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COMPARATIVE ANTIMICROBIAL STUDY OF ROHINYADI PACHANA KWATHA AND ROHINYADI PACHANA ARISHTA

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ABSTRACT

Introduction: Diarrhoea is defined as the passage of loose, liquid or watery stools. These liquid stools are usually passed more than three times a day. In diarrhoea ion channels and exchangers in epithelial cells can promote intestinal secretion, thereby enhancing gastrointestinal transit and promoting faecal excretion. *Atisaara,* one of the diseases mentioned in Ayurvedic Classics has its cardinal feature as frequency of loose stools which mimics the Diarrhoeal symptoms. *Kwatha* should be prepared and used instantly as it has limited shelf life. On the other hand, *Arishta* has prolonged shelf life, considering these factors, present study is planned to prepare *Rohinyadi Paachana Arista* from *Rohinyadi Paachana Kwatha,* for evaluation and validation of antimicrobial properties of two dosage forms on bacteria such as *Salmonella sp., Shigella flexneri and Escherichia coli.* **Methodology:** In antibacterial study by agar well diffusion method, the zone of inhibition of both the sample were tested against three bacteria namely Salmonella enterica, Shigella flexneri and Escherichia coli. **Result:** *Rohinyadi Pachana Arishta* exhibited zone of inhibition against all three tested organisms at all concentration. Rohinyadi Pachana Kashaya exhibited the zone of inhibition against *Shigella flexneri*.

KEYWORDS: Diarrhoea, *Rohinyadi Pachana Kwatha, Rohinyadi Pachana Arishta,* Antibacterial study.

INTRODUCTION

According to WHO, Diarrhoea is defined as "passage of three or more loose or liquid stools per day, or passing more stools than normal for the individual''. The major threat caused by diarrhoea is dehydration.[1] Globally, diarrhoea accounts for more than 5 to 8 million death annually, majority of them are infants and children below the age five.[2] In addition diarrhoea is a major cause of malnutrition ;diarrhoea and malnutrition, alone or together constitute major causes of morbidity and mortality among children.[3] *Salmonella, Shigella and Escherichia coli* are the most frequent bacterial cause of Diarrhoea.[4] In diarrhoea ion channels and exchangers in epithelial cells can promote intestinal secretion, thereby enhancing gastrointestinal transit and promoting faecal excretion.[5] *Atisaara,* one of the diseases mentioned in Ayurvedic Classics has its cardinal feature as frequency of loose stools which mimics the Diarrhoeal symptoms.[6] Moreover, unhealthy food consumption is mentioned as one of the *Nidaana* for *Atisaara* which is also mentioned in the case of Diarrhoea caused by food poisoning. *Rohinyadi Paachana Kwatha* is one of the formulations mentioned in *Brihat Niganthu Rathnakara* which is indicated in *Sarvatisaara.*[7] *Kwatha* should be prepared and used instantly as it has limited shelf life.[8] On the other hand *Arishta* has prolonged shelf life,[9] and is also easy for administration. Considering these factors, present study is planned to prepare *Rohinyadi Paachana Arista* from *Rohinyadi Paachana Kwatha*, for evaluation and validation of the pharmaceutical and analytical parameters and to compare antimicrobial properties of two dosage forms on bacteria such as *Salmonella sp., Shigella flexneri and Escherichia coli.*

AIM

To evaluate and compare in-vitro antimicrobial activity of *Rohinyadi Pachana Kwatha* and *Rohinyadi Pachana.*

MATERIALS AND METHODS

Antimicrobial susceptibility testing plays an important role in evaluating the effectiveness of antibacterial agents against microorganisms. There is various laboratory methods include agar dilution, disk diffusion and broth microdilution. The agar well diffusion method is a commonly used techniques for determining the antimicrobial activity of plant extracts.[10]

Source of data- The evaluation of anti-microbial activity of RPK and RPA was carried out in S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi.

Materials Required

- Test Strain- *E. coli, Salmonella enterica, Shigella flexneri.*
- Distilled water, Saline.
- Test tube, Incubator, Laminar air flow.
- Graduated micropipettes
- Growth medium-Nutrient Agar
- Sample- Rohinyadi Pachana Kwatha, Rohinyadi Pachana Arishta

Preparation of Nutrient Agar media

Beef extract (1g), yeast extract (2g), peptone (5 g) and Sodium Chloride (5g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally, 15 g agar was added to the media and autoclaved at 121℃ for 20 minutes.

Preparation of inoculum

Escherichia coli (MTCC 42), *Salmonella enterica* (MTCC 323) and *Shigella flexneri (*MTCC 1457) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 48h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum (Fig 1 and 2).

