PIJAR
Paryeshana International Journal of Ayuredic Reserach
www.pijar.org
ISSN:2456:4354

ANTIMICROBIAL ACTIVITY OF AQEUOUS & ETHANOLIC LEAF EXTRACT OF SHIMSHAPA (DALBERGIA SISSOO ROXB)-AN VITRO STUDY

DR.ASHWINI HIREMATH
ASSISTANT PROFESSOR , DEPT OF PG STUDIES IN DRAVYAGUNA,
SHRI JAGADGURU GAVISIDDESHWARA AYURVED MEDICAL COLLEGE & HOSPITAL, KOPPAL.

DOI: https://doi.org/10.47071/pijar.2021.v06i04.03

ABSTARCT:
Worldwide infectious diseases are becoming the leading cause for the death. Three infectious diseases were ranked in the top ten causes of death globally in the most recent survey by the WHO. They are lower respiratory infection (3.1 million deaths), HIV/AIDS (1.5 million deaths), and diarrhoeal diseases (1.5 million deaths).Medicinal plants have been in use since centuries as a remedy for human diseases, because they contain components of therapeutic value. *Shimshapa* (*Dalbergia sissoo Roxb)* is known as Indian rosewood. It is useful in many conditions like *Jwara, Netra roga, Atisara, Chardi, Sopha, Vrana, Krimi, Kushta, Basthivruk* etc.Aqueous and Ethanolic leaf extract of *Shimshapa* was analysed for both Antibacterial and antifungal activities by using two tests viz: MIC and disc diffusion method. This study is undertaken to evaluate antibacterial & antifunagl activity of leaf extract of *Shimshapa.*

Key words: *Shimshapa*, Antibacterial activity, Antifungal activity.

INTRODUCTION:

Infectious diseases are caused by pathogenic micro-organisms such as bacteria, viruses, fungi etc[1]. Worldwide infectious diseases are becoming the leading cause for the death. Three infectious diseases were ranked in the top ten causes of death globally in the most recent survey by the WHO. They are lower respiratory infection (3.1 million deaths), HIV/AIDS (1.5 million deaths), and diarrhoeal diseases (1.5 million deaths)[2]. To cure the infectious diseases search for medicines began long back when the people were aware about the existence of microbes. Antimicrobials which are available in the market have potentially serious adverse effects like toxicity, hyper sensitivity reaction, drug resistance, nutritional deficiencies and masking of infection etc. Hence it is necessary to

search for new antimicrobial properties of plants[3].

Shimshapa (*Dalbergia sissoo Roxb)* is known as Indian rosewood. It is useful in many conditions like *Jwara, Netra roga, Atisara*[6]*, Chardi*[7]*, Sopha*[8]*, Vrana, Krimi, Kushta*[9]*, Basthivruk*[7] etc. Different parts of *Shimshapa (Dalbergia sissoo Roxb)* are used in different formulations for the treatment in many diseases like *patra swarasa* used in eye disease, heart wood is used in fever, oil extraction is used in cutaneous infection, mucilage of the leaves used as application in excoriation. In acute stage of gonorrhea leaf decoction is used[10]. In India and Nepal rural people use *Shimshapa* leaves to treat animals suffering from non specific diarrhoea. Herbal preparation of *Shimshapa* with cow urine can be used as a potent antiseptic preparation for prevention and treatment of chronic bacterial infection[11].

In Ayurvedic science various drugs have been mentioned under Krimighna dravyas. Though Brihatrayi's have not mentioned Shimshapa under Krimighna varga, various Nighantus have mentioned the Krimighna karma of Shimshapa. Apart from that Shimshapa is also used in folklore practice for different purposes like Scabies, syphilis, eye and nose disorders, breast swelling, stomach problems etc.

MATER IALS & METHODS:

The drug *Shimshapa* was botanically identified and authenticated.

Source of Microorganisms

Gram positive Bacteria: Staphylococcus aureus, Enterococcus feacalis

Gram negative Bacteria: Escherichia coli, Pseudomonas aurigonsa,

Fungi: Candida albicans, Aspargillus Niger.

METHODS:

MIC Test (Aerobic)

Chemicals:

HIMEDIA M210-500G

BRAIN HEART INFUSION BROTH-500g

Ingredients of Brain heart infusion Broth

Ingredients	Gm/litre
Calf brain, infusion	200.00
Beef heart, infusion	250.00
Proteose peptone	10.00
Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate	2.50

P^{H} required : Final pH (at 25oC) 7.4+/- 0.2.

Procedure:

9 dilutions of each drug were done with BHI broth.In the initial tube

20microliter of drug was added into the 380microliter of BHI broth. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately.Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10-1 dilution.From 10-1 diluted tube 200microliter was transferred to second tube to make10-2 dilution.The serial dilution was repeated up to 10-9 dilution for each drug.From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200microliter of above culture suspension was added.The tubes were incubated for 24 hours and observed for turbidity.

