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RESEARCH ARTICLE

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Evaluation of Antibacterial Activity of Flowers of *Moringa Oleifera* Lam

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ABSTRACT

Background: *Moringa Oleifera Lam.*(*Shigru*) is a well-known drug in Ayurveda used for its Krimighna activity (ability to kill the pathogens). *Acharya Charaka* had mentioned *Shigru* in *Krimighna*[1] *Mahakashaya*. Whereas, some *nighantus* had specifically mentioned *Krimighna* activity of flowers of *Moringa Oleifera Lam*.viz. *Kaiyadeva*[2] *Nighantu* and *Shaligram*[3] *Nighantu*. Therefore,powder of flowers (*Shigru Pushpa Churna*) is selected for evaluation of anti-bacterial activity on the strains which affects a large number of population.

Methods: *Shigru Pushpa churna* was tested for anti-bacterial activity at different concentrations viz., 5µl, 10µl, 25µl, 50µl and 75µl, by Disc Diffusion method for 2 strains of Gram positive and 2 strains of Gram negative bacteria each, with DMSO (Dimethyl Sulphoxide) a neutral solvent.

Result: *Shigru Pushpa* inhibits growth of Staphylococcus aureus, Streptococcus mutans, Pseudomonas auringinosa and Escheria coli at higher concentrations of 50µl and 75µl whereas it is resistant at 5µl, 10µl and 25µl . Zone of inhibition was 13mm for Staphylococcus aureus, 13mm for Streptococcus mutans, 12mm for Pseudomonas auringinosa, 20mm for Escheria coli and activity index were 0.86, 0.43, 0.48 and 0.50, respectively.

Conclusion: *Shigru Pushpa* possess good anti bacterial activity against Staphylococcus aureus, Streptococcus mutans, Pseudomonas auringinosa and Escheria coli.

KEYWORDS

Shigru, Moringa oleifera Lam, Zone of Inhibition, Anti-bacterial, Activity Index, Bacteria

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INTRODUCTION

Moring Oleifera Lam. is slender and fast growing plant belonging to family moringaceae. Plant is indigenous in sub Himalayan tract. It is commonly cultivated throughout the country and grows almost throughout India[4].

It has corky bark; soft, white and spongy wood. Leaves are about 30-75 cms long, tripinnate in structure with petiole sheathing at base. Pinnate are 4-6 in pairs in which uppermost pinnate are opposite to each other. Foliate glands are present between each pair of pinnate and pinnulae. Ultimate leaflets are opposite to each other and about 0.85 to 1.7cms long entirely obovate or elliptical in nature, membranous and pale from beneath[5].

In *Ayurveda* plant is popurarly known as *Shigru (Sanskrit)*, Drum stick plant, Horse raddish tree (English), *Sahijana (Hindi), Saint, Sajjina (Bengali), Murunga (Tamil), Munuga (telagu), Shevaga, Sagata (Marathi).*

The plant (Fig 1) contains 4-hydroxymellein, vanillin, moringine, moringinine, bayrenol, indole acetic acid, indoleacitonitrile, benzylisothiocynate, pterogospermine exhibits antibiotic activity.

It has hypotensive, antibacterial, antifungal, antiviral, depressant, hepatoprotective, anti-inflammatory, anti-cancer, antibiotic, stimulant, anti tubercular, anti fertility action.Leaves are anti-inflammatory, anodyne, anti helmintic, ophthalmic rich in vitamin A and C[6].

Therefore, plant is selected for anti-bacterial activity.

Fig 1 Shigru Flowers

Figure 1 Description:

Flowers are about 2.5 cms in diameter, strongly honey scented, linear lanceolate in nature with sepals reflexed. Petals are about 1.7-2.5 cms long, linear sapulated, white in colour with yellow dot near base.

MATERIALS & METHODS

Plant Material: Flowers of Shigru were collected from Inchal village, Soundatti Tahasil, Belgavi and were authenticated at Central Research Facility, Analytical Laboratory, Belgavi with authentification number CRF/79/2015.

Preparation of Churna: Flowers were dried in the shade for 7 days and churna is

prepared with help of grinder which passes through 120 mesh.

Anti-bacterial activity: The bacterial strains selected were

- Gram Positive
  - ❖ Staphylococcus aureus
  - ❖ Streptococcus mutans
- Gram Negative
  - ❖ Pseudomonas auringinosa
  - ❖ Escherichia coli

The pathogenic strains of above bacteria were selected and anti-bacterial study was performed at Microbiology Department, Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre Belgaum.

Revival of microbial cultures:It was done by growing them in a flask in broth medium.

