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Antibacterial activity of some Indian Ayurvedic preparations against enteric bacterial pathogens

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ABSTRACT

In Ayurveda, various herbal preparations are clinically used to prevent or cure infectious diseases. Herbal preparations such as Triphala churna, Hareetaki churna, Dashmula churna, Manjistadi churna, Sukhsarak churna, Ajmodadi churna, Shivkshar pachan churna, Mahasudarshan churna, Swadist Virechan churna and Pipramool churna were investigated by preparing their organic solvent extract for antibacterial potential against enteric bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epidermidis, Salmonella typhimurium* and *Proteus vulgaris*, respectively. In the present study, Triphala churna, Hareetaki churna, Dashmula churna were potent antibacterial agents against *S. epidermidis, P. vulgaris, S. aureus, E. coli, P. aeruginosa* and *S. typhi.* The study supports the use of these herbal preparations not only as dietary supplements but also as agents to prevent or control enteric bacterial infections.

Key words: Antibacterial activity, dashmula churna, hareetaki churna, triphala churna

INTRODUCTION

In Ayurvedic medicine, many medicinal plants are useful in strengthening human health care system and the formulations based on such medicinal plants play an important role in modern medicine.[1] Ayurvedic practitioners also identified a number of herbal preparations for curing various ailments and diseases.[2] The primary benefits of using plant-derived medicine are relatively safer than synthetic drugs and offer profound therapeutic benefits.[3] Single and polyherbal preparations have diverse range of bioactive molecules and play a dominant role in the maintenance of human health since ancient times.[4] More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines.[5]

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The most frequently used type of herbal preparations are churnas. Churnas are preparations comprising of fine powders of medicinal plants and may be single or in combination. Combinations of medicinal plants may increase the antimicrobial spectrum and potency of the preparations. Enteric or diarrheal infections are major public health problems in developing countries and contribute to the death of 3.3-6.0 million children annually. Enteric bacteria comprised of *Salmonella* sp., *Shigella* spp., *Proteus* sp., *Klebsiella* sp., *Escherichia coli, Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* are the major etiological agents of sporadic and epidemic diarrhea both in children and adults.[6] Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs.[7-9] There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens, but a limited numbers of *in vitro* studies on herbal preparations have been published. It is need of the hour to identify antibacterial potential of herbal products based on diseases for which no medicine or only palliative therapy is available.[10,11] Hence an attempt was made to screen the antibacterial potential of herbal preparations in the control prevention of enteric bacterial infection.

MATERIALS AND METHODS

The commercial herbal preparations as given in Table 1

Table 1: Herbal preparation tested for antibacterial potential

Herbal preparations	Manufacturer	Therapeutic use	Ingredients
Triphala churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur	A remedy for constipation	*Terminalia chebula, Terminalia belerica, Emblica officinalis*
Hareetaki churna	The Ayurveedya Arkashala Ltd., Satara	Mild laxative, internal cleaner, detoxifier, improve digestive functions	*Terminalia chebula*, Ricinus communis, *Zingiber officinale*, Rock salt (Sodium chloride), sodium sulphate and sodium chloride, Piper longam
Mahasudarshan churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur	Excellent remedy for the complication associated with fever	*Terminalia chebula, Terminalia belerica, Emblica officinalis*, *Curcuma longa*, Berberis aristata, Solanum xanthocarpum, Solanum indicum, Zingiber officinale, Piper nigrum, Piper longum, Piper longum, Fagonia arabica, Picrorrhiza kurroa, *Holarrhena antidysenterica*, etc
Ajmodadi churna	Sheetal Medicare Products Pvt. Ltd., Thane (MS)	Cough, blood disorder	*Apium graveolens, Trachyspermum ammi,* Embelia ribes, Cedrus deodara, Plumbago zeylanica, Piper longum, Foeniculum vulgare, Piper nigrum, Terminalia chebula
Manjistadi churna	Ritesh Pharmaceutical, Vadodara (GJ)	Alterative, blood purifier, fever	Rubia cordifolia, Terminalia chebula, Rosa damascena, Ipomoea turpenthum, *Cassia augustifolia*
Dashmula churna	Ritesh Pharmaceutical	Cough, influenza, cold, fever and headache	*Aegle marmelos*, Oroxylum indicum, Gmelina arborea, Stereospermum suaveolens, Premna integrifolia, Desmodium gangeticum, Uraria picta, Solanum indicum, Solanum xanthocarpum, Tribulus terrestris
Pipramool churna	Sheetal Medi-Care Products Pvt. Ltd.	Cough, constipation, colic, dyspepsia, diarrhoea	Piper longum root
Shivkshar Pachan churna	Sheetal Medi-Care Products Pvt. Ltd.	Digestive disorder	*Terminalia chebula,* sodium carbonate, Zingiber officinale, Piper nigrum, Piper longum, Ptychotis ajowan, Cuminum cyminum, Carum carvi, Ferula asafoetida
Swadist Virechan churna	The Zandu Pharmaceutical Works Ltd., Vapi (GJ)	Laxative, digestive disorder, cough	Solanum indicum, Glycyrrhiza glabra, *Cassia augustifolia*
Sukhsarak churna	Omkar Ayurved Mandir, Mumbai	Digestive, laxative, blood purifier, liver disorder	*Cassia augustifolia, Zingiber officinale, Emblica officinalis,* Ipomoea turpenthum, Embelia ribes, *Curcuma longa*, Glycyrrhiza glabra