Well diffusion method

After cooling to 45-50℃, 20 ml of medium were placed into each sterile petri plates (Fig 3). One ml of the inoculum of strain was immediately poured to the plate and spread to ensure even dispersion. (Figure 4). A sterile borer was used to drill the well (Figure 5). The antibiotic and samples were administered into the wells (Figure 6). Plates were incubated overnight at 37℃ and inspected after 48 hours. Samples of *Rohinyadi Pacahana Kwatha* and Rohinyadi Pachana Arishta were taken in 25µl,50 µl,75 µl,100 µl concentrations, control (Distilled water)-50 µl, Standard (Ampicillin) 1 mg/1 ml -15 µl,30 µl respectively. The result was assessed based on the zone of inhibition (ZOI) around the lower (25 µ), moderate (50 µ and 75 µ) and Higher (100 µ) concentration of drug.

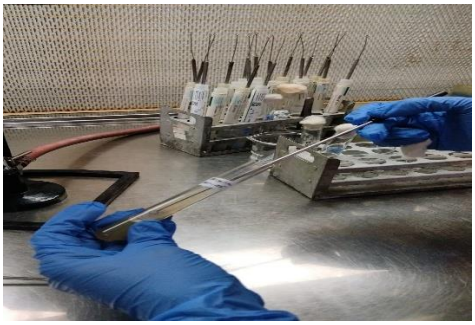

Fig. 1: Preparation of inoculum.

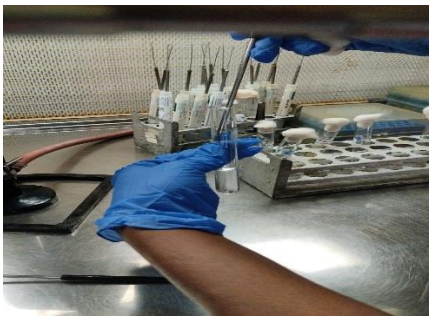

Fig. 2: Dilution of inoculum in saline.

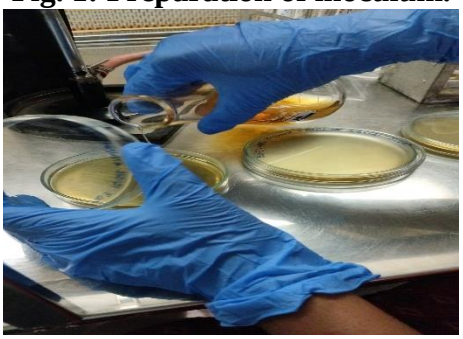

Fig. 3: Preparation of nutrient agar plate.

Fig. 4: Addition of inoculum.

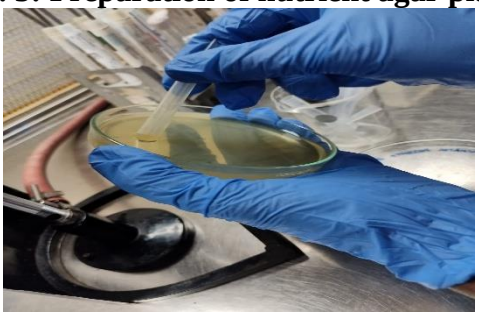

Fig. 5 Creating wells in petri plates.

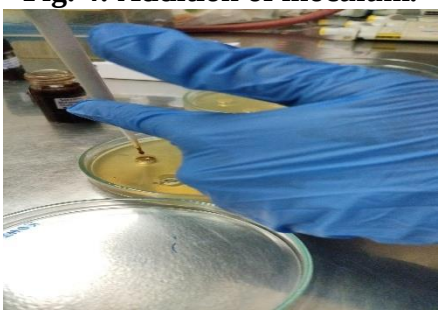

Fig. 6 Addition of samples.

RESULTS

Rohinyadi Pachana Arishta exhibited zone of inhibition against all three tested organisms at all concentration. Rohinyadi Pachana Kashaya exhibited the zone of inhibition against *Shigella flexneri*.

The result of invitro antimicrobial study of *Rohinyadi Pcahana kwatha* (RPK) and *Rohinyadi Pcahana Arishta* (RPA) against *E. coli, Salmonella enterica, Shigella flexneri* is given in table 1,2,3.