Nutrient broth medium, 20 ml was transferred to four 100 ml conical flasks one flask for each bacteria. The flasks were capped with cotton plug and autoclaved at 121ºC for 20 minutes at 15 lb pressure per square inch. Dried & frozen bacteria were transferred to conical flasks with nutrient broth media, kept at 37˚C to get cultures.

Preparation of media and media plates : Brain heart infusion agar was taken for all pathogens. Agar, 38 gms was dissolved in 1 litre of distilled water. The sterilized media was poured into sterile petri dishes aseptically. Agar acts a solidifying agent, when solidified the cups (holes) of 8mm diameter were bored using cork borer. After that solidifying plates were kept inverted at 37ºC overnight for checking any contamination. Bacterial cultures were applied to discs with the help of cotton swab stick. Prepared plates were incubated at 37ºC for 24 hours

Preparation of Test solution

Test compound was dissolved in DMSO (dimethyl sulphoxide) each 2 ml to give following concentrations.

1) 10 mg test compound dissolved in 2 ml of DMSO to get 5 µl. concentration
2) 20 mg test compound dissolved in 2 ml of DMSO to get 10 µl concentration
3) 50 mg test compound dissolved in 2 ml of DMSO to get 25 µl concentration
4) 100 mg test compound dissolved in 2 ml of DMSO to get 50 µl. concentration
5) 150 mg test compound dissolved in 2 ml of DMSO to get 75 µl. Concentration

Disc Diffusion method: For evaluation of anti-bacterial activity Disc Diffusion method was adopted.

Test solutions in 5 different concentrations viz. 5µl, 10µl, 25µl, 50µl and 75µl were placed in cups using sterilized pipettes with control and negative groups.

Petri plates were kept in a refrigerator for 2 hours to allow uniform diffusion of the solution then taken out from refrigerator and incubated fort 48 hours at 37ºC.

After incubation period was over, plates were observed for zone of inhibition and measured using transparent scale and readings were taken.

Group Design:

Test group: 5µl, 10µl, 25µl, 50µl and 75µl concentrations of *ShigruPushpaChurna* in DMSo.

Standard Group : 5% w/v ofloxacillin

Negative Group : Distilled water

Determination of activity index

Activity index of crude plant was calculated as[7]

Activity Index = Zone of inhibition of test drug / Zone of inhibition of standard drug.

RESULTS

Table 1 Table of Test drugs, Standard and Negative control group

Si. No.	Micro organism	Concentration of Flowers of MoringaOleifer Lam. (Test Drug)					Ofloxacillin (Standard Drug)	D/W (Negative Group)
		75 µl	50 µl	25 µl	10 µl	5 µl		
1.	Staphylococcus aureus	13 mm	10 mm	R	R	R	15 mm	R
2.	Steptococcus mutans	13 mm	12 mm	R	R	R	32 mm	R
3.	Psuedomonas auriginosa	12 mm	10 mm	R	R	R	30 mm	R
4.	Escheria coli	20 mm	15 mm	12 mm	R	R	30 mm	R

Note - R – Resistant

Table 1 Description:

The *Shigru Pushpa* shows 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.

The *Shigru Pushpa* shows zone of inhibition of 13 mm for 75 µl, 12 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Streptococcus mutans.

The *Shigru Pushpa* shows zone of inhibition of 12 mm for 75 µl, 10 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Psuedomonasauriginosa.

The *Shigru Pushpa* shows zone of inhibition of 20 mm for 75 µl, 15 mm for 50 µl, and 12mm 25 µl, for become resistant for 25 µl, 10 µl, and 5 µl for Escherichia coli.

Fig 2 Zone of Inhibition for *Staphylococcus aureus*

The *Shigru Pushpa* shows 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.

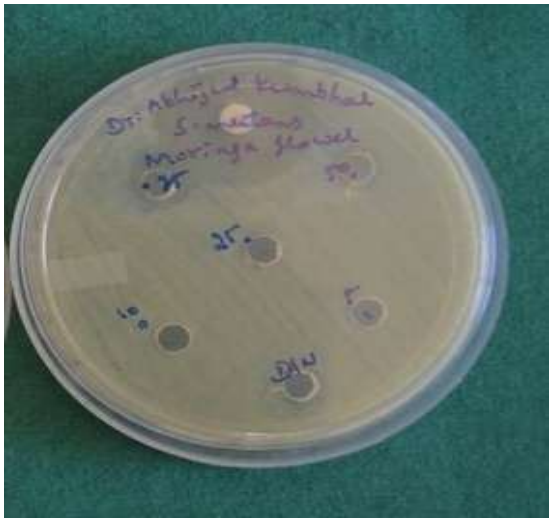


Fig 3 Zone of Inhibition for *Streptococcus mutans*

Figure 3 Description

The *Shigru Pushpa* shows zone of inhibition of 13 mm for 75 µl, 12 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Streptococcus mutans.

