



ANTIMICROBIAL STUDY OF PALASHABEEJADI CHURNA –AN INVITRO STUDY

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ABSTRACT

Ayurvedic medicine is considered to be world's oldest medical system. Starting with the references in the Atharvaveda, we have textual evidence of traditional use of medicinal plants in India . In Ayurvedic practice churna is the most widely prescribed dosage form . palasha beejadi churna is a poly herbal formulation mentioned in bhaishajya ratnavali in context of udaragata krimi.its ingredients are Palashabeeja,indrayava vidanga ,nimba ,bhunimba and guda.it is having properties like krimighna ,kandughna etc.hence in present study an attempt will be made to evaluate the antibacterial and antifungal activity of the same.

Key words: antimicrobial ,krimi,palasha beeja,churna.

INTRODUCTION

Now a days Microbial resistance, caused by irrational use of antibiotics, is a major issue in the treatment of infectious diseases. A new strategy to manage microbial resistance is the use of natural derivatives in pure form or in combination with antibiotics or chemotherapeutic agents which, in many cases, create a synergistic effect.

Microorganisms are the tiny entities which are invisible to naked eyes and microscopic in nature ,surviving in extreme climatic conditions. Other than hereditary genetic factors and lifestyle disorders most of the diseases are caused due to microorganisms.

Microbial resistance, caused by irrational use of antibiotics, is a major issue in the treatment of infectious diseases. Ayurvedic medicine is considered as the worlds oldest medical system,which is originated in india dating back over thousands of years.

Natural products are a potential supply for novel biologically active compounds that could lead to innovation of new theurapeutics.plant based antimicrobial are safer and eco friendly.

The discovery of new sources of natural antimicrobial agents requires the systematic screening of a large number of plants; however, ethnopharmacological data can reduce the time to discover the antimicrobials.

the term krimi is frequently uses in ancient Ayurvedic classics from vedic period to samhita period the word krimi by etymology means the one which causes suffering or ill health .krimi has been used in Ayurveda in broader sence i.e ist covers all the microbes and worms covering a wide range of infections and infestations,which includes bacteria, viruses ,parsites,and fungi many preparations has been mentioned in classics as krimighna and acts against microbes and they are yet to explore to replace the active compounds of allied sciences. Churna kalpana is the basic formulation in bhaishajya kalpana which is widely used and easy to give for theurapeutic benefits. palasha beejadi churna is one among them ,mentioned In Bhaishajya Ratnavali,Chapter Krimiroga Chikitsa Prakaranam .

AIMES AND OBJECTIVES

- To prepare Palasha Beejadi Churna as per classics
- To study the physico chemical and phyto chemical analysis of Palashabeejadi Churna.
- To evaluate the antimicrobial effect of Palashabeejadi Churna by invitro method.

MATERIALS AND METHODS

Test drug

The raw drugs required are collected from herbal garden and local market guda was collected one year prior to study and stored,authentication of raw drugs is done by experts of dravya guna department at NKJAMC Bidar. test drug was prepared at RasaShastra and Bhaishajya kalpana Department Rasashala of N.K.Jabshetty Ayurvedic Medical college & P.G Centre, Bidar. The sample Palasha beejadi churna¹ was prepared as per classics.

Table no.1 showing the ingredients of palashabeejadi churna.

Si no.	Drug	Part used	Quantity
1	Palasha ²	Seeds	1 part
2	Indrayava ³	Seeds	1part
3	Vidanga ⁴	Seeds	1 part
4	Nimba ⁵	Bark	1 part
5	Bhunimba ⁶	Wholeplant	1 part
6	Guda ⁷		10parts

Preparation of the churna⁸ :

250 grams of each drug palasha beeja, indrayava, vidanga, nimba and bhunimba are weighed before the churnikarana.

All the drugs are pounded in khalwa yantra separately till it becomes fine powder.