Table 01: In vitro antibacterial activity of *Rohinyadi Pachana Kwatha* and Arishta against *E. coli.*

Sample	Volume	Rohinyadi Pachana Kwatha ZOI (Radius in mm)		Rohinyadi Pachana Arishta ZOI (Radius in mm)	
Concentrations	**25 µl**	0	0	06	06
	50 µl	0	0	07	07
	75 µl	0	0	07	08
	100 µl	0	0	08	09
Controls	**100 µl**	0	0	0	0
Standard (Ampicillin) 1 mg/1 ml	**15µl(RPK) 30µl(RPA)**	22	24	10	10

Table 02: *In vitro* antibacterial activity of *Rohinyadi Pachana Kwatha* and Arishta against *Salmonella enterica.*

Sample	Volume	Rohinyadi Pachana Kwatha ZOI (Radius in mm)		Rohinyadi Pachana Arishta ZOI (Radius in mm)	
Concentrations	**25 µl**	0	0	05	06
	50 µl	0	0	08	07
	75 µl	0	0	08	07
	100 µl	0	0	09	08
Controls	**100 µl**	0	0	0	0
Standard (Ampicillin) 1 mg/1 ml	**15µl(RPK) 30µl(RPA)**	12	13	12	12

Table 03: *In vitro* antibacterial activity of *Rohinyadi Pachana Kwatha* and Arishta against *Shigella flexneri.*

Sample	Volume	Rohinyadi Pachana Kwatha ZOI (Radius in mm)		Rohinyadi Pachana Arishta ZOI (Radius in mm)	
Concentrations	**25 µl**	05	05	07	08
	50 µl	07	06	10	11
	75 µl	08	07	12	13
	100 µl	08	07	13	15
Controls	**100 µl**	0	0	0	0
Standard (Ampicillin) 1 mg/1 ml	**15µl(RPK) 30µl(RPA)**	15	16	11	11

DISCUSSION

Diarrhea is defined as the passage of loose, liquid or watery stools. These liquid stools are usually passed more than three times a day. The major threat caused by diarrhoea is dehydration. Globally, diarrhoea accounts for more than 5 to 8 million deaths annually, majority of them are infants and children below the age five. In addition, diarrhoea is a major

cause of malnutrition; diarrhoea and malnutrition, alone or together constitute major cause of morbidity and mortality among children**.** *Salmonella, Shigella and Escherichia coli* are the most frequent bacterial cause of Diarrhoea. In diarrhoea ion channels and exchangers in epithelial cells can promote intestinal secretion, thereby enhancing gastrointestinal transit and promoting faecal excretion. The main cause of diarrhoea is infection from virus, bacteria and other parasites. Rehydration therapy and preventive strategies are adopted.

The *Rohinyadi Pachana Kwatha* is mentioned in *Brihat Nighantu Rathnakara* for the treatment of *Atisara* consist of 5 ingredients. These ingredients are *Katuki, Ativisha, Pata, Vacha and Kushta.* The *Rohinyadi Pachana Kwatha* is specifically indicated for the management of *Sarvatisara,* a broad term used to describe various types of Diarrhoeas. The ingredients of *Rohinyadi pachana Kwatha* mentioned in *Brihat Nighantu Ratnakara* possesses various therapeutic properties like *Deepana, Pachana, Grahi, Krimigna, Vishagna, Atisarahara, Rakta Sthambaka and Shulahara* helps in pacifying the symptoms associated with *Atisara.*

Antibacterial drugs are substances that either kill bacteria or prevent them from multiplying and causing a disease. Antibacterial study is done to determine the antibacterial efficacy of a substance or formulation against specific bacterial strains. After selecting the relevant bacterial strains, using agar well diffusion method the efficacy of the drug or extract can be checked.

In this study the selected diarrhoea causing bacteria like *Salmonella enterica, Shigella flexneri* and *Escherichia coli* (*E. coli*) was cultured and by using Agar well diffusion method the zone of inhibition was assessed to check the efficacy of RPK and RPA in preventing the growth of bacteria. The antibacterial study of RPK and RPA utilized the Agar well diffusion method, initially inoculum was made and kept ready, nutrient agar medium was prepared and poured into the agar plates and inoculated with respective bacterial strains later wells were created in the plates and introduced with the samples of RPK and RPA. The plates were incubated and zone of inhibition around the wells were measured.

The antimicrobial study of RPK and RPA against 3 bacterial strains, RPK shows antibacterial activity on *Shigella flexneri* whereas RPA showed antibacterial activity against *E. coli, Salmonella enterica, Shigella flexneri*. This would suggest the efficacy of both RPK and RPA against the bacterial growth. *Rohinyadi Pachana Arishta* is a modified dosage form of

Rohinyadi Pachana Kwatha which is having prolonged shelf life and more palatable compared to RPK. This would be more beneficial in bacteria induced diarrhoea.

By this study it is evident that *Rohinyadi Pachana Arishta* shows better efficacy compared to *Rohinyadi Pachana Kwatha* in bacteria induced diarrhoea.

