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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *PENTABARK KASHAYA* AGAINST SELECTED BACTERIAL STRAINS CAUSING WOUND INFECTION: *IN-VITRO* STUDY

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Keywords:

Antibacterial activity, *Pentabark kashaya*, Wound infection, Agar well method

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ABSTRACT: Background: Wound infection occurs when one or more organisms invade the wound. Nosocomial infections are the most common hospital acquired infections responsible for mortality and morbidity. Multidrug resistance against bacterial strains is the most serious emerging situation in worldwide, warranting the search for other alternatives. In this regard *Pentabark kashaya was* developed which contains ingredients possessing antimicrobial, anti inflammatory, and wound healing activity. **Materials and Methods:** Antibacterial activity of *Pentabarkkashaya (PK)* was determined against three predominant bacterial strains one gram positive and two gram negative strain *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The sensitivity of the organisms were tested by both the agar well method and broth dilution method (MIC) and it was compared with the antibacterial activity of provide iodine solution 5%. **Result:** *Pentabark kashaya* showed antibacterial activity against all the test pathogens in both agar well and broth dilution method. The highest antimicrobial activity was observed against *Escherichia coli* and *Pseudomonas aeruginosa* in agar well diffusion method showed the inhibitory zone of 14 mm in both organisms. **Conclusion:** *Pentabark kashaya has* antibacterial property against all the test organisms.

INTRODUCTION: Wound infection is one of the most common and serious complications among the hospital acquired infections. It can increase the length of hospital stay and accounts for the mortality rate up to 70–80% [1]. In wounds, identifying and managing infection is an important aspect of primary care practice. Topical wounds require special attention as they are more prone for bacterial, fungal, and viral contaminations, thereby making them further susceptible to other types of secondary complications [2].

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Bacterial infections are serious problems to the successful treatment of the wounds resulting in the complications sometimes leading to fatal sepsis [3].

The common bacterial pathogens responsible for wound infections are *Staphylococcus aureus*, *Pseudomona aeruginosa*, and bacteria belonging to family Enterobacteriaceae [4]. These pathogens can seriously delay wound healing process by disrupting the normal clotting mechanisms and promoting disordered leukocyte function and poor quality granulation tissue formation, reduce tensile strength of connective tissue, and impair epithelization [5]. The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current anti-bacterial therapy [6]. The pharmaceutical industries have produced a number of new antibiotics but resistance to these drugs by microorganisms has

increased as bacteria has the genetic ability to transmit and acquire resistance to synthetic drugs that are utilized as therapeutic agents [7]. So, it is necessary to develop new drugs to control pathogenic microorganism. *Ayurveda* has described numerous medicinal plants and formulations which have wound healing, antibacterial, antifungal, and antiprotozoal effect that could be used either systemically or locally. Now a days medicinal property of plants have also been preferred throughout the world, due to their potent pharmaco-logical activities, low toxicity, and economic viability, when compared with synthetic drugs. Medicinal plants are rich in a wide varietyof bioactive secondary metabolites such as tannins, terpenpoids, alkaloids, saponins, flavonoids, and phenolic compounds that can produce a definite physiological action on the human body and helps in control of wound infection [8]. The ingredients of *Pentabark kashaya* are *Vata* (*Ficus bengalensis* Linn), *Udumbara* (*Ficus racemosa* Linn), *Ashwatha* (*Ficus religiosa* Linn), *Parish* (*Thesposia populnea* Soland.), *Plaksha* (*Ficus infectoria* Roxb), *Kasisa* (Ferrous Sulphate ($FeSO_47H_2O$), *Tuttha* (Copper Sulphate ($CuSO_47H_2O$), and *Spatika* (Potash Alum ($K_2SO_4Al_2SO_4)_324H_2O$) are having the antimicrobial, anti-inflammatory wound healing properties. In the present study antibacterial activity of *Pentabark kashaya* was evaluated against three predominant bacterial strains one gram-positive and two gram-negative strain *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

MATERIALS AND METHODS:

Source of Raw Drugs: Vata (*Ficus bengalensis* Linn), Udumbara (*Ficus racemosa* Linn), Ashwatha (*Ficus religiosa* Linn), Parish (*Thesposia populnea* Soland.), Plaksha (*Ficus infectoria* Roxb), Kasisa (Ferrous Sulphate ($FeSO_47H_2O$)), Tuttha (Copper Sulphate ($CuSO_47H_2O$)), and Spatika(Potash Alum ($K_2SO_4Al_2SO_4)_324H_2O$)) were procured from the GMP certified KLE Ayurved Pharmacy, Belagavi and authenticated at central research facility, Ayush approved drug testing laboratory of KAHER's Shri B.M.K Ayurveda Mahavidyalaya, Belagavi.

