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Analysis of the anti-microbial and probiotic action of *Pathyadi churna*

Research Article

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Abstract

Pathyadi churna is a Herbo-Mineral preparation mentioned in *Chakradutta*, which is indicated in all types of *Ajeerna* like *Ama, Vidagdha, Vishtabdha* and *Rasasesha. Pathya, Pippali* and *Souvarchala lavana* are the ingredients of the preparation. This study is conducted to find the action of Pathyadi Churna against microorganisms which causes digestive disorders and which helps in digestion. The antimicrobial study of *Pathyadi churna* revealed its action against S. aureus, C. albicans, Lactobacillus acidophilus and B. subtilis, but the probiotic action is revealed when the trial drug is given with its *Anupana Masthu.* Also found that *Pathyadi churna* doesn't act against E. coli bacteria. Overall, *Pathyadi Churna* showed anti-microbial action alone and probiotic action with its *Anupana Masthu.*

Key Words: *Pathyadi Churna,* Antimicrobial activity*, Probiotic activity, Masthu.*

Introduction

Ayurveda defines *Churna* as the powdered form of dry drugs (1). *Raja* and *Kshoda* are the synonyms of *churna* (2). *Pathyadi Churna* is a herbo-mineral formulation mentioned in the classical texts like *Chakradutta* (3) and *Bhaishajya Ratnavali* (4). It is indicated in digestive disorders like 4 types of *Ajeerna (Ama, Vidagdha, Vishtabdha and Rasasesha), Gulma, Aruchi* etc. *Ushnajala* and *Masthu* are the *Anupanas* mentioned for this preparation.

Objectives

To detect the antibacterial action using agar well diffusion method and detect the probiotic action of the same sample with *Masthu* as *Anupana*.

Materials and Methods

The literary details of the drugs were collected from the authentic classical textbooks like *Chakradutta* and *Bhaishajya Ratnavali.* For the analysis of antibacterial activity using agar well diffusion method, listed in Table no: 1 and analysis of probiotic action listed in Table no: 2.

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Sources of Data

The certified raw drugs prescribed in the formulation were collected from Ancheri Drugs, Nehru bazar, Thrissur. The drug identification were conducted at *Dravyaguna Vijnana* department, Vishnu Ayurveda College, Shoranur. The antimicrobial study (Table 3) and probiotic analysis (Table 4) along with pharmaceutical study (Table 5), were conducted in CARe keralam, KINFRA park, Thrissur.

Method of preparation

Pathyadi churna was prepared with 1:1:1 ratio of *Pathya, Pippali* and *Sauvarchala lavana.* Each drug was finely powdered with the help of machines separately and taken in the above said ratio and mixed together thoroughly.

Procedure of analysis of Antibacterial activity

Inoculum preparation: A Loopful of Bacterial culture was transferred from working stock slants to 5ml of SCDM (MRS broth for Lactobacillus acidophilus) and incubated at 37^{0}C till a visible turbidity equivalent to 0.5 MacFarland unit. For Candida, culture was carried out on YEPD broth and incubated at 25^{0}C.

25ml of respective agar media (for bacteria- MHA, MRS Agar, for fungi- MGYPA) were added to sterile petriplates and allowed to dry for 5 minutes. Then the cultures were inoculated on plates by swabbing on the surface of the media. Using sterile Cork Borer of 8mm diameter, prepare wells on the swabbed agar plates. 100µ sample (aqueous and ethanol extract- 5,00,000 ppm) was added to the well using micropipette. Antibiotic control (streptomycin 1000ppm & Fluconozole 500ppm) and solvent control ethanol

were also kept. Then kept the plates in the biosafety cabinet till the diffusion of sample occurs and after that incubated the bacterial plates at 37^{0}C for 24 hrs and fungal plates at 25^{0}C for 3 days. After incubation, measured the diameter (in mm) of the zone of inhibition using a ruler and recorded the results (Table no:3).