The powders of all the five ingredients are sieved separately through 80 number sieve as mentioned in ayurvedic formulary of india for fine powders.

again the powders are weighed and mixed together in equal quantity in khalwa yantra for the formation of homogenous mixture. guda is added in double quantity of churna and mixed well in khalwa yantra the final product is dark brown in colour with sweetish and bitter smell. the consistency with guda is moderately soft to touch. stored in air tight glass container.

Sources of Chemical and Reagents

All the chemical reagents and other requirements of experimental study used from stock of Skanda Life Science Private Limited, R & D Centre, Sri Shaila bramara Complex, Sy. No 47, No 10-12, Chandana layout, Sriganadakaval, Nagarbhavi, Bengaluru.

Test organisms

1. Staphylococcus aureus⁹
2. Streptococcus pyogenes¹⁰
3. Ecoli¹¹
4. Salmonella typhi
5. Candida albicans
6. Aspergillus niger.

Sample: palasha beejadi churna

Standard:

1. Ciprofloxacin (0.1 mg/ml) as antibacterial standard
2. Itraconazole (20 mg/ml) as anti fungal standard
3. control : methanol

Table no.2: materials for antibacterial study

Si no.	Particulars	source	Catalogue no.
1	Soyabean casein digest Agar	HiMedia	MH290-500G

Table no.3 materials for antifungal study

Si no	Particular	source	Catalogue no.
1	Soyabean casein digest Agar (SCDA)	Himedia	MH290-500G
2	Potato dextrose agar (PDA)	himedia	GM096-500G

PROCEDURE**Extraction of test sample**

- 10g of sample weighed in beaker and dissolved in 50ml of 70% alcohol.
- Then beakers were kept on water at 50°C along with magnetic stirrer for 4 hours.
- After 4 hours of incubation, the extract was filtered using Whatmann filter paper No1.
- Then the filtrate was kept at 50°C for few hours until the extract got completely dried and turned into semisolid form.
- The semisolid sample was weighed and yield was noted.

Agar well diffusion method:

Preparation of inoculum for antibacterial study: Staphylococcus aureus, Streptococcus pyogenes, E.coli, Salmonella typhi cell suspension were prepared from cultures grown on Tryptic soya broth (TSB) respectively for 16-18hr at 37°C for bacteria. Cell density is adjusted to 1×10^6 cells/ml using 0.5 McFarland Standard.

Preparation of Inoculum for antifungal study: Candida albicans and Aspergillusniger cell suspension were prepared from cultures grown on Tryptic soya broth (TSB) and Potato dextrose broth (PDB) for 16-18hr at 37°C for candida and 48-72hr at 28°C for Aspergillusniger respectively. Cell density is adjusted to 1×10^6 cells/ml using 0.5 McFarland Standard.

Table No.4 sample detail

Si.no	Test compound	Solvent	Stock
1	Sample(palasha beeajadichurna).	methanol	100mg/ml

Determination of anti bacterial activity.

- 100 µl Inoculum of was Staphylococcus aureus, Streptococcus pyogenes, E.coli and Salmonella typhi inoculated on Soyabean casein digest agar plates (90 mm). And 5mm well were made on agar plates.
- Test compounds (20 and 10µl; 100mg/ml), and ciprofloxacin (10 µl, 0.1 mg/mL) were impregnated on 5mm wells on agar plates.
- The plates were Incubated at 37°C for 24 hrs and observe for zone of inhibition around the well and measured in mm.

Determination of anti fungal activity

- 100 µl Candida albicans and Aspergillusniger Inoculum of were inoculated on Soyabean casein digest agar plates (90 mm) and Potato dextrose agar plate (90 mm) respectively. And 5mm well were made on agar plates.
- Test compounds (20 and 10µl; 100mg/ml), and Itraconazole (10 µl, 20 mg/mL) were added to 5mm wells on agar plates.
- The plates treated with Candida albicans and Aspergillusniger were Incubated at 37°C for 24 hrs and at 28°C for 72-96hrs respectively.

iv. After incubation, plates were observed for zone of inhibition around the well.