Preparation of Pentabark Kashaya: [9] Preparation of *Pentabark kashaya* was done in Rasashastra and Bhaishajya Kalpana department, KAHER's Shri B.M.K Ayurveda Mahavidyalaya, Belagavi.

TABLE 1: SHOWING INGREDIENTS OF 100 ML *PENTABARK KASHAYA*

S. no.	Name of Drug	Latin Name	Part used	Quantity
1	*Vata*	*Ficus bengalensis* Linn.	Bark	10gm
2	*Udumbara*	*Ficus racemosa* Linn.	Bark	10gm
3	*Ashwatha*	*Ficus religiosa* Linn.	Bark	10gm
4	*Parisha*	*Thesposia populnea* Soland.	Bark	10gm
5	*Plaksha*	*Ficus infectoria* Roxb.	Bark	10gm
6	*ShodhitaKasisa*	Ferrous Sulphate ($FeSO_47H_2O$)		1gm*
7	*ShodhitaTutta*	Copper Sulphate ($CuSO_47H_2O$)		25mg*
8	*ShodhitaSpatika*	Potash Alum ($K_2SO_4Al_2SO_4)_324H_2O$)		2.75 mg*
9	Sodium benzoate			10mg
10	Methyl paraben			100mg

*Note - Selection of quantity of *Shodhita Tutta* (0.012%), *Kasisa* (0.5 to 1 %) and *Spatika* (1 to 10%) was made on the basis of text book inorganic pharmaceutical Chemistry by Bently and Indian Pharmacopoeia.

Method of Preparation: *Panchavalkala (Vata, Udumber, Ashwatha, Parisha, Plaksha) Kashaya* 100ml was prepared as per standard operative procedure. Prepared *Kashaya* (Decoction) was taken in a steel vessel and it was mixed with *Shodhita Kasisa*, *Shodhita Tutta*, and *Shodhita Spatika* as quantity mentioned. Preservatives, Sodium benzoate and Methyl paraben were added individually and stirred well till they completely dissolved. *Kashaya* (Decoction) was filtered and stored in bottle.

Antibacterial Study: Antibacterial study was done in microbiology department of Mararha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre Belgavi.

Selection of Pathogens for the Study: Antibacterial activity of *Pentabark kashaya* was determined against three predominant bacterial strains one gram-positive and two gram-negative strain *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

TABLE 2: SHOWING THE ATCC NO OF MICRO-ORGANISM

S. no.	Micro- organism	Standard ATCC No.
1	*Staphylococcus aureus* (Gram +ve)	ATCC No.12598
2	*Escherichia coli* (Gram –ve)	ATCC No.25922
3	*Pseudomonas aeruginosa* (Gram –ve)	ATCC No.25619

Inoculums Preparation: Brain heart infusion broth medium was prepared by adding brain heart infusion broth into distilled water and sterilized in an autoclave. Using a loop or swab, colonies were inoculated to the broth. Visually turbidity of broth was adjusted to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, the suspension was standardized with a photometric device.

Antibacterial Activity by Agar Well Method:

Inoculation of Agar Plate: Brain infusion agar plate was prepared. Within 15 min of adjusting the inoculums to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculums and rotated it against the wall of the tube above the liquid to remove excess inoculums. Entire surface of agar plate was swabbed three times, rotating plates approximately 60º between streaking to ensure even distribution. Inoculated plate was allowed to stand for at least 3 min but no longer than 15 min before making wells. Hollow tube of 5mm diameter was taken, heated it. Press it on an inoculated agar plate to make a well in the plate. Likewise, five wells on each plate was made. With the help of micropipette 75µl, 50 µl, 25 µl, 10 µl and 5 µl of compound was added into the respective wells on each plate **Fig. 1, 2, 3**.

