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SCREENING OF SOME HERBAL PREPARATIONS USED IN INDIAN AYURVEDIC MEDICINE FOR ITS ANTIBACTERIAL POTENTIALS

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ABSTRACT

Keywords:

Antibacterial activity,
Ayurvedic preparations,
Enteric bacterial pathogens

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Ayurvedic medicine plays a crucial role in healthcare and serves the health need of a vast majority of people in developing countries. Some clinically used ayurvedic herbal preparations were investigated (solvent extract preparation) 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*. In present study Shatawaryadi churna, Panchasakar churna, Talisadi churna, Dashmula churna and Manjistadi churna were potent antibacterial against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Study suggested that these herbal preparations may be useful as an alternative medicine in the treatment of enteric bacterial infection.

INTRODUCTION: Herbal medicines are plant derived material or preparations, which contain raw or processed ingredient from one or more plants or its parts, with therapeutic value and used as dietary supplements to fight or prevent common disease in various systems of medicine such as Ayurveda, Unani and Siddha ¹. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities.

The traditional herbal combinations and extracts are known to improve health by combating or preventing microbial infections and curing various ailments and diseases ^{2,3}. The plant-derived medicines are relatively safer than synthetic drugs and offering profound therapeutic benefits by providing alternative and effective treatment for chronic disorders and various diseases ^{4,5}. More than 1500 herbal preparations are sold in India as dietary supplements or ethnic traditional medicine to treat the diseases but only a few of them have been scientifically explored for its antibacterial potentials ^{6,7}.

The most frequently used herbal preparations is churna; a preparations comprising of fine powders of medicinal plants and may be single or in combination. Combinations of medicinal plants may increases the antimicrobial spectrum and potency of activity. Enteric or diarrhoeal infections account for high proportion of health problems in the developing countries and

contribute to the death of 3.3 to 6.0 million children annually. Enteric bacteria such as *Salmonella sp.*, *Shigella sp.*, *Proteus sp.*, *Klebsiella sp.*, *E. coli*, *Pseudomonas sp.*, *Vibrio cholerae*, and *S. aureus* are major etiologic agents of sporadic and epidemic diarrhea in both children and adults ⁸. Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs. Available reports on lesser efficacy and more side effects of synthetic drugs need to search an alternative medicine 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. Therefore, there 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. At this juncture, it is of interest to determine the scientific basis for the traditional use of these herbal medicines and evaluate the antibacterial potential in significance to prevention of enteric bacterial infection.

Materials and Methods: The commercial herbal preparations as given in **Table 1** were purchased from the local market of Amravati. These herbal preparations have multiple botanical ingredients in addition to some chemical substances.

TABLE 1: HERBAL PREPARATION WITH ITS INGREDIENTS

Herbal Preparation	Manufacturer	Therapeutic use	Ingredients
Shatawaryadi churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd. Nagpur	Demulcent and nutritive tonic. used impotency, general debility, sterility	<i>Asparagus racemosus</i> , <i>Sida veronicaefolia</i> , <i>Pueraria tuberosa</i> , <i>Tribulus terrestris</i> , <i>Emblica officinalis</i>
Panchasakar churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur	Constipation and abdominal distention	<i>Terminalia chebula</i> , <i>Zingiber officinale</i> , <i>Foeniculum vulgare</i> , <i>Cassia angustifolia</i> Rock salt,

Talisadi churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur	Useful in cough and cold.	<i>Taxus baccata, Piper nigrum, Zingiber officinale, Piper longum, Bambusa arundinacea, Elettaria cardamomum, Cinnamomum zeylanicum</i>
Dashmula churna	Ritesh Pharmaceutical., Vadodara (GJ)	Cough, influenza, cold, fever and headache	<i>Aegle marmelos, Oroxylum indicum, Gmelina arborea, Stereospermum suaveolens, Premna integrifolia, Desmodium gangeticum, Uraria picta, Solanum indicum, Solanum xanthocarpum, Tribulus terrestris</i>
Manjistadi churna	Ritesh Pharmaceutical., Vadodara (GJ)	Alterative, blood purifier, fever	<i>Rubia cordifolia, Terminalia chebula, Rosa damascene, Ipomoea turpenthum, Cassia augustifolia, Sugar</i>
Bilwadi churna	Ritesh Pharmaceutical., Vadodara (GJ)	Astringent and alterative. indicated in sprue, Diarrhoea and dysentery	<i>Aegle marmelos, Cyperus rotundus, Woodfordia fruticosa, Zingiber officinale, Bombax malabaricum</i>
Jatiphaladi churna	Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur	Used in diarrhoea, dysmenorrhea, Cough.	<i>Myristica Fragrans, Syzygium aromaticum, Elettaria cardamomum, Cinnamomum tamala, Cinnamomum zeylanicum, Cinnamomum camphora, Bambusa arundinacea, Valeriana wallichii, Emblica officinalis etc</i>
Gokharu churna	Ritesh Pharmaceutical, Vadodara (GJ)	UTI infection, female disorder	<i>Pedelum murex</i>
Hingwashtak churna	Dabur India Ltd., Alwar (RJ)	Used in constipation, carminative and gastric stimulant.	<i>Zingiber officinale, Piper nigrum, Piper longum, Ptychotis ajowan, Rock salt, Cuminum cyminum Carum carvi, Ferula asafoetida</i>