were purchased from the local market of Amravati. These herbal preparations have multiple botanical ingredients in addition to some chemical substances.

Preparation of Extracts

The aqueous extract was prepared by adding 20 g of herbal preparations in 200 mL distilled water, boiled on low heat for 2 h, filtered through cloth and filtrate was evaporated to dry on sand bath. The organic solvent extracts were separately prepared by adding 20 g herbal preparation (powder) in 200 mL of respective organic solvent (acetone, ethanol and methanol) in screw-capped bottles, shaked at 190-220 rpm on a rotary shaker. After 24 h of shaking, it was filtrated, evaporated in vacuum and dried by rotary evaporator at 60°C.[12] Dried extracts were stored in labeled sterile screw capped bottles at 4°C and later used *in vitro* study.

Bacterial Cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study. The bacteria rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards[13] guideline. The pathogenic bacterial culture was inoculated into sterile nutrient broth and incubated at 37°C for 3 h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Preparation of Disc for Antibacterial Activities

The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc 5 mg of each extracts of herbal preparations. The prepared disc were dried in controlled temperature to remove excess of solvent and used for study.

Antibacterial Activity Using Disc Diffusion Method

The modified paper disc diffusion[13] was employed to determine the antibacterial activity of both aqueous and organic extract of the herbal preparations. In each test tube turbidity of inoculum was matched with McFarland turbidity standard. Inoculum was spread over the nutrient agar plate using a sterile cotton swab in order to obtain uniform microbial growth. Then the prepared antibacterial discs were kept over the lawn and pressed slightly along with positive and negative control. Ampicillin 10 mcg/disc (Hi-Media) were used as positive control while disc soaked in sterile distilled water and various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18 h at 37°C. The antibacterial activity was evaluated for 5 mg/disc and diameter of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as highly active (>20 mm), mild active (15-20 mm) and slightly active (12-15 mm) and less than 12 mm was taken as inactive. Antimicrobial Sensitivity Index (ASI) was calculated by following formula:

$$\text{Antimicrobial Sensitivity Index for herbal preparation} = \frac{\text{Total zone of growth inhibition}}{\text{No. of antimicrobial agents tested} \times \text{no. of bacterial pathogens}}$$

RESULTS AND DISCUSSION

In the present study, the samples namely Triphala churna, Hareetaki churna, Dashmula churna, Manjistadi churna, Sukhsarak churna, Ajmodadi churna, Shivkshar pachan churna, Mahasudarshan churna, Swadist Virechan churna and Pipramool churna were tested for their antibacterial properties against the various bacterial pathogens [Figure 1].

Results showed that Triphala churna possess strong antibacterial activity against *Staphylococcus epidermidis, S. aureus* while moderate against *Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and weak against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium* and *Bacillus subtilis*. Acetone and methanol extracts of Hareetaki churna was strong antibacterial agents against *S. epidermidis, S. aureus* at 5 mg/disc while moderate against *B. subtilis, P. vulgaris, S. typhi, P. aeruginosa* and mild against *K. pneumoniae, E. aerogenes, S. typhimurium* and *E. coli*. Hareetaki churna is used as mild laxative, detoxifier and improve digestive functions. Herbs used in Hareetaki churna possess antibacterial properties; *Terminalia chebula* antibacterial against *E. coli, S. aureus, P. aeruginosa, P. vulgaris, S. epidermidis, S. typhi, S. typhimurium* [14-16] and methicilin-resistant *S. aureus*.[17] Mahasudarshan churna is an excellent remedy for the complication associated with fever, liver enlargement, spleen, fatigue and nausea, etc. *Holarrhena antidysenterica* is one of the important ingredients of Mahasudarshan churna has reported antibacterial activity against *S. aureus, S. flexneri, S. typhosa, B. subtilis*.[18,19] Studies have indicated the antibacterial potential of Mahasudarshan churna against *S. typhi, S. epidermidis, E. coli, S. aureus, K. pneumoniae, P. vulgaris* and *P. aeruginosa* [Table 2]. Mahasudarshan churna is useful to control infectious diseases such as typhoid fever, intestinal infection, urinary tract infections and respiratory infection which are associated with the above-mentioned bacterial pathogens.