Procedure of analysis of probiotic activity

Took 3 test tubes containing 10ml SCDM (for bacillus subtilis). To the first tube,1gm *Churna* was added and to the second one, 1 gm *Churna* with *Masthu* homogenate (1gm *Churna* + 10ml *Masthu*) was added. Kept the third tube as blank control. Similarly did the same, using MRS Broth (Lactobacillus acidophilus). Inoculated 100ul culture of Bacillus subtilis and Lactobacillus acidophilus, having turbidity equivalent to 0.5 MacFarland unit. Into the SCDM and MRS Broth respectively. Incubated the tubes at 37^{0}C for 24 hours. Then performed pour plating using {CA (Bacillus subtilis) and MRS Agar (Lactobacillus acidophilus). Incubated the tubes at 37^{0}C for 24 hours. After incubation, observed the plates for Colony Forming Units (CFU) and recorded the results (Table no:4).

Observations

Table 1: Materials and medias required for analysis of Antibacterial activity using Agar well diffusion method

Sl. No	Materials & Medias
1	Conical flasks
2	Petriplates
3	Micropipettes
4	Test tubes
5	pH meter and Paper
6	Soyabean Casein Digest Medium (SCDM)
7	Yeast Extract Peptone Dextrose broth (YEPD)
8	Lactobacillus MRS Broth
9	Mueller Hinton Agar (MHA)
10	Lactobacillus MRS Agar
11	Maltose Glucose Yeast extract Peptone agar (MGYPA)
12	Standard drug (Streptomycin)
13	Standard drug (Fluconazole)
14	Biosafety cabinet
15	Bacteriological incubator (37^{0}C)
16	BOD incubator (25^{0}C)
17	Fungal culture (Candida albicans NCIM 3102)
18	Bacterial cultures (Staphylococcus aureus NCIM 2127, E.Coli NCIM 2065, Bacillus subtilis NCIM 2063, Lactobacillus acidophilus MTCC 10307)

Table 2: Materials & medias required for analysis of probiotic action testing

Sl. No:	Materials and medias
1	Conical flasks
2	Petriplates
3	Micropipettes
4	Test tubes
5	pH meter and paper
6	Soyabean Casein Digest Medium (SCDM)
7	Lactobacillus MRS Broth
8	Plate Count Agar (PCA)
9	Lactobacillus MRS Agar
10	Biosafety cabinet
11	Bacteriological incubator (37^{0}C)
12	Bacterial cultures (Bacillus subtilis NCIM 2063, Lactobacillus acidophilus MTCC 10307)

Table 3: Results of Antibacterial activity using Agar well diffusion method

Sl No:	Test Organism	Test result- Zone of Inhibition			Test method
		Ethanol extract (5,00,000 ppm)	Aqueous extract (5,00,000 ppm)	Standard drug	
1	Staphylococcus aureus	18 mm	21 mm	Streptomycin (1000 ppm)- 26 mm	Agar well diffusion method
2	Escherichia Coli	No Zone	No Zone	Streptomycin (1000 ppm)- 20 mm	
3	Candida albicans	16 mm	No Zone	Streptomycin (1000 ppm)- 36 mm	
4	Bacillus subtilis	15 mm	No Zone	Streptomycin (1000 ppm)- 35 mm	
5	Lactobacillus acidophilus	No Zone	No Zone	Streptomycin (1000 ppm)- 13 mm	

Table 4: Results of Probiotic action testing

Sl. No		1	2
Test organism		Bacillus subtilis	Lactobacillus
Test results- Colony Forming Unit (CFU)	Blank	40,000	3,80,00,000
	Pathyadi	30,000	60,000
	Pathyadi churna+	30,000	3,70,00,000
Test	CKL/MB/MOA-043		