FIG. 1: ANTIBACTERIAL ACTIVITY AGAINST *S. AUREUS*

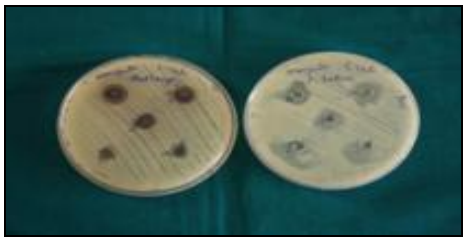

FIG. 2: ANTIBACTERIAL ACTIVITY AGAINST *E. COLI*

FIG. 3: ANTIMICROBIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGINOSA*

Incubate the plates for 18-24 h at 37 ºC in incubator. Measure the diameter of inhibition zone to the nearest whole mile meter by holding the measuring device.

Antibacterial Activity by Broth Dilution Method (MIC): 9 dilutions of each drug have to be done with BHI for MIC. In the initial tube 20 microliter of drug was added into the 380 microliter of BHI broth. For dilutions 200 microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 microliter was transferred to second tube to make 10^{-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 200 microliter of above culture suspension was added **Fig. 4, 5**. The tubes were incubated for 24 h and observed for turbidity.

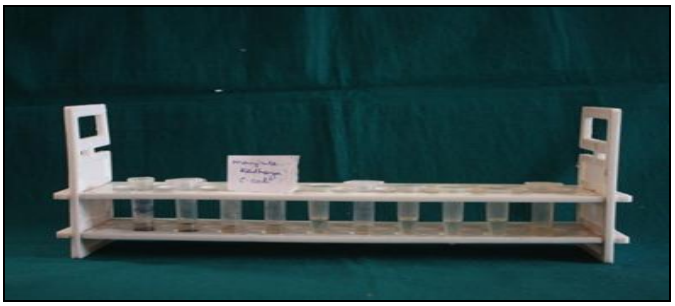

FIG. 4: MIC OF *PENTABARK KASHAYA*

FIG. 5: MIC OF POVIDONE IODINE SOLUTION

OBSERVATION AND RESULTS: *Pentabark kashaya* shows antibacterial activity against all the test pathogens in both agar well and broth dilution method.

TABLE 3: SHOWING ZONE OF INHIBITION OF *PENTABARK KASHAYA* AND POVIDONE IODINE SOLUTION IN AGAR WELL METHOD

S. no.	Samples	75 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml
			P. aerugenosa			
1	*Panchavalkaladi Kashaya*	14mm	12mm	10mm	R	R
2	Povidone Iodine	10mm	08mm	R	R	R
			S. aureus			
1	*Panchavalkaladi Kashaya*	12mm	10mm	R	R	R
2	Povidone Iodine	17mm	15mm	13mm	R	R
			E. coli			
1	*Panchavalkaladi Kashaya*	14mm	11mm	10mm	R	R
2	Povidone Iodine	13mm	10mm	R	R	R
			Observation: S – Sensitive R – Resistant			

Pentabark kashaya showed zone of inhibition of 10mm at 50µg/ml and 12mm at 75µg/ml dilutions against *S. aureus* whereas povidone iodine shows 13mm at 25µg/ml, 15mm at 50µg/ml and 17mm at 75µg/ml dilutions. *Pentabark kashaya* showed zone of inhibition of 10mm at 25µg/ml, 11mm at 50µg/ml and 14mm at 75µg/ml dilutions against *E. coli* whereas povidone iodine shows 10mm at 50µg/ml and 13mm at 75µg/ml dilutions. *Pentabark kashaya* showed zone of inhibition of 10mm at 25µg/ml, 12mm at 50µg/ml and 14mm at 75µg/ml dilutions against Pseudomonas whereas povidone iodine shows 8mm at 50µg/ml and 10mm at 75µg/ml dilutions. Povidone iodine was resistant at 25µg/ml dilution.

TABLE 4: SHOWING OBSERVATION OF MIC OF *PENTABARK KASHAYA* AND POVIDONE IODINE SOLUTION

S. no.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
					E. coli						
1	*Panchavalkaladi Kashaya*	S	S	S	S	S	S	R	R	R	R
2	Povidone Iodine	S	R	R	R	R	R	R	R	R	R
					S. aureus						
1	*PanchavalkaladiKashaya*	S	S	S	S	S	S	S	S	R	R
2	Povidone Iodine	S	R	R	R	R	R	R	R	R	R
					P. aerugenosa						
1	*PanchavalkaladiKashaya*	S	S	R	R	R	R	R	R	R	R
2	Povidone Iodine	S	R	R	R	R	R	R	R	R	R
					Observation: S – Sensitive R – Resistant						