Disc Diffusion Test

01) Media Used:-

Brain Heart Infusion agar

02) Temperature :-

Bring agar plates to room temperature before use.

03) Inoculum preparation :-

a. Using a loop or swab, transfer the colonies to the plates.

b. Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.

04) Inoculation of Agar plate:-

a. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.

b. Swab entire surface of agar plate three times, rotating plates approximately 60o between streaking to ensure even distribution. Avoid hitting sides of petriplate and creating aerosols.

c. Allow inoculated plate to stand for atleast 3 minutes but no longer than15 min before making wells.

05) Stock solution preparation:-

Prepare the stock solution weighing 10mg of compound and dissolve it in 1ml of DMSO

06) Addition of compound into plate:-

a. Take hollow tube of 5mm diameter, heat it. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate.

b. With the help of micropipette add 75µl, 50µl, 25µl of drug. And 100µl of standard drug.

07) Incubation:-

a. Incubate plates within 15 min of compound application.

b. Invert plates, and stack them no more than five high.

c. Incubate for 18-24 hrs at 37°C in incubator.

08) Reading plates:-

a. Read plates only if the lawn of growth is confluent or nearly confluent.

b. Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

Note:

a. In anti-fungal disc diffusion method, Sabouraud agar medium was used instead of Brain heart infusion agar.

RESULTS & DISCUSSION:

The results of both the extracts of Leaf of Shimshapa showed dose dependant Antibacterial & antifungal activity.

Disc Diffusion Results:

Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against E.coli

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**ciprofloxacin**
1	Aqueous extract	14mm	13mm	10mm	R	40mm
2	Ethanolic extract	15mm	12mm	R	R	38mm

Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against Staphylococcus aureus

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**Ciprofloxacin**
1	Aqueous extract	13mm	12mm	R	R	40mm
2	Ethanolic extract	15mm	10mm	R	R	38mm

Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against Enterococcus feacalis

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**Ciprofloxacin**
1	Aqueous extract	18mm	15mm	13mm	R	38mm
2	Ethanolic extract	15mm	12mm	R	R	35mm

Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against Pseudomonas aeruginosa

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**Ciprofloxacin**
1	Aqueous extract	12mm	R	R	R	50mm
2	Ethanolic extract	11mm	R	R	R	50mm

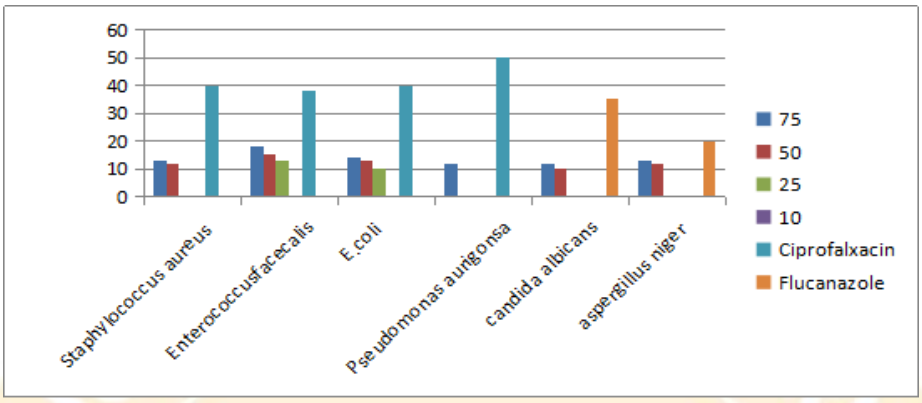
Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against Candida albicans

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**Flucanazole**
1	Aqueous extract	12mm	10mm	R	R	35mm
2	Ethanolic extract	15mm	10mm	R	R	30mm

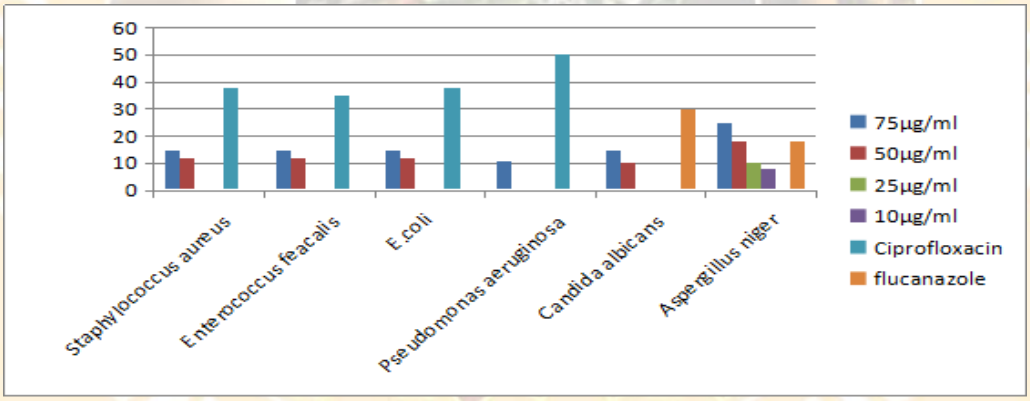
Showing Disc Diffusion Result of Aq. Extract and Ethanolic extract of Shimshapa leaves against Aspergillus Niger