Preparation of extracts: The aqueous extract was prepared by adding 20 g of herbal preparations in 200 mL distilled water, heated at 60°C for 2 h, filtered through cloth and the filtrate was evaporated on sand bath. The dry mass was then stored at 4°C. The organic solvent extract was prepared by adding 20 g herbal preparation (powder) in 200 mL of organic solvent (acetone, ethanol, and methanol) in screw-capped bottles; shake at 190-220 rpm on a rotary shaker. After 24h of shaking, the extract was filtered, evaporated in vacuum, and dried by rotary evaporator at 60°C¹¹. Dried extracts were stored in labeled sterile screw capped bottles at 4°C and later used for the *in vitro* study.

Bacterial cultures: The standard pathogenic bacterial cultures procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures 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. The inoculum size of the bacterial culture standardized according to the National committee for Clinical Laboratory Standards guideline¹². The pathogenic bacterial culture 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 standardized to 10⁵ 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 was 5 mg of each extracts of herbal preparations. The prepared discs dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial Activity using disc diffusion method:

The modified paper disc diffusion employed to determine the antibacterial activity of both aqueous and organic solvents extract of herbal preparations. Turbidity of inoculums matched with McFarland turbidity standard. Inoculums spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 µg/disc (Hi-Media, Mumbai) used as positive control while disc soaked in sterile distilled water or various organic solvents and dried placed on lawns as negative control.

The plates incubated for 18h at 37°C. The antibacterial activity evaluated for 5 mg/disc and diameter of inhibition zones measured. Experiment carried out in triplicate and the averages diameter of zone of inhibition recorded. The antibacterial activity was classified as strong (>20 mm), moderate (15-20 mm) and mild (12-15 mm) and less than 12 mm was taken as inactive. Antimicrobial Sensitivity Index (ASI) calculated by following formula¹³.

$$\text{Antimicrobial Sensitivity Index for Herbal preparation} = \frac{\text{Total zone of growth inhibition}}{\text{No. of Antimicrobial agents tested} \times \text{No. of bacterial Pathogens}}$$

RESULTS AND DISCUSSION: In the present study, herbal preparations such as Shatawaryadi churna, Panchasakar churna, Talisadi churna, Dashmula churna, Manjistadi churna, Bilwadi churna, Jatiphaladi churna, Gokharu churna, and Hingwashtak churna were screened for antibacterial potential against enteric bacterial pathogens. They exhibited significant antibacterial activity against *S. epidermidis*, *S. aureus*, *P. vulgaris*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, *E. aerogenes*, *S. typhimurium* and *S. typhi*.

As per antibacterial sensitivity index of herbal preparations (ASI) as shown in **fig. 1**, it was observed that the Shatawaryadi churna, Panchasakar churna, Talisadi churna, Dashmula churna and Manjistadu churna were potent antibacterial where as Bilwadi churna, Jatiphaladi churna, sukhsarak churna, Gokharu churna and Hingwashtak churna showed significant antibacterial activity against enteric bacterial pathogens. The Shatawaryadi churna, Panchasakar churna and Talisadi churna are potent antibacterial against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *S. typhimurium*, *K. pneumoniae*, *E. aerogenes* *P. vulgaris*, *B. subtilis* *S. typhi* and *E. coli* (**Table 2**).