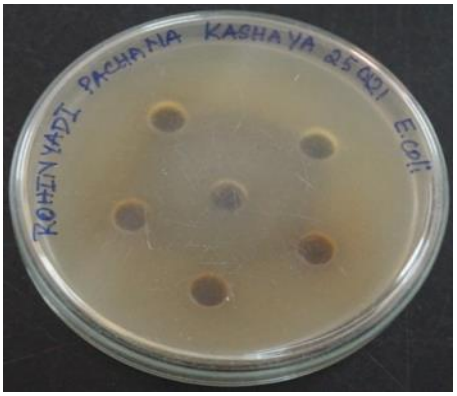

Fig. 7: ZOI in RPK in E. coli.

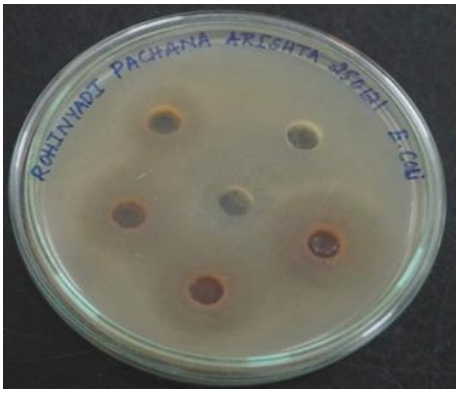

Fig. 8: ZOI in RPA in E. coli.

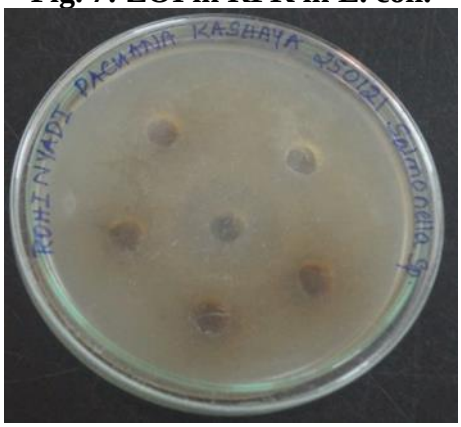

Fig. 9: ZOI in RPK in Salmonella sp.

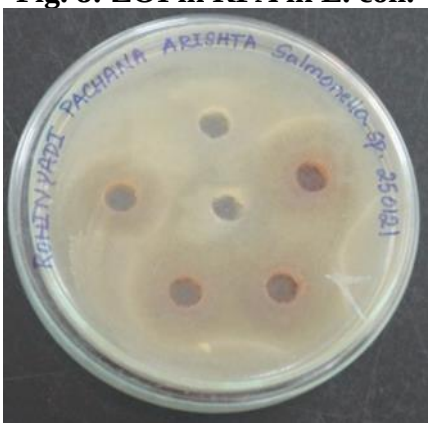

Fig. 10: ZOI of RPA in Salmonella sp.

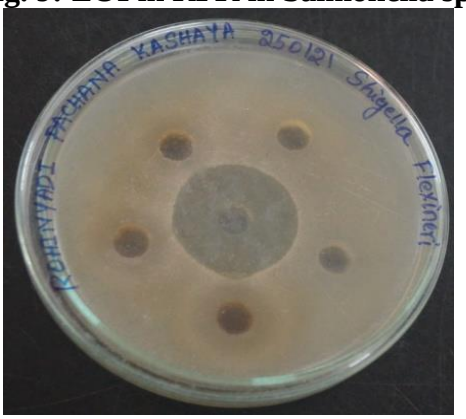

Fig.11 ZOI of RPK in Shigella flexneri.

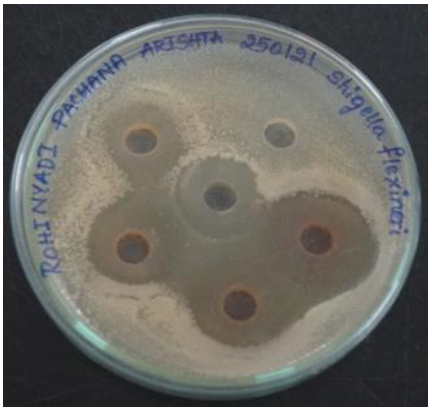

Fig. 12 ZOI of RPA in Shigella flexneri.

CONCLUSION

In the antibacterial study, comparing *Rohinyadi Pachana Kwatha* and *Rohinyadi Pachana* Arishta, it was observed that *Rohinyadi Pachana Arishta* exhibited zone of inhibition against all three tested organisms at all concentration. *Rohinyadi Pachana Kashaya* exhibited the

zone of inhibition against *Shigella flexneri*. The microbial load taken in the study may not be akin to the microbial load of gut flora. Clinical study can be carried out to know the human body interaction with the new dosage form. These studies will provide a more comprehensive understanding of its therapeutic potential and effectiveness in the management of specific diarrhoeal conditions.

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