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ANTIMICROBIAL ACTIVITY OF *PATOLADIKWATH*

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Abstract

Background*: Surgery is not without wounds and trauma. Healing is a complex phenomenon which includes resurfacing, reconstitution, and restoration of tensile strength of injured skin and it is major challenge to a surgeon which requires proper wound care. In Ayurveda Acharya Sushruta has emphasis more on Vrana and its chikitsa. He defines vrana as the one which causes gatravichurnana and produces thevivarnata of shareer. Shashtiupakrama are explained for the management of vrana. It is said that for proper wound healing one should prevent the wound from the infection. There are several formulations mentioned in the classics which are having antibacterial and anti-inflammatory activity. Among which Acharya Vagbhata has mentioned Patoladikwath for vranaprakshalan. It contains Patolpatra (Trichosanthesdioica Roxb) and Nimbapatra (Azadirachtaindica A. Juss) having the Jantughna, Tridoshashamaka, Dahaprashmana, vrana-shodhaka, Vrana-ropaka and Kandughna properties. In day today life infectious diseases are the major problem, among them the S.aureus, Pseudo monas, E. coli are the most common and dangerous organisms.The bacteria have become resistant to many antibiotics generated by modern science. It is the question, how much of the next generation antibiotics have to prepared. To overcome these problems it is necessary to see the effect of herbal drugs on these bacteria's and also in human body.*

Aims and Objective*: See the antimicrobial activity of Patoladikwath against Pyogenic Bacteria mainly on S.aureus, Pseudomonas and E. coli, by MIC and Disc diffusion method.*

Methods and Materials*: It was subjected to Laboratory based antimicrobial activity study was conducted in Microbiology laboratory of Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre Belgaum.*

Results*: In MIC- It is found that the Patoladikwathdrug is found to be Sensitive from 0.4 concentration onwards but Resistant at 0.2 concentration in E.coli, pseudomonas and s. aureus. In disc diffusion method– Patoladikwathdrug showed sensitive in E.coli at 25,50 and 75 concentration But Resistant at 5 and 10 concentration and Patoladi drug showed the to be resistance at all concentration i.e.5,10, 25, 50, 75 in pseudomonas and s. aureus*

Conclusions*: The plant extract displayed an activity against E. coli, S. aureus and Pseudomonas, which could support the traditional claim of the society.*

Key words: *Antibacterial activity, MIC, disc diffusion, broth dilution, Patoladikwath*

Introduction: Surgery is not without wounds and trauma. Healing is a complex phenomenon which includes resurfacing, reconstitution, and restoration of tensile strength of injured skin and it is major challenge to a surgeon which requires proper wound care. In A*yurveda Acharya Sushruta* has emphasis more on *Vrana* and its *chikitsa*. He defines *vrana* as the one which causes *gatravichurnana* and produces the *vivarnata* of *sharer* [1].*Shashti upakrama* [2] are explained for the management of *vrana.* It is said that for proper wound healing one should prevent the wound from the infection. There are several formulations mentioned in the classics which are having antibacterial and anti-inflammatory activity. Among which AcharyaVagbhata has mentioned Patoladikwath for vrana prakshalan [3]. It contains *Patolpatra* (*Trichosanthesdioica Roxb*) *and Nimbapatra* (*Azadirachtaindica A. Juss*) having the Jantughna, Tridoshashamaka, Dahaprashmana, vrana-shodhaka, Vrana-ropaka and Kandughna properties. [4]

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects [5,6].

As population is increasing to a higher side day by day, there are not enough supply of drugs, there are excessive cost of treatments or curing, side effects of several allopathic drugs and development of resistance to at present used drugs for infectious diseases have led to increased importance on the use of plant materials as a source of medicines for a wide variety of human ailments.

In day today life infectious diseases are the major problem, among them the S.aureus, Pseudo monas, E. coli are the most common and dangerous organisms. The bacteria have become resistant to many antibiotics generated by modern science. It is the question, how much of the next generation antibiotics have to prepared. To overcome these problems it is necessary to see the effect of herbal drugs on these bacteria's and also in human body [7].

So, the purpose of this study was to screen and evaluate antimicrobial activity of the PatoladiKwath to support the traditional therapeutic claim and to provide base line information for the scientific communities to carry on further study.

Aims and Objectives: See the antimicrobial activity of Patoladikwath against Pyogenic Bacteria mainly onS.aureus, Pseudomonas and E. coli, by MIC and Disc diffusion method.