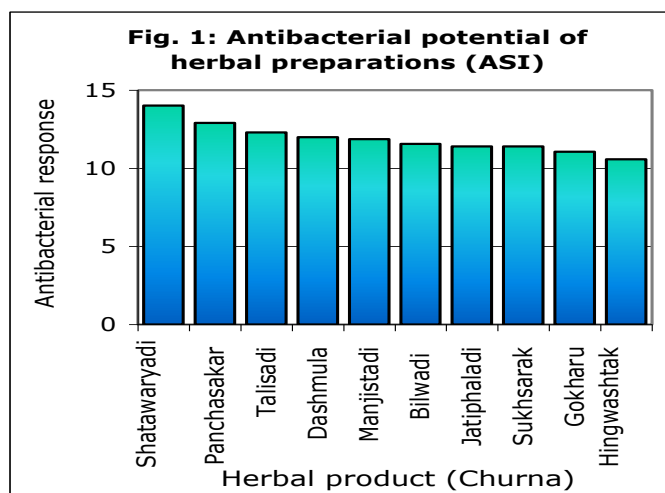


FIG. 1: ANTIBACTERIAL POTENTIAL OF HERBAL DRUGS (ASI)

Asparagus racemosus is important ingredients (Table 1) of Shatawaryadi churna is aphrodisiac, demulcent, nutritive, coolant, diuretic, alterative, useful in piles, eye infection, uterine disorder etc¹⁴. Uma *et al*,¹⁵ reported antimicrobial properties of *A. racemosus* against enterotoxigenic *E. coli*, enteropathogenic *E. coli*, *S. typhimurium*, *S. enteritidis*, *S. dysenteriae*, *S. flexineri*, *K. pneumoniae* and *P. aeruginosa*. *Emblica officinalis* exhibited potent antibacterial activity against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi* A, *S. paratyphi* B and *S. marcescens*¹⁶.

All the herbs used in Panchasakar churna possess antibacterial properties; Lo-Cantore *et al*,¹⁷ reported that *Foeniculum vulgare* and *Zingiber officinale* was antibacterial against *E. coli* and *B. megaterium*. *Terminalia chebula* exhibited antibacterial activity against enteric bacterial pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *S. epidermidis*, *S. typhi*, *S. typhimurium*^{18,19}. Talisadi churna is very useful respiratory discomfort and cough, acute exacerbation of asthma chronic and allergic bronchitis and content

shunthi and pippali. Shunthi is known as Vishvabhaishjya (an herb useful in all diseases) and pippali are rejuvenator for the respiratory and digestive system. *Piper longum* is antibacterial against *E. coli*, *P. aeruginosa*, *B. cereus*, *K. pneumoniae*, *S. typhi*, *S. marcescens*, *S. dysenteriae* and *S. aureus*. *Taxus baccata* (Talispatra) is antibacterial against *S. typhimurium* and *P. syringae*²⁰.

TABLE 2: ANTIBACTERIAL POTENTIAL OF HERBAL PREPARATIONS

Herbal Preparation	Extract	<i>P. vulgaris</i> (MTCC 426)	<i>S. epidermidis</i> (MTCC 435)	<i>S. aureus</i> (MTCC 96)	<i>E. coli</i> (MTCC 739)	<i>P. aeruginosa</i> (MTCC 424)	<i>B. subtilis</i> (MTCC 441)	<i>K. pneumoniae</i> (MTCC 109)	<i>S. typhi</i> (MTCC733)	<i>E. aerogenes</i> (MTCC 111)	<i>S. typhimurium</i> (MTCC 98)	ASI Antibacterial Sensitivity Index
Shatawaryadi churna	Aqueous	17	24	20	18	12	21	18	21	16	18	14
	Acetone	19	25	24	20	14	22	20	23	16	20	
	Ethanol	17	25	27	20	13	20	21	22	15	18	
	Methanol	19	25	27	20	16	21	21	23	17	20	
Panchasakar churna	Aqueous	15	24	30	12	16	22	13	15	16	13	12.9
	Acetone	16	25	32	11	16	18	22	22	14	21	
	Ethanol	16	25	28	11	15	18	18	17	13	19	
Talisadi churna	Methanol	18	27	32	19	20	20	17	21	15	15	12.2
	Aqueous	12	25	20	20	16	17	18	22	15	11	
	Acetone	17	27	21	23	15	18	17	20	13	13	
	Ethanol	14	22	18	17	14	17	20	18	16	15	
Dashmula churna	Methanol	19	28	24	22	19	20	22	23	16	15	11.9
	Aqueous	17	18	21	19	17	18	16	17	14	15	
	Acetone	20	21	24	16	17	18	17	20	14	15	
	Ethanol	19	20	20	16	15	15	17	20	16	17	
Manjistadi churna	Methanol	16	20	24	18	15	16	17	18	16	16	11.8
	Aqueous	15	25	26	18	16	23	13	15	14	15	
	Acetone	15	25	27	17	15	20	12	13	15	17	
	Ethanol	17	25	24	15	17	20	17	17	14	17	
Bilwadi churna	Methanol	15	22	26	16	19	18	18	16	16	19	11.5
	Aqueous	20	25	23	17	16	17	15	20	13	16	
	Acetone	21	27	25	18	16	17	15	22	15	14	
	Ethanol	21	23	24	17	17	16	15	20	14	16	
Jatiphaladi churna	Methanol	22	26	25	19	18	16	19	22	15	16	11.3
	Aqueous	17	23	27	17	14	17	13	17	20	17	
	Acetone	18	25	27	20	15	18	15	13	20	18	
	Ethanol	20	27	26	20	17	18	15	16	18	17	
	Methanol	17	23	22	18	15	17	15	13	20	19	