SI. no	**Samples**	**75µl/ml**	50 **µl/ml**	25 **µl/ml**	**10 µl/ml**	**Flucanazole**
1	Aqueous extract	13mm	12mm	R	R	20mm
2	Ethanolic extract	25mm	18mm	10mm	08mm	18mm

Result of Antimicrobial activity of aqueous leaf extract of Shimshapa by Disc diffusion method



Result of Antimicrobial activity of Ethanolic leaf extract of Shimshapa by Disc diffusion method



CONCLUSION:

Aqueous and Ethanolic leaf extract of *Shimshapa* showed moderate efficacy of Antibacterial activity against gram +ve bacteria Enterio feacal & gram -ve bacteria E. coli. Aqueous and Ethanolic leaf extract of *Shimshapa* showed greater efficacy of Antifungal activity against fungi Aspergillus niger. Therefore, further efficacy & safety studies are encouraged on this potential herb.

ACKNOWLEDGEMENT:

The author is thankful to Dr.Kishore G bhat, Professor & HOD, Dept of Microbiology, Ma ratha Mandal's NGH Institute of Dental Sciences & Research centre, Belgaum. . The author is sincerely thankful to Dr. S.S Sarawad, Professor Dept Of PG Studies in

Dravyaguna, B.V.V.S Ayurved Medical College & Hospital, Bagalkot.

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FIGURES:

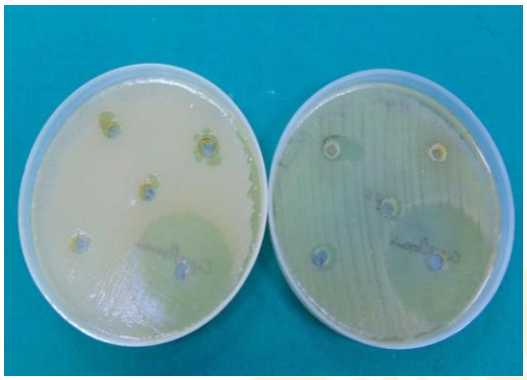

Figure 1: Disc diffusion test for Staphylococcus aureus

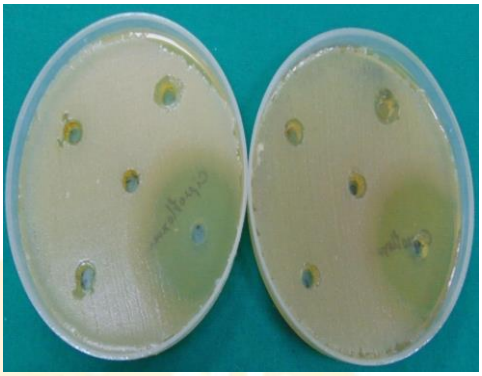

Figure 2: Disc diffusion test of E.feacalis

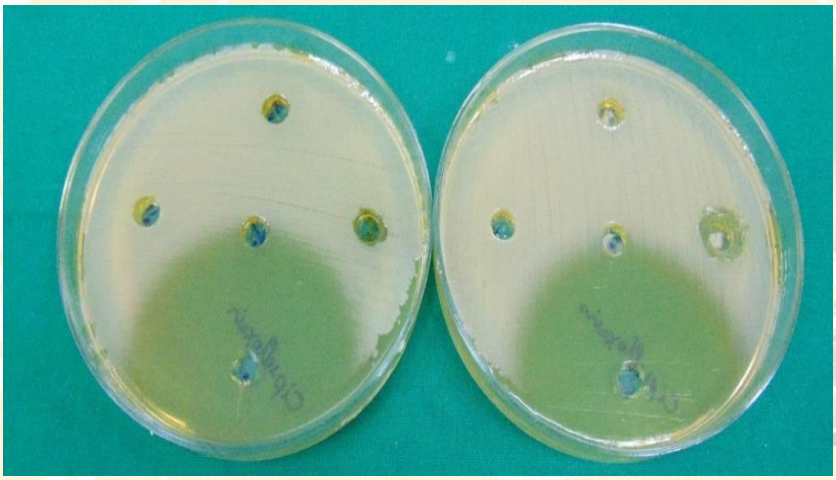

Figure 3: Disc diffusion test for Pseudomonas species

Corresponding author:
DR.ASHWINI HIREMATH
ASSISTANT PROFESSOR ,DEPT OF PG STUDIES IN DRAVYAGUNA,SHRI JAGADGURU GAVISIDDESHWARA AYURVED MEDICAL COLLEGE & HOSPITAL, KOPPAL
Email: ashwinihiremath1307@gmail.com

Source of Support: NIL
Conflict of Interest : None declared

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