



ANTIBACTERIAL POTENTIAL OF SOME HERBAL PREPARATION: AN ALTERNATIVE MEDICINE IN TREATMENT OF ENTERIC BACTERIAL INFECTION

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ABSTRACT

Ayurvedic herbal preparations commonly used in ayurvedic system of medicine in treatment of various infectious diseases such as Pashanbhed churna, Arjuna churna, Bilba churna, Amla churna, Gokharu churna, Panchasakar churna, Trikatu churna, Avipattikar churna, Chandanadi churna, Pushyanug churna were investigated for antibacterial potential by disc diffusion method against 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*. The study showed that Chandanadi churna, Bilba churna, Amla churna and Pushyanug churna were effective against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Hence, it is suggested that these herbal preparations not only supplement of the diet but also an alternative in the treatment and control of enteric bacterial infection.

Keywords: Herbal medicine, Ayurvedic preparations, Antibacterial activities, Ayurvedic churna, Enteric infections

INTRODUCTION

The ayurvedic system of medicine has described various herbal formulations in the treatment of diseases, which play an important role in modern health care and curing various ailments and diseases^{1,2}. The uses of herbal medicines are increasing as dietary supplements to fight or prevent common diseases³. It has great demand for primary health care because plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc. are thought to be non-toxic, have less side effects and easily available at affordable cost^{4,5}. The most frequently used type of herbal preparations is churna. Churn's are preparations comprising of fine powders of medicinal plants and may be single or in combination. The combinations of medicinal plants may increases the antimicrobial spectrum and potency.

Enteric or diarrhoeal infections are major public health problems in developing countries and contribute to the death of 3.3 to 6.0 million children annually. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae*, and *S. aureus*, which are major etiologic agents of sporadic and epidemic diarrhea both in children and in adults⁶. 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 as alternative medicine⁷⁻⁹. There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacteria. However, a limited numbers antimicrobial activity of *in vitro* studies on herbal preparation has been published; it has not been determined whether they are superior or equivalent to antibiotics. Therefore, scientific evaluation of these herbal preparations is the need of hour to check their antimicrobial activity and support their use as alternative medicine in treatment of enteric infections. Hence an attempt was made, to screen the antibacterial properties of herbal preparation available in India with significance in 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.

Preparation of extracts: The aqueous extract was prepared by adding 20g of herbal preparations in 200mL distilled water, boiled on low heat for 2h, filtered through cloth and filtrate was evaporated

to dry on sand bath. The dry mass was then sterilized and stored at 4°C. The organic solvent extract was prepared by adding 20g herbal preparation (powder) in 200mL of organic solvent (acetone, ethanol and methanol) in screw-capped bottles, shaken at 190-220 rpm on a rotary shaker. After 24h of shaking, it was filtrated, evaporated in vacuum and dried by rotary evaporator at 60°C¹⁰. 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 (Table 2). The bacteria rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18h 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 (NCCLS, 2002) guideline¹¹. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37°C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10⁵ CFU/mL with the help of SPC and Nephro-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 1, 2, 3, 4 and 5mg 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 (NCCLS, 2000) was employed to determine the antibacterial activity of both aqueous and solvent extract of herbal preparations. 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 get a uniform microbial growth. Then the prepared antibacterial disc 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 solvent and dried were placed on lawns as negative control. The plates were incubated for 18h at 37°C. The antibacterial activity was evaluated for 5mg/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 (>20mm), mild active (15-20mm) and slightly active (12-15mm) and less than 12mm was taken as inactive. Antimicrobial Sensitivity Index (ASI) was calculated by following formula