Table 5: Pharmaceutico analytical study of *Pathyadi Churna*

Parameter	Unit	Result	Specification	Detection limit	Test method
pH(10% aqueous solution)	-	4.31	-	-	API Part I Vol I
Loss on drying	%	4.39	-	-	API Part I Vol I
Total ash	%	36.29	-	-	IP 2018
Acid insoluble ash	%	0.33	-	-	IP 2018
Water soluble extract	%	59.12	-	-	IP 2018

Alcohol soluble extract	%	33.40	-	-	IP 2018
Sodium	%	13.86	-	-	AOAC 21st edition 2019
Potassium	%	1.07	-	-	AOAC 21st edition 2019
Calcium	%	1.09	-	-	AOAC 21st edition 2019
HPTLC			-	-	
Gallic acid	%	0.15	-	-	CKL/ANL/ HPTLC-001
Piperine	%	0.38	-	-	CKL/ANL/ HPTLC-001
Heavy metals			-	-	
Arsenic	Mg/ kg	0.19	NMT 3.0-	0.05	CKL/ANL/ AY-008
Cadmium	Mg/ kg	BDL	NMT 0.3	0.05	CKL/ANL/ AY-008
Lead	Mg/ kg	0.35	NMT 10.0	0.05	CKL/ANL/ AY-008
Mercury	Mg/ kg	Not Detected	NMT 1.0	0.05	CKL/ANL/ AY-008
Phytochemical screening			-	-	
Alkaloids	-	Present	-	-	Dragendroff's reagent test
Flavonoids	-	Absent	-	-	Shinoda test
Phenol	-	Present	-	-	Folin ciocalteu phenol reagent test
Tannins	-	Present	-	-	Ferric chloride test
Quantification			-	-	
Alkaloids	%	0.88	-	-	Experimental phyto pharmacognosy
Phenols	%	8.27	-	-	CKL/ANL/ UV-002
Tannins	%	7.39	-	-	CCRAS 40.3

Action of *Pathyadi churna* on S. aureus

Pathyadi churna showed 18mm zone of inhibition against Staphylococcus aureus with ethanol extract and 21mm zone of inhibition with aqueous extract. The standard drug Streptomycin showed 26mm of zone of inhibition (Image 1). This indicates that, *Pathyadi churna* restricts and reduces the growth of Staphylococcus aureus. Agar well diffusion method was used to analyze.

Image 1: Action of *Pathyadi churna* on S. aureus

Action of *Pathyadi churna* on E. Coli

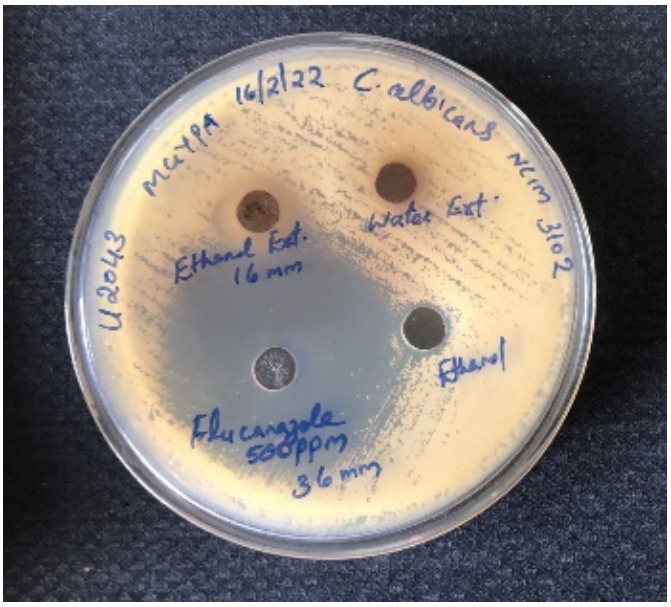
Both ethanol and aqueous extracts of *Pathyadi churna* didn't show any zone of inhibition against Escherichia coli and showed 20mm inhibition zone against standard medicine Streptomycin in agar well diffusion method (Image 2). This indicated that *Pathyadi churna* doesn't have any effect on E. Coli bacteria.

