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Evaluation of Antibacterial Activity of Shigru Patra Churna

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ABSTRACT

Background: Shigru (Moringa Oleifera Lam.) is a well known drug in Ayurveda used for its Krimighna activity (ability to kill the pathogens). Acharya Charaka had mentioned Shigru in Krimighna Mahakashaya1. Nighantus had specifically mentioned Krimighna activity of Shigru Patra viz. Kaiyadeva Nighantu2, Raj Nighantu3 and Shaligram4 Nighantu. Therefore Patra churna (powder of leaves) is selected for evaluation of anti bacterial activity on the strains which affects a large number of population.

Methods : Shigru Patra churna at different concentrations viz. 5µl, 10µl, 25µl, 50µl and 75µl were tested for anti bacterial activity 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. Zone of Inhibition was calculated.

Result : Shigru Patra inhibits growth of Staphylococcus aureus, Pseudomonas auringinosa and Escheria coli at higher concentrations of 50µl and 75µl and is resistant to Streptococcus mutans at all concentrations. Zone of inhibition was 13mm for Staphylococcus aureus, 12mm for Pseudomonas auringinosa and 15mm for Escheria coli and activity index were 0.86, 0.40 and 0.50 respectively.

Conclusion : Shigru Patra possess good anti bacterial activity against Staphylococcus aureus, 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 olant belonging to family moringaceae. Plant is indigenous in sub Himalayan tract. It is commonly cultivated throughout the country and grows almost throughout India[5].

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[6].

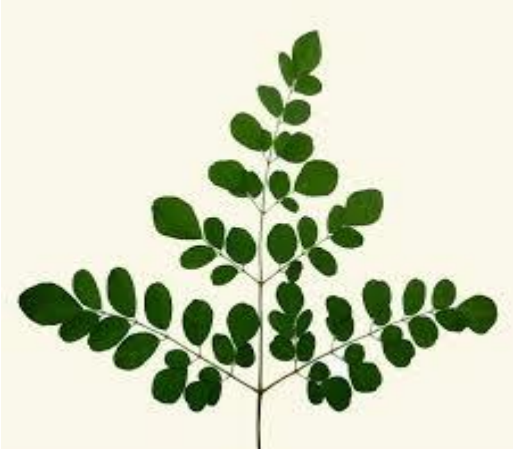
In Ayurveda plant is popurarly known as Shigru (Sanskrit), Drum stick plant, Horse raddish tree (English), Sahijana (Hindi), Saint, Sajjina (Bengali), Murunga (Tamil), Munuga (telagu), Shevaga, Sagata (Marathi).

The plant contais 4- hydroxymellein, vanillin, moringine, moringinine, bayrenol, indole acetic acid, indole acitonitrile, 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[7].

Therefore plant is selected for anti-bacterial activity.

Picture showing Shigru Leaves :



Materials and Methods :

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

Preparation of Churna: Leaves were dried in a shade for 7 days and powered with help of grinder passes through 120 mesh.

Anti bacterial activity : The bacterial strains selected were

- Gram Positive
  - ❖ Staphylococcus aureus
  - ❖ Streptococcus mutans
- Gram Negative
  - ❖ Pseudomonas auringinosa
  - ❖ Escheria col

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

Revival of microbial cultures : Microbes collected from Institute of Microbial Technology were in dried form. It needed to be revived. Like all other living forms, micro-organisms need suitable nutrients and favourable environments for growth. A simple way to obtain bacteria is to grow them in a flask in broth medium.

100 ml Nutrient broth medium were transferred in conical flasks (of quantity 100ml) 20ml each. 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 370C to get cultures.

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

Preparation of Test solution

Test compound was dissolved in 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 contro and negative group.

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 Shigru Patra Churna in DMSo.

Standard Group : 5% w/v ofloxacillin

Negative Group : Distilled water

Determination of activity index

Activity index of crude plant was calculated as[8]

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

Results :

Table of Test drugs, Standard and Negative control group

Si. No.	Micro organism	Shigru Patra concentration (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	R	R	R	R	R	32 mm	R
3.	Psuedomonas auriginosa	12 mm	10 mm	R	R	R	30 mm	R
4.	Escheria coli	15 mm	12 mm	R	R	R	30 mm	R