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

Table 1: Herbal preparation tested for antibacterial potential

| Herbal preparations | Manufacturer | Therapeutic use | Ingredients as per listed on package |
|---------------------|--|--|---|
| Pashanbhed churna | Dave Ayurved Bhavan, Panvel Mumbai | Diuretic, diarrhoea, cough, pulmonary affections and fever | <i>Bergenia ligulata</i> |
| Arjuna churna | United Pharmacy Pune (MS) | Skin disease, dysentery, syphilis, fever, cough | <i>Terminalia arjuna</i> |
| Bilba churna | Sheetal Medi-Care Products Pvt. Ltd. Thane (MS) | Constipation, typhoid, Intestinal disorder, diarrhoea, dysentery | <i>Aegle marmelos</i> |
| Amla churna | Ritesh Pharmaceutical Vadodara (GJ) | Oral disease, dental carries, anemia, cold, fever, constipation, digestive disorder and liver problems | <i>Embilica officinale</i> |
| Gokharu churna | Ritesh Pharmaceutical Vadodara (GJ) | UTI infection, female disorder | <i>Pedelum murex</i> |
| Panchasakar churna | Shree Baidyanath Ayurved Bhavan Pvt. Ltd. Nagpur | Constipation and abdominal distention | Rock salt, <i>Terminalia chebula</i> , <i>Zingiber officinale</i> , <i>oeniculum vulgare</i> , <i>Cassia angustifolia</i> |
| Trikatu churna | Ritesh Pharmaceutical Vadodara (GJ) | Digestive, tonic, stimulant. | <i>Piper longum</i> , <i>Piper nigrum</i> , <i>Zingiber officinale</i> |
| Avipattikar churna | Shree Baidyanath Ayurved Bhavan Pvt. Ltd. Nagpur | Purgative, carminative, hyperacidity, indigestion | <i>Zingiber officinale</i> , <i>Piper nigrum</i> , <i>Piper longum</i> , <i>Terminalia chebula</i> , <i>Terminalia belerica</i> , <i>Emblica officinalis</i> , <i>Cyperus rotundus</i> , <i>Elettaria cardamomum</i> |
| Chandanadi churna | Shree Baidyanath Ayurved Bhavan Pvt. Ltd. Nagpur | Urinary infection, antiseptic, gonorrhoea, cystitis, genito-urinary affections | <i>Santalum album</i> , <i>Acacia arabica</i> , <i>Syzygium cumini</i> , <i>Magnifera indica</i> , <i>Ptychotis ajowan</i> , <i>Tinosporia cordifolia</i> |
| Pushyanug churna | Shree Baidyanath Ayurved Bhavan Pvt. Ltd. Nagpur | Alterative, stringent, diuretic, tonic, leucorrhoea and other female complication | <i>Cyperus rotund</i> , <i>Cissampelos pareira</i> , <i>Syzygium cumini</i> , <i>Symplocos racemosa</i> , <i>Magnifera indica</i> , <i>Saxifera ligulata</i> Extract of <i>berberis aristata</i> , <i>Hibiscus sabdariffa</i> etc |

RESULTS AND DISCUSSION

In the present study, available herbal preparations were screened for antibacterial activity against *S. epidermidis*, *S. aureus*, *P. vulgaris*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, *E. aerogenes*, *S. typhimurium* and *S. typhi*. Among them Chandanadi churna, Bilba churna, Amla churna and Pushyanug churna were effective against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Antibacterial activity of herbal preparations (Table. 2), It was observed that Pashanbhed churna and Arjuna churna was strong antibacterial against *S. epidermidis*, *S. aureus* and moderate against *P. vulgaris* and mild against *E. aerogenes*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. typhimurium*. Pashanbhed churna is single plant preparation of *Bergenia ligulata* useful in diarrhea, cough, fever and pulmonary affections¹², ingredients of Arjuna churna; *Terminalia arjuna* possess antibacterial properties against *S. aureus*, *S. epidermidis*, *S. mutans*, *B. subtilis*, and *M. smegmatiss*, *K. pneumoniae* and *E. faecalis*¹³. These finding indicated the presence of antibacterial principle in Arjuna churna and Pashanbhed particularly strong antibacterial against *S. epidermidis*.

Bilba churna is an important ingredient of *Aegle marmelos*. Its various plant parts useful in constipation, diarrhea, and fruit are used in chronic diarrhoea and dysentery and have antibacterial and antifungal properties¹⁴. Amla churna contents *Embilica officinalis* are used to treat hemorrhage, diarrhea and dysentery¹⁵, it is antibacterial against *E. coli*, *K. pneumoniae*, *K. ozaenae*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, and *S. marescens*¹⁶. Similar results were observed in present study, Bilba churna and Amla churna found antibacterial against *S. aureus*, *S. epidermidis*, *E. coli* and *S. typhimurium*, *P. aeruginosa*, *S. typhi*, *P. vulgaris*, *B. subtilis*, *K. pneumonia* and *E. aerogenes*. Gokharu churna,