Image 2: Action of *Pathyadi churna* on E. Coli

Action of *Pathyadi churna* on Candida albicans

Ethanol extract of *Pathyadi churna* showed 16mm zone of inhibition against Candida albicans and aqueous extract didn't show any zone of inhibition. The standard medicine Fluconazole showed 36mm of zone of inhibition (Image 3). This indicates that, only ethanol extract of *Pathyadi churna* acts against Candida albicans.

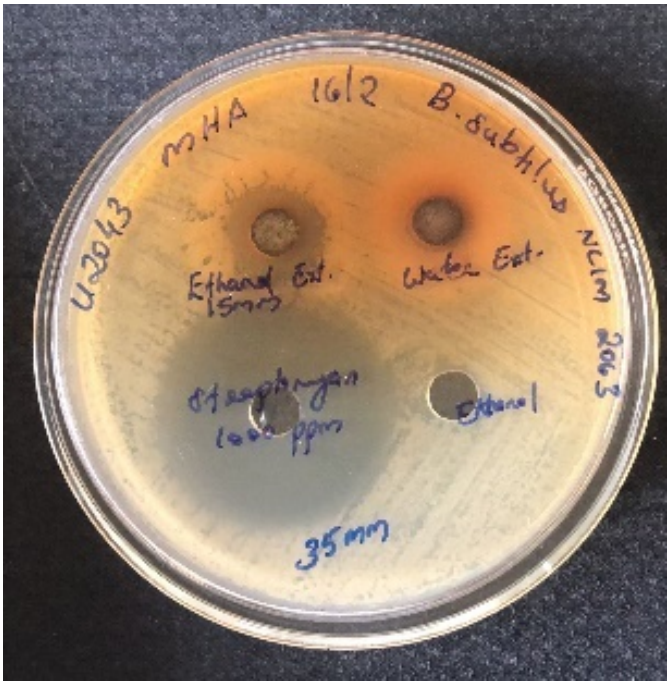
Image 3: Action of *Pathyadi churna* on Candida albicans



Action of *Pathyadi churna* on Bacillus subtilis

The ethanol extract of *Pathyadi churna* shows 15mm of inhibition zone against Bacillus subtilis, but the aqueous extract did not show any zone of inhibition. The standard medicine Streptomycin showed 35mm of inhibition zone (Image 4). This indicates, only ethanol extract of *Pathyadi churna* and streptomycin resist the growth of Bacillus subtilis.

Image 4: Action of *Pathyadi churna* on Bacillus subtilis



Action of *Pathyadi churna* on Lactobacillus acidophilus

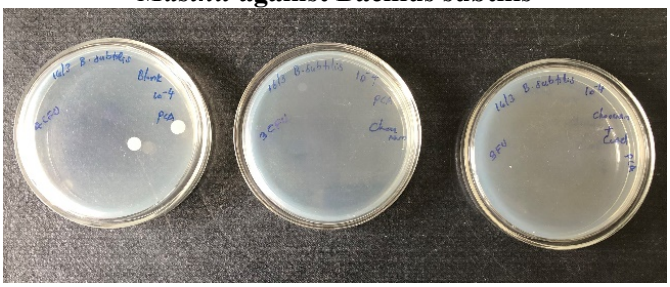
Both ethanol and aqueous extracts of *Pathyadi churna* show 11 mm of zone of inhibition against Lactobacillus acidophilus. The standard medicine streptomycin showed 13mm of zone of inhibition (Image 5). This says that *Pathyadi churna* resist the growth of Lactobacillus acidophilus.