Observations and results

Table 5 showing inhibitory activity of test sample against the fungal organisms taking itraconazole as standard.

Test sample	Organism	Conc.per well	Zone of inhibition in mm	Method used
Standard (itraconazole) Control (methanol) Sample	Candida albicans	0.2mg	20	Agar well diffusion
		10µl	–	
		1 mg	–	
		2mg	–	
Standard (itraconazole) Control (methanol) Sample	Aspergillusniger	0.2mg	12	Agar well diffusion
		10µl	–	
		1mg	–	
		2mg	–	




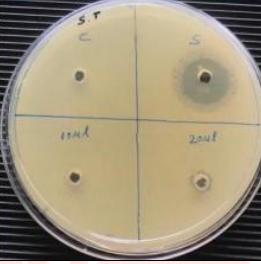
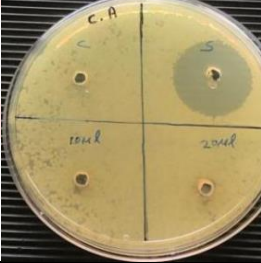
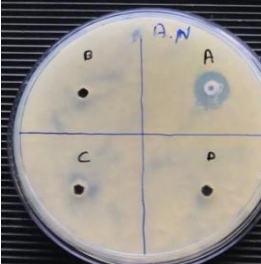
Table No.6 :inhibitory activity of sample against bacterial organismsm with ciprofloxacin as standard.

RESULTS

The tested sample showed inhibitory activity against, *Staphylococcus aureus*, *Streptococcus pyogenes*, *E.coli*, *Salmonella typhi* with a zone of inhibition of 7mm, 8mm, 6mm and 7mm whose standards were having zone of inhibition as 15,20,19 and 21 respectively.

Test sample	Test organism	Conc.per well	Zone of inhibition in mm	Figure
Test sample	Test organism	Conc.per well	Zone of inhibition inmm	Figure
Control (methanol)	s.aureus	10 µl	-	Fig
		1mg	-	
		2mg	7	
Sample				
Standard(ciprofloxacin	s.pyogenes	1 µg	20	Fig
Control (methanol)		10 µl	-	
		1mg	-	
Sample		2mg	8	
Standard(ciprofloxacin	E coli	1 µg	21	Fig
Control (methanol)		10 µl	-	
		1 mg	-	
Sample		2mg	6	
Standard(ciprofloxacin	Salmonella typhi	1 µg	19	Fig
Control (methanol)		10 µl	-	
		1mg	-	
Sample		2mg	7	

The zone of inhibitions is more in *Staphylococcus aureus* and *streptococcus pyogenes* it does not shows inhibitory effect against *candida albicans*, and *aspergillus niger*.

	<p>FIG:1 inhibitory activity of test sample against <i>S.aureus</i> S –standard (ciprofloxacin),c-control(methanol) Sample: 1mg/well,2mg/well.</p>
	<p>Fig:2 inhibitory activity of test sample against <i>S.pyogenes</i> S –standard (ciprofloxacin),c-control(methanol) Sample: 1mg/well,2mg/well.</p>
	<p>Fig:3inhibitory activity of test sample agains <i>E.coli</i> S –standard (ciprofloxacin),c-control(methanol) Sample: 1mg/well,2mg/well.</p>
	<p>Fig :4 inhibitory activity of test sample againt <i>S.typhi</i> S –standard (ciprofloxacin),c-control(methanol) Sample: 1mg/well,2mg/well.</p>
	<p>Fig:5inhibitory activity of test sample againt <i>C.albicans</i> S –standard (itraconazole),c-control(methanol) Sample: 1mg/well,2mg/well.</p>
	<p>Fig:6 inhibitory activity of test sample againt <i>A.niger</i> S –standard (itraconazole),c-control(methanol) Sample: 1mg/well,2mg/well.</p>

Discussion

Palashabeejadi churna is a poly herbal formulation described in the text Bhaishajya Ratnavali in the context of udaragata krimi its ingredients are Palashabeeja,Indrayava, Vidanga,Nimba ,Bhunimba And Guda.