Gokharu churna	Aqueous	22	22	21	18	14	-	14	15	13	13	11
	Acetone	21	20	21	22	16	14	17	18	16	15	
	Ethanol	21	21	22	18	16	13	15	16	14	13	
	Methanol	20	24	21	20	17	14	16	15	14	16	
Hingwashtak churna	Aqueous	15	20	22	17	14	-	16	17	16	13	10.5
	Acetone	20	23	24	18	15	13	20	20	17	13	
	Ethanol	16	20	22	20	15	13	17	19	14	14	
	Methanol	20	24	23	21	16	15	15	17	21	14	

(Zone of inhibition of growth in mm, Average of 3 reading)

Dashmula churna found antibacterial against *S. aureus*, *S. epidermidis*, *P. vulgaris*, *S. typhi*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* (fig. 2). Ingredients of Dashmula churna possess antibacterial properties. Mazumder *et al*,²¹ reported anti-diarrhoeal properties of *Aegle marmelos* root against *V. cholerae*, *E. coli* and *Shigella* sp. Manjistadi churna and Bilwadi churna was antibacterial against all tested bacterial pathogens *viz.* *S. epidermidis*, *S. aureus*, *B. subtilis*, *S. typhimurium*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, *S. typhi*, and *E. aerogenes*. Manjistadi churna might be useful in prevent the bacterial infections such as staphylococcal infections, enteric infections, urinary tract infections etc. All the ingredients of these churna possess antibacterial properties; *Rubia cordifolia* is clinically used for the purification of blood by the physicians of the Indian System of Medicine. It possesses antiinflammatory, immuneregulator, anticancer, anticlotting properties.

Aegle marmelos antibacterial against *E. coli*, *Klebsiella* sp, *Citrobacter* sp, *Enterobacter* sp, *S. typhi*, *S. paratyphi*, *Shigella* sp, *P. vulgaris*, *P. mirabilis* and *Pseudomonas* sp²². Jatiphaladi churna is antibacterial against *S. epidermidis*, *S. aureus*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, *P. vulgaris* and *E. aerogenes*. *Myristica fragrans* is important ingredients of Jatiphaladi churna and it is antibacterial against multi-drug resistant *S. typhi*, *E. coli*, serotypes of *Salmonella*, *L. monocytogenes* and *A. hydrophila*¹⁸. Gokharu churna is strong antibacterial against *S. aureus*, *S. epidermidis*, *P. vulgaris*, *E. coli*, *P.*

aeruginosa, *K. pneumoniae* and *S. typhi*. Gokharu churna is single plant preparation, (*Tribulus terrestris*), used in urinary tract infection, urinary calculosis, burning in micturation, urinary affections, gout, and kidney diseases and also have aphrodisiac, diuretic, demulcent properties. *Tribulus terrestris* is reported antibacterial against *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa* and antifungal against *C. albicans*²³. Hingwashtak churna was antibacterial against *S. aureus*, *S. epidermidis*, *P. vulgaris*, *B. subtilis*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* (Fig. 2). An ingredient of Hingwashtak churna possesses antibacterial activity. Rahman *et al*,²⁴ reported antimicrobial activities of *Ferula assafoetida* against *B. megaterium*, *B. subtilis*, *L. acidophilus*, *M. luteus*, *S. epidermidis*, *S. aureus*, *V. cholerae*, *E. coli*, *S. typhi* and *S. flexneri*.