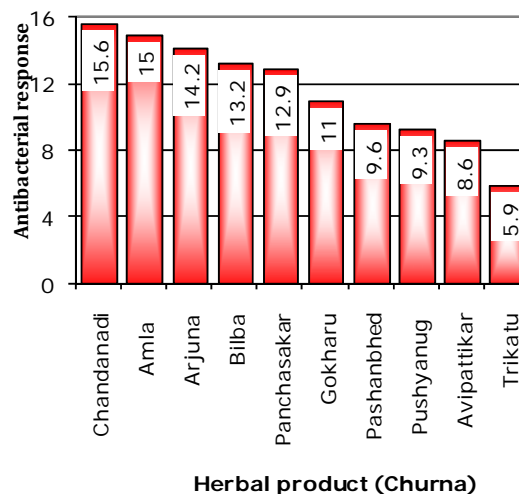


Fig. 1: Antibacterial response of herbal preparations

Panchasakar churna and Trikatu churna was strong antibacterial against *S. aureus*, *S. epidermidis*, *P. vulgaris*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi*. Ingredients of this preparation *Tribulus terrestris* is reported antibacterial against *E. coli*, *S. aureus* *B. cereus*, *P. aeruginosa* and antifungal against *C. albicans*¹⁷. *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*^{18,19,13}. Trikatu churna is an herbal preparation

contents three medicinal plants; *Piper longum*, *Piper nigrum*, *Zingiber officinale* (fig.1). Trikatu churna possess wide range of antibacterial properties against *S. epidermidis*, *S. aureus*, *P. vulgaris*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae*. Avipattikar is preparation of *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Terminalia chebula* etc. It was proved potent antibacterial agent against *S. epidermidis*, *S. aureus*, *P. vulgaris*, *K. pneumoniae*, *S. typhi*, *S. typhimurium*. Chandanadi churna and Pushyanug churna was potent antibacterial against *P. vulgaris*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *S. aureus*. *Santalum album* and *Holarrhena antidyserterica* are important ingredients of Chandanadi churna and Pushyanug churna and reported as antibacterial against *B. subtilis*, *S. epidermidis* *P. pseudoalcaligenes*, *P. vulgaris* and *S. typhimurium*^{20,10}.

From the study it was also observed that the compound herbal preparation was more antibacterial while single herbal preparation least antibacterial against bacterial pathogens this may be due to its synergistic or combine effects of medicinal plants. Chandanadi churna, Bilba churna, Amla churna and Pushyanug churna were strong antibacterial against *Staphylococcus epidermidis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* which are associated with different type of infections including urinary tract infection, wound infection, otitis media, gastroenteritis, and meningitis. Study proved its traditional applications are safe as ayurvedic medicine and therefore may be useful to prevent or control the enteric infections.

Table 3: Antibacterial activity of herbal preparations against various bacterial pathogens at 5 mg/disc (Zone of inhibition of growth in mm average of 3 reading)

