aseptic precaution.

2. Ethanolic extraction

Leaves were washed with distilled water and then cleaned by absolute alcohol and then rinsed by distilled water three times. The ethanolic extract will be done by using soxhlet extractor. Powdered dried leaves (50 gm) will be extracted with 250 ml of solvent.

3. Water Extract:- Leaves were washed with distilled water and then cleaned by absolute alcohol and then rinsed by distilled water three times. The ethanolic extract will be done by using soxhlet extractor. Powdered dried leaves (50 gm) will be extracted with 250 ml of solvent.

- **Antimicrobial Study**
- **The Antimicrobial Study will be carried out as follows**

Microbial strain: Antimicrobial activity for gram positive organisum, *Staphylococcus aureus* and gram negative organism, *Escherichia coli* responsible for wound infection.

- **Culture media**

Nutrient agar medium for bacterial strains will be used for the antimicrobial activity. Amoxicillin drug will be used as a standard for antimicrobial activity.

Method to asses antimicrobial activity

Antibacterial Activity by Agar well diffusion method: Each Petri dish containing nutrient agar medium was inoculated with one bacterial culture. The bore size was 10 mm. All plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The petri dishes were incubated at 37^0 C for 24 hrs. The Ethanol, water and crude extracts of plant were tested at different concentration. Amoxicillin was used as standard. The plates were incubated at room temperature for 24 hrs and zones of inhibition were measured. Diameter of the zone of inhibition was measured. The diameter obtained for the test samples were compared with standard amoxicillin. Size of zones (in mm) will be observed for different concentration (in µg/ml) i. e. 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 10 µg/ml.

- **Place of study**

1. Department of Dravyaguna, Mahatma Gandhi Ayurved College Hospital and Research centre, Wardha.
2. Central Research Laboratory of Mahatma Gandhi Ayurved College Hospital and Research centre, Salod (H) Wardha and CRL of JNMC Sawangi(M), Wardha.
3. IPER Wardha.

- **Study type:-** Laboratory Analytical study.

CALCULATIONS AND RESULT

Table No. 1: Zone of inhibition shown by standard and Ethanolic extract.

Sr. No	Cons. in µg	Zone of Inhibition in mm			
		Amoxicillin		Ethanolic Extract	
		E coli	*S. aureus*	*E. coli*	*S. aureus*
1	10	17	29	Nil	10
2	50	19	30	Nil	14
3	100	20	33	Nil	16
4	250	28	35	Nil	17
5	500	36	38	Nil	18
6	1000	38	40	Nil	20
7	Control (Water)	x	x	Nil	x

Effect of Ethanolic extract on S. Aureus at 1000 µg concentration

Table No. 2: Zone of inhibition shown by standard and Water extract.

Sr. No	Cons. in µg	Zone of Inhibition in mm			
		Amoxicillin		Water Extract	
		E coli	*S. aureus*	*E. coli*	*S. aureus*
1	10	17	29	11	15
2	50	19	30	12	16
3	100	20	33	13	19
4	250	28	35	14	21
5	500	36	38	16	22
6	1000	38	40	17	23
7	Control (Water)	x	x		

Effect of Water extract on E. Coli at 1000 µg concentration

Effect of Water extract on S. Aureus at 1000 µg concentration

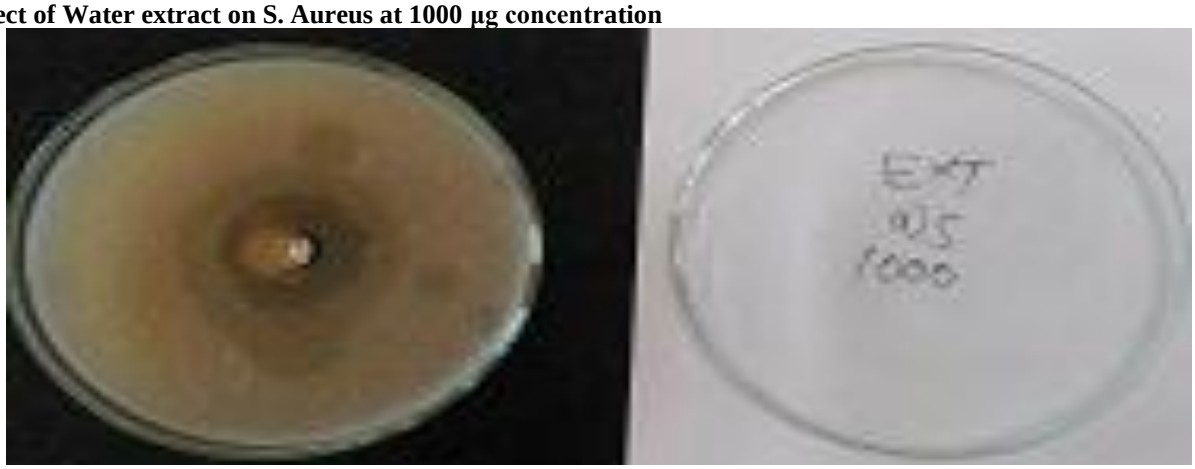
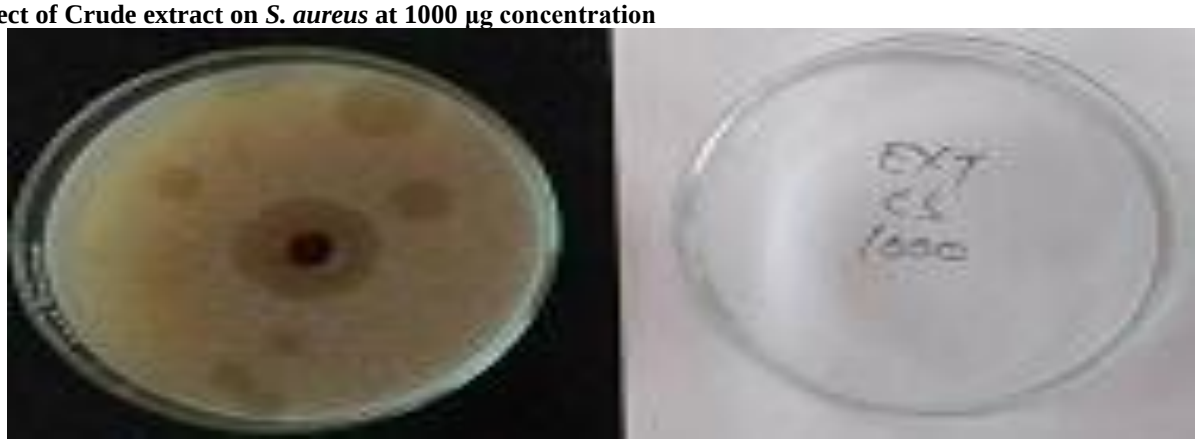