According to antibacterial sensitivity index of herbal preparations (ASI) as shown in fig 1, it was observed that the Shatwaryadi churna, Panchsakar churna, Talisadi churna, Dashmula churna and Manjistadu churna were potent antibacterial where as Bilwadi churna, Jatiphaladi churna, sukhsarak churna, Gokharu churna and Hingwashtak churna showed significant antibacterial activity against enteric bacterial pathogens. From the study, it was concluded that, the herbal preparation studied were strong antibacterial agent against bacteria associated with dysentery, diarrhoea, gastroenteritis, urinary tract infection, invasive disease, skin infections (impetigo, folliculitis), endocarditis, septicemia, respiratory tract infection, eye infections etc.

Scientific evaluation of these herbal preparations gives better information regarding the antibacterial efficacy of herbal medicine available in India. This study supports the use of these herbal

preparations in combating or controlling the bacterial infection.

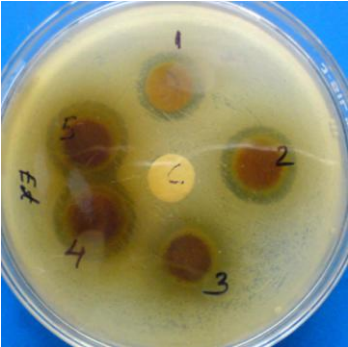
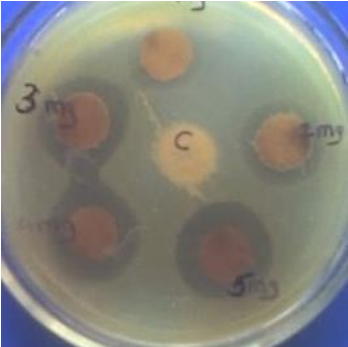
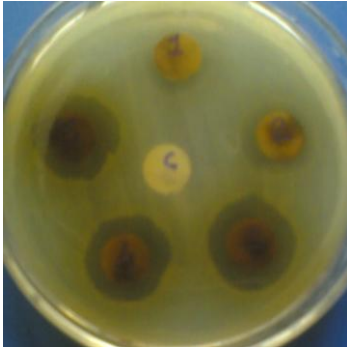
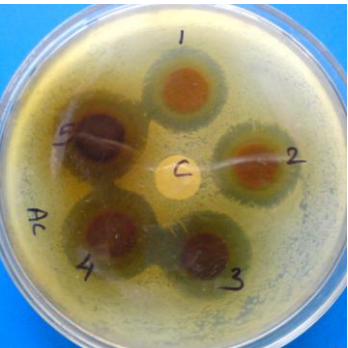
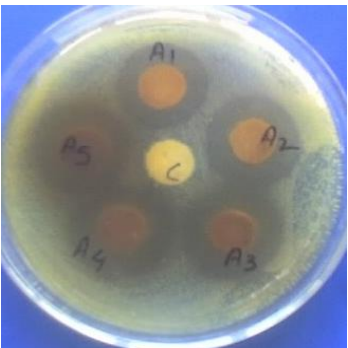
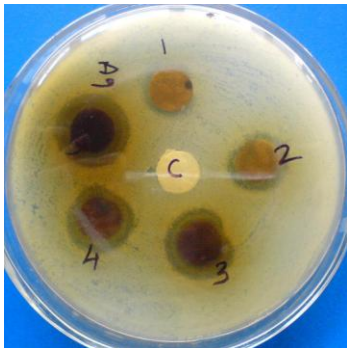
		
<p>Antibacterial activity of ethanol extract of Shatawaryadi churna against <i>P. vulgaris</i></p>	<p>Antibacterial activity of methanol extract of Panchasakar churna against <i>E. coli</i></p>	<p>Antibacterial activity of aqueous extract of Dashmula churna against <i>P. vulgaris</i></p>
		
<p>Antibacterial activity of Acetone extract of Manjistadi churna against <i>S. epidermidis</i></p>	<p>Antibacterial activity of acetone extract of Jatiphaladi churna against <i>S. aureus</i></p>	<p>Antibacterial activity of aqueous extract of Hingwashtak churna against <i>S. aureus</i></p>

FIG. 2: PHOTO PLATE SHOWING ANTIBACTERIAL ACTIVITY OF HERBAL PREPARATION AGAINST BACTERIAL PATHOGENS

CONCLUSION: Our findings suggest that, ayurvedic herbal preparations extracts have great potential as antimicrobial agent against many enteric bacterial pathogens and can be used in the treatment of infectious diseases. Scientific evaluation of these herbal preparations gives better information regarding the antibacterial

efficacy of herbal medicine available in India. This study 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. Further research is underway to isolate the compounds responsible for the antibacterial activity.

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