TABLE 5: SHOWING DISC DIFFUSION RESULT OF *PENTABARK KASHAYA*

S. no.	Organism	Standard	Povidone iodine	*Pentabark kashaya*
1	*Staphylococcus aureus* (Gram +ve)	26mm at 2µg/ml	13mm at 25µg/ml 15mm at 50µg/ml 17mm at 75µg/ml	10mm at 50µg/ml 12mm at 75µg/ml
2	*Escherichia coli* (Gram –ve)	32mm at 2µg/ml	10m at 50µg/ml 13mm at 75µg/ml	10mm at 25µg/ml 11mm at 50µg/ml 14mm at 75µg/ml
3	*Pseudomonas* (Gram –ve)	>21mm at 2µg/ml	08mm at 50µg/ml 10mm at 75µg/ml	10mm at 25µg/ml 12mm at 50µg/ml 14mm at 75µg/ml

TABLE 6: SHOWING MIC RESULT OF *PENTABARK KASHAYA*

S. no.	Organism	Standard	Povidone iodine	*Pentabark kashaya*
1	*Staphylococcus aureus* (Gram +ve)	2µg/ml	100 µg/ml	0.8 µg/ml
2	*Escherichia coli* (Gram –ve)	2µg/ml	100 µg/ml	3.12 µg/ml
3	*Pseudomonas* (Gram –ve)	<4µg/ml	100 µg/ml	50 µg/ml

The minimum inhibitory concentration (MIC) of *Pentabark kashaya* against *E. coli, S. areus* and *Pseudomonas* was 3.12µg/ml, 0.8µg/ml and 50µg/ml respectively whereas MIC of Povidone iodine was 100µg/ml in all test organisms.

DISCUSSION: Wound infections cause economic burden to the patients and also increases the hospital stay. Multidrug resistance against human pathogensis an emerging serious condition. Due to a high incidence of antibiotic resistance, evaluating the antibacterial effect of herbal medicines as potent agents for treating wound infections has a paramount importance. in addressing animal as well as human health problems [10].

In the present study *Pentabark kashaya* was evaluated for its antibacterial property against both gram negative and gram positive micro organisms. The results were compared with the antibacterial activity of povidon iodine 5% solution.

Pentabark kashaya showed maximum zone of inhibition of 14mm for *Pseudomonas* and *E. coli* organism and minimum zone of inhibition of 12mm for *S. aureus*. Povidon iodine showed maximum zone of inhibition of 17mm for *S. aureus* and 13mm for *E. coli* and minimum zone of inhibition of 10mm for *Pseudomonas* **Table 5**. This reveals that *Pentabark kashaya* was more active against *Pseudomonas* and *E. coli*.

The minimum inhibitory concentration (MIC) of *Pentabark kashaya* was 0.8µg/ml, 3.12µg/ml, and 50µg/ml against *S. areus, E. coli* and *Pseudomonas* respectively whereas Povidone iodine had MIC of 100µg/ml against all the three microorganisms **Table 6**. This result shows that *Pentabark kashaya* has anti-bacterial activity against all the three organisms more on *S. aureus*.

Pentabark kashaya has shown antibacterial activity against all the three test organisms' *i.e. E. coli, S. aureus, Pseudomonas* in both methods, this may be because of antimicrobial activity of *Panchavalkala* (five barks), *Kasisa* (Ferrous Sulphate ($FeSO_4 7H_2O$)), Tuttha (Copper Sulphate ($CuSO_4 7H_2O$)). As formulation contains tannins, alkaloids, saponins as a phytochemicals [11] which are known to have anti-inflammatory, astringent, and antimicrobial activities [12]. *Shodhita Tutta* (Copper Sulphate) has antibacterial activity on *E. coli, S. aureus* bacteria and antifungal activity on fungi *Candida albicans* [13]. *Spatika* (Potash alum) has bacteriostatic action with MIC of 2% conc. [14]

CONCLUSION: *In-vitro* antibacterial study showed *Pentabark kashaya* has antibacterial activity against all the three test organisms *S. aureus, E. coli* and *Pseudomonas aeruginosa*. So, this formulation can be used in wound management as an alternative medicine.

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CONFLICTS OF INTEREST: No competing financial interests exist.

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