Image 5: Action of *Pathyadi churna* on Lactobacillus acidophilus

Probiotic action of *Pathyadi churna* with *Masthu* against Bacillus subtilis

The blank control of Bacillus subtilis showed 40,000 CFU whereas *Pathyadi churna* with and without *Masthu* showed 30,000 CFU (Image 6). This indicates that the trial drug with and without *Masthu* doesn't promote the growth of Bacillus subtilis as expected, but at the same time, it doesn't decrease the growth as seen in *Pathyadi churna* alone.

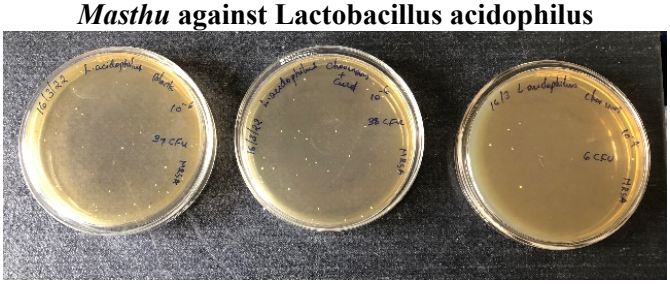
Image 6: Probiotic action of *Pathyadi churna* with *Masthu* against Bacillus subtilis



Probiotic action of *Pathyadi churna* with *Masthu* against Lactobacillus acidophilus

The blank control of Lactobacillus acidophilus showed 3,80,00,000 CFU whereas *Pathyadi churna* showed 60,000 CFU and *Pathyadi churna* with *Masthu* showed 3,70,00,000 CFU (Image 7). This indicates that *Pathyadi churna* with *Masthu* has more probiotic effect than *Pathyadi churna* alone.

Image 7: Probiotic action of *Pathyadi churna* with *Masthu* against Lactobacillus acidophilus



Discussion

It is rare to find a drug with anti-microbial action giving probiotic effect when associated with another compound. Here in this study, the anti-microbial effect of the drug alone and probiotic effect when combined with its *Anupana* is revealed. The chemical constituents of *Terminalia chebula Retz.* (*Pathya*) are Gallic acid, Eugenol, Chebulanin, Terchebulin, Ascorbic acid, Arjunolic acid etc (5). The HPTLC revealed that 0.15% oh Gallic acid is present in *Pathyadi churna*. There are various kinds of studies on the biological and pharmacological activities of Gallic Acid, including Antioxidant (6,7) Antimicrobial (8,9) Anticancer (10,11) Anti-inflammatory (12,13), Gastroprotective (14,15,16,17) and metabolic disease prevention activities (18,19,20). Studies in rats suggests that Gallic acid promotes the gut microbiota fermentation of both proteins and polysaccharides (21). The anti-ulcerogenic effect of Gallic acid also discovered in rats (22). So, these properties of Gallic acid may help to relive the symptoms of digestive disorders like dyspepsia and promote digestion.

The constituents of *Piper longum L* are Piperine, Piperettine, Asarinine, Pipercide, Piperidine etc (23). By HPTLC analysis, it is found that *Pathyadi churna* contains 0.38% of Piperine in it. Some studies proved the effect of Piperine in the process of digestion, antioxidant properties and the role in the management of various disorders (24,25,26). Some studies suggests that Piperine as a bio-enhancer of ciprofloxacin against staphylococcus aureus (27,28), so the digestive problems due to Staphylococcus aureus can be cured by Piperine. Also some studies found to make use of Piperine as a gastro-intestinal absorption enhancer (29). By looking into these facts, we can understand that Piperine enhances digestion and has antioxidant properties, intestinal absorption enhancement property, and antimicrobial properties which will help to cure the digestive disorders like dyspepsia.

Staphylococcus aureus colonizes the nose, throat, skin and gastro-Intestinal tract of humans. GI carriage of S. aureus is difficult to eradicate (30). The S. aureus is recognized as the causative organism of enterocolitis (31). Both ethanol and aqueous extract of *Pathyadi churna* showed 18mm and 21mm of zone of inhibition. This indicates that the trial drug actually resists the growth of S. aureus, which means the drug is acting against the bacteria. So, *Pathyadi churna* will be effective against the diseases caused by the S. aureus bacteria.