Materials and Methods

Study Design and Period: Laboratory based Experimental study design was conducted in Microbiology laboratory of Maratha Mandal'sNathajirao G. Halgekar Institute of Dental Sciences & Research Centre Belgaum from Sept, 5 to Sept 15, 2015.

Plant Material: Drugs (Patoladi Kwath Bharad) was collected from KLE's GMP Certified Ayurveda Pharmacy, Khasbag, Belgaum.identification and authentication has been done in CRF, K.L.E.s Ayurveda College, Belgaum. Analytical study was carried out in the CRF, KLEU BMK Ayurveda Mahavidyalaya, Belgaum

Preparation of Kwatha (Followed SOP): One part of drug was boiled with 16 parts of water, over a mandagni till the liquid reduces to 1/8th of the quantity taken [8].

Microorganisms: Escherichia coli, staphylococcus aureus, pseudomonas aerugenosa, which are commonly known to cause wound infection were used to screen the antibacterial activity of the Patoladikwath.

Materials & Equipment's: Distilled water, ferric chloride, hydrochloric acid, chloroform, sulphuric acid, sodium hydroxide, glacial acetic acid, ammonia solution, Grinder or blender, conical flask, test tubes, petri plates, laminar air flow, Incubator, autoclave, Freeze, hot air oven, water bath and heater

Antibacterial screening: Disc diffusion methods were employed for antimicrobial screening of the extracts.

Disc Diffusion Procedure[9]

1. **Media Used:** Brain Heart Infusion agar
2. **Temperature:** Bring agar plates to room temperature before use.
3. **Inoculum Preparation**

1. Using a loop or swab, transfer the colonies to the plates.
2. 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.

4. **Inoculation of Agar Plate**

1. 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.
2. 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.
3. Allow inoculated plate to stand for atleast 3 minutes but no longer than 15 min before making wells.

5. **Addition of Compound into Plate**

1. 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.
2. Add 75µl, 50 µl, 25 µl, 10 µl and 5 µl of compound into the respective wells on each plate.

6. **Incubation**

1. Incubate plates within 15 min of compound application.
2. Invert plates, and stack them no more than five high.

3. Incubate for 18-24 hrs at 37 ºC in incubator.
7. **Reading Plates**
1. Read plates only if the lawn of growth is confluent or nearly confluent.
2. Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

Note

1. In anti-fungal disc diffusion method, Sabouraud agar medium is used instead of Brain heart infusion agar.
2. For Facultative anaerobes, incubate plates in the Co2 Jar and keep the jar in the incubator at 37 ºC.
3. For Anaerobic organisms, incubate plates in the Anaerobic jar and keep the jar in the incubator at 37 ºC.

Determination of Minimum Inhibitory Concentration (MIC) [10]

MIC Procedure

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

Note: For facultative anaerobes, tubes were incubated at 37°C for 48-72 hrs in Co_2 Jar. For strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hrs.

HIMEDIA M210-500G

Brain Heart Infusion Broth(BHI)

500g

Ingredients	*Gms/litre*
Calf brain, infusion from	200.00
Beef heart, infusion from	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50
Final pH (at 25ºC) 7.4+/-0.2	

Results & Discussion

Results MIC

Kashaya	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
E.coli	S	S	S	S	S	S	S	S	S	R
Pseudomonas	S	S	S	S	S	S	S	S	S	R
S.aureus	S	S	S	S	S	S	S	S	S	R

Results Disc Diffusion

Kashaya	75	50	25	10	5
E.coli	18mm	15mm	10mm	R	R
Pseudomonas	R	R	R	R	R
S.aureus	R	R	R	R	R

Note: S-Sensitive; R-Resistant

Figure- 1

Figure-2

Figure-3

Figure 1,2,3. Zone of inhibition of different microorganism (E.coli, S. aureus, Pseudomonas) with a specific concentration of Patoladikwath as medicine

Figure-4

Figure-5

Figure-6

Figure:4,5,6 Showes Antimicrobial activity test by MIC Method

In MIC-It is found that the Patoladikwathdrug is found to be Sensitive from 0.4 concentration onwards but Resistant at 0.2 concentration in E.coli, pseudomonas and s. aureus.

In disc diffusion method– Patoladikwathdrug showed sensitive in E.coli at 25,50 and 75 concentration But Resistant at 5 and 10 concentration and Patoladi drug showed the to be resistance at all concentration i.e.5,10, 25, 50, 75 in pseudomonas and s. aureus

Conclusion: The plant extract displayed an activity against E. coli, S. aureus and Pseudomonas, which could support the traditional claim of the society. So based on the findings, the further study to be conducted concerning the chemical compositions and the structure elucidation of the active component of the plant.

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