Fig 4 Zone of Inhibitionfor *Psuedomonas auringinosa*

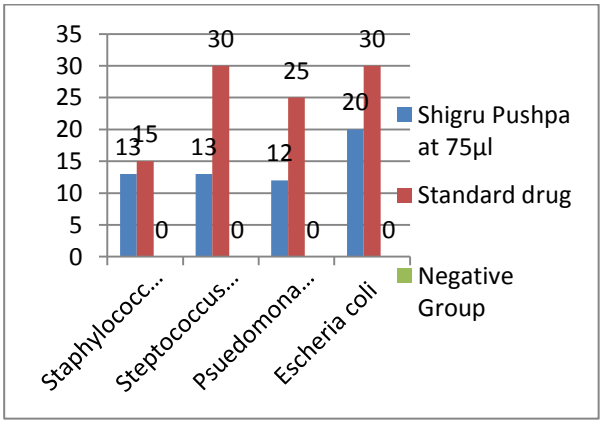
Figure 4 Description:

The *Shigru Pushpa* shows zone of inhibition of 12 mm for 75 µl, 10 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Psuedomonasauriginosa.

Fig 5 Zone of Inhibition for *Escheria coli*

Figure 5 Description**:**

The *Shigru Pushpa* shows zone of inhibition of 20 mm for 75 µl, 15 mm for 50 µl, and 12mm 25 µl, for become resistant for 25 µl, 10 µl, and 5 µl for Escherichia coli.



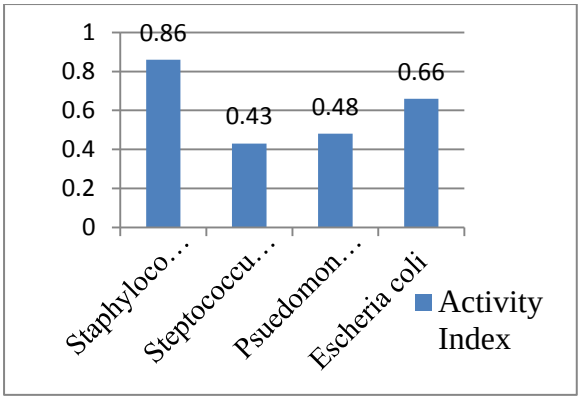
Graph 1 Graph of Test drugs, Standard and Negative control group

Table 2 Description:

The Activity Index of *Shigru Pushpa* was 0.86 for Staphylococcus aureus, 0.43 for Streptococcus mutans, 0.48 for Psuedomonas auringinosa ans 0.66 for Escheria coli.

Table 2 Table of Activity Index

Si. No.	Micro organism	*Shigru Pushpa* zone of inhibition in mm at 75 µl	Ofloxacillin zone of inhibition in mm	Activity Index
1.	Staphylococcus aureus	13 mm	15 mm	0.86
2.	Steptococcusmutans	13 mm	30 mm	0.43
3.	Psuedomonasauriginosa	12 mm	25 mm	0.48
4.	Escheria coli	15 mm	30 mm	0.66



Graph 2 Graph of Activity Index

DISCUSSION

The *Shigru Pushpa* shows 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.

The *Shigru Pushpa* shows zone of inhibition of 13 mm for 75 µl, 12 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Streptococcus mutans.

The *Shigru Pushpa* shows zone of inhibition of 12 mm for 75 µl, 10 mm for 50 µl and become resistant for 25 µl, 10 µl, and 5 µl for Psuedomonasauriginosa.

The *Shigru Pushpa* shows zone of inhibition of 20 mm for 75 µl, 15 mm for 50 µl, and 12mm 25 µl, for become resistant for 25 µl, 10 µl, and 5 µl for Escherichia coli.

The study shows higher zone of inhibition at 75 µl and the zone of inhibition lowers with the concentration and become resistant at 25 µl, 10 µl, and 5 µl of the test drug.

CONCLUSION

The difference in activity at different concentrations may be due to concentrations of phytoconstituents in the test drug sample. This indicates that the proper concentrations of phytoconstituents in other words the proper dose of the drug is essential for antibacterial activity, as the higher concentrations are giving more promising results. Higher (75 µl) concentration of test drug gives significantly good results as compared to 50 µl, 25 µl, 10 µl, and 5 µl concentration of the test drug. Out of four pathogens tested, all the four viz. Staphylococcus aureus, Streptococcus mutans, Psuedomonas auriginosa and Escheria coli are inhibited by Flowers of *Moringa Oleifera Lam.*Activity index for Staphylococcus aureus (0.86) was significantly higher than Streptococcus mutans, Psuedomonas auringinosa and Escheria coli.

This study concludes that powder of Flowers of *Moringaoleifere Lam.* possess good anti bacterial effect.

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