The drug palasha is having tikta kashaya rasa ,laghu ruksha guna katu vipaka and ushna veerya and does the karma like vrishya ,deepana,krimihara,arshahara,grahi and bhagna sandhanaka

Indrayava having tikta,kashaya rasa laghu,ruksha guna sheeta veerya and katu vipaka.Its Karma is kapha pitta shamaka,grahi and deepana.

vidanga is mentioned in various nighantus,it possess katu ,kashaya rasa ,laghu ruksha and teekshna guna,ushna veerya ,and katu vipaka.these qualities are favourable for destroying the bacteria and stopping its further growth.its karma are deepana,krimighna,vishaghna,anulomana and shirovirechana.Its used in vibandha,kushtha and krimi

It is having tikta,kashaya rasa,laghu guna,katu vipaka and sheeta veerya.due to it's rasa Nimba is kapha pitta shamaka,grahi,and katu vipaka

bhunimba with tikta rasa,sheeta veerya and katu vipaka does the actions like kapha pitta nashana and deepana. In ayurvedic pharmaceuticals concept of churna is well established for medicinal purpose as well as in forming the base of other formulations,like vati,gutika and granules,etc.as most of the ingredients are having katu tikta rasa ,laghu ruksha guna and katu vipaka ,helps the formulation to impart its krimighna effect.

The final product is dark brown in colour with sweetish bitter taste and smell and moderately soft to touch .Ph of the sample is acidic in nature which aids its krimighna action.

In the present study the agar well diffusion method was used to know the antimicrobial activity of test sample palasha beejadi churna against the organisms ,staphylococcus aureus, streptococcus pyogenes,(gram positive) e-coli,salmonella typhi.(gram negative)and candida albicans and aspergillus niger(fungi).

Staphylococcus aureus, Streptococcus pyogenes, E.coli, Salmonella typhi cell suspension were prepared from cultures grown on Tryptic soya broth (TSB) respectively for 16-18hr at 37°C for bacteria. Cell density is adjusted to 1×10^6 cells/ml using 0.5 McFarland Standard.

Candida albicans and Aspergillus niger cell suspension were prepared from cultures grown on Tryptic soya broth (TSB) and Potato dextrose broth (PDB) for 16-18hr at 37°C for candida and 48-72hr at 28°C for Aspergillus niger respectively. Cell density is adjusted to 1×10^6 cells/ml using McFarland Standard.

Standard ciprofloxacin,itraconazole test samples and control methanol were added to 5 mm well in petri plates,the plates were incubated for 24 hours and zone of inhibition around the well is measured in mm .

The tested sample showed inhibitory activity against, Staphylococcus aureus,Streptococcus pyogenes, E.coli, Salmonella typhi with a zone of inhibition of 7mm, 8mm, 6mm and 7mm whose standards were having zone of inhibition as 15,20,19 and 21 respectively.

Conclusion:

Palasha beejadi churna is a polyherbal formulation explained in Bhaishajya ratnavali in krimirogachikitsa adhikara Churna is prepared by pounding in khalwa and homogenous mixing of the churnas Palasha Beeja ,Nimbatwak ,Indrayava Beeja ,Vidanga Beeja And Panchanga Of Bhunimba And Guda. Antimicrobial study of 70 % methanolic extract of the palashabeejadi churna showed antimicrobial activity against gram positive bacteria Staphylococcus aureus,Streptococcus pyogenes, and gram negative bacteria E.coli , Salmonella typhi It does not shows any inhibitory activities against fungal organisms candida albicans and aspergillus niger Hence ,it can be concluded that The formulation palasha beejadi churna proved to be antibacterial more than antifungal in the experimental study.

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