Note :- R – Resistant

Pictures showing Zone of Inhibition

Staphylococcus aureus

Steptococcus mutans

Psuedomonas auriginosa

Escheria coli

Graph of Test drugs, Standard and Negative control group

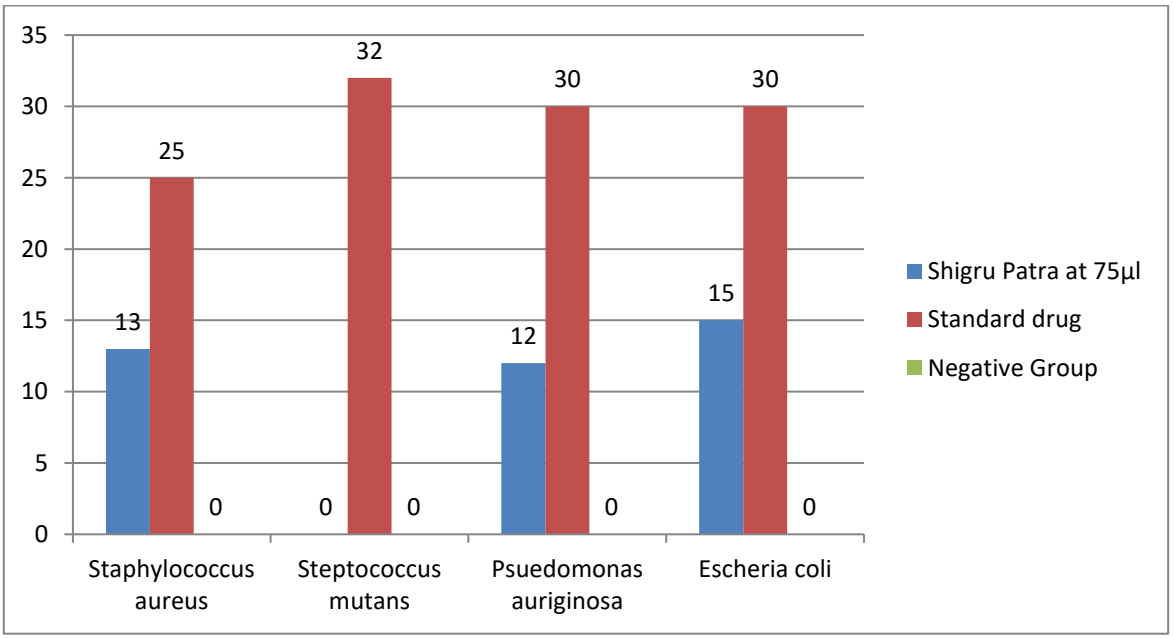
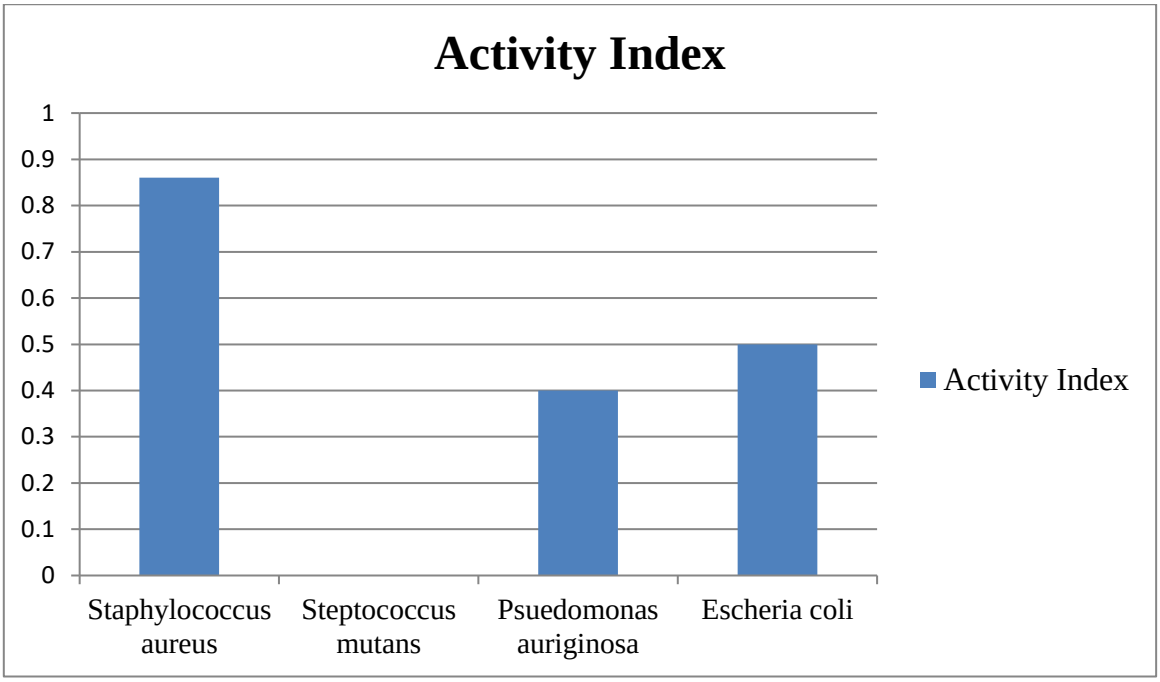


Table of Activity Index :

Si. No.	Micro organism	Shigru Patra 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.	Steptococcus mutans	R	32 mm	0.00
3.	Psuedomonas auriginosa	12 mm	30 mm	0.40
4.	Escheria coli	15 mm	30 mm	0.50

Graph of Activity Index



Discussion :

The Shigru Patra 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 Patra shows total resistance for Streptococcus mutans.

The Shigru Patra 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 Psuedomonas auriginosa.

The Shigru Patra 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 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.

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 bacterias tested Staphylococcus aureus, Psuedomonas auriginosa and Escheria

coli are inhibited by Shigru Patra but Steptococcus mutans was resistant.

Activity index for Staphylococcus aureus (0.86) was significantly higher than Psuedomonas auriginosa and Escheria coli.

This study concludes that Shigtu Patra Churna possess good anti bacterial effect.

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