| Herbal preparations | Solvent extracts | | | | | | | | | | |
|---------------------|----------------------------|---------------------------------|------------------------------------|------------------------------|-----------------------------|-----------------------------------|---------------------------------|-----------------------------------|-------------------------------|----------------------------------|-----------------------------------|
| | | <i>P. vulgaris</i> (MTCC426) | <i>S. epidermidis</i> (MTCC435) | <i>S. aureus</i> (MTCC96) | <i>E. coli</i> (MTCC139) | <i>P. aeruginosa</i> (MTCC424) | <i>B. subtilis</i> (MTCC441) | <i>K. pneumoniae</i> (MTCC109) | <i>S. typhi</i> (MTCC1733) | <i>E. aerogenes</i> (MTCC111) | <i>S. typhimurium</i> (MTCC98) |
| Panchasakar churna | Aqueous | 15 | 24 | 30 | 12 | 16 | 22 | 13 | 15 | 16 | 13 |
| | Ethanol | 16 | 25 | 28 | 11 | 15 | 18 | 18 | 17 | 13 | 19 |
| | Methanol | 18 | 27 | 32 | 19 | 20 | 20 | 17 | 21 | 15 | 15 |
| | Acetone | 16 | 25 | 32 | 11 | 16 | 18 | 22 | 22 | 14 | 21 |
| Pushyanug churna | Aqueous | 18 | 29 | 18 | 14 | 15 | 13 | 12 | - | 13 | 12 |
| | Ethanol | 16 | 25 | 24 | 13 | 16 | 13 | - | - | 15 | 12 |
| | Methanol | 18 | 27 | 27 | 12 | 15 | 15 | 11 | - | 19 | 15 |
| | Acetone | 20 | 26 | 25 | 14 | 16 | 15 | 12 | - | 16 | 15 |
| Pashanbhed churna | Aqueous | 17 | 28 | 22 | 16 | 16 | 12 | - | - | 16 | 12 |
| | Ethanol | 17 | 29 | 19 | 16 | 16 | 12 | - | - | 15 | 12 |
| | Methanol | 20 | 29 | 22 | 13 | 16 | 14 | - | - | 17 | 14 |
| | Acetone | 19 | 31 | 20 | 17 | 16 | 16 | - | - | 18 | 13 |
| Gokharu churna | Aqueous | 22 | 22 | 21 | 18 | 14 | - | 14 | 15 | 13 | 13 |
| | Ethanol | 21 | 21 | 22 | 18 | 16 | 13 | 15 | 16 | 14 | 13 |
| | Methanol | 20 | 24 | 21 | 20 | 17 | 14 | 16 | 15 | 14 | 16 |
| | Acetone | 21 | 20 | 21 | 22 | 16 | 14 | 17 | 18 | 16 | 15 |
| Trikatu churna | Aqueous | 17 | 14 | 15 | 13 | 17 | 18 | 13 | - | - | - |
| | Ethanol | 17 | 19 | 17 | 12 | 16 | 17 | 12 | - | - | - |
| | Methanol | 16 | 18 | 17 | 12 | 16 | 16 | 13 | - | - | - |
| | Acetone | 16 | 18 | 19 | 14 | 16 | 17 | 14 | - | - | - |
| Amla churna | Aqueous | 17 | 20 | 24 | 20 | 18 | 17 | 15 | 16 | 15 | 18 |
| | Ethanol | 20 | 20 | 28 | 19 | 18 | 16 | 16 | 19 | 17 | 18 |
| | Methanol | 22 | 23 | 29 | 22 | 18 | 20 | 21 | 19 | 16 | 20 |
| | Acetone | 19 | 24 | 29 | 21 | 19 | 21 | 16 | 20 | 15 | 21 |
| Avipattikar churna | Aqueous | 17 | 22 | 20 | 15 | - | 12 | 14 | 15 | 12 | 17 |
| | Ethanol | 15 | 18 | 18 | 14 | - | 14 | 17 | 16 | 12 | 14 |
| | Methanol | 22 | 20 | 17 | 13 | 13 | 16 | 18 | 17 | 12 | 15 |
| | Acetone | 18 | 23 | 20 | 14 | 13 | 15 | 16 | 15 | 13 | 16 |
| Bilba churna | Aqueous | 17 | 22 | 24 | 19 | 18 | 15 | 23 | 19 | 14 | 19 |
| | Ethanol | 18 | 23 | 25 | 17 | 16 | 18 | 23 | 16 | 15 | 17 |
| | Methanol | 20 | 25 | 26 | 22 | 16 | 19 | 23 | 18 | 16 | 20 |
| | Acetone | 21 | 24 | 23 | 22 | 17 | 17 | 24 | 16 | 17 | 18 |
| Arjuna churna | Aqueous | 19 | 23 | 24 | 16 | 17 | 16 | 19 | 18 | 17 | 15 |
| | Ethanol | 21 | 25 | 23 | 20 | 19 | 18 | 20 | 19 | 17 | 15 |
| | Methanol | 21 | 25 | 26 | 23 | 21 | 18 | 21 | 21 | 20 | 17 |
| | Acetone | 20 | 25 | 27 | 22 | 19 | 20 | 22 | 17 | 16 | 15 |
| Chandanadi churna | Aqueous | 20 | 26 | 24 | 21 | 13 | 17 | 20 | 20 | 13 | 20 |
| | Ethanol | 20 | 28 | 25 | 20 | 14 | 20 | 21 | 21 | 17 | 18 |
| | Methanol | 21 | 28 | 27 | 22 | 20 | 23 | 20 | 21 | 21 | 19 |
| | Acetone | 22 | 30 | 24 | 21 | 19 | 19 | 20 | 20 | 20 | 22 |
| Negative control | Water | - | - | - | - | - | - | - | - | - | - |
| | Ethanol | - | - | - | - | - | - | - | - | - | - |
| | Methanol | - | - | - | - | - | - | - | - | - | - |
| | Acetone | - | - | - | - | - | - | - | - | - | - |
| Positive control | Ampicillin (10mcg/disc) | 16 | 25 | 24 | 11 | 16 | 18 | 30 | 18 | 14 | 19 |

CONCLUSION

The present study suggested that, selected ayurvedic herbal preparations have great potential as antimicrobial agent against enteric bacterial pathogens and they can be used as alternative medicine in the treatment of enteric bacterial. 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.

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