Table No. 3: Zone of inhibition shown by standard and Crude extract.

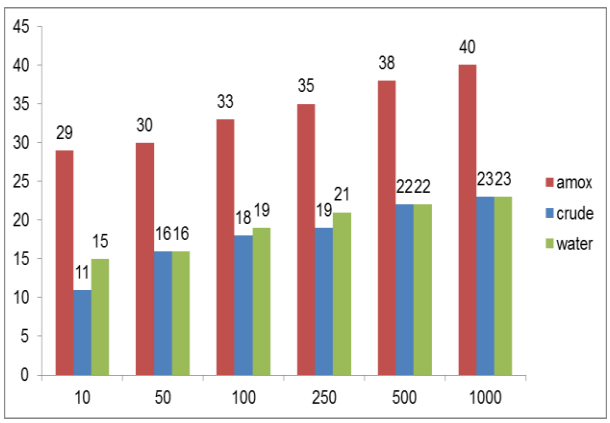
Sr. No	Cons. in µg	Zone of Inhibition in mm			
		Amoxicillin		Crude Extract	
		E coli	*S. aureus*	*E. coli*	*S. aureus*
1	10	17	29	10	11
2	50	19	30	12	16
3	100	20	33	13	18
4	250	28	35	14	19
5	500	36	38	15	22
6	1000	38	40	16	23
7	Control (Water)	x	x		

Effect of Crude extract on E. Coli at 1000 µg concentration

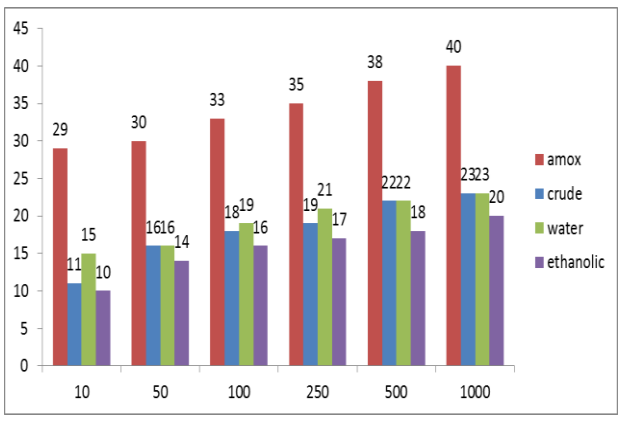
Effect of Crude extract on *S. aureus* at 1000 µg concentration



Zone of inhibition (in mm on Y – axis) on E. Coli of standard Amoxicillin, crude and water extract in various concentration(in **µg on X- axis)**



Zone of inhibition (in mm on Y – axis) on S.aureus of standard Amoxicillin, crude, water and ethanolic extract in various concentrations (in **µg on X- axis).**



DISCUSSION

- As per the above observations, amoxicillin, the standard compound have shown the maximum zone of inhibition against both the selected species of microorganism i.e. S. aureus and E. coli. at all the concentrations (10 to 1000 ug/ml) when compared with the ethanolic, crude and water extracts of Khandu Chakka.
- The zone of inhibition produced by the Ethanolic extract on S. aureus was significant at lower concentration but did increase significantly with increase in the concentration of extract. This extract was found to be ineffective against E. coli at all concentrations.
- The moderate antimicrobial activity was produced by crude and water extract at all concentrations against E. coli when compared with the Amoxicillin.
- As the extract is a mixture of many components, the activity shown by its Phytochemical components was at very low concentration. The same activity determined by the isolated constituents against same pathogens may be more than the standard compound.

CONCLUSION

- Antimicrobial activity of Crude and water extract of Khandu Chakka of was found to be better in S. aureus as compared to E. coli.
- Antimicrobial activity of Crude and Water extract of Khandu Chakka was found to better on *S. aureus* as compared to ethanolic extract.
- No antimicrobial activity was found in Ethanolic extract on *E.coli*.
- Anti microbial activity of water extract of Khandu Chakka was found to be best amongst three extracts.
- This study has generated an evidence for anti microbial activity, which will provide cost effective option for treating the wound infection.
- Also it can help to promote the cultivation of Khandu Chakka as a medicinal plant. This will ultimately aid in improving the economy of farmer to some extends.

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