Methanol and acetone extracts of Ajmodadi churna were strong antibacterial agents against *S. aureus*, *S. epidermidis, S. typhi, B. subtilis, E. coli* and *P. vulgaris* while aqueous extract was less antibacterial. Ajmodadi churna is rich formulation of carminative and antispasmodic herbal ingredients. All the ingredients of Ajmodadi

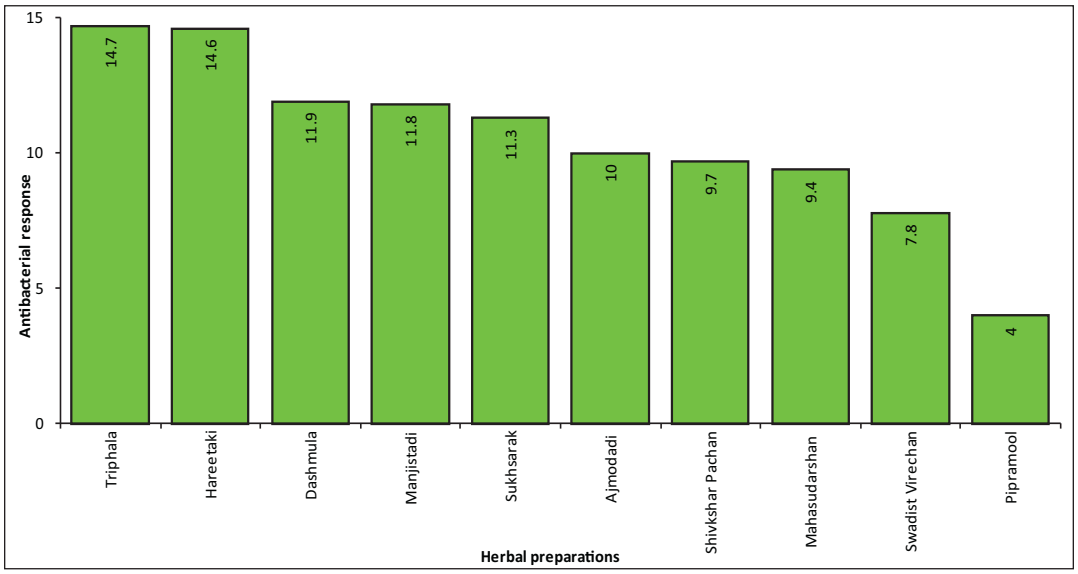

Figure 1: Antibacterial potential of herbal preparations

Table 2: Antibacterial activities of herbal preparation against various bacterial pathogens

Herbal Preparation	Solvent extract	*P. vulgaris* (MTCC426)	*S. epidermidis* (MTCC435)	*S. aureus* (MTCC96)	*E. coli* (MTCCi739)	*P. aeruginosa* (MTCC424)	*B. subtilis* (MTCC441)	*K. pneumoniae* (MTCC 109)	*S. typhi* (MTCC i733)	*E. aerogenes* (MTCC 111)	*S. typhimurium* (MTCC 98)
Triphala churna	Aqueous	20	26	27	-	21	-	-	17	-	-
	Ethanol	22	26	25	21	21	19	17	19	-	23
	Methanol	22	32	27	22	23	17	19	19	-	18
	Acetone	22	32	27	21	21	17	19	19	-	17
Hareetaki churna	Aqueous	17	32	27	-	20	17	-	17	-	-
	Ethanol	22	32	26	-	15	20	19	18	22	16
	Methanol	17	32	30	15	16	19	18	18	18	16
	Acetone	22	32	24	17	17	20	17	18	17	16
Mahasudarshan churna	Aqueous	-	17	14	16	-	-	-	17	-	-
	Ethanol	19	18	17	20	16	-	15	21	12	-
	Methanol	14	20	19	17	14	14	15	23	-	-
	Acetone	16	24	23	20	15	15	21	20	-	-
Ajmodadi churna	Aqueous	15	24	30	18	15	15	16	20	13	12
	Ethanol	16	20	23	14	13	18	15	15	14	12
	Methanol	18	24	30	16	15	21	14	21	15	13
	Acetone	16	25	25	17	16	17	13	17	12	12
Sukhsarak churna	Aqueous	14	27	30	18	16	17	22	18	12	20
	Ethanol	15	26	29	17	14	18	17	14	14	17
	Methanol	16	29	32	20	14	16	18	16	13	17
	Acetone	18	25	29	19	15	18	13	15	14	14
Swadist Virechan churna	Aqueous	14	20	18	13	15	15	12	-	-	-
	Ethanol	17	25	25	-	16	18	-	-	-	-
	Methanol	19	33	31	-	14	21	-	-	-	-
	Acetone	18	30	26	14	16	20	-	13	-	-
Manjistadi churna	Aqueous	15	25	26	18	16	23	13	15	14	15
	Ethanol	17	25	24	15	17	20	17	17	14	17
	Methanol	15	22	26	16	19	18	18	16	16	19
	Acetone	15	25	27	17	15	20	12	13	15	17
Dashmula churna	Aqueous	17	18	21	19	17	18	16	17	14	15
	Ethanol	19	20	20	16	15	15	17	20	16	17
	Methanol	16	20	24	18	15	16	17	18	16	16
	Acetone	20	21	24	16	17	18	17	20	14	15
Pipramool churna	Aqueous	15	16	15	15	-	14	-	-	-	-
	Ethanol	13	17	18	19	-	17	-	-	-	-
	Methanol	16	18	18	16	-	14	-	-	-	-
	Acetone	16	15	16	17	-	17	-	-	-	-
Shivkshar Pachan churna	Aqueous	15	24	25	12	18	15	15	14	14	16
	Ethanol	17	20	25	13	21	18	15	15	14	16
	Methanol	18	20	24	16	22	16	15	15	16	16
	Acetone	17	23	24	16	20	16	14	14	15	17
Positive control	Ampicillin (10mcg/disc)	16	25	24	11	16	18	30	18	14	19