Pathyadi churna didn't show any antimicrobial activity against E. Coli bacteria. There was no zone of inhibition of E. Coli against *Pathyadi churna*. It concludes that *Pathyadi churna* did not stop the growth of E. Coli bacteria. So, this drug cannot cure any disease caused by E. Coli.

The ethanol extract of *Pathyadi churna* showed 16mm of zone of inhibition against andida albicans. Aqueous extract didn't show any zone of inhibition. Candida albicans is a human commensal that is also responsible for chronic gastritis and peptic ulcerous disease (32). Some studies showed positivity for particular C. albicans genotypes could signify the susceptibility of dyspepsia (33). Recent studies indicate that the over growth of Candida albicans is associated with several diseases of the Gastro-Intestinal tract, including ulcerative colitis and crohn's disease (34). The growth of C. albicans which cause the digestive disorder like dyspepsia, crohn's disease, ulcerative colitis etc, are inhibited by the *Pathyadi churna*. So, these diseases are subsided by *Pathyadi churna*.

Bacillus subtilis, known as the Hay bacillus or Grass bacillus, is a gram positive, catalase positive bacterium found in soil and the Gastro-Intestinal tract of ruminants, humans and marine sponges (35). Subtilis is an ideal multifunctional probiotic, with great potential for preventing the growth of pathogenic bacteria and enhancing nutrition assimilation (36). The aqueous extract of *Pathyadi churna* didn't show any inhibition in the growth of B. subtilis, whereas the ethanol extract showed 15mm of zone of inhibition. So, the drug actually reduced the probiotic B. subtilis bacteria if given alone. But when *Pathyadi churna* is given along with its *Anupana Masthu* (curd water), the bacteria is found to be same CFU (Colony Forming Unit) as blank control. This indicates that, if the drug is given with *Anupana Masthu*, the chance of antibacterial effect against B. subtilis is reduced which is good for enhancement of digestion.

Lactobacillus acidophilus is a type of bacteria found in our intestines (37). This bacteria helps the digestive system to breakdown sugars such as lactose into lactic acid (38). Yogurt is a rich source of L. acidophilus (39). *Pathyadi churna* with *Masthu* doesn't have any inhibition zone against lactobacillus acidophilus, which means the drug doesn't decrease the growth of L. acidophilus. Both blank group and *Pathyadi churna* with *Masthu* showed same result. But, at the same time, *Pathyadi churna* alone decreased the L. acidophilus. This indicates that the drug *Pathyadi churna* with *Masthu* promotes digestion than *Pathyadi churna* alone. *Pathyadi churna* alone is destroys probiotic organisms also, whereas if it is taken with *Masthu*, the probiotic organisms don't reduce in gut and helps in digestion and curing the disease.

Conclusion

Pathyadi churna is a fine powder indicated in digestive disorders like Ajeerna. The antimicrobial activity of *Pathyadi churna* helps to inhibit the growth of micro-organisms like S. aureus, C. albicans which cause digestive disturbances. And when taken along with *Masthu*, the count of micro-organisms like B. subtilis and Lactobacillus acidophilus will not be reduced and they will promote the proper digestion. Usually after antibiotic consumption, probiotic drugs like Florajen, Acidophilus are also taken to regenerate the health of gut microbes. Similar effect will be generated when we give Pathyadi Churna along with *Masthu*. Because, the Churna restricts growth of micro-organisms which causes the digestive disorders and doesn't trouble, but enhance the growth of the micro-

organisms which helps in digestion. So, we can conclude that Churna has anti-microbial and probiotic action against gut microbes. This indicates the importance of *Acharyokta Anupana.* Thus, this drug can be suggested for gastro intestinal disorders caused by these micro-organisms.

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