(Quantity per disc- 5 mg) (Zone of inhibition in mm average of three reading)

churna such as *Trachyspermum ammi* is carminative, antispasmodic and antibacterial against *E. coli*, *S. aureus* and *P. aeruginosa*.[20,21] *Plumbago zeylanica* is antibacterial against *E. coli* and *P. aeruginosa*.[22] Sukhsarak churna is a laxative and blood purifier, useful in digestive and liver disorder. In present study it was found strong antibacterial against *S. aureus, S. epidermidis, B. subtilis, S. typhimurium, E. coli, K. pneumoniae, S. typhi* and *P. vulgaris*. Methanol extract of Swadist Virechan churna was a strong antibacterial agent against *S. aureus, B. subtilis, P. vulgaris, P. aeruginosa* and *B. subtilis* [Figure 1]. The use of Swadist Virechan churna may be helpful to reduce bacterial infections caused by above test pathogens. Manjistadi churna indicated as alterative and blood purifier, useful in fever. Study indicated that Manjistadi churna was antibacterial against *S. epidermidis, S. aureus, B. subtilis, S. typhimurium, E. coli, P. aeruginosa, K. pneumonia, P. vulgaris, S. typhi* and *E. aerogenes*. Dashmula churna is a herbal preparation of 10 important medicinal plants [Table 1]. Study also proved the antibacterial potential of Dashmula churna against *S. aureus, S. epidermidis, P. vulgaris, S. typhi, B. subtilis, E. coli, K. pneumonia, E. aerogenes* and *P. aeruginosa* and useful in treatment of the bacterial infections [Table 2]. Almost all the ingredients of Dashmula churna were reported as potent antibacterial agent against various bacterial pathogens. *Aegle marmelos*, an important ingredient of Dashmula churna root, have antibacterial properties against *V. cholerae*, *E. coli* and *Shigella* sp.[23] Pipramool churna was also showed significant antibacterial activity against *S. aureus, S. epidermidis, E. coli, B. subtilis* and *P. vulgaris*. Shivkshar Pachan churna is the fortified form of Hingwashtak churna. *Ferula asafetida* is an important ingredient of Shivkshar Pachan churna and reported as antibacterial agent against *Bacillus megaterium, B. subtilis, L. acidophilus, M. luteus, S. epidermidis, S. aureus, E. coli, S. typhi* and *S. flexneri*.[24,25] Present study indicated that both the aqueous and organic extracts of Shivkshar Pachan churna is potent antibacterial agent against *S. aureus, S. epidermidis, B. subtilis, P. aeruginosa* and *P. vulgaris*. Thus clinically used herbal preparations proved to posses antibacterial potential against *S. aureus, S. epidermidis, P. aeruginosa, E. coli, P. vulgaris, K. pneumoniae* and *S. typhimurium*. Hence diseases caused by test organism such as skin infections (impetigo, folliculitis), invasive diseases (wound infections, osteomyelitis, bacteremia), wound infection, urinary tract infections, endocarditis, septicemia, respiratory tract infection, eye infections, etc. may be prevented or controlled by the use of above tested ayurvedic preparations.

CONCLUSIONS

Our findings suggested that, ayurvedic herbal preparations extracts have great potential as antimicrobial activity against enteric bacterial pathogens and they can be used in the treatment of infectious diseases. The data obtained in these studies justify the use of these ayurvedic herbal preparations in medical practice by majority of the populations in India. The study also supports the use of these herbal preparations not only as the dietary supplement but also as agent to prevent or control the enteric bacterial infections.

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